The polio-eradication programme and issues of the end game

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Poliovirus causes paralytic poliomyelitis, an ancient disease of humans that became a major public-health issue in the 20th century. The primary site of infection is the gut, where virus replication is entirely harmless; the two very effective vaccines developed in the 1950s (oral polio vaccine, or OPV, and inactivated polio vaccine, or IPV) induce humoral immunity, which prevents viraemic spread and disease. The success of vaccination in middle-income and developing countries encouraged the World Health Organization to commit itself to an eradication programme, which has made great advances. The features of the infection, including its largely silent nature and the ability of the live vaccine (OPV) to evolve and change in vaccine recipients and their contacts, make eradication particularly challenging. Understanding the pathogenesis and virology of the infection is of major significance as the programme reaches its conclusion.

Introduction

Poliomyelitis is caused by infection of the motor neurones of the central nervous system (CNS) by poliovirus. The motor neurones of the anterior horn of the spinal cord that are the target comprise the grey matter of the spinal cord, and this tropism gives the disease its name, from polios and myelos, Greek for ‘grey’ and ‘matter’, respectively. The brain can also be affected. The funerary stele of the Egyptian priest Rom (Fig. 1) from about 1300 BCE is considered the earliest-documented instance of poliomyelitis, showing the characteristic downflexed foot and withered limb associated with muscle atrophy following loss of motor innervation of the leg. Given the presentation, it is striking that reports of cases are rare until the end of the 19th and beginning of the 20th centuries; by the mid-20th century, however, poliomyelitis was one of the most-feared diseases in developed countries (Kluger, 2004). The efforts to understand it and to develop countermeasures are described in the classic book by Paul (1971).

The virus and its pathogenesis

Polio was first shown to be caused by a virus by Landsteiner & Popper (1909) when they transmitted the disease successfully from the spinal cord of a fatal human case to a monkey. Rodents were not susceptible and, until the late 1940s and the development of cell-culture methods, the assay of the virus and understanding of its pathogenesis depended on the use of Old World primates. Poliovirus is a positive-strand RNA virus of the family Picornaviridae, now classified with many coxsackievirus A types as a species C human enterovirus (http://www.picornaviridae.com). The genome is approximately 7500 nt long, with a protein (VPg) linked covalently to the 5’ end; its layout is shown in Fig. 2. It comprises a highly structured 5’ non-coding region (NCR) of about 750 nt that functions as an internal ribosome entry site (IRES) (Pelletier & Sonenberg, 1988) preceding the single ORF, which is divided conventionally into three regions: P1, P2 and P3. The four structural proteins are encoded by P1, the 5’ portion of the ORF, and consist of VP1, VP2, VP3 and VP4; VP2 and VP4 are formed by cleavage of the precursor VP0 in the last stage of the maturation of the virus particle after packaging the RNA. The non-structural proteins derived from the P2 and P3 regions are encoded by the 3’ two-thirds of the genome and are involved in RNA replication, protein processing and the cell biology of virus infection. The functions are complex and not central to the eradication programme; however, whilst the activity associated with P3C is the main protease that processes the viral proteins to their functional form, protein P2A cleaves the structural from the non-structural portion of the genome during translation, has a major function in shut-off of host-cell synthesis and, in some way, influences the function of the IRES. This will be mentioned later. The genome terminates in an NCR of about 70 nt before the polyadenylate tract.

The virion is composed of 60 copies of each of the four capsid proteins arranged with icosahedral symmetry, and the atomic structures of several polioviruses have been solved (Filman et al., 1989; Hogle et al., 1985). The virus uses a specific cellular receptor termed CD155 or PVR, which is found on cells from Old World monkeys and higher primates, including chimpanzees and humans (Mendelsohn et al., 1989). Transgenic mice carrying the receptor gene are susceptible to infection by parenteral but not oral routes, developing a disease closely resembling...
poliomyelitis in humans (Koike et al., 1991; Ohka et al., 2007; Ren et al., 1990). Humans are the only known natural host for the virus.

There are three serotypes of the virus, designated types 1, 2 and 3, all of which use the same cellular receptor; it is not obvious why other serotypes do not exist, given the close relationship between polioviruses and the other species C enteroviruses and the extensive recombination between them. Immunity to one serotype does not protect adequately against the other two, so vaccines contain single strains of each serotype. The vaccine strains were isolated at least 50 years ago. Whilst there is no evidence for antigenic variation on a scale affecting vaccine efficacy, there are clearly genetically identifiable, geographical clusters of virus strains (Rico-Hesse et al., 1987). Sequence determination has played a major role in identifying the source of viruses causing outbreaks; for example, the epidemics in Indonesia and the Yemen in 2005, described below, were caused by strains imported from Nigeria.

The earliest major epidemics of poliomyelitis occurred in Sweden. In 1905, Wickman (Minor, 1997a) showed by epidemiological studies that paralysis only developed in about 1% of infections. Infection can be completely silent, or lead to a systemic infection termed the minor disease (or abortive poliomyelitis), including sore throat and fever, or to the major (paralytic) disease. Typically, the minor disease occurs 7 days and the major disease 30 days after infection. Depending on the site of virus replication in the CNS, the major disease may affect the legs (usually hemilaterally), the respiratory centres (bulbar poliomyelitis) or, on occasion, the brain itself, leading to encephalitis. Based on experience in developed countries in the 1950s, about 10% of patients recover completely, 5–10% die and the remainder have some residual paralysis, but recover some function. Post-polio syndrome, where paralysis re-emerges late after some recovery from the original disease, probably reflects the presence of declining neuronal backup, as neurones are lost as a result of ageing (Jubelt & Agre, 2000).

By 1912, it had been shown that poliovirus infectivity could be found in faeces as well as in CNS tissue (Minor, 1997a); unfortunately, the view had taken root that polio was an exclusively neurological disease, and the monkey model supported that view. The virus was passaged by intranasal inoculation, leading to invasion via the olfactory lobes and spread to the spine (Minor, 1997a), and the favoured laboratory virus was in fact strongly adapted to neural tissue by this process. This gave a misleading view of pathogenesis and has often been cited as an example of the inadequacy of animal models of human diseases. Natural virus infection of humans is mainly of the gut, with an initial viraemic phase leading to infection of distal lymphoid tissue such as the tonsils. A version of pathogenesis attributable to Sabin (1956) is shown in Fig. 3. The model of Bodian (1955) is more usually cited and proposes that the primary site of replication is lymphatic tissue, and the Peyer’s patches of the gut in particular. Replication in gut tissue is central to both

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**Fig. 1.** Funerary stele of the Egyptian priest Rom, about 1300 BCE, showing withered limb and downflexed foot typical of poliomyelitis. Reprinted with permission of the Carlsberg Museum, Copenhagen.

