Chapter 4
Current Status and Future of Polio Vaccines and Vaccination

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Abstract The history of polio vaccines and their use illustrates the concept of evolution of vaccines driven by changing epidemiological and socioeconomic conditions. The development of two vaccines against poliomyelitis—inactivated Salk vaccine (IPV) and live oral Sabin vaccine (OPV)—is among the most consequential achievements of prophylactic medicine of the past century. Each with their own strengths and weaknesses, they were used over the past 50 years in different settings and different regimens and combinations. This resulted in virtual elimination of the disease in almost the entire world with the exception of a few countries. Continuation of the eradication campaign coordinated by WHO may soon result in complete cessation of wild poliovirus transmission, and poliovirus may join smallpox virus in the club of extinct pathogens. However, unlike smallpox vaccination that was stopped after the interruption of virus circulation, vaccination against poliomyelitis will have to continue into the foreseeable future, due to significant differences in the nature and epidemiology of the viruses. This chapter reviews the reasons for the need to maintain high population immunity against polioviruses, makes the case for developing a new generation of polio vaccines, and discusses their desirable properties as well as new vaccine technologies that could be used to create polio vaccines for the post-eradication environment.

4.1 Introduction

Vaccines occupy a unique place among medical biotechnology products. Among the oldest of such products, some vaccines were developed and are still manufactured using centuries-old methods. Increasing demands for safety, efficacy, and manufacturing efficiency create strong pressures to use modern technologies for vaccine manufacture requiring introduction of innovative approaches. Vaccines against poliomyelitis are among the most widely used and successful vaccines ever,

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and thus they represent a paradigm for other vaccines. Their introduction almost 60 years ago resulted in virtual elimination of the dreadful disease from the face of the Earth. This dramatic change in the epidemiology of poliomyelitis and shifts in societal perception of the risk-benefit balance triggered several important changes in polio immunization policies. Potential complete eradication of the disease in the foreseeable future may require replacement of the currently used vaccines with products having a new target profile more suitable for post-eradication environments. This process represents a clear illustration of the evolution of vaccines in response to epidemiological and socioeconomic changes and the need to continuously work on updating vaccine manufacturing technology. This chapter will review the history of polio vaccines and discuss the reasons for developing new products. It will also review some innovative approaches that are now being explored for polio vaccines and could be also used for development of other products.

4.2 Natural History of Poliomyelitis and Milestones in Discovery of Polio Vaccines

Poliomyelitis is a neurological disease that manifests itself by flaccid paralysis that follows a few days of febrile illness and in many cases lasts for the rest of the life of its victims. In the most severe bulbar cases, death ensues due to paralysis of respiratory muscles (Baker 1949). The disease was first described in the eighteenth century by a British doctor Michael Underwood (Underwood 1789), but it was known for many centuries before that, as evidenced by ancient images found in Egypt depicting typical victims of poliomyelitis. However, for most human history poliomyelitis occurred as a sporadic disease that occasionally afflicted children and young adults, giving it its other name "infantile paralysis" (Badham 1834–35; Heine 1840; Cornil 1863; Jacobi 1874–75). At the turn of the twentieth century, the nature of the disease changed, and it gradually became an epidemic disease with a global reach (Putnam and Taylor 1893; Flexner and Clark 1912–13; Frost 1913).

The reasons for this transformation were changes in socioeconomic conditions that led to improved hygiene. In the past most children were infected with poliovirus in infancy and early childhood while they were still protected by maternal antibodies and were less susceptible to the virus. Because of a very low attack rate (one out of several hundred infected individuals), this early encounter with the virus led to a relatively small number of clinical cases but left the rest of those who were exposed to the virus with a life-long immunity. Therefore, wild polioviruses were vaccinating the human population against themselves and thus restricted their own spread. With improved sanitation and hygiene, the first encounter with poliovirus occurred later when children were no longer protected by maternal antibodies, and as a result the number of paralytic cases increased. Lower population immunity created the possibility for virus to spread rapidly and cause outbreaks of increasing size and severity.

The first isolation of poliovirus was reported in 1909 by Austrian scientists Karl Landsteiner and Erwin Popper (Landsteiner and Popper 1909). At the same time Flexner and Lewis demonstrated that monkeys can be infected with the virus (Flexner and Lewis 1909) and that they can be made resistant to the virus by either passive transfer of antibodies from immune animals or active immunization (Flexner and Lewis 1910). Subsequent studies revealed that there are three distinct serotypes of poliovirus (Burnet and Macnamara 1931; Bodian et al. 1949; Kessel and Pait 1949) that belong to the human *Enterovirus* genus (Pallansch et al. 2013) within the Picornaviridae family (Racaniello 2013). These small RNA viruses contain a single molecule of positive-strand RNA of about 7,440 nucleotides inside an icosahedral protein capsid composed of 60 copies of each of four structural proteins. The virus attaches to a protein receptor called CD155 expressed on the surface of susceptible cells, penetrates the cells through endocytosis, and releases its genomic RNA into the cytoplasm to direct synthesis of all viral proteins. All poliovirus proteins are synthesized as a single precursor polypeptide chain of about 2,200 amino acids, which is then autocatalytically cleaved to generate a variety of proteins with different functions needed to synthesize viral progeny and subvert host metabolism and defense systems. Poliovirus infection is highly productive yielding thousands of infectious particles from each infected cell, which then dies and lyses; however, in some rare cases the virus may establish chronic infection. The mechanisms of chronic infection and its role in viral pathogenesis are not fully understood. This aspect will be briefly touched upon later in this chapter.

The increasingly severe nature of polio outbreaks in the twentieth century attracted the attention of both the general public and scientists who sought to develop measures against the disease. A boost to public awareness was the fact that US President Franklin D. Roosevelt had contracted poliomyelitis at the age of 39 leaving him partially paralyzed for life. Together with his friend Basil O'Connor, he helped to establish the National Foundation for Infantile Paralysis that would later become known as the March of Dimes. This charitable organization raised money to help polio victims and also to fund research leading to the prevention of the disease.

Many leading scientists became involved in the work on poliomyelitis that enabled the development of anti-polio vaccines. Demonstration that serum from convalescents can protect from poliomyelitis (Kramer et al. 1932) and that monkeys can be immunized by inactivated virus (Brodie 1934) led to the attempt to actively immunize humans (Brodie and Park 1935). These early trials were unsuccessful and several recipients of this vaccine developed paralytic disease (Leake 1935).

In 1949 a significant breakthrough was achieved by John F. Enders, Thomas H. Weller, and Fred C. Robbins who developed in vitro cell cultures and demonstrated that they could support growth of poliovirus in the laboratory (Enders et al. 1949). For this discovery that opened a route to laboratory research on poliovirus, including development of vaccines, they were awarded the 1954 Nobel Prize in physiology and medicine.

Other key studies were pursued by William Hammon and others who explored the use of serum from people immune to poliomyelitis to protect against the

disease. A large clinical study showed that gamma globulin from these sera completely protected against paralysis (Hammon et al. 1952). This provided decisive proof that humoral immunity is sufficient for protection, and therefore that creation of a vaccine that induced such an immune response might be possible.

The work on vaccines progressed in two directions. The first was led by Dr. Jonas Salk and his associates who developed a protocol for formalin inactivation of poliovirus grown in cell cultures. Under carefully controlled conditions, virus lost infectivity while retaining immunogenicity. The vaccine was administered as an intramuscular injection. The results of clinical trials of this vaccine were publicly revealed in April of 1955. They demonstrated very high protective efficacy of the vaccine, which was subsequently confirmed by its mass use that immediately followed this announcement.

Other groups were pursuing creation of live attenuated vaccines. They aimed to select strains of polioviruses that would replicate in vaccine recipients but would not be able to infect the central nervous system. Enders, Weller, and Robins demonstrated that passaging of virus in cultured cells led to reduction in its neurovirulence (Enders et al. 1952). Hilary Koprowski was developing a live vaccine based on mouse-adapted strains (Koprowski et al. 1952; Koprowski 1958). The most successful strains were developed by Albert Sabin (Sabin 1954a, b, 1955a, b). Live vaccine was administered orally by putting a drop of vaccine directly into a child's mouth or in small sugar cubes. Use of this oral polio vaccine (OPV) made from these strains was hampered by the existence of Salk's inactivated polio vaccine (IPV) and by lingering doubts about the safety of vaccine made from live virus. However, large-scale clinical studies conducted in the former Soviet Union and some other countries in Eastern Europe demonstrated its safety and high efficacy as well as low production costs and ease of administration (Chumakov 1960; Sabin 1961a). The next section of this chapter will compare properties of OPV and IPV in detail. Here we will just mention that these properties determined the ultimate overwhelming dominance of OPV in public health systems worldwide for the next 50 years. Another factor leading to increasing acceptance of OPV despite availability and high efficacy of IPV was the so-called Cutter incident (Nathanson and Langmuir 1963a, b, c; Offit 2005). Just 2 weeks after IPV licensure, it was found that some batches of the vaccine produced by Cutter Laboratories contained residual live virus that had escaped inactivation, leading to several paralytic and even lethal cases caused by vaccination. The Cutter incident had a profound and long-lasting effect on regulation of vaccines and led to creation of a legal framework for compensation of victims of vaccine-related injuries. More importantly, this tragic episode had a silver lining by opening the door to OPV that became the instrument for not only disease control but possibly for its complete eradication.

