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BRIEF REVIEW

100 years poliovirus: from discovery to eradication. A meeting report

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Abstract Just over hundred years ago, Karl Landsteiner and Erwin Popper identified a virus, later termed poliovirus, as the causative agent of poliomyelitis. This groundbreaking discovery simultaneously provided the basis for the measures that today prevent the outbreaks of the terrible epidemics caused by poliovirus. In 1988, the WHO started its eradication program to eliminate the virus from the planet. The symposium celebrated the discovery of poliovirus and discussed our current state of knowledge of poliovirus biology. Prospects for the eradication program were evaluated, with particular emphasis being placed on why certain countries still have not succeeding in interrupting wild-type transmission of poliovirus. Discussion also centred on the role of inactivated poliovirus vaccines in the eradication program and the maintenance of a poliovirus-free world, whenever this goal should be achieved.

Introduction

In 1909, Landsteiner and Popper reported the identification of a virus, subsequently called poliovirus (PV), as the causative agent of poliomyelitis [20]. To celebrate 100 years of this seminal discovery, one of the first to show that a virus could cause a human disease, an international symposium was held on the 20 November 2009, in Vienna, Austria (http://www.meduniwien.ac.at/100yearspolio). The invited scientists reviewed the pathfinding role of PV in virology, evaluated the progress made in eradicating this devastating human pathogen and discussed strategies to achieve the goal of a polio-free world. The speakers were Georg Stingl (Medical University of Vienna, Austria), Neal Nathanson (University of Pennsylvania, USA), Jeffrey Almond (Sanofi Pasteur, Lyon, France), James Hogle (Harvard University, USA), Eckard Wimmer (Stony Brook University, USA), Vadim Agol (Moscow State University, Russia), Rudolf Tangermann (World Health Organisation, Geneva, Switzerland), Robert S. Scott (Rotary Foundation of Rotary International, Evanston, USA), Tim Petersen (Bill and Melinda Gates Foundation, Seattle, USA), Olen M. Kew (Centers for Disease Control and Prevention, Atlanta, USA) and Konstantin Chumakov (US Food and Drug Administration, Maryland, USA). At the end of the symposium, Neal Nathanson also chaired an open discussion entitled "The Endgame of Poliovirus Eradication" with Chumakov, Kew and Tangermann on the panel.

Discovery and epidemiology of poliovirus

Karl Landsteiner's discovery of poliovirus

In the opening talk, Georg Stingl explained the background to the discovery of PV, which was actually made in 1908. Karl Landsteiner studied medicine at the University of Vienna and trained subsequently in chemistry and biochemistry in the laboratories of Bamberger and Fischer. In 1898, Landsteiner began a 10-year stint at the University of Vienna, where he described for the first time the ABO blood groups, work that subsequently brought him a Nobel Prize, and also worked on infectious diseases, including *T. pallidum*, the causative agent of syphilis.

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In 1908, Landsteiner took a better-paid appointment as prosector at Vienna's "Wilhelminenspital". Here, he continued his work on the classification of the different constituents of the ABO blood groups as well as discovering that poliomyelitis was caused by a transmissible agent. This discovery began with the admission of a 9-year-old boy with an unremarkable medical history to the Wilhelminenspital. Suddenly, the boy developed flu-like symptoms and headaches; 2 days later, he began to suffer from paralysis, leading to respiratory distress and, very rapidly, death. Landsteiner and his colleague Erwin Popper, a paediatrician, performed an autopsy and noted symptoms in the grey matter of the brain that were very reminiscent of those of poliomyelitis. To identify a possible infectious agent, he looked without success for the presence of bacteria in the fluid of the cerebro-spinal fluid or in the CNS. In addition, attempts to grow bacteria from homogenates of material from the CNS on blood agar and such like were also unsuccessful. Landsteiner finally injected a homogenate of the CNS tissue intra-peritoneally into rabbits, guinea pigs, mice and monkeys. No effect on the health of the first three species was observed. However, both monkeys injected developed paralysis and subsequently died. Upon autopsy, the pathology of the brains of the monkeys was very similar to that seen in the boy from which the homogenates had originally been taken.

Landsteiner and Popper correctly interpreted these observations as demonstrating that poliomyelitis was caused by a transmissible agent smaller in nature than bacteria and thus most probably a virus. Landsteiner confirmed and extended these observations in a 7-month stay in Paris, the work giving rise to series of subsequent papers [21, 22]. The work, published in French, was performed in Paris because the institutions had relatively easy access to monkeys from the French overseas colonies.

The epidemiology of poliovirus

Landsteiner's seminal discovery occurred in an era when there was a tremendous increase in the number of cases of poliomyelitis in the first decades of the twentieth century [23, 32]. In his presentation, Neal Nathanson first reviewed this period in the Americas and then contrasted the situation with that in the second half of the century when control of the disease started and ultimately led to the eradication of wild-type PV in the Americas. His overview reflected on several epidemiological enigmas in the rise and fall of poliomyelitis, put forward hypotheses to resolve the enigmas and provided evidence to support them.