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**Fig. 2.** Organization of the genome of poliovirus, showing the genome-linked protein (VPg), long 5’ NCR, the capsid proteins (VP4, 2, 3 and 1) and the non-structural proteins (P2A, P2B and P2C; P3A, 3B, 3C and 3D). The non-structural proteins are involved in replication. Proteins 2A and 3C have protease activity and the genome is approximately 7430 bases in length.
models. There are still many unknowns (Nathanson, 2008); among other things, it is not known where, or by what cell, the virus that is shed in the stool is produced: it could be lymphoid tissue, gut epithelium of some sort, or another site. Most infections in the world are believed to occur by the faecal–oral route, although a respiratory route may also be important. The virus spreads from the gut to secondary-infection sites as a result of viraemia, and from there to the CNS. Gut infections are silent, the secondary-infection sites correspond to the minor disease and CNS infections cause the major (paralytic) disease. This suggests that humoral antibody sufficient to prevent viraemia would protect against disease by confining infection to the gut, which is the accepted model for the rarity of poliomyelitis until the end of the 19th century. Thus, when hygiene is poor, exposure to infected faecal material will occur while the individual is still protected by maternal antibody and infection will be safely confined to the gut. As standards improve and exposure is less common, infection will happen slightly later in life after the loss of maternal immunity and viremia will result. This gave rise to the alternative name of infantile paralysis for polio. This is also consistent with the occurrence of vaccine-associated poliomyelitis in the UK, which was rare when the first dose of OPV was given at 4 months of age, but became undetectable when the schedule was changed to give the first dose at 2 months, when levels of maternal antibodies would be expected to be consistently higher (Minor, 1997b).

This explanation of the emergence of polio only as standards of hygiene improve seems to require that maternal antibody should protect poorly, if at all, against gut infection. If maternal antibody extends to the gut, an exposed child will not become infected while protected by maternal antibody and therefore cannot become immune as a result. As maternal antibody declines, the child will become fully susceptible to infection and disease. On the other hand, if maternal immunity does not extend to the gut, infection could occur, but viraemia and thus disease would be prevented. The child could continue to excrete virus safely until their own immune system develops a response to eliminate the virus and leave the child immune. The fact that humoral antibody can protect from disease was shown in a large trial of the effect of passive immunoglobulin in the 1950s (Hammon et al., 1953). Any vaccine able to induce humoral immunity should therefore reduce polio cases. This is supported by the data in Fig. 4, which shows the incidence of poliomyelitis in the USA from 1950 to 1980. Both inactivated polio vaccine (IPV), as developed by Salk, and oral polio vaccine (OPV), as developed by Sabin, had profound effects on the incidence of disease.

**Polio in the 1970s/1980s**

By the mid-1970s, polio had been controlled and essentially eradicated in developed countries including the USA, UK and most of Europe. Both OPV and IPV were effective and safe; the UK used OPV and the Netherlands IPV, and

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**Fig. 3.** Pathogenesis of poliomyelitis, showing poliovirus entry by the oral route and spread by viraemia to secondary sites and thence to further sites (Sabin, 1956).
wild-type viruses were not circulating in either country. This was unexpected at the time for a country using IPV; it was thought that OPV would break transmission of the virus, because it would imitate natural infection and therefore induce gut immunity, whereas IPV would not do so as its effect was on serum humoral immunity. Whilst IPV might not prevent infection of the gut, however, it can prevent viraemia and the consequent infection of distal sites such as the tonsils and pharynx by inducing humoral immunity. Interruption of the circulation of wild-type virus was therefore explained by assuming that, where hygiene standards are high, exposure to infection is by the respiratory route, so that transmission is interrupted without affecting the ability of the gut to be infected. This has still not been demonstrated to be the true explanation, and the effect of IPV in areas of poor hygiene has not been established satisfactorily. In contrast, OPV has been clearly shown to break transmission and remains the vaccine of choice in epidemics (John, 2009).

In both the UK and the Netherlands, however, there were pockets of unimmunized individuals susceptible to imported virus who developed poliomyelitis. In the Netherlands, there were outbreaks in communities refusing vaccination on religious grounds (Mulders et al., 1997; Oostvogel et al., 1994). Neither the disease nor infection with the virus spread to the main immune populations. In Finland in the early 1980s, serological studies showed that the immunity of the population to type 3 poliovirus was poor (Lapinleimu, 1984). This was probably caused by the use of old-style, low-potency IPV and, in 1986, an outbreak of type 3 poliomyelitis caused eight cases; there is reason to believe that the majority of the population was infected, based on widespread isolation of the virus from sewage and later serological studies (Pöyry et al., 1988).

Unless poliovirus is eradicated, it is obvious that the absence of the disease requires a high-quality immunization programme that must be maintained indefinitely. In the 1970s/1980s, the cost of IPV and its supposed inability to interrupt faecal–oral transmission made it unsuitable for use on a global scale. In contrast, whilst OPV imitated infection and would therefore protect against subsequent infection by wild-type virus, it was thought to work only poorly in tropical countries (Ofosu-Amaah, 1984). This conclusion was based on the low impact of immunization on reported case numbers. However, surveillance was poor and it is doubtful that the vaccine given had been properly looked after or administered correctly. In addition, polio is less seasonal in tropical countries than in temperate climates. When vaccine is administered at set ages in routine immunization programmes, all children born in the low season are protected from infection, so the pool able to maintain the wild-type virus in preparation for the next high season is reduced; the low season is shorter in tropical countries, so the effect on the susceptible pool is lower and virus elimination is less likely (Nathanson, 1984).

This can be overcome by the use of vaccine in mass campaigns.

The eradication programme

Sabin (1986) had shown that the use of OPV in mass campaigns was likely to interrupt transmission by abruptly reducing the pool of those susceptible to infection and, in the 1980s, campaigns were introduced in South America with dramatic effect. By 1988, the southernmost countries, including Argentina, Chile and Uruguay, were all free of poliomyelitis, and the World Health Organization (WHO)
was encouraged to commit to the eradication of poliomyelitis from the world by 2000 (WHO, 1988). Whilst it failed to meet this target, by the end of 2000, the whole of the Pacific region, including China, had eradicated the disease, as had the Americas and most of South-East Asia; the countries with continuing endemic transmission in 2000 were chiefly in the Indian subcontinent and central Africa. National Immunization Days (NIDs) played a major role in eradication, the aim being to immunize all children under the age of 5 years in a region or country, ideally in a single day. China immunized its entire child cohort (one-quarter of the world’s children at the time) in 1 week, and India has regularly immunized 120 million children at a time, with immunization events occurring every other month.