4.3 OPV vs. IPV

IPV was licensed on April 12, 1955, 10 years to the day after the passing of polio's most famous victim—Franklin D. Roosevelt. Its introduction in the USA and European countries led to a spectacular decline of the incidence of paralytic poliomyelitis. However, immunization with IPV does not induce sterilizing immunity, meaning that while being completely protected against paralysis, vaccine recipients can be successfully infected with poliovirus and pass on the virus to their contacts. In other words, IPV is not very effective in preventing spread of the virus and breaking chains of its transmission. On the other hand, immunization with OPV makes the intestinal tract of vaccine recipients refractory to subsequent infection, virus replication, and shedding of the virus in stool. Another attractive property of OPV is its ability to cause a "herd effect" by spreading the vaccine virus from a primary vaccine recipient to his/her contacts—siblings, playmates, etc.—and thus immunizing them against the disease. These are perhaps the biggest advantages of live vaccine over inactivated. Combined with some other benefits of OPV such as lower cost and easier administration, these advantages led, after licensure of OPV in the early 1960s, to a dramatic shift from the use of IPV to almost exclusive use of OPV. With the exception of three countries in Scandinavia that by then had eliminated poliomyelitis and therefore had no incentive to switch to another vaccine, all other countries replaced IPV in their immunization schedules with OPV. The additional advantages of OPV include a significantly lower production cost and ease of administration. While IPV is given through intramuscular injections and therefore requires qualified medical personnel, OPV is given orally by depositing a drop of the vaccine into the mouth of a child. Removing the need for trained medical personnel to administer vaccine is a major advantage especially in resource-limited countries. The shift from IPV to OPV was also facilitated by no-cost licensure by Sabin of his attenuated strains to any manufacturer who would agree to follow his advice on the manufacturing process. In 1972 he donated his strains and granted control of their use to the World Health Organization.

Despite several obvious advantages of OPV, its mass worldwide use revealed some troubling weaknesses. The first was discovered relatively early after reports of rare cases of paralytic poliomyelitis following administration of OPV (Chang et al. 1966; Feigin et al. 1971; Wright et al. 1977). The link between these cases of vaccine-associated paralytic polio and OPV was long suspected but hard to prove until the introduction of molecular genetic methods and nucleotide sequencing (Nottay et al. 1981). These tests unambiguously proved that vaccine-associated paralytic polio (VAPP) is caused by a mutated form of the vaccine virus that regained neurovirulent properties (reversion to virulence). The incidence of VAPP varied in different countries, but one of the most representative studies conducted in the USA showed that paralysis occurred once per roughly 600,000 first doses of the vaccine (Alexander et al. 2004). Therefore, in the USA there were 5–10 cases of VAPP per year. As long as the morbidity caused by wild polioviruses was significantly higher, this level of adverse reactions did not attract broad attention. However, at some point VAPP became the leading cause of poliomyelitis

in the country and made the continued use of OPV ethically tenuous. This point was reached in the 1990s, when a new generation of IPV became available, and made it possible for some countries to switch from OPV to sequential use of IPV and OPV and then to the exclusive use of inactivated vaccine.

Another sobering discovery that was made relatively late in the use of OPV was the realization that reverted poliovirus can not only cause paralysis in vaccine recipients and their immediate contacts but it can also establish chains of transmission in populations and cause outbreaks of paralytic polio. The first discovery of the so-called circulating vaccine-derived polioviruses (cVDPV) was made in Hispaniola in the year 2000 (Kew et al. 2002), but other earlier outbreaks caused by cVDPV were revealed retrospectively by comparing nucleotide sequences of the virus isolates (Centers for Disease Control and Prevention 2001). Since 2000, dozens of outbreaks caused by cVDPV of all three serotypes were identified (Kew et al. 2004; Centers for Disease Control and Prevention 2012). Most often, though, such outbreaks are caused by derivatives of the Sabin type 2 strain (Fig. 4.1). The largest cVDPV outbreak started in Nigeria in 2006 and is still not over at the time of this writing (Wassilak et al. 2011) and was triggered by viruses that emerged independently from multiple sources (Burns et al. 2013). The events that triggered this outbreak were first suspension of all vaccination activities for several months, followed by resumption of vaccination campaigns. A significant cohort of nonimmune children that emerged during the pause in polio immunizations may have provided a fertile ground for emergence and spread of cVDPV. Similar events took place on a much smaller scale in the former Soviet Union in the 1960s (Korotkova et al. 2003).

Yet another observation that increased doubts about continued use of OPV was the discovery of another type of vaccine-derived polioviruses, namely, those that

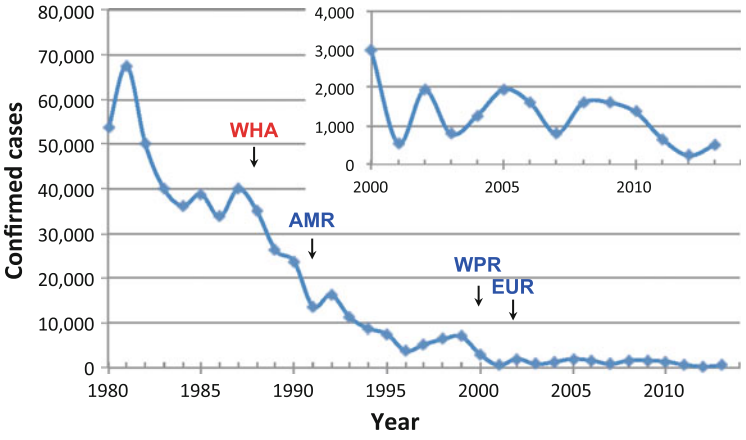

Fig. 4.1 Worldwide number of confirmed paralytic cases of poliomyelitis caused by wild polioviruses based on official WHO reports. Insert shows the incidence in the past 15 years. *Arrows* indicate the timing of the WHA decision to launch global polio eradication campaign and regional certifications in the America, Western Pacific, and European regions

were found in persons chronically infected with poliovirus (Feigin et al. 1971; Lopez et al. 1974; Davis et al. 1977; Minor 2001). Patients with some types of primary immunodeficiencies characterized by failure to produce antibodies (agammaglobulinemia) can become persistently infected with vaccine poliovirus during immunization and proceed to chronically excrete poliovirus for a prolonged period of time, often for years. Prolonged excretion of poliovirus was also observed in otherwise healthy people (Martín et al. 2004). These immunodeficiency-associated vaccine-derived polioviruses (iVDPV) may also regain virulence and in some cases were found to cause paralysis of their carriers (Hidalgo et al. 2003). Obviously, besides a threat to the patients, iVDPV are capable of reseeding the environment with virulent polioviruses and potentially restart virus circulation in regions where it has already been stopped (Minor 2009). Finally, one more type of vaccine-derived polioviruses, called ambiguous (aVDPVs), has been isolated from environmental samples (sewage, water, etc.) (Blomqvist et al. 2004; Cernáková et al. 2005; Centers for Disease Control and Prevention 2009; Roivainen et al. 2010). The origin of these viruses is unknown. However, since there is no natural reservoir for poliovirus except for humans, it is believed that aVDPVs are excreted by either unknown immunodeficient carrier(s) or are a result of cryptic circulation of cVDPV that continues undetected because of the absence of paralytic disease. In either case this phenomenon represents a significant concern, and discovery of the three types of VDPV has put to rest the previous dogma that Sabin viruses can revert only partially. It is now universally recognized that VDPVs can be as virulent as wild strains. The inevitability with which they emerge in countries using OPV has become a compelling justification for stopping OPV use in countries that eliminated transmission of wild poliovirus strains and replacing it with IPV.

The development that made the switch from OPV to IPV possible was the production in the 1980s by the Dutch National Institute for Public Health and the Environment (RIVM) of the enhanced potency IPV (eIPV) (van Wezel et al. 1984). Unlike the classical Salk vaccine that was made by formaldehyde inactivation of virus contained in harvests from cell cultures infected with poliovirus, eIPV was prepared by a more sophisticated process. First, instead of conventional monolayer cell cultures, cells were grown on a suspension of microcarrier beads in bioreactors. This resulted in a much higher cell density and increased virus yields. Second, the virus was purified from the harvest by a combination of size-exclusion and ion-exchange chromatographies and was largely free from most cellular components. As a result each dose of IPV could contain a greater amount of antigen leading to its higher potency. This new technology that was developed by a government public health institution was then quickly adopted by a number of large vaccine manufacturers and is now the basis for all IPV produced in the world. This technological breakthrough resulting from the successful interplay of public and private sectors was described in detail in an excellent review by Blume (2005).

The process of gradual replacement of OPV with eIPV is continuing as circulation of wild polioviruses is stopped in more countries and as they improve their economical circumstances making a more expensive IPV option a viable alternative to OPV. Replacement of OPV with IPV was facilitated by the introduction of

combination vaccines in which IPV is added to other antigens, including vaccines against diphtheria, tetanus, acellular pertussis vaccine, as well as hepatitis B or *Haemophilus influenzae*. This allowed IPV to be introduced without adding more injections to the existing immunization schedules. Describing further development of polio vaccines and the reasons behind it requires us to cover one of the most important public health endeavors of the past 25 years, namely, the worldwide polio eradication campaign.

