Chapter 1

An Introduction to Poliovirus: Pathogenesis, Vaccination, and the Endgame for Global Eradication

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Abstract

Poliomyelitis is caused by poliovirus, which is a positive strand non-enveloped virus that occurs in three distinct serotypes (1, 2, and 3). Infection is mainly by the fecal–oral route and can be confined to the gut by antibodies induced either by vaccine, previous infection or maternally acquired. Vaccines include the live attenuated strains developed by Sabin and the inactivated vaccines developed by Salk; the live attenuated vaccine (Oral Polio Vaccine or OPV) has been the main tool in the Global Program of Polio eradication of the World Health Organisation. Wild type 2 virus has not caused a case since 1999 and type 3 since 2012 and eradication seems near. However most infections are entirely silent so that sophisticated environmental surveillance may be needed to ensure that the virus has been eradicated, and the live vaccine can sometimes revert to virulent circulating forms under conditions that are not wholly understood. Cessation of vaccination is therefore an increasingly important issue and inactivated polio vaccine (IPV) is playing a larger part in the end game.

Key words Poliovirus, Picornaviruses, Human enterovirus C species pathogenesis, Live and killed vaccines, The Global Poliomyelitis Eradication Initiative, Vaccine derived polioviruses, Stopping vaccination

1 Introduction

Poliovirus is a positive strand RNA virus classified with certain Coxsackie A viruses within the Human Enterovirus C species of the picornaviridae. The genome is about 7500 bases in length enclosed in a non-enveloped capsid consisting of 60 copies each of four proteins designated VP1, 2, 3, and 4 and there are three serotypes (1, 2, and 3) as shown first in the 1950s by the demonstration that infection of monkeys with one serotype did not protect against the other two. The serotypes are most generally identified by their reaction with specific sera. Polio virus was shown to be a transmissible agent in 1909 by Landsteiner and Popper by infection and paralysis of old world monkeys [1] and this remained the only

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method of detection until the 1950s. Subsequently cell culture methods were developed, and currently molecular methods play a central role in detecting and accurately identifying strains.

2 Pathogenesis and History

There are very few recognizable cases of paralytic poliomyelitis reported in Western medical literature until the end of the nineteenth century when large seasonal epidemics in young children began to be recognized. However, the first case generally considered to be poliomyelitis is depicted on the funerary stele of the priest Rom from about 1300 BCE (Fig. 1) which shows the typical withered limb and down flexed foot that follows destruction of motor innervation and muscle wasting. Poliovirus derives its name from the fact that it specifically attacks the grey matter (Greek polios = grey, myelos = matter) of the anterior horn of the spinal cord. The clinical presentation is so striking that it seems reasonable to conclude that cases were very rare in the western world [2] until the epidemic pattern became established at the end of the nineteenth century and beginning of the twentieth.

Fig. 1 Funerary stele of the priest Rom ca. 1300 BCE showing the withered limb and down flexed foot typical of poliomyelitis. Printed by permission of the Ny Carlsberg Glyptotek, Copenhagen

Early epidemiological studies in Sweden showed that as well as frank paralysis (the major disease) there was often an earlier milder syndrome of sore throat and malaise typical of a general viral infection (the minor disease). Most infections were entirely silent, however. In fact the accepted view is that there are about 200 silent infections for every paralytic case depending on the virus type and strain, with type 1 being the most dangerous and type 2 the least. Initially because of the use of particular animal models of infection and the disease presentation it was thought that polio grew mostly in the central nervous system. However in the 1940s it became clear that this was not the case and in fact there was good evidence from very early on that the virus grows chiefly in the gut. From there it can break out into the blood and spread to unknown systemic sites [3] where it replicates before spreading to other areas including the Central Nervous system. There are therefore two viremic steps, one immediately following infection of the gut and the second about a week later which spreads the infection to other sites including typically the throat and tonsils. Viremic spread is greatly inhibited if not prevented altogether by the presence of humoral antibody. A study in the 1950s showed that passive antibody was sufficient to give protection from disease [4]. This in turn provides an explanation of the emergence of polio in major epidemics. Where a baby is exposed to the virus while protected by maternal antibody, infection is confined to the gut and thus harmless. As hygiene standards improve as at the end of the nineteenth century exposure of the baby to the virus happens later in life when maternal protection has been lost, allowing disease to occur. This is almost certainly an oversimplification as in areas where poliovirus circulates intensively, such as in Northern India before eradication, there were a very large number of cases despite the high level of serum antibodies in the mothers and the early exposure of the babies. It could be that maternal protection can be overwhelmed by high intensity exposure to the virus.