If executed successfully, there should be no unimmunized children or susceptible guts in the country for the wild-type virus to colonize, so that transmission is interrupted and the virus and disease are eradicated. Usually two or three rounds of vaccination have the desired effect, but they are supplemented by mopping-up programmes, routine programmes to maintain immunity and sub-NIDs (SNIDs) in limited areas to keep the virus under control. In fact, the relationship between eradication of the wild-type virus and the elimination of detectable cases of disease is more complicated and there are several examples where disease has been undetectable, while wild-type virus continues to be found in sewage (El Bassioni et al., 2003; Manor et al., 1999; Más Lago et al., 2003; Tambini et al., 1993). This raises obvious questions about the confidence with which polio can be said to be eradicated when the time comes and what post-eradication strategies would be adequate. Reliable surveillance to identify cases is essential and sometimes questionable. However, the last wild-type 2 virus in the world was isolated in October 1999; surveillance for type 2 is convincing, because circulating vaccine-derived strains of type 2 are regularly identified, as described later, and because of two incidents in India. In 2000, there were two cases of poliomyelitis attributed to type 2 virus, and in 2002–2003, a further six cases. Five of these patients had received OPV from a particular Indian manufacturer before disease onset, but the virus isolated from them was a virulent laboratory strain designated MEF; two closely related MEF strains were in fact identified (Deshpande et al., 2003). This suggests that, had wild-type 2 poliovirus still been circulating, it would have been detected.

By 2003, it was thought that polio was still circulating in seven endemic countries: India, Pakistan, Afghanistan, Nigeria, Niger, Egypt and Ethiopia. Other countries had sporadic cases from virus imported from these endemic countries (Fig. 5a). In 2003–2004, the immunization programme in northern Nigeria came to a halt as the local leaders withdrew support from the efforts, amid rumours that the vaccine contained steroids intended to sterilize girl children. This was combined with a lessen ing of immunization activities in the surrounding countries because of a shortage of funds; the result was an increase in the number of cases in Nigeria and spread of the virus across central Africa. In addition, strains that had been thought to be eradicated were identified again, notably in Sudan, pointing to imperfections in the surveillance programmes (Fig. 5b). Northern Nigeria is predominantly Muslim and, as the virus re-established itself, the time of the pilgrimage to Mecca approached. Polio outbreaks attributable to Nigerian strains occurred in Yemen and Indonesia in 2004–2005, with Yemen having the most cases of any country in that period (Fig. 5c). The virus presumably arrived there from Nigeria via Mecca. The outbreaks were brought under control by efforts across the whole of central Africa; Yemen and Indonesia eradicated the virus for a second time (WHO, 2011a).

This event demonstrates that, if one country has poliovirus, the world is at risk. This has been confirmed by outbreaks caused by virus imported from northern India into Tajikistan in 2009, repeatedly from northern India into Angola and from there into the Democratic Republic of Congo, and from Pakistan to China in 2011. It is possible that the importations from Nigeria via Mecca and into Angola from northern India do not involve children, as pilgrimage and work migrations involve mainly adults.

Some of the remaining endemic areas, including northern India, were particularly difficult to deal with for reasons that are not established. Reviews of the programme in these areas by WHO have shown convincingly that the vaccination programmes are executed very well, with SNIDs being conducted repeatedly and regularly to a high standard (WHO, 2011a). Cases have been reported in children who have had ten or more doses of vaccine confirmed by immunization records. The force of infection is very high, with very high concentrations of people, high birth rates and poor hygiene. Under these circumstances, it could be argued that there should be no polio at all, if polio is absent where exposure is at a young age and mothers have high antibody levels. One speculative possibility is that the force of infection is so high that it overcomes maternal immunity, but there is no real evidence for this.

One practical consequence was to change the type of OPV given. The most widely used form of OPV in the programme was a trivalent mixture of types 1, 2 and 3, but monovalent type 1 and type 3 and bivalent mixtures of types 1 and 3 are now used extensively, so that the presence of irrelevant serotypes does not interfere with gut infection by the vaccine virus of the type of particular concern. There is evidence that this is more effective (Grassly et al., 2007).

Politically, attitudes in Nigeria changed when the Saudi authorities introduced a requirement for all visitors to have evidence of immunization before they would be admitted; Nigeria and Saudi Arabia continue to have a thriving relationship. The result has been that polio is decreasing in both northern India and Nigeria. At the time of writing (June 2011), there have been no cases of polio in Uttar Pradesh or Bihar for >6 months and there has been only one case of poliomyelitis in the whole of India in 2011.
These areas are thought to be the most difficult from the point of view of the biology of the virus, although regions of conflict such as Afghanistan or Pakistan have greater problems of access. Programmatic issues have been political, economic, sociological and logistic, rather than virological. However, as the live vaccine controls disease due to the wild-type virus, the rare cases associated with the vaccine and the occurrence of circulating vaccine-derived polioviruses that cause outbreaks of disease have reintroduced the need to understand the virus and the vaccine better.

**OPV**

Many live-attenuated vaccine strains were developed in the 1950s, but all strains currently used worldwide are derived from the Sabin strains (Sabin & Boulger, 1973). They were developed by growth of virulent virus under a variety of conditions, including different cells and cell lines, multiplicities of infection and temperatures, the aim being to generate variants that would not cause paralysis in animal models; colossal numbers of primates were used to identify and characterize them (Sabin, 1956). The strains had to be
sufficiently fit to infect vaccine recipients, whilst being sufficiently weakened and stable on passage that they did not cause disease.

