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Poliovirus vaccination: current understanding of poliovirus interactions in humans and implications for the eradication of poliomyelitis

Philip D. Minor

Poliomyelitis is a paralytic disease of the motor neurones of the central nervous system, which is caused by poliovirus. The virus is transmitted by the faecal–oral route, and if virus replication is confined to the gut, it is harmless. Poliomyelitis is an ancient human disease, but was rare until the beginning of the 20th century, when children began to be exposed to the virus at older ages and were, therefore, no longer protected by maternal antibody, which had already been lost. Inactivated polio vaccines are increasingly being used in those countries in which poliomyelitis has been brought under control; however, live vaccines are still the most widely used types and the World Health Organization (WHO) have set the goal of using such vaccines to eliminate the wild-type virus throughout the world by the year 2000. Substantial progress has been made to this end; however, the strains of poliovirus that are used as vaccines are able to adapt rapidly to the human gut, losing their attenuated (weakened) character within a few weeks. Currently, there is urgent debate about the best method of stopping vaccination against poliomyelitis once the wild-type poliovirus has been eliminated completely, so that the vaccine-strain virus will also be eliminated. Proposed strategies include the abrupt cessation of vaccination with the live virus worldwide, followed by the optional use of inactivated vaccines for an appropriate period. Further information about both the epidemiology and the pathogenesis of the disease is required before an informed choice can be made. The topics covered in this article include a brief history of studies of the disease, its pathogenesis and its control by vaccination, the molecular biology of the live vaccines, which have been extremely successful in controlling poliomyelitis so far, and the concerns that are raised as the eradication of the wild-type virus approaches.

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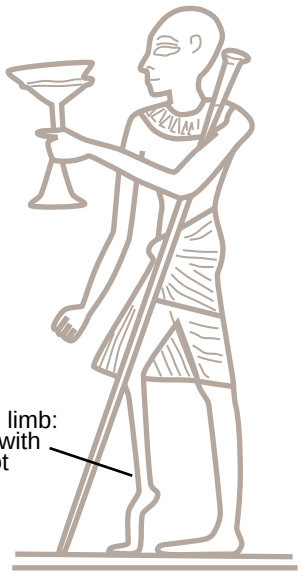
The most common clinical presentation of poliomyelitis is as a paralytic disease in which the motor neurones of the central nervous system are selectively destroyed by poliovirus, which belongs to the picornavirus family. The sensory neurones of the central nervous system are left intact and functional. The typical symptoms of the disease include paralysis of the lower limbs rather than the arms, and of one side of the body rather than both. The name poliomyelitis derives from the Greek for 'grey marrow', indicating the destruction of the grey matter, in the form of the motor neurones, in the spinal cord.

History of poliomyelitis

The early history of poliomyelitis has been reviewed in a classic book written by Paul in 1971 (Ref. 1). The first documented evidence of any human disease being attributed to a virus infection is believed to be a funerary stele from Middle Kingdom Egypt, dated at ~1300 BC (Fig. 1), which depicts the priest Rom with the classical withered limb and down-flexed foot that is characteristic of poliomyelitis. There are very few recognisable references to poliomyelitis in the medical literature published during the 3000 years following Rom's death; thus, in view of its striking and characteristic clinical appearance, it is generally accepted that poliomyelitis was rare during this period. However, towards the end of the 19th century and at the beginning of the 20th century, epidemics of paralytic poliomyelitis began to occur in infants (hence its alternative name infantile paralysis), initially in Sweden, but later in the USA and other countries (Ref. 1). This emergence is attributed to improvements in the standards of hygiene, so that children were older when they were first exposed to poliovirus infection and, therefore, no longer protected by the antibodies that they had passively acquired from their mothers. In 1905, Wickman showed that most poliovirus infections did not cause even the mildest symptoms; he also showed that if the paralytic disease were to occur, it became apparent 8–30 days after infection. Later, it was shown that there was a period of 3–4 days between infection and the first manifestation of any symptoms; such symptoms, which are collectively termed the minor disease, are those of a mild and generalised viral infection, including a rash and sore throat. The minor disease can lead on to the various forms of serious poliomyelitis, which are collectively referred to as the major



Early evidence of poliomyelitis from Middle Kingdom Egypt ca. 1300 BC

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Figure 1. Early evidence of poliomyelitis from Middle Kingdom Egypt ca. 1300 BC. A tracing of one of the figures from the funerary stele of the priest Rom, which is currently in the Carlsberg Museum, Copenhagen, Denmark. The figure has a withered right leg and dropped foot, which are typical of poliomyelitis **(fig001pmn)**.

disease, and include paralysis, as described below. Frequently, however, the minor disease is the only sign of infection. In 1909, Landsteiner and Popper showed that monkeys injected intracerebrally with human spinal cord taken from fatal cases of poliomyelitis suffered similar clinical signs to those seen in human cases, thus providing a means of both detecting the virus and studying its pathogenesis experimentally (Ref. 2). In 1912, Kling and colleagues described the presence of poliovirus infectivity in the contents of the intestines as well as in the nervous tissue taken from fatal cases, thus suggesting that the site of virus replication was the intestine, and the probable route of transmission oral–faecal (Ref. 3).

There are three serotypes of poliovirus, defined by the fact that infection with one serotype does not confer protection against disease caused by either of the other two. Within a serotype, different strains can also be recognised, chiefly on the

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basis of differences in nucleic acid sequence. In general, ≤1% of infections with a serotype 1 virus progress to clinical poliomyelitis, the figure being tenfold lower for serotype 3, and even lower for serotype 2. The reasons for the differences between the serotypes and why some individuals succumb to poliomyelitis, whereas most people are asymptomatic, are not known. The symptoms of the major disease of poliomyelitis are the same for all three serotypes. Meningitis, which is also known as abortive poliomyelitis, can occur. Of paralytic cases, as many as 5–10% are fatal, 10% recover completely and the remainder show some residual paralysis. The actual clinical signs depend on which region of the central nervous system is affected. First, spinal paralysis results from the infection of the lower motor neurones and typically affects only one leg. Second, the more serious bulbar poliomyelitis involves neurones in the brainstem and, therefore, affects breathing. It was this latter manifestation of the disease that led to the development of the iron lung as an artificial respirator during the 1950s. Third, encephalitis results from the infection of the brain itself; it makes up ~1% of all cases and is usually fatal.

Poliomyelitis is preventable either by vaccination or by the administration of immunoglobulins containing antibodies that are specific for poliovirus. The rationale of either approach is to prevent the virus reaching the nervous system and producing irrevocable damage. Once the symptoms of the major disease appear there is currently still no treatment.

Pathogenesis of poliomyelitis

What is known about the pathogenesis of poliomyelitis has been recently reviewed (Ref. 4).

The Flexner model

Early studies of poliomyelitis were dogged by the mistaken view that it was largely or exclusively a disease of the neurones. This view was promulgated by Flexner, who showed in 1910 that monkeys could be infected by the nasal route with extracts of spinal cord taken from fatal cases of poliomyelitis (Ref. 5), such that the poliovirus spread through the brain and into the spinal cord. He concluded that the natural route of transmission in human disease was, therefore, via the nose. Although it is likely that poliomyelitis could be induced in humans by the nasal route by such a procedure, it is now known that the main route of transmission is oral–faecal, as suggested by the early research carried out in Sweden (Ref. 3); nonetheless, the Flexner model dominated poliovirus studies for 30 years.

The Bodian model

The model of pathogenesis that was proposed by Bodian in the 1950s hypothesised that after the poliovirus has been ingested it establishes an infection in those lymph nodes that are local to the gastrointestinal tract, including the tonsils and the Peyer's patches. From these primary sites, the infection spreads to more distant lymph nodes, then enters the bloodstream and from there infects other susceptible tissues including the central nervous system (Ref. 6).