The model requires that a child's gut becomes infected with poliovirus in the presence of protective levels of maternal antibody until the child's immune system develops. Maternal antibody cannot provide full protection against infection of the infant gut or the child would become infected only when maternal antibody had declined and would therefore be susceptible to disease at the same time. Improving hygiene standards would then only delay disease not cause an increase in cases or an epidemic pattern to emerge. It is not known whether passive antibodies will protect against gut infection. More important to the eradication program, the effectiveness of immunity induced by inactivated vaccine (IPV) at preventing gut infection remains at least a matter for debate as IPV may be chiefly associated with the induction of humoral antibodies.

In summary poliovirus is shed in the feces and the main route of spread is fecal–oral, although respiratory routes are possible; most infections are silent and if the level of immunity is high, the

proportion of symptomatic infections may be even lower unless immunity prevents gut infection. Counting cases of poliomyelitis is an indication of virus circulation but not necessarily the most secure, sensitive, or reliable method in the end stages of eradication.

3 Antigenic Forms of Polio Virus

In the 1950s it was shown that when a culture of poliovirus was fractionated on a sucrose gradient of five steps (A to E) there were two peaks of antigenic activity, one associated with the C step, the other with the D. The C antigen reacted more strongly with acute phase human sera and the D antigen with convalescent sera [5]. The two antigens were therefore termed C and D antigen and correspond to a first approximation to empty capsids and full infectious virus particles respectively. Because a convalescent patient is assumed to be protected, it was concluded that antibodies against the D antigen were protective while those against the C antigen were not; antibodies reacting with D antigen neutralize infectivity. It was also concluded that only D antigen could stimulate protective antibody; it is now known that neutralizing antibodies exist that react with both C and D antigen which can be induced by either particle type, so that this is not the case although it sometimes assumed that antigenicity and immunogenicity are interchangeable. As most IPV is now purified to exclude empty capsids, potency may be based on measurement of D antigen content. The D antigen is converted into a C or C like form by relatively mild treatments such as heating, adsorption to surfaces, or mild UV treatment [6].

4 Vaccines Against Poliomyelitis-Inactivated Polio Vaccine (IPV)

Vaccines were developed in the 1950s and are the key to the control of poliomyelitis and ultimately the Global Polio Eradication Initiative. The first to be licensed was the inactivated vaccine developed by Jonas Salk, which consisted of largely unpurified preparations of tissue culture grown virus treated with diluted formalin under careful conditions so that while infectivity was lost, the ability to induce a protective immune response was retained. There were many issues associated with the vaccine initially. The first batches included preparations that were not fully inactivated and caused poliomyelitis in recipients [7].This was a failure of the production process; specifically there was a need for adequate filtration of the vaccine before and after inactivation to make sure that aggregates of virus not penetrated by the formaldehyde were removed. Introduction of filtration steps ensured that this did not happen again, but also reduced the amount of antigen present leading to the need to measure and standardize the potency by some means. Initially this was done by

assessing immunogenicity in animal models such as guinea pigs or young chickens, assays that are variable and not always quantitative. The virus was originally grown on primary monkey kidney cells from wild caught old world monkeys, mostly rhesus macaques. They are susceptible to many simian viruses and one polyoma virus, SV40, was present in many batches and only poorly inactivated by the formalin treatment. It was also hard to detect as it did not cause a cytopathic effect in rhesus monkey cells, although it did so in cells from cynomolgus monkeys. SV40 is able to cause tumors in certain animal models and it is clear that many batches of IPV contained live virus. The data suggest that the contamination had no long term consequences in practice but it was a major concern which has still not been resolved to everyone's satisfaction [8]. Supply was also a practical issue, because of the scale of production required. Finally there was a long running debate between those who favored the killed vaccines and those who preferred live vaccines that would imitate natural infection.

Current production technology for IPV was developed in the 1980s; it depends on large scale cell cultures usually in continuous cell lines, giving high titre preparations. Assay of potency is most commonly by measurement of D antigen content or immunogenicity in rats, in which the response is quantitative. The issues with the original IPV of safety, supply, contamination and assay have therefore been dealt with.