4.4 Polio Eradication

The introduction of IPV in 1955 triggered a significant decline in polio incidence, and the switch to OPV in most countries in the early 1960s continued this trend so that by the next decade, polio was no longer a significant problem in developed parts of the world. However, it continued to actively spread in resource-limited countries mostly because of the inadequate vaccine coverage. The idea of polio eradication was proposed by Albert Sabin based on the absence of an animal reservoir for the virus (Sabin 1961b, c, 1965; Hampton 2009). His strategy envisioned the use of OPV in mass campaigns conducted during a short time, often just 1 day when all children in the target age group (usually between 0 and 4 or 5 years old) would receive vaccine simultaneously regardless of their prior immunization status. These campaigns, which were later called National Immunization Days (NID), were aimed at stopping circulation of wild polioviruses.

The first organized polio eradication campaign was proposed by the Pan American Health Organization in 1985, which resolved to completely eliminate polio from the Americas (de Quadros 1992, 1997). The initiative was strongly supported by the Rotary International organization that continuously remained one of the key players in the worldwide campaign, US Agency for International Development (USAID), UNICEF, Inter-American Development Bank, and other donors. In addition to the NIDs, the campaign relied on extensive epidemiological monitoring based on acute flaccid paralysis (AFP) surveillance (Andrus, de Quadros et al. 1992). AFP is the primary clinical manifestation of poliomyelitis but can also result from other infectious and noninfectious causes. Its incidence throughout the world is rather uniform (1–2 cases a year per 100,000 of population). This creates a possibility to evaluate the quality of local surveillance systems: reported rate below this level indicates the need for improved surveillance. Each case of AFP is followed up, including virological examination to confirm or reject the diagnosis of poliomyelitis. Further differentiation between wild and vaccine polioviruses and among serotypes is performed by immunological tests and nucleotide sequencing that also enables to determination of the phylogenetic relatedness of the isolates. This powerful molecular epidemiology approach helps to trace virus transmission and identify the source of virus that caused each paralytic case (Kew et al. 1990).

The campaign in the Americas was highly successful and resulted in complete elimination of polio in 1991—just 6 years after the start of the program. This prompted the World Health Assembly, the governing body of the WHO, to resolve

in 1988 that polio should be eradicated worldwide by the year 2000. The strategy was similar to that used in the Americas (Chumakov and Kew 2010). The world was divided into six regions that coordinated immunization campaigns, tracked their progress, and reported it to the WHO headquarters. Stopping wild polio circulation in each region followed by a period of extensive surveillance leads to regional certification. After all regions are certified free from circulation of wild viruses, poliovirus would be declared eradicated worldwide after 2 years with no paralytic cases or isolation of wild poliovirus from patients or the environment. During the 12 years during which global eradication was expected to be completed, there was a dramatic decline in the incidence of disease (Fig. 4.2). The number of endemic countries declined from 125 in 1988 to 20 in 2000 and to just 3 at the time of this writing. The transmission of wild type 2 polioviruses was completely interrupted in 1999, and type 3 appears to have been eliminated in 2012. The number of independent genetic lineages has significantly decreased. All these indicators suggested that the program was moving in the right direction, but progress was stalled at the turn of the century because of a variety of factors that will be discussed below. As a result 25 years after the inception of the eradication campaign, there are still three countries in which transmission has never been interrupted (Pakistan, Afghanistan, and Nigeria), and progress in some regions is compensated by unexpected outbreaks of the disease in others. In May of 2014 this prompted WHO to declare poliovirus spread a public health emergency of International concern.

The new global strategy adopted in 2013 envisions that wild polioviruses circulation will be interrupted in 2015 and that the final certification could be achieved in 2018 (WHO 2013). These optimistic projections are based on the recent progress, but since many similar predictions in the past have turned out to be incorrect, we must remain cautious.

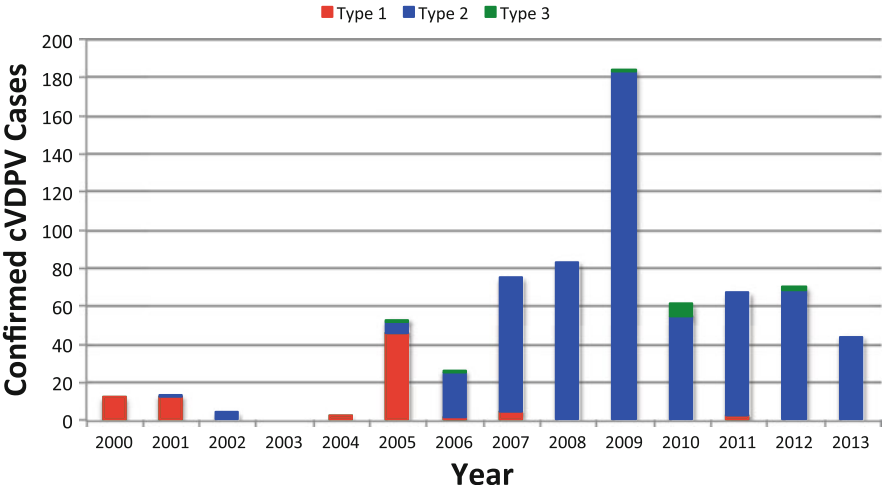

Fig. 4.2 Worldwide number of confirmed paralytic cases of poliomyelitis caused by circulating vaccine-derived polioviruses. Data from http://www.polioeradication.org

The reasons for the failure to eradicate poliovirus during the originally projected timeframe include previously unknown aspects of poliovirus biology, as well as complex social, economical, and religious factors, and the deteriorating security situation in many regions of the world. Vaccination of children, and especially conducting NIDs, is complicated if not impossible in the areas with active military conflicts. Prejudice against vaccination that exists and even promoted in some societies requires significant efforts on the part of the campaign to overcome active resistance to the immunization activities. Protracted campaigns also contribute to the fatigue of local public health workers that gradually lose faith its ultimate success. All these factors are beyond medicine or science and are difficult to overcome. In this chapter we will only review the new scientific knowledge that was derived from developments of the past 15 years that are relevant to the future strategy of dealing with polio, including creation of new vaccines.

One important factor contributing to the slowdown of progress of polio eradication was an unexpectedly low efficacy of OPV in some regions. For instance, in some states in northern India the per-dose seroconversion rate was found to be less than 10 %, requiring multiple repeated vaccinations to reach the population immunity level of 85–90 % needed to stop virus transmission (Patriarca et al. 1991; Grassly et al. 2006, 2007; O'Reilly et al. 2012). At this low level of immunogenicity, more than 15 doses of vaccine were needed to immunize most children, which takes up to 2 years. In some states in India with the most resilient circulation of wild polioviruses, every child under 5 years of age was immunized 10 times a year, bringing the total number of doses to 50 (!). Combined with extremely high birth rates, a significant susceptible population of children remained despite extraordinary immunization efforts. The only solution to this problem could be to increase the immunogenicity of OPV. Part of the reason for the low efficacy was interference among the three serotypes of vaccine virus after administration of trivalent OPV. To minimize the interference, monovalent vaccines against serotypes 1 and 3 were used (Nasr El-Sayed et al. 2008; John et al. 2011) supplementary to routine vaccination with trivalent OPV. The rationale behind this change was that type 2 poliovirus is the most robust of the three Sabin strains and strongly competes with the other two. In addition, wild type 2 poliovirus was eradicated in 1999, and therefore maintaining high immunity against type 2 poliovirus was a lower priority than stopping transmission of types 1 and 3. Introduction of monovalent OPV1 and OPV3 and then bivalent OPV1 + 3 vaccine succeeded, and the circulation of wild polioviruses in India was interrupted in 2011 (John and Vashishtha 2012; Kaura and Biswas 2012; O'Reilly et al. 2012).

The next unexpected revelation about the biology of poliovirus was the discovery in year 2000 of circulating vaccine-derived polioviruses that was already discussed above. There is a consensus among scientists that VDPVs are as virulent as wild strains of the virus, and must be looked at similarly (Agol 2006; Dowdle and Kew 2006; Minor 2009). Therefore, eradication of poliovirus must include not only wild strains but also VDPVs. Furthermore, since the only way to avoid emergence of VDPV is to stop the use of OPV, eradication can only be possible when the use of vaccine that led to eradication is terminated as well. The original solution to this

central paradox of polio eradication was to stop OPV use synchronously after global certification (Dowdle et al. 2003). The safety of this approach, however, is untestable and is fraught with danger. Serious doubts about the prudence of this approach were strengthened by the discovery of the so-called orphan polioviruses isolated in regions that were believed to be free from polio circulation for several years and that were genetically linked to the old local strains by using a "molecular clock" method (Jorba et al. 2008). This could be a result of either breaches in surveillance or a cryptic circulation of poliovirus in communities without overt clinical manifestations or a combination of both. Regardless of the reasons, the phenomenon of orphan polioviruses cannot be discounted in discussions of the strategy of OPV withdrawal because it is very hard to reach absolute certainty that polio is no longer present in a given community. Therefore, the current strategy envisions that availability and universal introduction of IPV is a prerequisite to withdrawal of OPV (WHO 2013). Since wild type 2 polioviruses were eliminated in 1999, the only source of type 2 paralytic polio is attributed to VDPV. Therefore, replacement of trivalent OPV by bivalent OPV1 + 3 for routine immunization could eliminate these cases and also be a test case for eventual withdrawal of all OPV vaccinations. Since wide use of bivalent OPV to stop transmission in endemic countries may have been linked to increased incidence of type 2 cVDPVs as a result of the diminished population immunity to this serotype (Arita and Francis 2011; Arya and Agarwal 2011), the switch from tOPV to bOPV must occur only in the context of maintaining high population immunity by switching to IPV.