The Sabin model

The model that was proposed by Sabin at the same time as the Bodian model was more subtle and probably closer to the actual situation (Ref. 7). In this model, the poliovirus was considered to establish an infection on the mucosal surfaces of the pharynx and intestinal tract; this explains the continued excretion of the virus in the faeces for weeks after it is undetectable in the lymphoid tissue. It also explains why virus can be isolated from the throats of infected individuals who have had their tonsils and adenoids removed. The virus can spread from the mucosa to the local lymph nodes, from which it can be isolated, but need not necessarily replicate there. From the local lymph nodes, it can spread via the blood in a low-grade undetectable primary viraemia to infect more distant lymphatic tissues or other unknown susceptible sites. It is the replication in these secondary sites that produces the secondary viraemia, where virus can be detected in the blood and which can be prevented from infecting the central nervous system by passive antibodies in the form of immunoglobulin given by injection (Ref. 8) or prior vaccination.

Vaccines against poliomyelitis

Clinical trials of vaccines that were made from the ground-up spinal cords of infected monkeys were carried out in 1935. They were of limited success, causing poliomyelitis at a high frequency in recipients. The first successful vaccine was the formalin-killed preparation of Salk (also known as inactivated polio vaccine or IPV), which was first licensed for human use in 1955. Although the inactivated vaccines that are in current use are of

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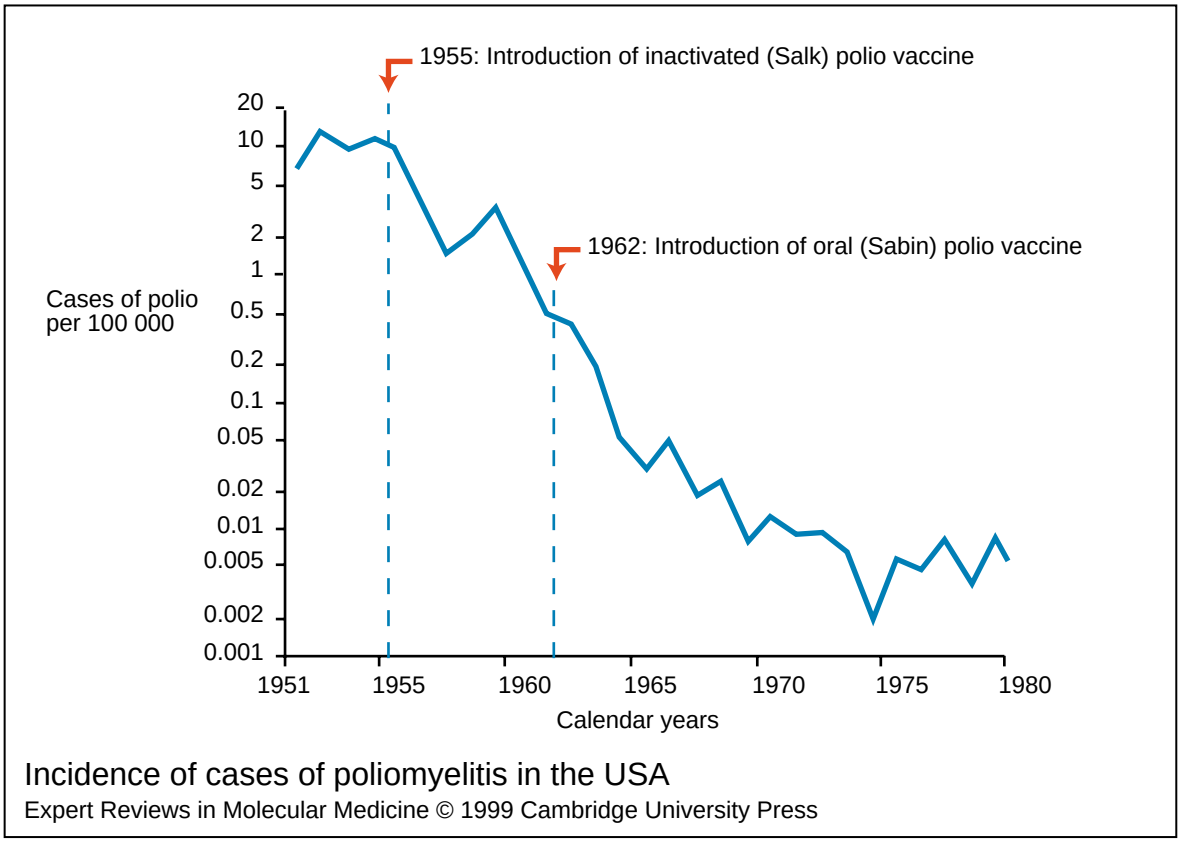

Figure 2. Incidence of cases of poliomyelitis in the USA. Cases are recorded per 100 000 head of population, for the period 1951–1979. The graph shows the decline in incidence of poliomyelitis after the introduction of the inactivated poliovaccine (in 1955) and the subsequent introduction of the live attenuated poliovaccine (in 1962). The incidence is shown on a logarithmic scale. The inactivated vaccine reduced the number of cases by 90% over a period of 7 years **(fig002pmn)**.

higher purity and potency than those produced during the 1950s, they are manufactured using essentially the same procedure as that used to make the early Salk vaccines. It is likely that this type of vaccine will assume a greater prominence in the future as the worldwide eradication of polio approaches. The Salk-type vaccine is already the only vaccine that is being used in many European countries; it is also a component of the polio vaccination programme in the USA. However, the most dramatic effects on the incidence of poliomyelitis worldwide have been due to the use of the attenuated vaccines, which were developed by Sabin and first licensed for human use in 1962 (Ref. 9). Attenuated vaccines infect recipients and produce immunity without causing disease.

Although the merits of the two different types of vaccine have been the subject of heated debate, both were clearly highly effective. The falling incidence of poliomyelitis in the USA between 1955 and 1979 is shown in Figure 2. The introduction of the inactivated vaccine reduced the number of cases by 90%, and this decline continued after the introduction of the live polio vaccine during the early 1960s. The main reason for the switch to the use of live vaccines was that smaller quantities of the virus are needed to infect and, therefore, immunise recipients than are required for immunisation using the non-replicating inactivated vaccine. Consequently, sufficient quantities of the live (but not of the killed) vaccine could be manufactured to meet the demand. In the current global vaccination programmes, where supply is not an issue because of modern production methods, the advantage of using the live vaccine is that only this vaccine is thought to be able to break virus transmission in conditions of poor hygiene, as described below. However, even where poliomyelitis has been controlled by the use of the live vaccine, a few

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cases remain, as shown in Figure 2. It has been demonstrated unambiguously that some of these cases are caused by the vaccine virus itself. In the USA, the incidence of vaccine-associated poliomyelitis is estimated to be ~1 in 530 000 for first-time vaccinees or 1 in 1 200 000 for vaccinees overall (Ref. 10). The number of cases of poliomyelitis occurring in recipients of the vaccine is roughly equal to the number of cases occurring in those who have contact with recipients, such as siblings or parents; however, in contrast to the situation in unvaccinated populations, only 10% of vaccine-associated cases are attributable to the serotype 1 strain, whereas the serotype 2 and serotype 3 strains account for approximately equal numbers of the rest. In the UK, there is less than one case of vaccine-associated poliomyelitis per year. During the 1950s, the annual rate of poliomyelitis was in the range of 1000–10 000 cases.