The effect of IPV on the incidence of poliomyelitis in the USA is shown in Fig. 2 where cases were reduced by over 95 % and the downward trend continued. Nonetheless in the early 1960s the

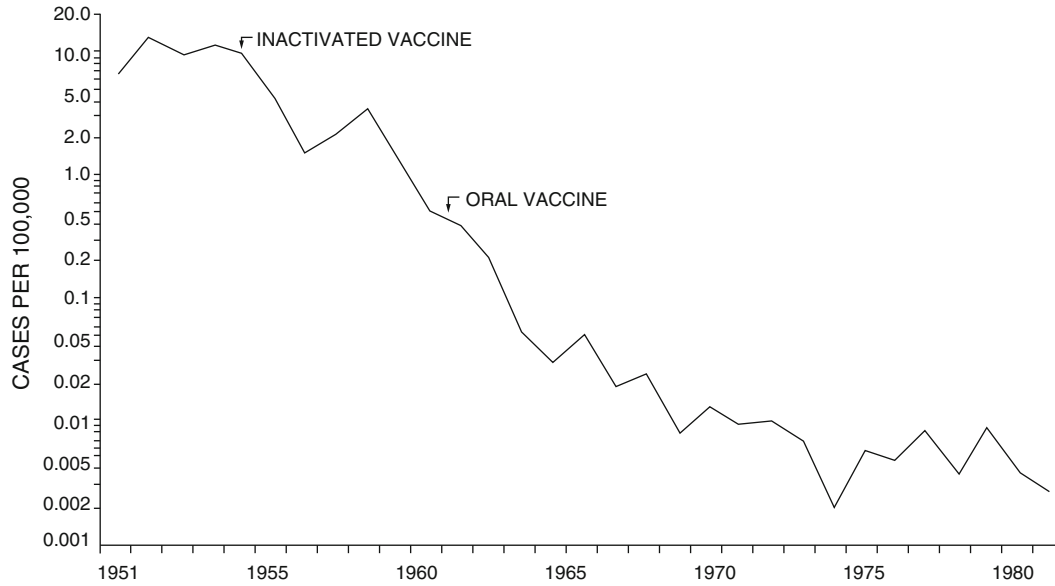

Fig. 2 Incidence of poliomyelitis in the USA from 1950 to 1970s showing the effect of killed and live vaccines

live attenuated vaccines developed by Albert Sabin replaced IPV, and they have formed the main tool in the Global Polio Eradication program of WHO. However IPV is playing an increasingly significant role in the end of polio.

5 Live Attenuated Polio Vaccines (OPV)

There was a long running argument between those who favored the killed vaccines of the Salk type and those who favored a live attenuated vaccine that would mimic natural infection. The view was that live vaccines would break transmission by inducing the full range of immunity including gut immunity where IPV would not. It was also argued that a live vaccine that infected the recipient and was shed for some time could also infect and immunize contacts and therefore have a bigger effect than IPV. Spread was therefore thought to be a positive argument for OPV.

By the late 1970s polio was largely eliminated from developed countries, most of whom used OPV as developed by Sabin. There was a residual number of cases, about 1000-fold less than in the pre-vaccine era and it was shown that apart from a few imported cases they were caused by the vaccine strains, either in recipients or in their immediate contacts. The vaccine could therefore revert and the spread to others was a mixed blessing. There is thus a need to test the vaccine before it is used to ensure that it is safe, and for many years this involved primates, following modified versions of the tests used by Sabin in developing the strains in the first place. Currently there are alternatives in the form of transgenic mice bearing the polio receptor so that unlike normal mice they are susceptible to infection, or molecular tests for consistency.

The vaccine replicates for an average of about 4 weeks in recipients [9] and over that time the properties of the virus excreted change. The type 2 and type 3 components become more virulent in animal models and many changes can be followed at the molecular level. Nonetheless OPV was remarkably safe and very effective in temperate climates.