In fact, from the early trials it was clear that a single passage through the human gut, particularly of the type 3 strain, produced a virus of increased neurovirulence; as the vaccine came into use, there were reports of poliomyelitis associated temporally with vaccine, usually explained by assuming that the victim had been exposed to a wild-type strain. It was not demonstrated unequivocally that the vaccine could revert in vaccinees and cause poliomyelitis until molecular methods of identification were applied in the 1980s (Minor, 1982; Minor et al., 1982; Nottay et al., 1981). The frequency is low, estimated at one in 500 000 first-time vaccinees, and the incidence in recipients of the second dose is far smaller. Cases occur in recipients and in their contacts; it is considered that the type 3 strain causes more cases in recipients, while the type 2 strain causes more cases in their contacts (Nkowane et al., 1987). The type 1 strain gives a frequency of vaccine-associated cases about tenfold lower than those of the other two types combined; in contrast, most wild-type cases are caused by type 1, with type 3 giving a tenfold-lower frequency and type 2 lower still. The vaccine is clearly safe under current conditions, but it is possible that the profile would be different if maternal antibody levels were low. This could be an issue if there is a need to respond to an outbreak for any reason in a post-eradication, post-immunization world (Tebbens et al., 2010).

The potential for vaccine-associated cases of poliomyelitis made it necessary to test the vaccine for virulence in primates and led to detailed molecular study of the basis for the attenuation of the Sabin strains (reviewed by Minor, 1992). The approach used to identify attenuating mutations exploited the infectiousness of cloned copies of the genome (Racaniello & Baltimore, 1981). Closely related strains of very different neurovirulence were compared and inter-strain recombinants or specific mutants were constructed. Virus was recovered from the manipulated cloned copies of the genome and then tested in non-human primates or later in transgenic mice. The data are summarized in Table 1. In all cases, it was concluded that there is a significant mutation in the 5’ NCR that affects the thermodynamic stability of a highly structured region (Fig. 6). The mutation in type 3 affects a 3 bp stem, replacing a triple-hydrogen-bonded GC pair with a single-hydrogen-bonded GU (Fig. 6c). The type 2 mutation appears not to affect a structure at all; however, it is possible that, by changing the G into an A, an AU pair forms between bases 481 and 511, so weakening the long base-paired region from bases 474 to 480 (Fig. 6b). The type 1 base involves a substitution of a G for an A at the end of the long stem structure, replacing a double-hydrogen-bonded AU for a single-hydrogen-bonded GU (Fig. 6a). All base pairs that are predicted to differ between wild-type and vaccine strains are allowed, but result in a weaker twin-stemmed structure in the attenuated virus from about base 470 to 480; the structure forms part of the IRES and is therefore important in the initiation of protein synthesis.

The mutation found in the type 3 vaccine strain is predicted to have a greater effect on the thermodynamic stability than that found in type 1. This is reflected in the effects of the mutations on in vitro initiation of protein translation (Svitkin et al., 1990), where the difference between the efficiency of translation of RNAs from the Sabin type 1 strain and the wild-type Mahoney strain is less than the difference between RNAs from the Sabin type 3 strain and its virulent precursor Leon. The mutations can render virus growth sensitive to elevated temperatures in certain cell types, and the effect also parallels the predicted reduction in stability (Macadam et al., 1992). The effects of the 5’ non-coding mutations can be suppressed in African green monkey cells such as Vero or BGM by a wide range of mutations in the viral protease 2A (Rowe et al., 2000). The observation could be of practical significance, as much of the polio vaccine used in the world is now made in Vero cells. The mutations in 2A do not appear to affect the phenotype in human cells or in mouse cells stably expressing the human receptor for poliovirus; few 2A mutations have been reported in virus isolates from vaccinees. They do not measurably affect the virulence of the virus in either monkeys or transgenic mice where this has been tested, and the mechanism of action has not been established (Rowe et al., 2000).

In addition to the 5’ non-coding mutations, each vaccine strain has at least one attenuating mutation located in the capsid region. In type 3, the capsid mutation alters an amino acid from a serine in the virulent Leon strain to a phenylalanine in the vaccine strain and makes virus growth sensitive to high temperature in culture; in type 2, a mutation in VP1 at residue 142 has an effect on neurovirulence, although other in vitro phenotypic effects have not been established and, while the situation in type 1

**Table 1. Changes that attenuate the Sabin vaccine strains of poliovirus**

The base or amino acid in the virulent virus is shown first. Mutations believed to have a slight effect are shown in parentheses.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>5’ NCR</th>
<th>Structural proteins</th>
<th>Non-structural proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>480 A-U</td>
<td>Many changes implicated</td>
<td>(3D pol 73 Tyr–His)</td>
</tr>
<tr>
<td>2</td>
<td>481 G-A</td>
<td>VP1 143 Ile–Val; (VP4 41 Ala–Ser)</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>472 C-U</td>
<td>VP3 91 Ser–Phe; (VP1 6 Ile–Thr)</td>
<td>None</td>
</tr>
</tbody>
</table>
is complicated by the large number of differences between the vaccine strain and the Mahoney strain, it is believed that mutations in VP1 and VP4 have effects (Bouchard et al., 1995; McGoldrick et al., 1995; Omata et al., 1986). Most of what will be discussed below concerns the type 3 strain, which is the best characterized.

The virus phenotype is the product of complex interactions. However, the mutations that attenuate the virulence of a particular serotype in animal models appear to be significant in humans, as they can be shown to have reverted or to have been phenotypically suppressed in isolates from vaccine-associated cases or in virus excreted by healthy vaccinees.

**Ecology of virus in the human gut**

The type 3 vaccine strain changes over time in vaccine recipients (Evans et al., 1985; Minor et al., 1986), first reverting the 5’ NCR mutation at base 472, then suppressing the effects of the VP3 amino acid change at residue 91. The process in two children in one family is shown in Fig. 7, but is typical (Minor et al., 2005). For all three serotypes, recombinant viruses are generated, in addition to the point mutations introduced. The type 3 5’ non-coding mutation, in which a cytosine in the wild type is substituted for a uridine, reverts most rapidly of the three serotypes, generally within a few days of vaccination. The type 2 strain mutation reverts rather less rapidly and the type 1 strain slowest of all, not changing in about half of recipients (Dunn et al., 1990; Laassri et al., 2006; Minor et al., 2005). Moreover, the type 1 mutation, in contrast to the other serotypes, can be suppressed either by a change of residue 525 from a U to a C, generating a triple-hydrogen-bonded GC base pair with residue 480, or by mutation elsewhere in the long stem at base 476, where a UU mismatch between 476 and 529 is converted into an AU base pair (Fig. 6a). The order of genetic stability in the gut thus reflects the order of thermodynamic stability, with the more stable structures reverting most slowly and incompletely or by second-site mutation.