Interruption of the transmission of poliovirus by vaccination

The effect of the use of live attenuated polio vaccine in the temperate climates of the developed world was evident early on; however, in tropical countries, it had no impact on disease. The reason for this is probably related, in part, to the quality of the vaccines used, which in some cases are known to have been almost completely inactivated as a result of poor handling in hot climates. The nature of the exposure of susceptible individuals to the virus was also different. In temperate climates, exposure tended to be greatest during the warmer summer months, so that there were definite times during winter when it was easy to immunise individuals, before they were infected by the wild-type virus. By reducing the susceptible population in this way, it was possible to break poliovirus transmission. In contrast, there is less seasonal variation in exposure to infection in tropical countries, such that there is a far greater chance of infection with the wild-type virus before vaccination at all times of the year. The solution, suggested by Sabin in 1966 but not really taken up seriously on a global scale until the late 1980s, was to saturate the population with live vaccine over a brief period, so that every susceptible human gastrointestinal tract in the area would be occupied by the live vaccine virus, making infection with the wild-type virus impossible. Thus, the spread and survival of the wild-type virus would be prevented. In practical terms, this takes the form of National Immunisation Days (or NIDs). In India in January 1998, some one-hundred million children were immunised in a single, vast campaign. In 1992, a quarter of the world's children were immunised during a single week in China. The enormity of such exercises is difficult to imagine; the economic effects of stopping a country for a day to immunise its children and the logistical aspects of delivering the vaccine on such a scale are truly extraordinary. The effects of such efforts are shown in Figure 3, which classifies countries according to the number of polio cases reported in 1988 and in 1996. For example, in the Americas, the last case of polio was reported in Peru in 1992, and the entire land mass is now considered to be free of the wild-type virus, although not of the live vaccine strain of poliovirus that is currently still used. China is now regarded to be free of poliomyelitis, except for some imported cases in Mongolia. In fact, the Western Pacific region (which includes Vietnam, Cambodia and Malaysia) has been free of the wild-type poliovirus from mid-1997 to at least mid-1999. The use of polio vaccines in large campaigns complements their use in: (1) routine immunisation programmes, which identify individual children at the specific age that is considered most appropriate for immunisation in the country concerned and (2) mopping-up campaigns, which target particular populations that are still at risk or have not previously been reached.

Eradication and surveillance

The elimination of poliomyelitis depends crucially on accurate and honest surveillance in a country to assess the effects of the programmes on the presence of the wild-type poliovirus. Surveillance can take the form of examining isolates of poliovirus from whatever source, including environmental and clinical specimens, to establish if they are derived from the vaccine strains or are wild-type viruses. This is usually easily achieved by: (1) antigenic characterisation, using neutralisation assays or enzyme-linked immunosorbent assays (ELISAs) with strain-specific anti-polio antibodies and/or (2) genetic characterisation, by using either probe hybridisation or determining the sequence of short stretches of the virus genome to establish if they closely resemble the known sequences of the vaccine strains. Alternatively, schemes

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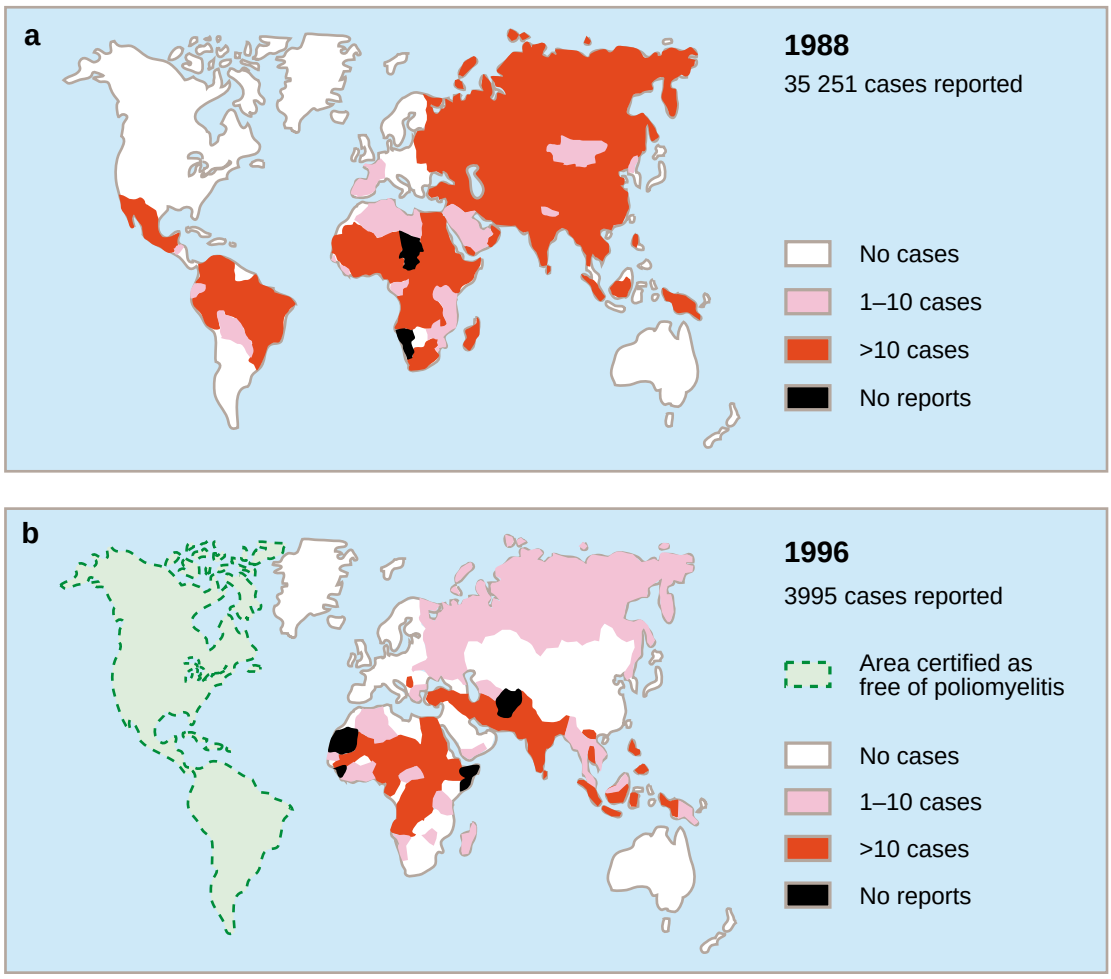

Incidence of cases of poliomyelitis worldwide (1988 and 1996)

Figure 3. Incidence of cases of poliomyelitis worldwide (1988 and 1996). Panel (a) shows the incidence of poliomyelitis in 1988, and panel (b) in 1996. White areas indicate those geographical areas that have been certified as being free of poliomyelitis (no cases were reported); pale-pink areas indicate 1–10 cases reported per year; dark-red areas indicate >10 cases per year and black areas indicate those areas where there was no surveillance (or no reports). In 1996, the Americas (North, Central and South) were certified to be free of poliomyelitis (shown by a green, dotted perimeter). In 1988, the total number of cases reported worldwide was 35 251; by 1996, this number had declined to 3995. However, the actual number of cases will be higher than these figures because not all cases of poliomyelitis are reported **(fig003pmn)**.

for detecting cases of acute flaccid paralysis (which can include poliomyelitis cases) can be set up and patients examined carefully to establish whether poliovirus is present. Currently, in most developing countries, the incidence of cases of acute flaccid paralysis other than those caused by poliovirus is believed to be ~1 in 100 000 children who are <15 years of age. This serves as an essential internal control for the adequacy of the surveillance.