There is no consensus at this time about whether the replacement of OPV with IPV must be done on an interim basis until there is more certainty that all wild viruses and VDPVs are removed from circulation and all stocks of poliovirus (including OPV) are securely contained or destroyed, or the use of IPV must continue indefinitely (Agol et al. 2005; Chumakov et al. 2007; Ehrenfeld et al. 2008). The arguments in favor of the first solution are based on saving of public health resources, which was the primary justification of the entire eradication campaign. On the other hand withdrawal of all protection against poliovirus will create an unprecedented epidemiological situation with the entire population born after OPV cessation being susceptible to the disease. This would create a significant vulnerability to accidental or intentional release of poliovirus back into circulation that could trigger a new pandemic of unpredictable proportions. This scenario becomes even scarier considering that poliomyelitis acquired by nonimmune adults is clinically more severe than the disease in infants and children. Therefore, after passage of some time, the entire population would become susceptible to a highly contagious and deadly/crippling disease, and poliovirus could become an ideal bioterrorism weapon. Containment of poliovirus and even complete destruction of all its stocks can diminish these concerns, but cannot resolve them completely. First, it is very difficult if not impossible to verify containment and destruction, but more importantly, modern technology allows live poliovirus to be synthesized from chemicals within a short time and at a very low cost (Cello et al. 2002). Thus, to many experts in the field, it appears increasingly likely that immunization against poliovirus must continue indefinitely.

It may be appropriate at this point to draw some parallels with the eradication of smallpox. While being a more deadly and contagious disease, eradication of smallpox was by far a more straightforward endeavor. The main distinction is that the diagnosis of smallpox is much easier, can be based on a quick examination, and does not require sophisticated laboratory procedures including nucleotide sequencing as is the case for poliovirus. The second difference is the very high disease to infection rate for smallpox: most susceptible individuals who were infected with variola virus developed the disease with its characteristic symptoms. In contrast, only one of a few hundred children infected with poliovirus proceed to develop any symptoms, making it very hard to quickly identify outbreaks of the disease. A good example is the first outbreak of cVDPV in Haiti and Dominican Republic that went undetected for the first 1½ years (Kew et al. 2002). These differences, combined with frequent and often severe adverse reactions to smallpox vaccinations, were a compelling reason to stop immunization against smallpox. However, decades later concerns about bioterrorism led to the development and stockpiling of a new generation of smallpox vaccines with an improved safety profile that is now ready to be used in case of emergency. Theoretically, a similar approach could be used for poliomyelitis, but difficulties in timely diagnosis will make such emergency response ineffective and will likely result in a new pandemic of poliomyelitis unless a sufficient level of population immunity is maintained universally.

These considerations take us to the next question of what is the ultimate objective of any eradication campaign and what is the strict definition of the term. Dealing with any infectious disease can go through three phases (Dowdle and Birmingham 1997; Dowdle 1998). First is control, i.e., application of preventive measures (e.g., vaccination) that lead to reduction of the disease burden to a socially acceptable level, which is maintained by continuous prophylaxis. The next phase is elimination, which is similar to control but reduces the morbidity to zero. Elimination is sustained by continuous vaccination to maintain high immunity levels that prevents the spread of the pathogen. Finally, eradication also means the complete absence of morbidity, but unlike elimination, it no longer requires preventive measures and vaccination. From the considerations presented above, it is clear that complete stopping of all polio vaccination is not prudent in the foreseeable future, and therefore in the strict sense of the word, the campaign should rather be called elimination but is referred to as eradication mostly for historical reasons.

4.5 New Generation of Polio Vaccines

As discussed above, continued use of OPV has become unacceptable because of safety and ethical considerations. However, its replacement with IPV involves significant challenges. The most important of these include its higher cost and the need for intramuscular injections delivered by qualified medical personnel. Another problem is that the lower ability of IPV to induce mucosal immunity precludes the

ability to break chains of transmission of the virus (Anis et al. 2013). Finally, current IPV is manufactured from highly virulent virus, which poses production biosecurity risks. Therefore, a new generation of polio vaccines is being explored for use after eradication, with properties that include lower cost, increased ability to induce mucosal immune responses, and addressing the biosecurity concerns (Ehrenfeld et al. 2009). For a live vaccine, a more genetically stable virus that would not revert to virulence would be essential. The current research and development efforts described below include both new OPV and IPV vaccines.

Elucidation in the 1980s and 1990s of the molecular mechanisms of poliovirus attenuation and reversion to virulence led to several efforts to create attenuated strains with higher genetic stability. Most of these efforts were aimed at restricting the emergence and accumulation of point mutations responsible for reversion. Since most VDPV strains are recombinants between Sabin strains and other non-polio enteroviruses, it is believed that recombination may also play a role in reversion to virulence. Evaluation of genetic stability is performed in vitro (in cultured cells) and in vivo (in animal experiments), but ultimately vaccine safety must be confirmed in humans. While several studies reported increased stability as measured in vitro, proving it in clinical studies represents a major challenge. Given the relatively low frequency of vaccine-induced complications (1 in about 600,000 first doses), to achieve the statistical power needed for definitive conclusions about the superiority of a new strain would require a clinical study of unprecedented size. Another consideration that complicates the development of a more stable attenuated strain is the absence of reliable in vitro or animal biomarkers of poliovirus safety. For this reason there have not been many studies in this direction until the creation of a consortium of several laboratories funded by the Bill and Melinda Gates Foundation that was tasked to develop a more genetically stable strain of type 2 OPV. At the time of this writing, the work is still ongoing. Therefore, we can only describe the general principles employed in this work.

One of the determinants of virulence and attenuation are mutations in a stem-loop domain (designated the F-domain of stem-loop VI) of the 5′-untranslated region. This domain is part of an internal ribosome entry site (IRES) and is believed to be involved in the interactions between translation initiation factors and the ribosome and the viral RNA molecule (Guest et al. 2004; Kauder and Racaniello 2004). It was reported that some of these factors are tissue-specific, and thus mutations in this region may affect tissue tropism and restrict virus replication in neuronal cells. Recombinants in which this region of poliovirus was replaced with the homologous element from human rhinoviruses were found to be strongly attenuated (Gromeier et al. 1996; Chumakov et al. 2001). These rhinovirus-poliovirus chimeras are now studied for their use as oncolytic agents against gliomas (Dobrikova et al. 2012). Such chimeric viruses could potentially be used as vaccines with improved stability.

Another approach aimed at the same attenuation determinant takes advantage of the observation that structural stabilization of this stem-loop structure leads to increased virulence, while its destabilization leads to attenuation. For instance, attenuation of type 3 poliovirus was achieved by mutating a stable G:C pair to a

weak G:U pair that destabilized the entire hairpin structure. During reversion, this G:U pair is replaced by the original G:C pair. Stability of A:U pairs is intermediate between G:C and G:U, so if the RNA hairpin is reengineered by replacing G:C and G:U pairs with A:U, the overall stability of the structure will remain roughly unchanged, and the virulence of such virus will also stay the same. This change, however, will result in higher genetic stability because it takes two mutations to convert an A:U pair to a more stable G:C pair, and the intermediates in this process (either G:U or A:C pairs) have a lower structural stability and hence lower fitness. A number of constructs created based on this principle were shown to have superior genetic stability and are now being considered as candidates for a more genetically stable vaccine virus (Macadam et al. 2001, 2006; Rowe et al. 2001).

Another way to impair the function of the IRES element is to delete or insert additional nucleotides, which leads to distortion of its overall conformation. Such manipulations, however, are not stable because virus can easily restore fitness by excising the inserts or filling the deletions with an unrelated piece of RNA of similar size from other sources. A way to overcome this instability was proposed by Wimmer and his colleagues, who took advantage of a cis-acting replicative element (*cre*) in viral RNA. Normally located in the center of the RNA molecule, it is critically important for initiation of RNA replication. Transplantation of the *cre* element from its normal position to the IRES region in the 5′-UTR strongly attenuated the virus (Toyoda et al. 2007). Since *cre* plays a critical role in RNA replication, the virus cannot excise this element, and thus the resulting attenuated constructs are genetically stable.

Viral RNA replicases are notoriously error prone, generating a lot of mutations and being one of the reasons for the genetic instability of viral RNA genomes. Despite the obvious problems created by high mutation rates, the ability to rapidly generate mutations gives viruses some advantages by allowing them to rapidly adapt to growth in new or changing environments. Therefore, the fidelity of viral replicases is optimized not to be very high or very low. This was demonstrated by the discovery of mutations in the polymerase gene that result in mutant replicase with increased fidelity (Pfeiffer and Kirkegaard 2003), which had an impaired ability to infect animals (Vignuzzi et al. 2006, 2008). This observation suggested the use of high-fidelity polymerase mutants to (1) decrease the rate of reversion and (2) provide an additional mechanism of attenuation.

All organisms including polioviruses have a certain bias in the use of synonymous codons. This is widely used in biotechnology when a foreign protein is expressed in a heterologous system. To maximize the yield of its product, the gene coding for the target protein is recoded by using codons most frequently used in the expression system. This process is called codon optimization. In experiments with poliovirus it was found that the reverse process—codon deoptimization (i.e., engineering viral genomes to use codons that are normally avoided in the poliovirus genome)—reduces viral fitness and decreases the yield of infectious virus (Burns et al. 2006). The resulting crippled virus cannot easily revert to restore its fitness because the change was a result of multiple mutations in different parts of the genome.