In some studies, the rate of reversion was found to be increased by previous immunization with IPV (Abraham et al., 1993). This was not always the case (Laassri et al., 2006) and, in one study, an effect was only found after the second dose of OPV (Minor et al., 2005), where it was also associated with a shorter period of virus excretion. This is consistent with the view that the virus is able to counter constraints on growth in the gut indirectly by becoming fitter in its general growth properties. Potentially, it provides an indication of constraints such as gut immunity induced by IPV in addition to prevention of virus excretion, where contradictory studies have been published (Faden et al., 1990; Laassri et al., 2006; Minor et al., 2005).

The other major attenuating mutation in the type 3 strain is at residue 91 of VP3, where a serine in the wild-type precursor is replaced by a phenylalanine in the vaccine strain. The assembly pathway is illustrated in Fig. 8; the mutated residue that lies at the interface between the pentamers that form the pentamer makes assembly of the pentameric subunits and virus growth temperature-sensitive (Macadam et al., 1991; Minor et al., 1989). The effect of the mutation is often suppressed in vaccine recipients and in vaccine-associated cases by compensating mutations at the interface (Fig. 9a). Strict back mutations of VP3 91 are less common. Moreover, mutations at other sites are also found, notably at the interface between pentamers that link to form the entire capsid (Fig. 9b), suggesting that the stability of the intact capsid particle or the speed of the transition from pentamer to virion may be enhanced; the assembly of the protomer is unaffected. A third type of mutation occurs on the internal
Fig. 7. Changes in the genetic and phenotypic properties of type 3 virus excreted after vaccination with trivalent vaccine in two children of the same family. Child 1 was immunized at 15 weeks and child 2 at 18 weeks of age (Minor et al., 1986).

Fig. 8. Assembly pathway of a polio virion.
The development of immunity and the response of the virus to more hostile conditions could explain the reproducible sequence of events shown in Fig. 7. In the non-immune recipient, the phenotype associated with the mutation in VP3 is stable and does not hamper virus growth, thus more constraints in the form of an immune response are required before it becomes significant. If this is correct, the immune response in the gut can be evaded by improving the fitness of the virus. Therefore, the recombination that occurs concurrently (Cammack et al., 1988) would also be predicted to make virus growth in the gut more efficient. This has not been tested. If the effect can be avoided by suppressing the weak effects of the \(ts\) phenotype, it suggests that the effect of the immune response is also quite mild.

The observation that type 1 vaccine causes fewer vaccine-associated cases than either type 2 or type 3 has been explained in a number of ways. In the studies to determine attenuating mutations, the number of differences between the vaccine viruses and the virulent viruses with which they were compared is associated inversely with the frequency of vaccine-associated cases. There are 55 differences between the type 1 vaccine strain and the virulent Mahoney strain (Nomoto et al., 1982), 23 between the type 2 strain and the revertant 117 strain isolated from a vaccine-associated case (Pollard et al., 1989), and 10 between the type 3 strain and the virulent precursor strain Leon (Minor, 1992). However, for type 2, most of the revertant phenotype can be produced with three mutations, so the necessity for the remaining 20 has not been demonstrated. The hypothesis is more plausible for type 1, where many mutations may have incremental effects, leading to problems in identifying them with confidence because of the slight effect that they have on their own.

In addition, the ecology of the virus in the gut may play a role. Where a virus is minimally attenuated and growth is slightly restricted, there will be little pressure for it to revert, as for the type 1 5’ NCR mutation or the mutation in VP3 of type 3, as argued above. Whatever attenuated phenotype is associated with the mutation will therefore be retained for longer. Conversely, a mutation with a major effect on growth, such as the 5’ NCR mutation of type 3, will be strongly selected against and lost rapidly, regenerating the virulent phenotype potentially before an immune response can be induced. Paradoxically, the more attenuated virus could therefore be less attenuated in practice. If this is correct, it indicates that there is a narrow phenotypic window for satisfactory attenuation of a vaccine, where the mutations must not affect virus fitness so severely that they are lost rapidly, but must be

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**Fig. 9.** Atomic structure of a polio virion, showing location of mutation in Sabin type 3 strain at residue 91 of VP3 (cyan) and suppressor mutations found in viruses excreted by healthy vaccinees or vaccine-associated cases (shown in white). VP1 is shown in blue, VP2 in yellow, VP3 in red and VP4 in green. Data were derived from Minor et al. (1989); structures were redrafted here. (a) External view down onto the pentameric axis of symmetry, showing mutations at the interface between protomers making up a single pentamer. (b) External view of the interactions between three pentamers that form the whole capsid; mutations in VP2 and VP1 are shown. (c) View from the inside of the virion, showing suppressor mutation in VP1 away from any obvious interface. The myristoyl residue attached to VP4 is shown in pink.
collectively sufficient to prevent the virus from causing disease. In animal tests, the type 1 vaccine strain is the least attenuated, despite its better safety record in humans (Dragunsky et al., 1996). By introducing many mutations with slight attenuating effects, a stable strain could be produced where loss of any one mutation would have only a marginal effect on virus fitness. A reasonable explanation for the lower rate of vaccine-associated polio with the type 1 strain than the type 2 and 3 strains may therefore be that the type 1 strain has many mutations with only slight effects that are stable in the vaccine recipient.

Patients unable to mount a humoral response are at increased risk of paralytic polio, as they are unable to prevent the viraemic spread of virus to the CNS. There are several examples of long-term shedding of virus by such patients, but they are rare. In one early study, two of 30 patients excreted virus for 18 months and 3 years, respectively; the others stopped shedding virus on a timescale similar to that in immunocompetent individuals, typically 5 weeks and rarely more than 10 weeks (MacCallum, 1971). Moreover, while there is an overall rise in faecal IgA levels at the time of cessation of virus excretion, it is neither a necessary nor a sufficient condition for virus production to stop (Savilahti et al., 1988). It can be hypothesized that the immune response imposes a pressure on virus replication that can cause it to stop, but that it may also stop spontaneously.