The scale of the achievement of the eradication programme is truly extraordinary. There is every likelihood that it will eventually be completely successful and that poliomyelitis caused by the wild-type virus will disappear from the planet, if not by 2000, then a few years later. Strategies for

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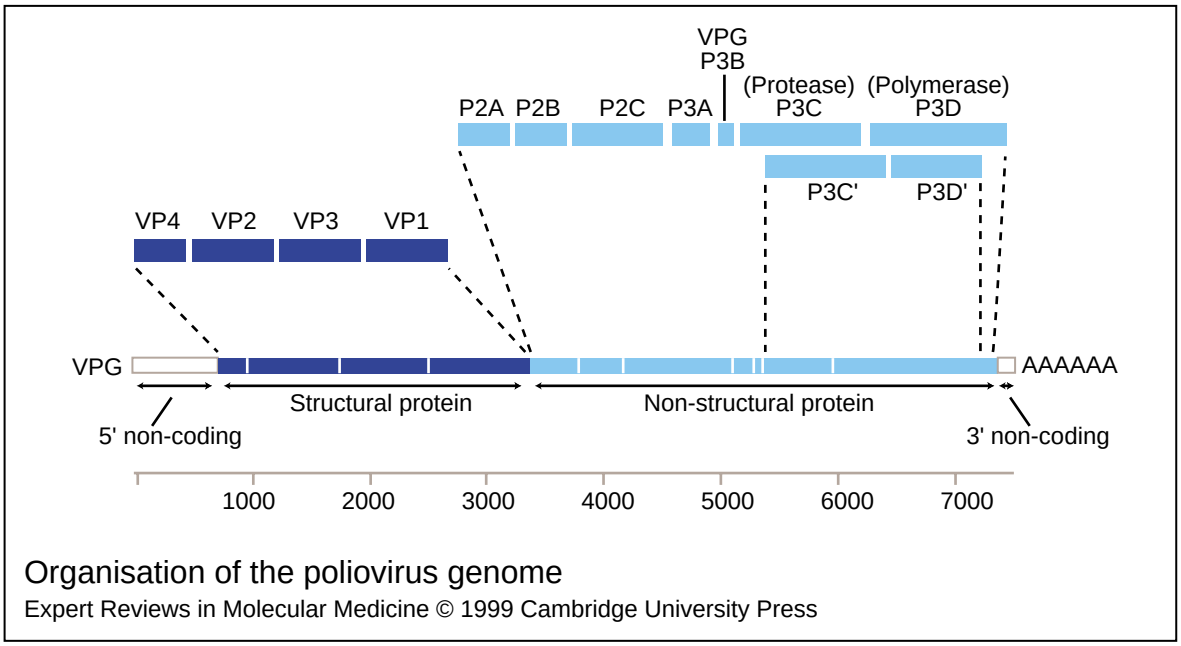

Figure 4. Organisation of the poliovirus genome. Non-coding regions are shown as empty boxes, regions that encode structural capsid proteins are shown in dark blue and regions that encode non-structural proteins are shown in light blue. The RNA genome is ~7500 bases in length (scale bar at the bottom of the figure); the 5' end is covalently linked to the virus-encoded protein VPG, and the 3' end possesses a polyadenylate tail (shown as AAAAAA) **(fig004pmn)**.

stopping the vaccination of humans against poliovirus then become important; thus, the nature of the virus and the vaccine and its interaction with its host are of great interest.

The poliovirus

The molecular biology of poliovirus vaccines has been reviewed in 1992 (Ref. 11). The organisation of the poliovirus genome is shown in Figure 4. It consists of a single strand of positive-sense RNA (~7500 bases in length); the 5' end is covalently linked to a virus-encoded protein (VPG) and the 3' end possesses a polyadenylate tail. A long non-coding region (of ~750 bases) at the 5' end precedes a single, large open-reading frame, which is translated as a single protein, the polyprotein. The polyprotein is cleaved as it is translated by virus-encoded proteases (P2A, P3C and uncleaved P3CD), producing the active proteins that are involved in virus replication. The structural proteins (VP1, VP2, VP3 and VP4) that form the shell of the virus particle are encoded before the non-structural proteins (P2A, P2B, P2C, P3A, P3B, P3C and P3D), which are involved in the replication of the genome of the poliovirus and subverting the machinery of the host cell to make viral proteins. Because the poliovirus genome is of positive sense [i.e. it is equivalent to messenger RNA (mRNA) and can therefore be translated directly into virus proteins], the RNA itself is infectious when introduced into cells. In studies of the virus, full-length complementary DNA (cDNA) cloned copies of the virus genome can be made, manipulated, transcribed into RNA in vitro and infectious virus recovered; the properties of the in-vitro-produced poliovirus can then be studied in vitro or in animal models as required.

Molecular basis of the attenuation of Sabin vaccine strains of live attenuated poliovirus

A serotype of poliovirus is defined by its gross serological properties and its failure to induce cross-protection between serotypes. Each serotype encompasses many strains that are distinguished by more subtle properties including the sequence of their genomes and differences in their antigenic properties, which, although detectable, do not result in a failure of cross-protection between strains of the same serotype. Strains can also differ greatly with regard to virulence. Because a strain belonging to one serotype, by definition, does not protect against disease caused by a different serotype, it follows that an effective vaccine must

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contain a representative of each serotype. The three Sabin vaccine strains (one corresponding to each of the three serotypes) were prepared by growth of natural wild-type isolates under different conditions, and each has its own unique history of laboratory growth (Ref. 9). The basis of attenuation (whereby the virus becomes less able to cause disease) or the reversion of the vaccine strains (whereby a virus recovers its ability to cause disease) has been studied by comparing the vaccine strain of each serotype with a closely related strain, either a precursor of the vaccine strain or an isolate from a vaccine-associated case of poliomyelitis. The sequence comparisons for the pairs of strains that have been used for the three serotypes are shown in Figure 5.

Viruses, in which either segments of the genomes had been exchanged or specific mutations had been introduced, were then prepared by the manipulation of full-length cDNA copies of the viral genomes. Viruses were recovered and studied for virulence. The serotype 3 strains that were compared were the Sabin vaccine strain and its virulent precursor, Leon, which was originally isolated from a fatal case of poliomyelitis in 1937. The number of differences in the sequences of the two strains is surprisingly small (Fig. 5a). Of the nine (definite) differences seen, only that at position 472 in the 5' non-coding region and that at base 2034 (which produces an amino acid change at residue 91 of the virus coat protein VP3 from a serine to a phenylalanine) were required to attenuate the virus in an animal model (Ref. 12). There were more differences between the Sabin serotype 2 vaccine strain of poliovirus and the virulent 117 serotype 2 isolate from a vaccine-associated case (Fig. 5b); however, only the mutation at residue 481 in the 5' non-coding region and the mutation at base 2908 (which produces an amino acid change at residue 143 of the virus coat protein VP1 from an isoleucine to a valine) were required to change the virulence of the virus (Refs 13, 14). The situation with serotype 1 (Fig. 5c) was more complex (Ref. 15): the mutations that caused attenuation included those at residue 480 in the 5' non-coding region, although others in this region seemed to have an effect. Mutations in the structural proteins at residues 65 of VP4, 225 of VP3, and 106 and 134 of VP1 all had an attenuating effect. None of the mutations in any of the non-structural proteins has been implicated as a major factor in changes of poliovirus virulence for any of the serotypes.

Molecular changes in poliovirus vaccine strains in vaccine recipients

If the analysis of the molecular basis of attenuation as described here is correct, then when isolates of poliovirus derived from the rare cases of vaccine-associated disease are examined, the mutations implicated in the attenuation of polio vaccine viruses should have been lost, so that the particular bases are the same as those found in the virulent strain used for comparison; alternatively, the effects of the mutations should have been compensated for (or suppressed) by mutations at other sites (i.e. second-site suppression). Mutations that are known to attenuate the vaccine strains of poliovirus have been shown to be reverted or suppressed in isolates taken from vaccine-associated cases, which is consistent with their having an effect in humans as well as in animal models. However, the same changes have also been shown to occur in normal vaccinees; in fact, for type 3 strains and many type 2 strains, the vaccine-related poliovirus isolates derived from poliomyelitis cases and those derived from healthy recipients are indistinguishable in their properties (Ref. 11).