The mechanism by which codon deoptimization reduces viral fitness may be more complex than simply using rare codons. Besides codon usage bias, most organisms also manifest a codon pair bias (Gutman and Hatfield 1989). It means that there is a preference in the way codons coding for neighboring amino acids are selected: some codon pairs are used more frequently than others. If this order is changed by swapping different synonymous codons in the sequence, the result is similar to codon deoptimization, even though the overall codon usage remains unchanged (Coleman et al. 2008). The reason behind codon pair bias is yet to be established. To complicate the situation even further, it was found that in the poliovirus genome the frequency of G following C (the presence of dinucleotide CpG) and A following U (UpA) is lower than would be expected in a random sequence. If poliovirus RNA is recoded into a sequence with a higher number of CpG and UpA dinucleotides, the size of its plaques decreases proportionally to the number of changes introduced (Burns et al. 2009). For viruses generated by all these "genome scrambling" approaches, the yield of infectious virus decreases significantly, while the yield of physical particles is affected to a smaller degree. The biological mechanisms behind these phenomena are still unknown, as well as it is unclear whether all these observations represent the same phenomenon or have distinct reasons behind them. Nevertheless genome scrambling may have important applications in the development of attenuated and inactivated vaccines (Mueller et al. 2010).

So far we have described novel rational ways to attenuate virus in a more stable way and to restrict reversion by preventing point mutations. Another aspect of the search for a more genetically stable poliovirus is to try to restrict its ability to recombine with other viruses. Poliovirus and enteroviruses in general are highly promiscuous and recombine with high frequency (Cooper 1977; Furione et al. 1993; Agol 1997; Combelas et al. 2011). This property is highly advantageous because it allows them to evolve rapidly and to mitigate the damage caused by point mutations by replacing defective parts of their genome with functional pieces hijacked from other viruses. It appears likely that recombination helps vaccine viruses to replace parts of their genome that were crippled by attenuation and as a result to regain some fitness. Therefore, restricted recombination frequency may be a desirable property for an improved vaccine strain.

The work in this direction is complicated by our limited knowledge about the mechanisms of recombination. It is believed that homologous recombination plays an important role for poliovirus. Therefore, recoding relevant portions of the vaccine poliovirus genome to minimize homology with other viruses may reduce recombination frequency. Finding polymerase mutations with lower intrinsic recombination frequency could also be helpful in limiting the ability of viruses to exchange parts of their genome (Runckel et al. 2013). However, the ultimate utility of these approaches is unknown. It is still unclear whether recombination events themselves or selection based on fitness are the rate-limiting step that determines the frequency of the emergence of recombinant viruses. Work in these directions is ongoing and as a minimum promises to produce new knowledge about this fascinating aspect of poliovirus biology.

The list of shortcomings of the current IPV includes its relatively high cost, the need for intramuscular injections, and the lower mucosal immune response. In the post-eradication environment, it will also be joined by biosecurity concerns since it is manufactured from highly virulent strains that must be grown in large quantities. Despite all best efforts to contain the virus, there will always be a small chance of accidental or intentional release of live virus into the environment, the consequences of which could be catastrophic. Therefore, it has been proposed that IPV manufacture should be based on attenuated strains with a better biosafety profile.

This work is being pursued in several directions. One obvious solution would be to make inactivated vaccine from the attenuated Sabin strains to produce what is now known as Sabin IPV (sIPV). An additional advantage of this solution would be to maintain a "warm base" for OPV manufacture, in case there should be a need to restart its production in the future. This work started in the early 1990s (Doi et al. 2001) and demonstrated that while the immunogenicity of type 1 Sabin IPV was at least as good as the immunogenicity of conventional IPV (cIPV) made from the wild Mahoney strain, the immunogenicity of IPV made from the two other serotypes of Sabin viruses, especially of type 2, was inferior to wild-type IPV (Dragunsky et al. 2004, 2006; Tano et al. 2007). Further development revealed that the amount of type 1 sIPV antigen needed to induce an immune response comparable to that of cIPV prepared from the Mahoney strain was significantly lower. The reverse was true for type 2 viruses (Westdijk et al. 2011). As a result the optimal composition of trivalent sIPV was different from that in the cIPV. As of this writing, sIPV was licensed in Japan (Shimizu 2012) and phase 3 clinical evaluation was completed in China. In Japan it is produced by Kaketsuken and Biken in the form of combination vaccines with diphtheria, tetanus, and pertussis (DTP) antigens for subcutaneous administration. Since there is no poliomyelitis in China or Japan, clinical studies of sIPV were performed using a seroconversion endpoint that demonstrated that with appropriate formulation its efficacy is comparable to conventional IPV. The Institute for Translational Vaccinology in the Netherlands (formerly a part of RIVM and NVI) supported by the World Health Organization has developed an sIPV production process (Verdijk et al. 2011) and licensed it to a number of manufacturers in developing countries. Therefore, the first of the new generation IPV is Sabin IPV, manufacture of which is believed to carry lower biosecurity risks.

There are still important questions about sIPV that need to be resolved. Some of them are related to standardization of this new class of IPV, selection of appropriate potency testing methods, and reference reagents. Other issues that need further studies are related to quantification of biosecurity risks associated with its production and the types of safety tests that should be a part of its manufacture. While intuitively it appears that using attenuated virus for making inactivated vaccine is safer than using wild strains, this risk is not easily quantifiable, because if released into circulation, Sabin viruses can easily regain their virulence (see discussion about VDPV). In addition, according to the current Global Action Plan adopted by the WHO (World Health Organization 2004), after wild virus circulation is stopped and OPV use is terminated, Sabin strains must be contained under the same

strict conditions as wild strains. Therefore, sIPV manufacturing facilities will have to be upgraded to BSL3/polio containment level, defeating a significant part of the reason behind its development and introduction. Therefore, while being a step in the right direction, sIPV may not be the ultimate solution for the future.

A number of research groups are also working on development of even safer alternative strains that could be used for IPV production. The main requirement for such strains is that they must be completely apathogenic and that this attenuated phenotype be stable in vitro and in vivo, so that they could not revert to virulence and restart circulation even if they were released into the environment. The approaches used for generating such stably attenuated viruses are similar to those that were discussed above in the section describing development of new OPV2. They include replacement of reversion-prone IRES elements of Sabin polioviruses with homologous regions from non-neurotropic viruses such as human rhinoviruses (Gromeier et al. 1996; Chumakov et al. 2001; Dobrikova et al. 2012), stabilization of attenuating domains in the IRES by reengineering the F-domain stem-loop using A:U pairs (Macadam et al. 2006), moving the *cre* element to the 5′-UTR (Toyoda et al. 2007), introduction of high-fidelity mutations in the polymerase gene (Vignuzzi et al. 2008), and scrambling coding sequences to alter codon usage bias, codon pair bias (Toyoda et al. 2007), or the number of CpG and UpA dinucleotides (Burns et al. 2009). Proof of principle studies performed for all these approaches in vitro showed that the resulting virus may have a higher genetic stability. However, whether they could be used for manufacture of a sufficient quantity of poliovirus antigen needed for IPV production and whether they will be more stable in vivo (and thus more acceptable from the biosecurity standpoint) are yet to be established. Obtaining reliable information about the latter aspect is quite challenging because there is no adequate preclinical (animal) model of poliovirus transmissibility and genetic stability in vivo.

The ideal solution to biosecurity concerns would be a manufacturing process that does not require any infectious virus. While antigens for many other vaccines can be successfully produced in various expression systems such as baculovirus, yeasts, etc. the difficulty for using this approach for poliovirus vaccine is that most if not all of its protective epitopes are formed by secondary or even tertiary interactions between stretches of amino acids from different polypeptide chains. Their activity is highly sensitive to conformational changes, and therefore only native virus particles can elicit protective immune response. There is no effective in vitro system of poliovirus assembly that could be used to produce amounts of poliovirus particles needed for vaccine manufacture. The assembly process of poliovirus capsids is quite complex and is not fully understood. However, it is known that it involves auto-proteolytic cleavage of one of the protein precursors that takes place only after RNA is encapsidated inside these particles and “locks” the entire structure in a proper conformation. Empty particles containing no poliovirus RNA that are produced during virus replication or during expression of poliovirus proteins are quite unstable. Stabilization by protein engineering may potentially solve this problem (Porta et al. 2013). This could open a way to producing immunogenic empty capsids to be used as vaccines in a process that would require no live poliovirus.

Another avenue of research and development of new inactivated poliovirus vaccines aims to lower the cost and/or improve their immunogenicity (thereby reducing the dose of antigen needed for inducing a protective immune response). Cost reduction could be achieved by increasing the yields of virus by introducing new manufacturing processes and cell substrates. It has been reported that use of suspension cultures of PerC6 cells in serum-free medium allows cells to grow to a much higher densities and results in a higher virus yields (Sanders et al. 2013). Another way to reduce vaccine cost is to use alternative routes of delivery that would increase immunogenicity and allow dose-sparing. Adding adjuvants is a well-known solution to increase immunogenicity and dose-sparing, and there are a number of groups actively exploring the use of various conventional and novel adjuvants in combination with poliovirus vaccines. Among conventional adjuvants, alum was shown to increase immunogenicity of IPV (Verdijk et al. 2013; Westdijk et al. 2013). Novel adjuvants such as oil-in-water adjuvants (Baldwin et al. 2011) and agonists of toll-like receptors and other components of the innate immune system are also under investigation. Some adjuvants were shown to also increase the mucosal immune response after intramuscular administration, and this aspect is also is under study (Ivanov et al. 2006).