On the other hand the effect of OPV in developing countries and tropical climates was poor in so far as it could be tracked. Part of this may have been due to vaccine quality and delivery systems, but part was probably due to the epidemiology of polio virus infections, which are seasonal, particularly in temperate climates. Thus immunizing in a routine program in the low season in temperate climates can reduce the number of susceptibles below a sustainable level so that transmission is reduced in the high season. Where transmission is year round and high it is a matter of chance whether the vaccinee gets the wild type or the vaccine first, so transmission continues. The solution was to run a campaign of immunization where a large proportion of

susceptibles were immunized at the same time so reducing the pool rapidly. This method had been used in the Southern States of America as "Sabin Sundays," but it was introduced into Latin America in the form of National Immunisation Days. The effect was dramatic and by 1988 some Latin American countries were polio free and others were making clear inroads. In 1988, the 41st World Health Assembly recognized the progress made and adopted resolution 41–28 that committed WHO to the eradication of polio by the year 2000. While this target was obviously not met, by 2003 endemic transmission of polio was confined to four countries: India, Pakistan, Afghanistan, and Nigeria. There were repeated instances of reintroduction of polio into countries that had eliminated; in one instance immunization in Northern Nigeria was stopped for a period for local reasons and polio spread across the whole of Central Africa and was exported to Yemen and Indonesia. It is clear that if one country has polio, the entire world is at risk.

At the time of writing however there has not been a case of type 2 polio caused by a wild type strain since 1999 other than some cases in India as a result of contamination of OPV with a laboratory strain. It is very likely that type 2 polio in the wild has been eradicated. Similarly there has not been a case of type 3 polio in the world for the last 8 months, and it is conceivable that it has also been eradicated. The last case of type 1 polio in India was in 2011 which given the epidemiological circumstances was a colossal achievement. Polio eradication is therefore moving into the endgame. If it is eventually achieved it will have been the most ambitious vaccination activity of all time, with a cost so far of about $9 billion.

6 The Endgame

It is hard to justify continuing a vaccination program against a disease that has been eradicated, but the cessation of vaccination against polio will be a very complex process. Firstly it is necessary to be sure that the disease has truly been eradicated, and this means eradication of the virus. Effective surveillance for poliovirus will therefore be essential after eradication is declared and may focus increasingly on environmental searches rather than recording cases of disease. Until it is certain that the virus has gone, vaccine production must continue and as this currently requires growth of poliovirus this risks reintroduction. The apparent eradication of wild type 2 virus suggests that vaccination could stop one serotype at a time; in fact the type 2 strain of OPV dominates the response to vaccination and most campaigns now use bivalent vaccine containing only type 1 and 3 and serious consideration is being given to stopping all usage of type 2 OPV in both routine and campaign based programs.

In addition a major feature of OPV is that the strains which are already capable of some transmission from recipients to contacts can evolve to become as transmissible as the wild type. Circulating vaccine derived polioviruses (cVDPVs) have caused numerous outbreaks in areas where vaccine coverage is imperfect although the conditions required for their emergence are still not really clear. In addition individuals deficient in humoral immunity (hypogammaglobulinemics) can become excrete the vaccine strains for periods of many years during which the virus typically becomes virulent. Surveillance of sewage in many countries has also revealed the presence of strains of derived from the vaccine but of unknown specific origin; they are probably from unidentified long term excreters. The use of OPV therefore poses a hazard to the eradication of polio and the need for surveillance covers the vaccine derived strains as well as the wild type.

Consequently countries are moving to the use of IPV. Western Europe now uses IPV exclusively as does Northern America; countries of South America and Russia are also adopting it. Current IPV production requires the growth of strains that are known from accidental past exposures to be highly paralytic; production must therefore be strictly contained in a polio free world, and given the scale of virus growth required this is extremely difficult. There are currently three main producers of IPV in the world of which two provide the majority. It is hard to believe that other countries and producers will fail to become involved.

The World Health Organisation has developed a number of relevant documents to address some of the issues of what needs to be contained, when containment is required and how it should be done.

The eradication plan (Draft of February 2013) lists four phased objectives based on the hypothesis that wild type polio will be eradicated by the end of 2014.

1. Poliovirus detection and interruption; Plan for the last wild type poliovirus for the end of 2014, followed by outbreak responses, particularly for cVDPVs.
2. Strengthen routine immunization, address the prerequisites for withdrawal of type 2 OPV. From end 2015 to end 2016 complete IPV introduction and type 2 OPV withdrawal.
   From end 2016 IPV and bOPV are in routine use.
3. Finalize containment plans by the end of 2015, complete containment by end 2018. It is implicit that this applies only to the wild type as OPV will still be in use.
4. Legacy planning.

Global certification would be at the end of 2018, and bOPV use would cease during 2019.

The global action plan describes risk elimination and risk management procedures once polio is eradicated. It relates specifically to containment and safe laboratory use of poliovirus and the production of IPV.