In recipients of polio vaccine who excrete type 3 vaccine strains, the base at position 472 in the 5' non-coding region of type 3 poliovirus always reverts to the base that is found in the virulent form within 6 days, and usually by day 3 after vaccination. Individuals typically excrete virus for 5–6 weeks following vaccination, and 1% continue to do so for 10 weeks. By day 11 post-vaccination, the type 3 virus loses all or part of its temperature-sensitive growth phenotype, which can be shown to be due to the other major attenuating mutation, that at residue 91 of VP3. Moreover, to date, the virus that has been excreted at this stage has always been a recombinant that comprises structural proteins and some of the non-structural proteins derived from type 3 poliovirus and the remainder of the genome derived from either type 2 or type 1 virus. If the recombinant is one that has been formed between type 3 and type 2 viruses (i.e. a type 3/2 recombinant) and excretion of virus continues, a second recombination event invariably occurs in which the extreme 3' end of the type 2 segment of the recombinant virus is exchanged for that from either a type 3 or type 1 virus, leading to a 3/2/3 or a 3/2/1 recombinant, respectively. The probable

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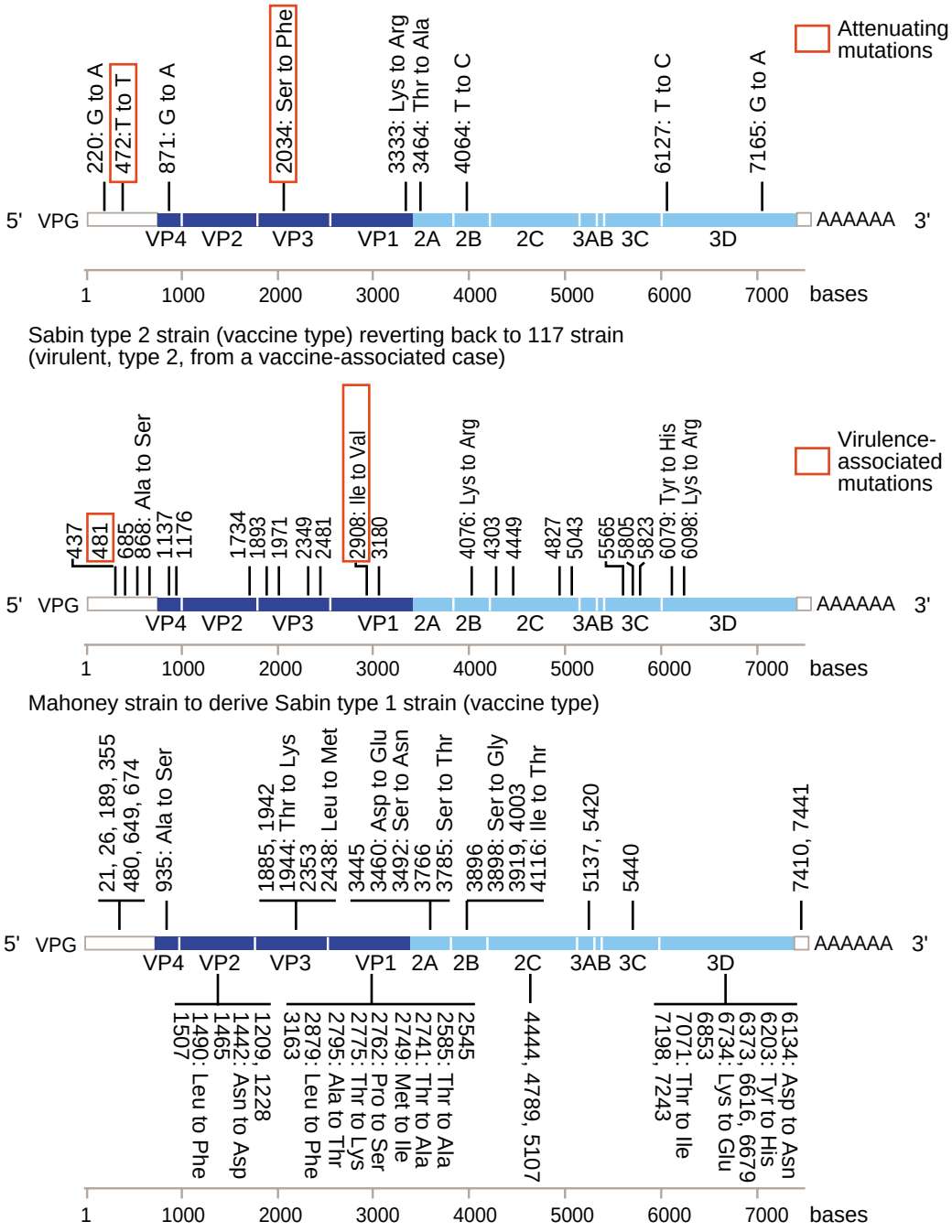

Comparisons of the sequences of polioviruses used to study the basis of attenuation and reversion of the Sabin vaccine strains of poliovirus

Figure 5. Comparisons of the sequences of polioviruses used to study the basis of attenuation and reversion of the Sabin vaccine strains of poliovirus *(see next page for legend)* **(fig005pmn).**

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Figure 5. Comparisons of the sequences of polioviruses used to study the basis of attenuation and reversion of the Sabin vaccine strains of poliovirus. (a) Mutations that are involved in deriving the Sabin type 3 vaccine strain from the Leon strain. (b) Mutations that are involved in reverting the Sabin type 2 strain back to the 117 strain, an isolate from a vaccine-associated case. (c) Mutations that are involved in deriving the Sabin type 1 strain from the Mahoney strain. The numerical positions of nucleotide changes on the cloned DNA copy of the poliovirus genome are shown. The most common base changes (where known) and the amino acid differences produced in the encoded protein are also shown, where relevant. Abbreviations used: AAAAAA = polyadenylate tail; VP = virus protein; VPG = a poliovirus-encoded protein; Amino acids: Ala = alanine; Arg = arginine; Asn = asparagine; Asp = aspartic acid; Glu = glutamic acid; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Phe = phenyalanine; Pro = proline; Ser = serine; Thr = threonine; Tyr = tyrosine; Val = valine; Nucleosides: A = adenosine; C = cytidine; G = guanosine; T = thymidine **(fig005pmn)**.

mechanism involves a recombination event between a type 3 and a type 2 virus, followed by a second recombination event between the first recombinant virus and a second virus, which is the product of a recombination event between a type 2 virus and either a type 3 or type 1 virus, and retains the structural proteins of type 2. These observations imply that growth in the gut selects against some feature of the central part of the genome of the type 3 vaccine strain and some feature of the extreme 3' end of the genome of the type 2 vaccine strain, and that the surviving virus escapes whatever selection pressure is applied by removing the offending parts of the genome by recombination. The nature of the selective pressures involved remains unclear. Type 2/3 recombinants have been isolated from vaccine-associated cases of poliomyelitis, but not usually from healthy recipients of the polio vaccine. Thus, by day 11, the type 3 component has lost the effects of both mutations that have been identified as attenuating the virus, and recombined its genome extremely rapidly and precisely. This occurs in all vaccine recipients who continue to excrete virus (Ref. 16).

The type 1 and type 2 strains of poliovirus also evolve rapidly (Ref. 17). The 5' non-coding attenuating mutation of type 2 viruses at base 481 is lost ~7 days after immunisation, slightly later than the corresponding mutation in type 3 viruses; in type 1 viruses, the equivalent mutation (at base 480) is lost in ~50% of the recipients, sometimes by second-site suppression, as described below. The attenuating mutation in the type 2 strain at residue 143 of VP1 is lost in some of the isolates derived from healthy vaccinees. As would be expected if this mutation had an attenuating effect, it is lost from all isolates derived from vaccine-associated cases of poliomyelitis (Ref. 14).