During an epidemic, the sequence of the virus drifts at an extremely steady rate of about 1% of all nucleotides or 2.7% of non-coding (third-base) changes (Jorba et al., 2008; Martín et al., 2000a). This provides a means of deciding when strains diverged. The rate of drift implies that the virus population is being sampled continuously; in the laboratory, this would normally be done by plaque-to-plaque passage. The equivalent process in an epidemic could be the passage from individual to individual; however, virus isolated from the long-term excreters of poliovirus drifts over time at exactly the same rate (Martín et al., 2000b, 2004). The rate is independent of serotype or, as far as can be told, the individual, and indicates that the stochastic sampling of the population required to generate the drift of sequence (Agol et al., 2001) happens because of bottlenecks in the infected individual, and not in the transmission from person to person. Drift in the gut cannot be explained by the equivalent of plaque-to-plaque passage in vivo because recombination requires multiple infections of a single cell and is very common both between vaccine strains and in the wild (Lukashev et al., 2003; Minor et al., 1986). On the other hand, the observations can be explained by the existence of a very small number of infectable cells sampling an excess of virus, which would have the same effect. The amount of virus excreted is about $4 \log_{10}$ (g faeces)$^{-1}$ (unpublished data) or $5 \log_{10}$ (g faeces)$^{-1}$ (Fine & Carneiro, 1999); assuming that the infection of a cell results in lysis, at a burst size of 200 p.f.u. per cell, as in cells in culture (unpublished data), and an unlikely faecal production rate of 1 kg day$^{-1}$, this implies that the maximum number of cells producing infectious virus is of the order of 50 000–500 000 in the entire length of the infectable gut in one 24 h period. Whilst cells in vivo could be infected chronically rather than lytically, persistent infection of cells would be hard to reconcile with the high rate of drift in the population. Attempts to superinfect patients who are already infected are usually unsuccessful (MacCallum, 1971). A possible inference is therefore that all cells that can be infected are infected. It might be a more effective strategy for chemotherapy to reduce the already small number of infectable cells (for example, by dosing them with polymerase or protease inhibitors to make them resistant before they are exposed to virus) than to reduce the amount of virus by dosing with capsid-binding compounds that reduce virus infectivity, which is in excess. The cell type infected has not been identified, but must have a limited function at the time of infection, as most polio infections are entirely asymptomatic.

Whilst the immune response may be slow to clear established infections, studies in which recipients are given two successive doses of vaccine show that a virus type that caused infection the first time around does not usually infect the second time (Minor et al., 2005). As other serotypes are able to infect, the effect is mediated by immunity, not by an effect on the number of infectable cells.

Epidemiologically significant vaccine-derived strains

In the period from about 1988 to 1992, poliovirus strains isolated from cases of polio in Egypt included type 2 viruses that were subsequently found to be related closely to the vaccine strain; they had been misidentified as wild type at the time (Yang et al., 2003). The existence of circulating vaccine-derived strains was first recognized in an outbreak of 22 cases in Hispaniola in 2000–2001 (Kew et al., 2002). The island comprises Haiti and the Dominican Republic and the strains were shown to be related closely to the Sabin type 1 strain; based on the degree of drift, they had been circulating unnoticed for about 2 years. They were also recombinants between the vaccine strains and unidentified C type enteroviruses, which contributed sequences in the 3’ half of the genome; several different recombinants were identified, including some that were clearly related and generated by second recombination events, having some sequences in common, but distinctive other regions. The relationship between the different recombinants is not known, specifically which came first, and the partners have not been identified. The strains were neurovirulent in animal models.

Since then, there have been numerous isolations of such strains, which are defined operationally and arbitrarily as similar to, but >1% different in sequence from, the vaccine strains in the VP1 gene, and therefore are assumed to have been circulating for over a year. Almost all of the strains are recombinants with an unidentified species C enterovirus; type 2 isolates are more common than type 1,
Table 2. Poliomyelitis cases caused by cVDPVs to October 2010

<table>
<thead>
<tr>
<th>Country/geographical region</th>
<th>Year</th>
<th>Serotype</th>
<th>No. of cases</th>
</tr>
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<tbody>
<tr>
<td>Peru</td>
<td>1983</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Egypt</td>
<td>1988–1993</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>Pakistan</td>
<td>2000</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Hispaniola</td>
<td>2000</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>Madagascar</td>
<td>2001–2002</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Philippines</td>
<td>2001</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>China</td>
<td>2004</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Indonesia</td>
<td>2005</td>
<td>1</td>
<td>46</td>
</tr>
<tr>
<td>Madagascar</td>
<td>2005</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Madagascar</td>
<td>2005</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Cambodia</td>
<td>2005–2006</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Myanmar</td>
<td>2006–2007</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Nigeria</td>
<td>2005–2010</td>
<td>2</td>
<td>319</td>
</tr>
<tr>
<td>Niger*</td>
<td>2006</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>2008–2009</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>2008–2009</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Somalia</td>
<td>2008–2009</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>DR Congo</td>
<td>2008–2010</td>
<td>2</td>
<td>26</td>
</tr>
<tr>
<td>Guinea*</td>
<td>2009</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>India</td>
<td>2009–2010</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>Afghanistan</td>
<td>2009–2010</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

*Importation from Nigeria.

which are in turn more common than type 3. Reported outbreaks are summarized in Table 2 (CDC, 2009, 2011).

The circumstances required to generate circulating vaccine-derived polioviruses (cVDPVs) are not clear; although it may be hypothesized that they arise and circulate in partially immune populations, it is not obvious what level of susceptibility is required. In Hispaniola, the vaccine coverage had fallen to about 50% overall, suggesting that equal numbers of vaccinated and unimmunized children might be mixing, providing ideal conditions for the selection of transmissible strains. In other regions, such as the Philippines, overall coverage was far better, but may have been poor in more localized areas. Circulating strains have not been identified everywhere, but they occur at an uncomfortably high frequency. If the strategy for the end game is to simply stop vaccinating, they are expected to appear post-eradication (Fine & Carneiro, 1999).

Long-term excretion of poliovirus is documented globally in patients deficient in humoral immunity. Usually it continues for a relatively defined period and, whilst the classification is arbitrary, a long-term excreter is defined as an individual who has shed virus for <5 years but >6 months, whereas a chronic excreter has shed for >5 years. Active searches among hypogammaglobulinaemic patients have been mostly unproductive, but there are now many long-term excreters and some chronic excreters, most identified because the patients developed paralytic poliomyelitis. Examples are given in Table 3 (Buttinelli et al., 2003; Halsey et al., 2004; MacCallum, 1971; MacLennan et al., 2004) and, whilst initially this was considered mostly a problem for developed countries, it is now clear that cases occur throughout the world. One UK patient is believed to have been excreting type 2 virus for over 25 years and isolates are available from 1995 to date. They are virulent in animal models, but the patient is so far healthy. Unlike cVDPVs, these types of vaccine-associated virus tend not to be recombinants with type C enteroviruses, although polio–polio recombinants do occur (Shahmahmoodi et al., 2008; Yang et al., 2003); they are termed iVDPVs (immunodeficiency vaccine-derived polioviruses). They have never been demonstrated to have caused an outbreak, although there seems to be no reason why they should not, and there is some evidence that they can spread to others. Some cases have arisen because the hypogammaglobulinaemic patient has been infected by a close contact, as in a recent case in Minnesota, USA (DeVries et al., 2011). As they pose a long-term threat to the eradication programme, there are efforts to develop effective antiviral treatments; attempts to stop excretion by other methods have not been very successful (MacCallum, 1971; MacLennan et al., 2004). Although one patient was reported to have stopped shedding after treatment with an antenteroviral drug (Buttinelli et al., 2003), the very-long-term excreter described above was treated with a variety of agents, including breast milk and ribavirin, leading to a short-term reduction in virus detection in stools, which was immediately reversed when treatment stopped. The sequence of the virus isolated as excretion resumed was very similar to that before treatment, so it seems likely that virus replication was not suppressed to the point where it was near extinction, as this might be expected to select a subpopulation. This suggests that the treatment was not particularly effective (MacLennan et al., 2004).