The skin is the first line of defense against many pathogens and therefore contains many immunologically active cells, including dendritic cells and macrophages that scout for invading pathogens. Therefore, intradermal administration of antigens is believed to be more effective compared to intramuscular administration. Clinical trials with intradermal delivery of a fractional dose of IPV demonstrated that this is indeed the case, but the dose-sparing effect fell short of the target 1/5 of the intramuscular dose (Resik et al. 2010; Cadorna-Carlos et al. 2012; Nelson et al. 2012; Soonawala et al. 2013). Effective priming immunization after one intradermal dose of IPV was demonstrated by an anamnestic response to a booster dose of the vaccine (Resik et al. 2013). Therefore, intradermal delivery is a viable option that can also eliminate the need for injections if needle-less devices are used. Another possibility for intradermal delivery is the use of "microneedle patches" (Hiraishi et al. 2011; del Pilar Martin et al. 2012; Kim et al. 2012; Edens et al. 2013). These small arrays of dissolvable plastic microneedles coated with antigen can be painlessly applied to the skin similar to a Band-Aid to deliver IPV intradermally. The utility and efficiency of this approach are now under investigation.

All these new developments relate to stand-alone IPV that may play a role in the endgame of polio eradication and help to transition from OPV to IPV. However, in the long-term perspective, IPV will be used in combination with other antigens in the form of tetravalent (DTaP-IPV), pentavalent (DTaP-IPV-HiB or DTaP-IPV-HepB), or hexavalent vaccines combining all these antigens. Combination vaccines provide the maximum public health benefit while minimizing cost and the number of injections needed for vaccine delivery. Such vaccines are already used in developed countries, and affordable versions of combination products needed for the rest of the world may use some of the approaches described above.

Closely related to the development of novel poliovirus vaccines are attempts to create new tools that could mitigate their adverse effects. As discussed above, OPV can induce chronic infection in immunodeficient patients. At present there is no cure that would help these patients clear infection and stop shedding of virulent iVDPVs. Development of antiviral drugs is underway that promise to not only be useful for this purpose but that could potentially help in an emergency response to protect people if an outbreak occurs after eradication is complete or to treat people accidentally exposed to poliovirus (Collett et al. 2008). Passive immunotherapy could also be used for these purposes either alone or in combination with anti-poliovirus drug(s). Its efficacy was well-demonstrated in the pre-vaccine era (Hammon et al. 1952), but it was not used because of the difficulty of producing intravenous immunoglobulin. Monoclonal antibody technology has made it possible to create human antibodies highly effective against poliovirus (Chen et al. 2011, 2013), and their utility is being studied along with antiviral drugs.

4.6 Conclusions

The history of poliovirus vaccines represents a fascinating story of an evolving relationship between two highly effective vaccines, each having their advantages and disadvantages (Fig. 4.3). Being the first of two, IPV triumphantly demonstrated that polio can be successfully prevented but opened the Pandora's box of vaccine-

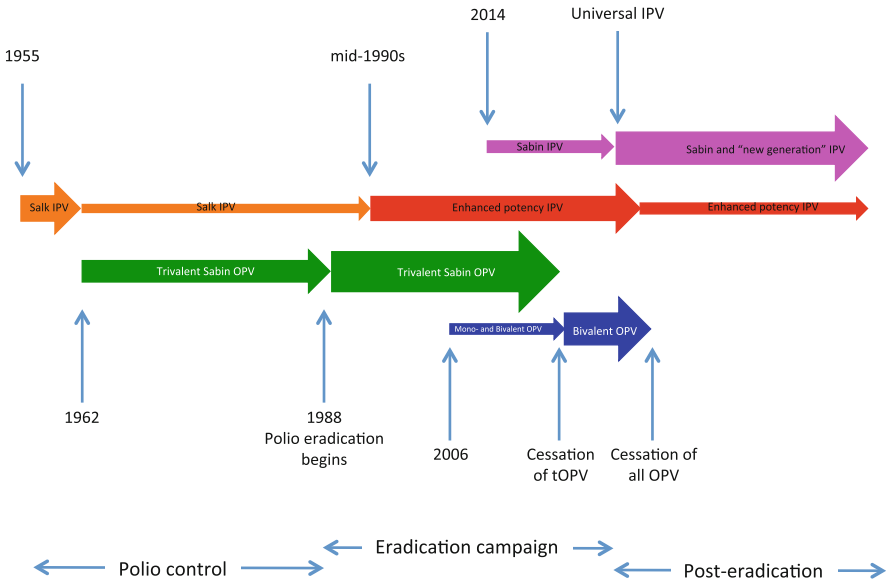

Fig. 4.3 Timeline illustrating evolution of polio vaccines

induced injuries that in turn led to the emergence of the modern regulatory and legal framework for vaccine development and use. This also opened the door for OPV that for many years was the vaccine of choice and led to remarkable progress in the control of poliomyelitis. This success of OPV will inevitably lead to its own demise and the need to be replaced by a safer inactivated vaccine. However, the new IPV is likely to be different from the IPV that we know now. Thus, the ever-changing epidemiological and socioeconomic landscape determines the need to continuously update the existing vaccines and to introduce innovative products that meet new challenges.

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References

Agol VI (1997) Recombination and other genomic rearrangements in picornaviruses. Semin Virol 8:77–84

Agol VI (2006) Vaccine-derived polioviruses. Biologicals 34(2):103–108

Agol VI, Chumakov K et al (2005) Don't drop current vaccine until we have new ones. Nature 435 (7044):881

Alexander LN, Seward JF et al (2004) Vaccine policy changes and epidemiology of poliomyelitis in the United States. JAMA 292(14):1696–1701

Andrus JK, de Quadros C et al (1992) Screening of cases of acute flaccid paralysis for poliomyelitis eradication: ways to improve specificity. Bull World Health Organ 70:591–596

Anis E, Kopel E et al (2013) Insidious reintroduction of wild poliovirus into Israel, 2013. Euro Surveill 18(38):1–5

Arita I, Francis DP (2011) Safe landing for global polio eradication: a perspective. Vaccine 29 (48):8827–8834

Arya SC, Agarwal N (2011) Bivalent live poliovirus vaccine: a blessing or a curse. Hum Vaccin 7 (7):800

Badham J (1834–35) Paralysis in childhood; four remarkable cases of suddenly induced paralysis in the extremities, occurring in children, without any apparent cerebral or cerebro-spinal lesion. Lond Med Gazzette 17:215

Baker AB (1949) Bulbar poliomyelitis: its mechanism and treatment. Am J Med 6:614–619

Baldwin SL, Fox CB et al (2011) Increased potency of an inactivated trivalent polio vaccine with oil-in-water emulsions. Vaccine 29(4):644–649

Blomqvist S, Savolainen C et al (2004) Characterization of a highly evolved vaccine-derived poliovirus type 3 isolated from sewage in Estonia. J Virol 78(9):4876–4883

Blume SS (2005) Lock in, the state and vaccine development: lessons from the history of the polio vaccines. Res Policy 34:159–173

Bodian D, Morgan IM et al (1949) Differentiation of types of poliomyelitis viruses. III. The grouping of fourteen strains into three basic immunologic types. Am J Hyg 49:234–245

Brodie M (1934) Active immunization in monkeys against poliomyelitis with germicidally inactivated virus. Science 79:594–595

Brodie M, Park WH (1935) Active immunization against poliomyelitis. J Am Med Assoc 105:9

Burnet FM, Macnamara J (1931) Immunological differences between strains of poliomyelitic virus. Br J Exp Pathol 12:57–61