1. Primary safeguards include containment, management structures in the facilities, protection of staff through immunization and contingency plans in the event of an incident. Containment covers design of the plant including airflows, showering, autoclaves where the demands of GMP and containment are not all coincident (e.g., for GMP airflows may need to be positive to prevent contamination of the product, while containment would require negative pressure to ensure that nothing escaped).
2. Secondary safeguards relate to the epidemiology in the country which should have high (>90 %) vaccine coverage and an effective immunization program for children. This should reduce spread in the event of an escape.
3. Tertiary safeguards relate to public hygiene including sewage systems and treatment.

There are four phases to the implementation of polio containment:

1. National surveys and an inventory of wild type polio virus holdings and destruction of unnecessary stocks.
2. Establishment of long term policy relating to the need to retain polio capability; WHO envisage no more than 20 laboratories worldwide still holding and using polio.
3. Global destruction and containment of wild poliovirus to start 1 year after the last wild type poliovirus is isolated. Implementation of primary secondary and tertiary safeguards for laboratories still using the wild type strains.
4. Recall and destroy OPV stocks. Implement primary and secondary safeguards for use of OPV/Sabin strains at the time of cessation of OPV use.

Primary, secondary and tertiary safe guards are required for wild type virus after eradication. Primary and secondary safeguards are required for OPV/Sabin strains after cessation of routine OPV use.

Guidelines for the safe production and quality control of inactivated poliomyelitis vaccine manufactured from wild poliovirus (Addendum, 2003, to the Recommendations for the Production and Quality Control of Poliomyelitis Vaccine (Inactivated)) provides detailed guidance for the development of BSL3 (polio) facilities. They are very stringent.

The issues of containment have raised the possibility that the Sabin strains might be a safer way to make IPV. Apart from differences in immunogenicity that have become apparent the safety added

is by no means clear given the issues with cVDPVs and other vaccine derived strains. The guideline on vaccine production refers explicitly to production using the Sabin strains, which do not require BSL3/IPV containment provided they are produced under conditions that would make them suitable for oral vaccine use; this implies testing which may introduce unacceptable extra burdens on production as the wild type currently requires no control of this kind.

Consequently there is interest in developing alternative strains and procedures for vaccine production, including strains that are genetically stable, that are attenuated by so many small mutations that there reversion is essentially impossible or eventually stable empty capsids. All are at a relatively early stage of development and the choice of approach and the containment required remain to be determined.

7 Summary and Conclusions

Poliomyelitis was a major public health issue for developed countries in the middle of the twentieth century. Vaccines make its eradication possible and the progress to this end is outstanding. The last steps in the process involve containment among other things and are complex and challenging. In view of the stage of the eradication program and the changes in vaccination they need to be addressed.

References

1. Landsteiner K, Popper E (1909) Ubertragung der poliomyelitis scuta auf affen. Z Immunitatdforsch Exp Therap Orig 2:377–390
2. Paul JR (1971) A history of poliomyelitis. Yale University Press, New Haven
3. Minor PD (1997) Poliovirus. In: Nathanson N, Ahmed R, Ganzalez-Scarano F, Griffin DE, Holmes KV, Murphy FA, Robinson HL (eds) Viral pathogenesis. Lipincott Raven, Philadelphia, pp 555–574
4. Hammon WD, Coriell LL, Wehrle PF, Stokes J (1953) Evaluation of Red Cross gammaglobulin as a prophylactic agent for poliomyelitis. 4. Final report of results based on clinical diagnosis. JAMA 151:1272–1285
5. Mayer MM, Rapp HJ, Roizman B, Klein SW, Cowan KM, Lukens D, Schwerdt CE, Schaffer FL, Charney J (1957) The purification of poliomyelitis virus as studied by complement fixation. J Immunol 78:435–455
6. Le Bouvier GL (1959) The modification of poliovirus antigens by heat and ultraviolet light. Lancet 269(6898):1013–1016
7. Offit P (2005) The cutter incident: how America's first polio vaccine led to the growing vaccine crisis. Yale University Press, New Haven. ISBN 0-300-10864-8
8. Shah K, Nathanson N (1976) Human exposure to SV40: review and comment. Am J Epidemiol 103:1–12
9. Minor PD (2012) The polio eradication programme and issues of the endgame. J Gen Virol 93:457–474