Environmental surveillance, as opposed to surveillance of cases, has not been pursued uniformly because of the resources required. However, several laboratories have successfully monitored sewage for polioviruses and reported vaccine-derived viruses (Cherkasova et al., 2003; Korotkova et al., 2003; Shulman et al., 2000). In some cases, the viruses are highly drifted and may represent either cVDPVs or

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Prolonged</th>
<th>Chronic</th>
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<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
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<tr>
<td>1+2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2+3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
iVDPVs. They have been designated atypical (a)VDPVs and characteristically their origin is not known; they are not associated with cases of poliomyelitis. They are obviously a threat to the eradication of poliomyelitis.

**Barriers to eradication**

Infection with poliovirus is inapparent; even where the infection leads to disease, there is typically a preclinical phase of 7–30 days, and most infections are entirely silent. Quarantine is ineffective in influencing epidemics (Paul, 1971) for this reason, and surveillance of paralytic cases detects very few of the total number of infections. This raises major difficulties for an eradication programme where, if one area has the virus, the entire world is at risk. Even where immunization programmes have been of high quality, outbreaks may still occur, as demonstrated recently in China, where ten cases were caused by virus originating in Pakistan (WHO, 2011a). As discussed earlier, there are examples of virus being detected even where cases were absent. Ensuring eradication means that the virus and not just the disease must be eradicated.

There are major practical difficulties in immunizing the population numbers needed; the vaccine is a product of high technology, unlike for instance the smallpox vaccine, and is heat-labile; again, unlike smallpox, there are no natural visible indicators of successful immunization. (In mass campaigns, the individuals who have received a dose of vaccine may be given an indelible mark on the finger to identify them later.) Finance is a major issue. Between 1988 and 2010, the polio-eradication programme cost US$9 billion. It will require about US$1 billion in 2011–2012. The amount saved in terms of vaccine costs and higher life productivity should polio be eradicated is plausibly far greater than the cost of eradication itself (Thompson & Tebbens, 2007).

The major problems, however, are to decide when the virus has been eradicated and how to stop vaccinating, when the vaccine itself can become a circulating virus that can paralyse. The belief is that the virus will die out faster than susceptible build-up, and surveillance is clearly key.

Some have suggested that the problems of the post-eradication era are so great and eradication so uncertain that attempts to eradicate the virus should be abandoned and efforts redirected to control of the disease (Arita et al., 2006; Chumakov et al., 2007; Thompson & Tebbens, 2007). This might include improving global vaccine uptake and coverage to high levels, which will be challenging. As an extreme example of the improvement needed, in 2009 coverage for three doses of diphtheria, tetanus and pertussis in Chad in sub-Saharan Africa was of the order of 24 %, which for polio would be nowhere near good enough to protect those who are not immunized through herd immunity (WHO, 2011b). Improvement of vaccination coverage is very desirable but, in the current situation, there would inevitably be outbreaks unless the virus was eradicated.

**Post-eradication strategies**

The strategy to be adopted after eradication of all wild-type polioviruses is declared remains fluid as the situation changes and, in any case, the immediate priority of the programme is eradication of the wild type. Eradication will be declared when 3 years have elapsed since isolation of the last wild-type poliovirus, and laboratory and production strains must be contained (Dowdle & Wolff, 2006). There has been substantial effort in all regions to identify laboratories that have poliovirus and, as far as possible, reduce their number, but production of IPV must continue and requires wild-type virus in very large amounts. At present, containment applies only to wild-type strains, defined by their capsid region, and excludes the Sabin vaccines. In view of the ability of the Sabin strains to give rise to epidemiologically significant polioviruses, this distinction seems increasingly artificial.

Many countries rely on polio vaccines purchased by United Nations agencies and this source of supply is likely to cease when eradication is declared, raising many concerns. Firstly, wild-type virus may not have been eradicated, despite failure to detect it for 3 years; secondly, containment may not be perfect; and thirdly, the vaccine-derived viruses (cVDPVs, iVDPVs and aVDPVs) will certainly be present. Longer-term issues include the use of poliovirus as a bioterrorist agent; poliovirus is a very poor battlefield weapon, as only 1 % of those infected develop disease, but this also makes it a good terrorist weapon if the idea is to spread fear once immunity has declined to low levels. Responses to post-eradication outbreaks from whatever source have been developed, although it is not clear at all what would trigger the major actions that are envisaged (Jenkins & Modlin, 2006; Tebbens et al., 2006).

Various models of the post-vaccination world have been constructed to answer questions on the cost-effectiveness of the whole programme, the likelihood of cVDPV outbreaks, the need to stockpile vaccines in case of an outbreak and how the use of stockpiled vaccines would be triggered in practice. They highlight the uncertain nature of cessation of immunization against polio and in particular the consequences of just stopping abruptly, where the response to subsequent outbreaks is very problematic (Fine & Carneiro, 1999; Tebbens et al., 2010).

In practice, there are developments at the national level, where there is increasing use of IPV over OPV. Over the last 10 years, all member states of the European Union have changed independently to the use of IPV in preference to OPV, at least partly because of the cost of vaccine-associated cases. The whole of the North American continent now uses IPV exclusively, and Mexico and Russia have recently changed to IPV from OPV (WHO, 2011a). It seems at least possible that the world will increasingly adopt IPV, in which case the ability of IPV to prevent transmission in areas of poor hygiene becomes important, in addition to the overall coverage with vaccines. It is conceivable that good global routine immunization programmes delivering a combination vaccine of
which IPV is a part could provide insurance for the post-eradication era. In any event, the use of IPV may be expected to increase. At the moment, there are four licensed producers of IPV, all in western Europe; two provide the majority of the vaccine, which is manufactured under high containment to prevent re-introduction of polio after eradication. As the world economy changes, new manufacturers are likely to enter the market, and the containment of the paralytic wild-type strains in the massive quantities required is a major concern.