Burns CC, Shaw J et al (2006) Modulation of poliovirus replicative fitness in HeLa cells by deoptimization of synonymous codon usage in the capsid region. J Virol 80(7):3259–3272
Burns CC, Campagnoli R et al (2009) Genetic inactivation of poliovirus infectivity by increasing the frequencies of CpG and UpA dinucleotides within and across synonymous capsid region codons. J Virol 83(19):9957–9969
Burns CC, Shaw J et al (2013) Multiple independent emergences of type 2 vaccine-derived polioviruses during a large outbreak in northern Nigeria. J Virol 87(9):4907–4922
Cadorna-Carlos J, Vidor E et al (2012) Randomized controlled study of fractional doses of inactivated poliovirus vaccine administered intradermally with a needle in the Philippines. Int J Infect Dis 16(2):e110–e116
Cello J, Paul AV et al (2002) Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template. Science 297:1016–1018
Centers for Disease Control and Prevention (2001) Circulation of a type 2 vaccine-derived poliovirus – Egypt, 1982–1993. Morb Mortal Wkly Rep 50:41–42, 51
Centers for Disease Control and Prevention (2009) Update on vaccine-derived polioviruses–worldwide, January 2008–June 2009. Morb Mortal Wkly Rep 58(36):1002–1006
Centers for Disease Control and Prevention (2012) Update on vaccine-derived polioviruses–worldwide, April 2011-June 2012. MMWR Morb Mortal Wkly Rep 61:741–746
Cernáková B, Sobotová Z et al (2005) Isolation of vaccine-derived polioviruses in the Slovak Republic. Eur J Clin Microbiol Infect Dis 24:438–439
Chang T-W, Weinstein L et al (1966) Paralytic poliomyelitis in a child with hypogammaglobulinemia: probable implication of type 1 vaccine strain. Pediatrics 37:630–636
Chen Z, Chumakov K et al (2011) Chimpanzee-human monoclonal antibodies for treatment of chronic poliovirus excretors and emergency postexposure prophylaxis. J Virol 85 (9):4354–4362
Chen Z, Fischer ER et al (2013) Cross-neutralizing human anti-poliovirus antibodies bind the recognition site for cellular receptor. Proc Natl Acad Sci U S A 110(50):20242–20247
Chumakov MP (1960) The effect of mass peroral immunisation by live vaccines from Sabin strains on the epidemiological process of poliomyelitis. J Hyg Epidemiol Microbiol Immunol 4:287–288
Chumakov K, Kew OM (2010) The poliovirus eradication initiative. In: Ehrenfeld E, Domingo E, Roos RP (eds) The picornaviruses. ASMscience, Washington, DC, pp 449–459
Chumakov K, Dragunsky E et al (2001) Inactivated vaccines based on alternatives to wild-type seed virus. Dev Biol (Basel) 105:171–177
Chumakov K, Ehrenfeld E et al (2007) Vaccination against polio should not be stopped. Nat Rev Microbiol 5(12):952–958
Coleman JR, Papamichail D et al (2008) Virus attenuation by genome-scale changes in codon pair bias. Science 320(5884):1784–1787
Collett MS, Neyts J et al (2008) A case for developing antiviral drugs against polio. Antiviral Res 79(3):179–187
Combelas N, Holmblat B et al (2011) Recombination between poliovirus and coxsackie A viruses of species C: a model of viral genetic plasticity and emergence. Viruses 3(8):1460–1484
Cooper PD (1977) Genetics of picornaviruses. In: Fraenkel-Conrat H, Wagner R (eds) Comprehensive virology, vol 9. Plenum, New York, NY, pp 133–207
Cornil V (1863) Paralysie infantile; cancer les seins; autopsie; altérations de la moelle épinière, des nerfs et des muscles; généralisation du cancer. C R Soc Biol (Paris) 5:187
Davis LE, Bodian D et al (1977) Chronic progressive poliomyelitis secondary to vaccination of an immunodeficient child. N Engl J Med 297(5):241–245
de Quadros CA, Andrus JK et al (1992) Polio eradication from the Western Hemisphere. Annu Rev Public Health 13:239–252
de Quadros CA, Hersh BS et al (1997) Eradication of wild poliovirus from the Americas: acute flaccid paralysis surveillance, 1988-1995. J Infect Dis 175(Suppl 1):S37–S42

del Pilar Martin M, Weldon WC et al (2012) Local response to microneedle-based influenza immunization in the skin. MBio 3(2):e00012-12
Dobrikova EY, Goetz C et al (2012) Attenuation of neurovirulence, biodistribution, and shedding of a poliovirus:rhinovirus chimera after intrathalamic inoculation in Macaca fascicularis. J Virol 86(5):2750–2759
Doi Y, Abe S et al (2001) Progress with inactivated poliovirus vaccines derived from the Sabin strains. Dev Biol 105:163–169
Dowdle WR (1998) The principles of disease elimination and eradication. Bull World Health Organ 76(Suppl 2):22–25
Dowdle WR, Birmingham ME (1997) The biologic principles of poliovirus eradication. J Infect Dis 175(Suppl 1):S286–S292
Dowdle W, Kew O (2006) Vaccine-derived polioviruses: is it time to stop using the word "rare"? J Infect Dis 194:539–541
Dowdle WR, de Gourville E et al (2003) Polio eradication: the OPV paradox. Rev Med Virol 13:277–291
Dragunsky EM, Ivanov AP et al (2004) Evaluation of immunogenicity and protective properties of inactivated poliovirus vaccines: a new surrogate method for predicting vaccine efficacy. J Infect Dis 190(8):1404–1412
Dragunsky EM, Ivanov AP et al (2006) Further development of a new transgenic mouse test for the evaluation of the immunogenicity and protective properties of inactivated poliovirus vaccine. J Infect Dis 194(6):804–807
Edens C, Collins ML et al (2013) Measles vaccination using a microneedle patch. Vaccine 31 (34):3403–3409
Ehrenfeld E, Glass RI et al (2008) Immunisation against poliomyelitis: moving forward. Lancet 371(9621):1385–1387
Ehrenfeld E, Modlin J et al (2009) Future of polio vaccines. Expert Rev Vaccines 8(7):899–905
Enders JF, Weller TH et al (1949) Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissue. Science 109:85–87
Enders JF, Weller TH et al (1952) Alterations in pathogenicity for monkeys of Brunhilde strain of poliovirus following cultivation in human tissues. Fed Proc 11:467
Feigin RD, Guggenheim MA et al (1971) Vaccine-related paralytic poliomyelitis in an immunodeficient child. J Pediatr 79(4):642–647
Flexner S, Clark PF (1912–13) A note on the mode of infection in epidemic poliomyelitis. Proc Soc Exp Biol Med 10:1
Flexner S, Lewis PA (1909) The transmission of poliomyelitis to monkeys. J Am Med Assoc 53:1639
Flexner S, Lewis PA (1910) Experimental poliomyelitis in monkeys; active immunization and passive serum protection. J Am Med Assoc 54:1780
Frost WH (1913) Epidemiologic studies of acute anterior poliomyelitis. Hyg Lab Bull, No 90
Furione M, Guillot S et al (1993) Polioviruses with natural recombinant genomes isolated from vaccine-associated poliomyelitis. Virology 196:199–208
Grassly NC, Fraser C et al (2006) New strategies for the elimination of polio from India. Science 314:1150–1153
Grassly NC, Wenger J et al (2007) Protective efficacy of a monovalent oral type 1 poliovirus vaccine: a case-control study [see comment][erratum appears in Lancet. 2007 May 26;369 (9575):1790]. Lancet 369(9570):1356–1362
Gromeier M, Alexander L et al (1996) Internal ribosomal entry site substitution eliminates neurovirulence in intergeneric poliovirus recombinants. Proc Nat Acad Sci U S A 93:2370–2375
Guest S, Pilipenko E et al (2004) Molecular mechanisms of attenuation of the Sabin strain of poliovirus type 3. J Virol 78(20):11097–11107
Gutman GA, Hatfield GW (1989) Nonrandom utilization of codon pairs in Escherichia coli. Proc Natl Acad Sci U S A 86(10):3699–3703

Hammon WM, Coriell LI et al (1952) Evaluation of Red Cross gamma globulin as a prophylactic agent for poliomyelitis. J Am Med Assoc 150:139
Hampton L (2009) Albert Sabin and the coalition to eliminate polio from the Americas. Am J Public Health 99(1):34–44
Heine J (1840) Beobachtungen über Lähmungszustände der unteren Extremitäten und deren Behandlung. Köhler, Stuttgart
Hidalgo S, Garcia Erro M et al (2003) Paralytic poliomyelitis caused by a vaccine-derived polio virus in an antibody-deficient Argentinean child. Pediatr Infect Dis J 22(6):570–572
Hiraishi Y, Nandakumar S et al (2011) Bacillus Calmette-Guerin vaccination using a microneedle patch. Vaccine 29(14):2626–2636
Ivanov AP, Dragunsky EM et al (2006) 1,25-dihydroxyvitamin d3 enhances systemic and mucosal immune responses to inactivated poliovirus vaccine in mice. J Infect Dis 193(4):598–600
Jacobi M (1874–75) Pathogeny of infantile paralysis. Am J Obstet 7:1
John TJ, Vashishtha VM (2012) Path to polio eradication in India: a major milestone. Indian Pediatr 49(2):95–98
John TJ, Jain H et al (2011) Monovalent type 1 oral poliovirus vaccine among infants in India: report of two randomized double-blind controlled clinical trials. Vaccine 29(34):5793–5801
Jorba J, Campagnoli R et al (2008) Calibration of multiple poliovirus molecular clocks covering an extended evolutionary range. J Virol 82(9):4429–4440
Kauder SE, Racaniello VR (2004) Poliovirus tropism and attenuation are determined after internal ribosome entry. J Clin Investig 113(12):1743–1753
Kaura G, Biswas T (2012) India reaches milestone of no cases of wild poliovirus for 12 months. BMJ 344:e1328
Kessel JF, Pait CF (1949) Differentiation of three groups of poliomyelitis virus. Proc Soc Exp Biol Med 70:315–316
Kew OM, Nottay BK et al (1990) Molecular epidemiology of wild poliovirus transmission. In: Kurstak E, Marusyk RG, Murphy FA, Van Regenmortel MHV (eds) Applied virology research, vol 2. Plenum, New York, NY, pp 199–221
Kew O, Morris-Glasgow V et al (2002) Outbreak of poliomyelitis in Hispaniola associated with circulating type 1 vaccine-derived poliovirus. Science 296:356–359
Kew OM, Wright PF et al (2004) Circulating vaccine-derived polioviruses: current state of knowledge. Bull World Health Organ 82:16–23
Kim YC, Song JM et al (2012) Increased immunogenicity of avian influenza DNA vaccine delivered to the skin using a microneedle patch. Eur J Pharm Biopharm 81(2):239–247
Koprowski H (1958) Vaccination with modified active viruses. Poliomyelitis. In: Papers and discussion presented at the fourth international poliomyelitis conference. J. B. Lippincott, Philadelphia, PA
Koprowski H, Jervis GA et al (1952) Immune responses in human volunteers upon oral administration of a rodent-adapted strain of poliomyelitis virus. Am J Hyg 55:108–126
Korotkova EA, Park R et al (2003) Retrospective analysis of a local cessation of vaccination against poliomyelitis: a possible scenario for the future. J Virol 77:12460–12465
Kramer SD, Aycock WL et al (1932) Convalescent serum therapy in preparalytic poliomyelitis. N Engl J Med 206:432
Landsteiner K, Popper E (1909) Übertragung der Poliomyelitis acuta auf Affen. Zeitschrift für Immunitätsforshung 2:377–390
Leake JP (1935) Poliomyelitis following vaccination against the disease. J Am Med Assoc 105:2152
Lopez C, Biggar WD et al (1974) Nonparalytic poliovirus infections in patients with severe combined immunodeficiency disease. J Pediatr 84(4):497–502
Macadam AJ, Ferguson G et al (2001) Live-attenuated strains of improved genetic stability. Dev Biol 105:179–187