The molecular consequences of attenuating and reverting changes in the 5' non-coding region of poliovirus

The 5' non-coding region of poliovirus has been predicted to have a well-defined secondary structure, based in part on computer modelling, and in part on the susceptibility of the viral RNA to chemical and enzymatic treatments (Ref. 18). A third line of evidence involved comparisons of the sequences of different poliovirus strains, which showed that when a base difference occurred in a sequence of the RNA that is predicted to be in a stem region, a compensating difference was found in the base with which it was predicted to pair. This strongly suggests that the structure has a real physiological significance in the growth of the virus. The resulting predicted structure for the 5' non-coding region of poliovirus is shown in Figure 6. The mutations that are associated with attenuation in the 5' non-coding region of the Sabin vaccine strains of poliovirus occur in domain V of this RNA structure, and their location is shown in more detail in Figure 7. The numbering of the nucleotides differs slightly between strains because of the existence of deletions and insertions in the sequence preceeding that shown in the figure. There are differences in the nucleotide sequences of the strains of the three serotypes shown, but it can be seen that, in general, the base pairing of the structure is maintained in each case. Differences between the type 2 and type 1 strains and the type 3 strain are indicated. The mutations that are involved in attenuation can all be considered to weaken the base-paired RNA structure. The attenuating mutation in type 3 (Fig. 7a) at residue 472 changes a strong G–C base pair to a weaker but still allowed G–U base pair in a stem comprising three base pairs; the mutation that is involved in attenuating the type 1 vaccine strain (Fig. 7c) at residue 480 similarly converts an A–U

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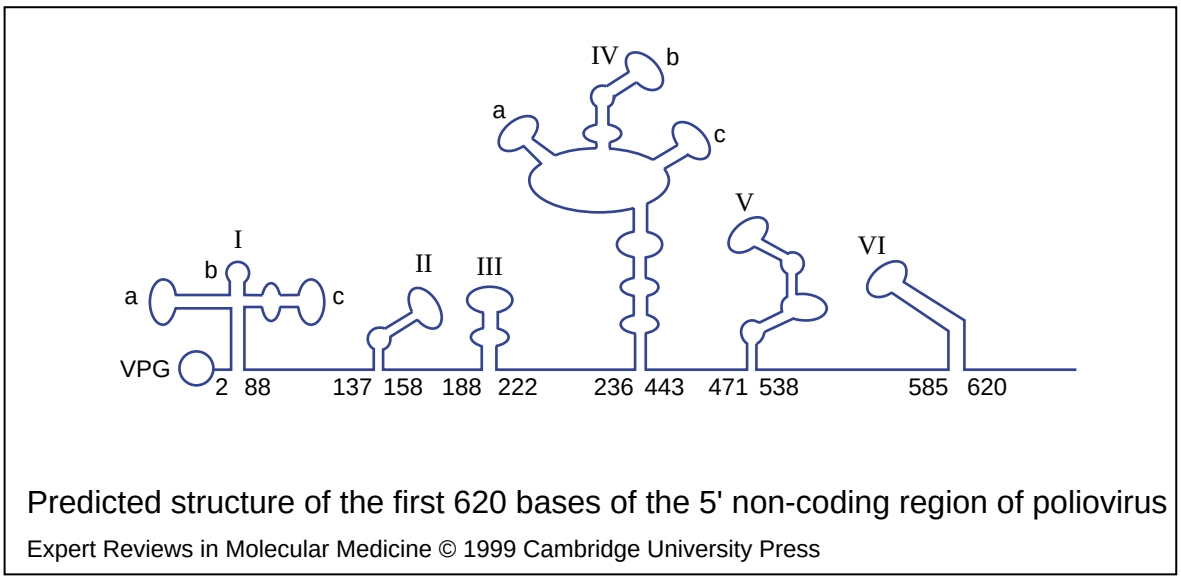

Figure 6. Predicted structure of the first 620 bases of the 5' non-coding region of poliovirus. The RNA sequence can be folded into six domains (I–VI) based on computer predictions, sequence comparisons between different polioviruses and sensitivity to chemical or enzymatic treatments that can distinguish between single-stranded and double-stranded structures **(fig006pmn)**.

base pair into a weaker but allowed G–U base pair. The structures are, therefore, weakened without being disrupted. The mutation that is involved in attenuating the type 2 vaccine strain (Fig. 7b) at residue 481 converts a G into an A, and could permit a new base pair to form between residue 481 and residue 511, which would weaken the same structures. In vaccinees, those mutations that are found in the type 2 and type 3 vaccine strains revert rapidly to the base that is found in the wild-type strain (Refs 16, 17). However, in the type 1 vaccine strain, only 50% of vaccinees excrete reverted virus; whereas most of these cases involve a direct reversion at base 480 to an A, many have a change in the base at the complementary position 525 (which mutates from a U to a C, thus creating a strong G–C base pair between residues 480 and 525). Furthermore, in ~10% of cases, neither of these bases changes; instead, the base at residue 476 is mutated to an A, changing a mismatched U–U base pair to an A–U base pair, which strengthens the overall RNA structure.

The mutations that are associated with the attenuation of the Sabin vaccine strains of poliovirus are all consistent with the predicted RNA structure, in so far as none of them introduces a major mismatch into a base-paired stem. Moreover, the fact that mutations that compensate for the effect of the attenuating mutation can occur at a variety of sites in the type 1 vaccine strain, all of which serve to strengthen the structure, is strong evidence that the RNA structure itself has a real physiological significance in the natural growth of the virus. It also illustrates the variety of ways in which the virus can alter to circumvent any constraints that might affect its fitness.

The molecular consequences of attenuating and reverting changes in the structural proteins of the type 3 Sabin vaccine strain

The diversity of ways in which the poliovirus can alter itself is also illustrated by the suppression of the attenuating mutation in the structural proteins of the type 3 Sabin vaccine strain (Ref. 19), which is shown in Figure 8. The attenuating mutation results in a serine being changed to a phenylalanine in VP3. The protein shell of polioviruses comprises the four capsid proteins VP1, VP2, VP3 and VP4; single copies of each of these four proteins form the basic building block, termed a protomer. Five such protomers associate to form a pentamer, and 12 pentamers associate to form the intact virus shell or capsid, which, therefore, contains 60 copies of each of the constituent virus structural proteins. The attenuating mutation in the Sabin type 3 strain introduces a bulky hydrophobic amino acid

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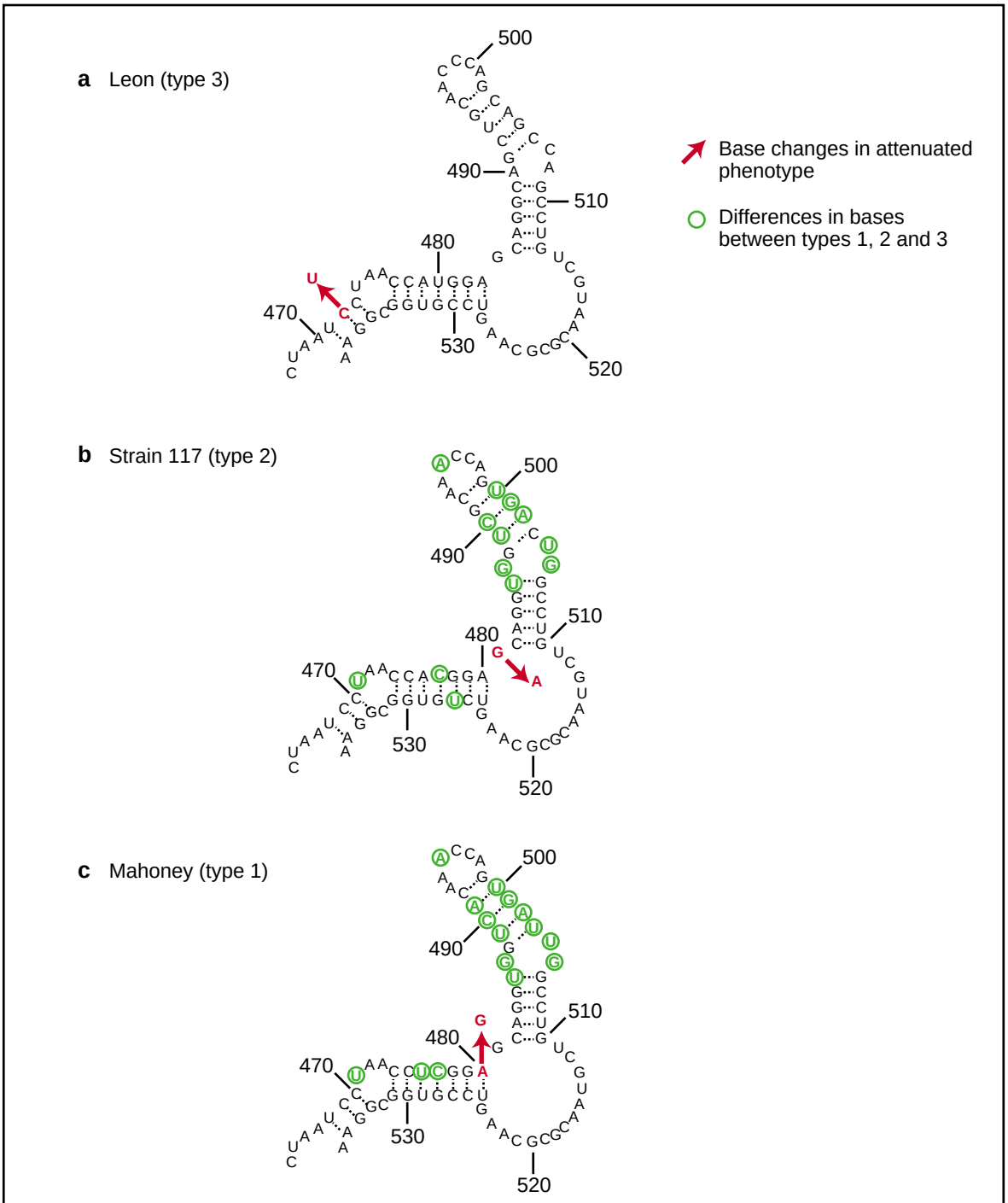