**Safer strains for vaccine production**

Once polio is eradicated, licensing a new vaccine will be difficult if protective efficacy in the target species (humans) is required. It is established that humoral immunity generated by inactivated vaccine is sufficient to protect from disease, and this may be an adequate surrogate marker, particularly if generated in response to antigens indistinguishable from the currently used IPV. A new live vaccine is unlikely to be developed after eradication, although there is current interest.

There is a tension between the conditions required to assure safety and those that allow production to take place (Dowdle & Wolff, 2006), and it seems possible that safer strains for IPV production could be devised. One possibility is to apply the Sabin vaccine strains used in manufacture of OPV to the manufacture of IPV.

It is obvious that the Sabin strains are less virulent than the wild-type strains but, given their ability to infect and evolve to different phenotypes, the degree of assurance that their use would offer is hard to judge. A secondary argument is that, should polio re-emerge, OPV might be the best way to counter the epidemic; manufacture of Sabin strain IPV would offer a base from which full-scale production could be developed quickly. Manufacture of IPV based on the Sabin strains (sIPV) would introduce extra tests and constraints on production conditions that would make it very unattractive for an established manufacturer, adding considerably to the costs. Passage of the virus from the seed stocks is restricted for OPV but not for IPV, and yields of the OPV strain are usually lower than for the wild type strains. In addition, the immunogenicity of the Sabin strains when inoculated as IPV is significantly different from that of the equivalent wild type; in particular, it takes about ten times more of the type 2 is significantly different from that of the equivalent wild type; in particular, it takes about ten times more of the type 2

Burns and co-workers and Mueller and co-workers have explored the possibility of codon de-optimization to reduce the fitness of the virus and therefore attenuate it (Burns et al., 2006, 2009; Coleman et al., 2008; Mueller et al., 2006). This approach has been made possible by the ability to synthesize entire viral genomes from which virus can be recovered (Wimmer & Paul, 2011). Viruses with a very high particle: infectivity ratio and poor growth have been generated by modifying the capsid region of the genome so that it contains rarely used codons; by inserting many mutations into the capsid region, it is unlikely that the virus will revert to the full wild type. The phenotype can be adjusted to the desired level of fitness and the virus cannot revert by recombination without ceasing to become a poliovirus. The mechanism of action may not be simply dependent on translation efficiency, but on the frequency of CpG and ApU codon pairs (Burns et al., 2006). However, in systems where factors and tRNAs are limiting rather than the optimized in vitro systems usually used, there is a large effect on translation (Mueller et al., 2006). The effect on virulence in vivo is complex, but where comparable quantities of virus particles are inoculated, the viruses are attenuated compared with the wild type. If similar amounts of infectious virus are used, the effect is the same as that of the unmodified strains. The consequences of this should the strains infect a human host are not clear.

An alternative approach has been to explore the quasispecies, where restricting the breadth of the sequence distribution by generating a high-fidelity polymerase attenuates the virus; this has been suggested as a generic method for producing attenuated vaccines (Vignuzzi et al., 2006) and has a clear effect on polio in the transgenic mouse model.

A further approach involves modification of the 5’ NCR. Based on the studies in vaccinees described above, where the attenuating mutations are strongly selected against in the human gut, it should be possible to generate a virus that is stable genetically and can grow in culture under production conditions, but cannot infect humans. Such a virus could be handled freely, but could only be used as a seed strain for IPV production. We have been working towards this goal by progressively modifying the 5’ non-coding structure involved in the attenuation of the Sabin vaccine strains. It has been shown that weakening the structure increases the attenuation of the strain in animal models, in direct proportion to the thermodynamic stability and temperature sensitivity of virus growth (Macadam et al., 1992, 2006). The rapid reversion of the
type 3 strain in the gut and the slower reversion of the other two types, depending on the degree of disruption of the structure, suggest that infection and attenuation go in parallel, so that if the structure were modified sufficiently, the strain would be non-infectious for the human gut, but still replicate in cell culture. Moreover, by weakening the structure by substituting AU for GC base pairs, the mutations will be stabilized, as two simultaneous changes are required to strengthen the structure. The constructs that have been produced are shown in Fig. 10 (Macadam et al., 2006; A. J. Macadam, personal communication). Their ability to cause paralysis in experimental models relates to the disruption of the structure in so far as it can be measured; it has proved impossible to generate a sufficiently concentrated preparation of construct S19 to cause paralysis at all. The structures are stable on passage at high temperatures, although second-site mutations in 2A are seen when they are grown in Vero cells. A cassette has been constructed in which the genome is derived from the Sabin type 3 strain, modified to contain the S19 5′ NCR structure shown in Fig. 10, into which the portion of the genome encoding the desired capsid proteins is inserted. Constructs include capsids from the wild-type strains used in current production and the Sabin vaccine strains. The attraction is that the product preparation should be structurally identical to existing wild-type or proposed Sabin IPV vaccine and therefore induce the same immune response; this should simplify clinical development. The type 3 strain has been used because the basis of attenuation and reversion is well understood. The strains require assessment for their suitability for production and their ability to infect people.

Conclusions and summary

The global polio-eradication programme has eliminated the disease from most of the world, apparently including India, where the challenge has been greatest. However, the agent must be eradicated to eradicate the disease and, where silent circulation is possible, as for poliovirus, this is not easy to track. The remaining issues of principle concern the vaccine, where the oral vaccine that has eradicated disease can itself cause outbreaks, and also the wild-type strains for the production of IPV. Vaccination must be possible after eradication is declared, if only to take care of long-term excreters of virus and the possibility of re-emergence. More progress towards eradication has been made than many thought possible and, while the way ahead is not certain, the alternative to eradication is to

![Fig. 10. Structure of 5′ NCR domain 5 encompassing approximately bases 465–540, showing mutations inserted to progressively weaken the structure and render it genetically stable; S15 approximates the thermodynamic stability of the Sabin type 3 strain (Macadam et al., 2006).](http://vir.sgmjournals.org)
allow polio to continue or to re-emerge, which will burden the world indefinitely.

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