Macadam AJ, Ferguson G et al (2006) Rational design of genetically stable, live-attenuated poliovirus vaccines of all three serotypes: relevance to poliomyelitis eradication. J Virol 80 (17):8653–8663
Martín J, Odoom K et al (2004) Long-term excretion of vaccine-derived poliovirus by a healthy child. J Virol 78:13839–13847
Minor P (2001) Characteristics of poliovirus strains from long-term excretors with primary immunodeficiencies. Dev Biol 105:75–80
Minor P (2009) Vaccine-derived poliovirus (VDPV): impact on poliomyelitis eradication. Vaccine 27(20):2649–2652
Mueller S, Coleman JR et al (2010) Live attenuated influenza virus vaccines by computer-aided rational design. Nat Biotechnol 28(7):723–726
Nasr El-Sayed N, El-Gamal Y et al (2008) Randomized controlled clinical trial of monovalent type 1 oral poliovirus vaccine. N Engl J Med 359:1655–1665
Nathanson N, Langmuir AD (1963a) The cutter incident. Poliomyelitis following formaldehyde-inactivated poliovirus vaccination in the United States in the spring of 1955. I. Background. Am J Hyg 78:16–28
Nathanson N, Langmuir AD (1963b) The Cutter incident. Poliomyelitis following formaldehyde-inactivated poliovirus vaccination in the United States in the spring of 1955. II. Relationship of poliomyelitis to Cutter vaccine. Am J Hyg 78:29–60
Nathanson N, Langmuir AD (1963c) The Cutter incident. Poliomyelitis following formaldehyde-inactivated poliovirus vaccination in the United States in the spring of 1955. III. Comparison of the clinical character of vaccinated and contact cases occurring after use of high rate lots of Cutter vaccine. Am J Hyg 78:61–81
Nelson KS, Janssen JM et al (2012) Intradermal fractional dose inactivated polio vaccine: a review of the literature. Vaccine 30(2):121–125
Nottay BK, Kew OM et al (1981) Molecular variation of type 1 vaccine-related and wild polioviruses during replication in humans. Virology 108:405–423
O'Reilly KM, Durry E et al (2012) The effect of mass immunisation campaigns and new oral poliovirus vaccines on the incidence of poliomyelitis in Pakistan and Afghanistan, 2001-11: a retrospective analysis. Lancet 380(9840):491–498
Offit PA (2005) The Cutter incident: how America's first polio vaccine led to the growing vaccine crisis. Yale University Press, New Haven
Pallansch MA, Oberste MS et al (2013) Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. In: Knipe DM, Howley PM (eds) Fields virology, vol 1. Lippincott Williams & Wilkins, Philadelphia, PA, pp 490–530
Patriarca PA, Wright PF et al (1991) Factors affecting the immunogenicity of oral poliovirus vaccine in developing countries: review. Rev Infect Dis 13:926–939
Pfeiffer JK, Kirkegaard K (2003) A single mutation in poliovirus RNA-dependent RNA polymerase confers resistance to mutagenic nucleotide analogs via increased fidelity. Proc Natl Acad Sci U S A 100(12):7289–7294
Porta C, Kotecha A et al (2013) Rational engineering of recombinant picornavirus capsids to produce safe, protective vaccine antigen. PLoS Pathog 9(3):e1003255
Putnam JJ, Taylor EW (1893) Is acute poliomyelitis unusually prevalent this season. Bost Med Surg J 129:509–519
Racaniello VR (2013) Picornaviridae: the viruses and their replication. In: Knipe DM, Howley PM (eds) Fields virology, vol 1. Lippincott Williams & Wilkins, Philadelphia, PA, pp 453–489
Resik S, Tejeda A et al (2010) Randomized controlled clinical trial of fractional doses of inactivated poliovirus vaccine administered intradermally by needle-free device in Cuba. J Infect Dis 201(9):1344–1352
Resik S, Tejeda A et al (2013) Priming after a fractional dose of inactivated poliovirus vaccine. N Engl J Med 368(5):416–424

Roivainen M, Blomqvist S et al (2010) Highly divergent neurovirulent vaccine-derived polioviruses of all three serotypes are recurrently detected in Finnish sewage. Euro surveill 15(19):pii/19566
Rowe A, Burlison J et al (2001) Functional formation of domain V of the poliovirus noncoding region: significance of unpaired bases. Virology 289(1):45–53
Runckel C, Westesson O et al (2013) Identification and manipulation of the molecular determinants influencing poliovirus recombination. PLoS Pathog 9(2):e1003164
Sabin AB (1954a) Current status of research on vaccination against poliomyelitis. J Mich State Med Soc 53(9):985, passim
Sabin AB (1954b) On the trail of avirulent viruses for immunization against poliomyelitis. Bibl Paediatr 58:429–436
Sabin AB (1955a) Behavior of chimpanzee avirulent poliomyelitis viruses in experimentally infected human volunteers. Am J Med Sci 230(1):1–8
Sabin AB (1955b) Characteristics and genetic potentialities of experimentally produced and naturally occurring variants of poliomyelitis virus. Ann NY Acad Sci 61(4):924–938, discussion 938–929
Sabin AB (1961a) Eradication of poliomyelitis. Ann Intern Med 55:353–357
Sabin AB (1961b) Poliomyelitis in Brazil, Uruguay, Argentina and Chile. Data of importance in planning for elimination of the disease. Yale J Biol Med 34:399–420
Sabin AB (1961c) Poliomyelitis incidence in the Soviet Union in 1960. JAMA 176:231–232
Sabin AB (1965) Oral poliovirus vaccine. History of its development and prospects for eradication of poliomyelitis. JAMA 194(8):872–876
Sanders BP, Edo-Matas D et al (2013) PER.C6((R)) cells as a serum-free suspension cell platform for the production of high titer poliovirus: a potential low cost of goods option for world supply of inactivated poliovirus vaccine. Vaccine 31(5):850–856
Shimizu H (2012) Poliovirus vaccine. Uirusu 62(1):57–65
Soonawala D, Verdijk P et al (2013) Intradermal fractional booster dose of inactivated poliomyelitis vaccine with a jet injector in healthy adults. Vaccine 31(36):3688–3694
Tano Y, Shimizu H et al (2007) Antigenic characterization of a formalin-inactivated poliovirus vaccine derived from live-attenuated Sabin strains. Vaccine 25(41):7041–7046
Toyoda H, Yin J et al (2007) Oncolytic treatment and cure of neuroblastoma by a novel attenuated poliovirus in a novel poliovirus-susceptible animal model. Cancer Res 67(6):2857–2864
Underwood M (1789) A treatise on diseases of children with general directions for the management of infants from the birth. Mathews, London
van Wezel AL, van Steenis G et al (1984) Inactivated poliovirus vaccine: current production methods and new developments. Rev Infect Dis 6(Suppl 2):S335–S340
Verdijk P, Rots NY et al (2011) Clinical development of a novel inactivated poliomyelitis vaccine based on attenuated Sabin poliovirus strains. Expert Rev Vaccines 10(5):635–644
Verdijk P, Rots NY et al (2013) Safety and immunogenicity of inactivated poliovirus vaccine based on Sabin strains with and without aluminum hydroxide: a phase I trial in healthy adults. Vaccine 31(47):5531–5536
Vignuzzi M, Stone JK et al (2006) Quasispecies diversity determines pathogenesis through cooperative interactions in a viral population. Nature 439(7074):344–348
Vignuzzi M, Wendt E et al (2008) Engineering attenuated virus vaccines by controlling replication fidelity. Nat Med 14(2):154–161
Wassilak S, Pate MA et al (2011) Outbreak of type 2 vaccine-derived poliovirus in Nigeria: emergence and widespread circulation in an underimmunized population. J Infect Dis 203 (7):898–909
Westdijk J, Brugmans D et al (2011) Characterization and standardization of Sabin based inactivated polio vaccine: proposal for a new antigen unit for inactivated polio vaccines. Vaccine 29(18):3390–3397
Westdijk J, Koedam P et al (2013) Antigen sparing with adjuvanted inactivated polio vaccine based on Sabin strains. Vaccine 31(9):1298–1304

WHO (2013) Polio Eradication & Endgame Strategic Plan 2013-2018. World Health Organization, Geneva

World Health Organization (2004) Global action plan for laboratory containment of wild polioviruses, 3rd edn. World Health Organization, Geneva

Wright PF, Hatch MH et al (1977) Vaccine-associated poliomyelitis in a child with sex-linked agammaglobulinemia. J Pediatr 91:408–412