Structures of domain V of the 5' non-coding region of the three virulent strains of poliovirus: Leon (type 3), 117 (type 2) and Mahoney (type 1)

Figure 7. Structures of domain V of the 5' non-coding region of the three virulent strains of poliovirus: Leon (type 3), strain 117 (type 2) and Mahoney (type 1) *(see next page for legend)* **(fig007pmn)**.

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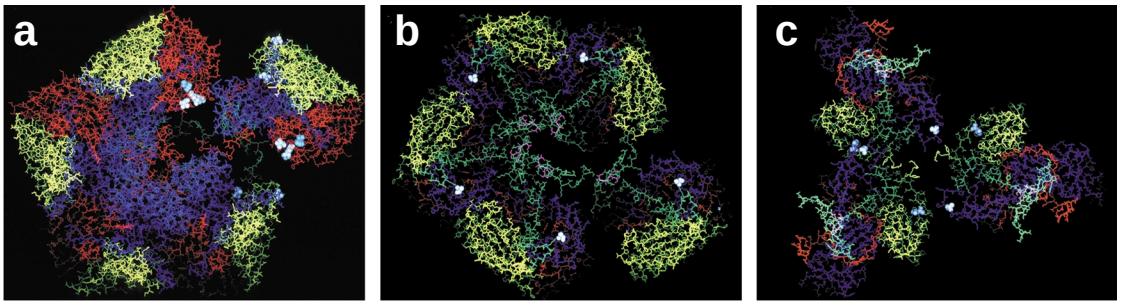
Figure 7. Structures of domain V of the 5' non-coding region of the three virulent strains of poliovirus: Leon (type 3), strain 117 (type 2) and Mahoney (type 1). The numbering of the nucleotides varies slightly between strains owing to substitutions and deletions in the sequence preceeding that shown. Base changes that have been implicated in the attenuated phenotype are shown by the thick, red arrrows at bases 472 [for Leon in (a)], 481 [for 117 in (b)] and 480 [for Mahoney in (c)]. The few differences in bases between the type 1 and type 2 strains and the type 3 strain are circled in green, to illustrate the general conservation of base pairing in domain V of the three serotypes of poliovirus. These three strains and their associated mutations are also depicted in Figure 5 **(fig007pmn)**.

at the interface between protomeric subunits in the attenuated virus, which makes the pentamer less stable. Consequently, the first step of virus assembly in the Sabin vaccine strain of poliovirus, which is the association of the protomers into pentamers, is prevented at high temperatures (i.e. at 39°C rather than 37°C) and virus multiplication stops. The temperature-sensitive phenotype is suppressed in most of the isolates that are obtained from vaccinees >11 days post-immunisation (Ref. 19); furthermore, most of the amino acid differences between these isolates and the Sabin vaccine strain of type 3 poliovirus from which they have been derived cluster at the interface between the protomers, as might be expected (Fig. 8a). The amino acid changes allow the interacting protomers to fit together better, despite the continued presence of the bulky phenylalanine residue at position 91 of VP3. However, some mutations have been found on the inner surface of the virion, away from the protomer interface, in a region that is thought to be involved in the structural transitions that take place during the assembly and uncoating of the poliovirus (Fig. 8b). A third type of mutation that suppresses the effect of the mutation at residue 91 is shown in Figure 8c; it involves a substitution in the region that links adjacent pentamers in the viral capsid. This mutation is believed to accelerate the transition of the pentamers into a complete capsid, and thus compensates for the instability of the pentamers. All of the mutations illustrated in Figure 8 have been found in poliovirus isolates derived both from healthy vaccinees and from vaccine-associated cases of poliomyelitis. It is of interest that the direct back



Structure of the capsid of type 3 poliovirus

Figure 8. Structure of the capsid of type 3 poliovirus. The figure shows the location of the amino acid changes that are involved in the temperature-sensitive and attenuated phenotype and its suppression (Ref. 23). In all three panels (a–c), the following colours have been used to represent the four different capsid coat proteins: blue = VP1, yellow = VP2, red = VP3 and green = VP4. The mauve structure is myristilate covalently attached to VP4. The attenuating mutation at residue 91 in VP3 is shown in cyan, and all suppressor mutations are shown in white. (a) A pentamer, viewed from outside the capsid, looking down at an icosahedral apex (the fivefold axis of symmetry). One subunit (top right) has been displaced to display the location of the amino acid substitutions (in cyan and white). (b) A pentamer, viewed from inside the capsid, showing VP4 (in green) wrapped around the inside of the icosahedral apex. A subunit (bottom right) has been displaced to make the interacting surfaces clearer; (c) view from inside the capsid, along the pseudo sixfold axis, showing the interaction between adjacent pentamers. The subunit to the right has been displaced to show the location of a suppressor mutation (in white) **(fig008pmn)**.

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mutation of residue 91 of VP3 to a serine is relatively rare, and is always accompanied by a second mutation at residue 54 of VP1, which on its own also affects the temperature-sensitive growth properties of the vaccine strain of poliovirus. It is likely that this preponderance of second-site suppresser mutations is due to the fine-tuning of the growth of the virus to the temperature of the gut, which is 37°C. Leon, the virus that contains a serine residue and is the laboratory precursor of the Sabin vaccine strain that has been used to analyse virulence and temperature-sensitive growth properties, has a temperature optimum of ~39°C, compared with 37°C for the revertants. This illustrates the fact that poliovirus has a wide spectrum of ways in which it can revert but also that it can do so with great specificity, depending on the selection pressures it faces.

The eradication of poliomyelitis: the end game

The first stage in the eradication of poliomyelitis involves the elimination of wild-type poliovirus strains by the use of the live vaccines that were developed by Sabin. The progress towards this goal has been extraordinary, and there is every possibility that eradication will be accomplished early in the new millennium. Once the eradication of wild-type viruses has been achieved, the only polioviruses that are circulating in the population will be the vaccine strains, which will then have to be eradicated. Which strategy should be followed is not, however, as obvious as might be hoped. One possibility is simply to stop vaccinating with live vaccine on a worldwide basis at a particular time. However, such a strategy would be successful only if any circulating poliovirus died out faster than the pool of susceptible individuals built up. There is some evidence that this might indeed happen, chiefly because the vaccine strains are believed to be poorly transmissible compared with the wild type. Following a poliovaccine campaign, it is difficult to isolate poliovirus from sewage after 6 months. Such vaccine campaigns include those that were designed to control epidemics of poliomyelitis (as in Finland in 1984) and those in which poliovaccine is given only in the context of a campaign (as in Cuba, where the vaccine is given twice a year). This suggests that the transmission of polio from vaccine recipients to their contacts is not extensive. Moreover, epidemics of poliomyelitis can occur in countries that have good polio immunisation programmes, if subpopulations are not immunised for religious reasons. So far, such epidemics have been caused only by wild-type poliovirus strains, despite the heavy use of the live vaccines in adjacent regions or countries. This is true of two outbreaks in the Netherlands, and of outbreaks in the Amish community in Pennsylvania, USA (Ref. 20). The outbreak in Finland in 1984 was also caused by a wild-type strain rather than a vaccine strain (Ref. 21). This evidence suggests that although vaccine strains can revert wholly or partially to a virulent form, they do not cause epidemics of poliomyelitis. Studies carried out during the 1950s suggested that usually when a child in a family was vaccinated, the poliovirus could be passed on to a second individual, who in turn could pass it on to a third person before the infection died out. It would be interesting to repeat such studies in various parts of the world to see if this finding is still true under modern conditions of hygiene. Although the vaccine strains are considered to be poorly transmissible, it should be remembered that each vaccine strain was ultimately derived from a wild-type strain (presumably one that was transmissible), and could in principle recover transmissibility as easily as it was lost.

The strategy of stopping vaccination worldwide at the same time has the advantages of simplicity and ease of implementation. One possible disadvantage is the occurrence of long-term excretors of poliovirus, which has been well documented since the early 1960s (Ref. 22). Individuals who are genetically unable to mount a normal humoral immune response can become infected with the vaccine strains of poliovirus and excrete the virus for several years; currently, the longest period of virus excretion has been estimated to be 14 years, but increasing. The administration of intravenous immunoglobulin is used to keep this group of patients healthy as far as most infections are concerned; it also serves to protect them from poliomyelitis, without necessarily clearing the infection from their intestinal tract. Such individuals are especially susceptible to poliomyelitis in general and vaccine-associated poliomyelitis in particular, and although every effort is made to avoid giving them live poliovaccine, many of them are not diagnosed as having this genetic immunodeficiency until after the normal age at which polio vaccination occurs, by which

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time it is too late to avoid exposure. It is not clear how many individuals who are persistently infected with poliovirus there are in any region of the world; however, it is known that most of the genetically immunodeficient patients who have been studied to date were not infected with poliovirus. The duration of the excretion of poliovirus can be estimated by determining the differences between the RNA sequence of the poliovirus that is being excreted and that of the vaccine strain of poliovirus; for any infection with poliovirus, whether by wild-type strain or vaccine strain, the rate of change of the RNA sequence is ~1% per year. The virus that is excreted has been shown to be virulent for susceptible experimental animals (which is not surprising in view of the findings with normal recipients of poliovaccine), and would presumably also be virulent for humans who had not been immunised. If the virus that is excreted by such immunodeficient individuals is transmissible, the long-term excretion of this virus would provide a possible source of infection, which could ultimately lead to poliomyelitis, for other individuals over a long period of time. It would, therefore, be necessary to maintain a high level of anti-poliovirus immunity within the population even in the absence of wild-type poliovirus, and without introducing additional live vaccine strain poliovirus into the population. One possible strategy might be the continued use of the inactivated poliovaccine until it was certain that all of the long-term excreters of poliovirus had stopped shedding poliovirus. This might require the use of inactivated vaccine for 50 years or more. Doubt has been expressed about the feasibility of switching to inactivated poliovaccine worldwide, and this approach would certainly present major logistic and production difficulties, even if society could be persuaded of the need to continue vaccination against a non-existent disease.

As well as those individuals who are genetically immunodeficient and are known to be capable of long-term infection with poliovirus, it is possible, although not yet established, that individuals who are infected with human immunodeficiency virus (HIV) might also become carriers of poliovirus, even during the period before they develop obvious symptoms of immune deficiency. It is conceivable that such groups of individuals might also maintain the live vaccine strain of poliovirus.

Research in progress and unanswered research questions

Persistence of poliovirus

One of the most pressing issues of poliovirus research is to determine the number of chronic excreters worldwide and then to devise some means of clearing them of the poliovirus, thus preventing them from forming a reservoir of infectious virus that could spread to others. Possible therapeutic approaches include the oral administration of anti-poliovirus immunoglobulin. However, it might prove to be difficult to achieve levels of antibody that are high enough to have an effect on the virus using this approach. Alternatively, chemotherapeutic agents, of which there are some that are specific for picornaviruses, might be effective.

The pathogenesis of poliovirus

The likely effectiveness of oral immunoglobulin therapy, apart from the difficulty of achieving sufficiently high titres in the intestine, will be affected by fundamental unanswered questions about the pathogenesis of poliovirus infections in humans. In particular, if the mucosal surface of the intestinal tract is the main site of the infected tissue (as proposed in the Sabin model described earlier), the infection might be accessible to orally ingested antibodies in the intestines; however, this would not be the case if the lymphoid tissues are the main sites of the infected tissue (as proposed by Bodian).

The logistics of poliovirus containment

It will be difficult to guarantee the safe usage of polioviruses in laboratories and ensure that adequate precautions are taken to prevent the viruses spreading from the laboratory to the world at large, because this virus is an extremely common laboratory agent; for example, it might be present unsuspected in a range of clinical specimens, such as faecal specimens that are being tested for the presence rotavirus or bacterial intestinal infections.

New poliovaccines

If poliomyelitis reappeared after a period during which the live poliovaccines were not used, epidemics would most likely have to be stopped by the use of live poliovaccine; this would set back attempts to eradicate the poliovirus roughly to the time when the wild-type virus was first eliminated. A genetically stable, non-

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transmissible live vaccine would, therefore, be a very useful addition to the anti-poliovirus armoury. Work to develop such strains is proceeding in several laboratories.

The safety of poliovaccines is a major concern and, ultimately, can be tested only by clinical usage. In the light of our current understanding of the molecular biology of poliovirus infection of humans, it might be possible to identify markers of reversion that are sufficiently precise, such that the scale (which is currently huge) of the clinical trials that are required to assure safety can be greatly reduced. This is a major challenge for the testing of any novel live poliovaccine.

Serotypes and the evolution of poliovirus

There are only three serotypes of poliovirus; however, other picornaviruses (including some rhinoviruses and some coxackievirus A types) are considered to be closely related to polioviruses because of similarities in their genomic sequences. Polioviruses were classically defined in terms of their ability to cause poliomyelitis, and their antigenic properties. An additional factor is now their use of a common receptor site by which they enter the target cell. The receptor site is a three-domain protein of the immunoglobulin superfamily, variously termed CD155 or PVR (for poliovirus receptor), which is currently of unknown function. It is not clear why only three antigenically distinct forms of picornavirus are able to use this means of entry, and it is possible that other strains could evolve to do so, such that the eradication of poliovirus might be only temporary. Studies of the usage of this receptor site are, therefore, important as well as interesting in their own right.

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Further reading, other resources and useful contacts

The World Health Organization (WHO) Polio Eradication website. This official WHO site provides information on all aspects of the global initiative to eradicate poliomyelitis, including technical information on polio, vaccines against polio, the current status of eradication by WHO Region, and much background material for further reading.
http://www.who.int/gpv-polio

Features associated with this article

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Figure 3. Incidence of cases of poliomyelitis worldwide (1988 and 1996) (fig003pmn).
Figure 4. Organisation of the poliovirus genome (fig004pmn).
Figure 5. Comparisons of the sequences of polioviruses used to study the basis of attenuation and reversion of the Sabin vaccine strains of poliovirus (fig005pmn).
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Figure 8. Structure of the capsid of type 3 poliovirus (fig008pmn).

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