Why did poliomyelitis emerge as an epidemic disease in European countries and the US between 1890 and 1910? The reason was neither a rise in the number of PV infections nor an increase in the virulence of PV. Instead, the origin of the massive outbreaks seems to lie in a later exposure of children to PV through improved public and private hygiene. Prior to the 1890s, children were exposed to PV in their first years of life, at a time when they were protected by maternal antibodies to the virus. Indeed, Nathanson presented data from 1957 showing that a serum titer of just 1:4 or greater was sufficient to protect against paralytic poliomyelitis [11]. In contrast, children over 12 months were not protected and were therefore at risk for infection and the development of poliomyelitis. Thus, in the initial epidemics of poliomyelitis, almost 70% of the cases were in children in the first 4 years of life [6]. In the following years, however, a marked increase in the age distribution of the affected children was observed. Thus, by about 1940, almost half of the cases were in young adolescents over the age of 10, with only 15% of the cases being in infants from birth to 4 years [6].

Nathanson explained again the apparent enigma through the increase and improvement in public sanitation, such as the provision of non-contaminated water and improved wastewater disposal as well as in private hygiene during the first half of the twentieth century. These investments in public health led to a concomitant delay in the occurrence of the first infection of the population, thus shifting the age distribution of the epidemic. An obvious illustration of this phenomenon was the American President Franklin D. Roosevelt, who contracted poliomyelitis in his late twenties.

The epidemiology of poliomyelitis also revealed a strong seasonality in countries with temperate climates. In such countries, the peak of poliomyelitis was in the months of August and September (hence the old reference "Sommergrippe" or "summer flu"), with hardly any cases being observed in the winter months [32]. However, comparisons between different climatic regions revealed that the important parameter was the humidity and not the temperature. Thus, although PV remains infective at 20°C at humidity levels over 50%, the virus loses its activity within a few minutes at a relative humidity of 40% [14].

This peak of poliomyelitis in the summer months was one of the factors that made the infection so feared. Children who had been active and healthy before the summer break would return to school paralysed and unable to walk properly. Furthermore, the life-long paralysis provided a permanent reminder of the power of the infection and was a decisive factor in the decision in 1988 to commence the campaign to eradicate PV from the planet.

With the availability of the inactivated Salk vaccine from 1955 and the live Sabin vaccine from 1961, the number of cases of paralytic poliomyelitis per year in the USA fell rapidly from 10,000 in the mid-1950s to around 10 in the mid-1970s [32]. Remarkably, by 1973, the wild-type virus had indeed been eradicated from the USA [33]. Any remaining cases of poliomyelitis were caused by

vaccine-associated infections or by PV imported by infected persons from other countries. The eradication of the wild-type viruses was an unexpected surprise, as epidemiological calculations had estimated that in 1970 about 5 million people in the USA would still have been vulnerable to PV infection [45]. This number was thought to have been sufficient to maintain the circulation of the wild-type virus. However, in Nathanson's opinion, two factors appear to have been decisive in preventing the circulation. The first was that the vaccination with the live and inactivated viruses did, despite predictions to the contrary, provide a sufficient level of "herd immunity". Secondly, this level of immunity was sufficient to keep the infections in the winter seasons at a very low level so that the virus could not survive until warmer weather arrived in the following spring.

The molecular biology of poliovirus

During the second half of the twentieth century, PV became the subject of intense scrutiny, initially to understand how the virus caused poliomyelitis and how the disease could be prevented. Subsequently, it became clear that knowledge gleaned from PV could also be applied to many other RNA viruses so that with time PV became a pathfinder in the rapidly developing field of virology. Three talks of the symposium were devoted to this aspect of PV.

Poliovirus cell entry and structural changes leading to uncoating

Infection commences with attachment of the virus to specific cell-surface receptors. The subsequent release of its genetic material into the cytoplasm requires the breaching of cellular membranes, which usually occurs from within endocytic compartments. The breaching of the cell membrane is poorly understood for non-enveloped viruses and, as pointed out by Hogle, PV is an excellent model for studying the processes involved. However, the high ratio between physical and infectious particles in PV preparations constitutes a serious problem and requires that microscopic observation be combined with functional assays, as cell entry by the majority of the particles might in fact reflect an unproductive pathway not leading to infection. To overcome this difficulty, Hogle presented the results of a collaboration with Xiaowei Zhuang (Department of Chemistry and Chemical Biology, Harvard University) in which a fluorescent dye was non-covalently attached to the viral genome. This allowed tracing of single virus particles under the fluorescence microscope, revealing that loss of label correlated with RNA release and infection. In addition, neutral-red-containing virus was inactivated by light as long as the RNA had not left the virion. This setup allowed the group to demonstrate that PV entry into HeLa cells occurs through multiple, possibly non-canonical, pathways. These are independent of clathrin, caveolin, dynamin, flotillin and microtubules; instead, they require energy, actin, intermediate filaments and an as yet unidentified tyrosine kinase.

Conversion of native virus to 135S subviral particles commences upon binding of the virion to the receptor. Total internal reflection-fluorescence microscopy showed that RNA release is efficient and occurs within 30 min in close vicinity (within 100–200 nm) to the plasma membrane. However, the RNA only accesses the cytosol from within vesicles and not from the plasma membrane. As demonstrated with a pH-sensitive probe attached to the viral capsid, this vesicular compartment is inaccessible to the extracellular milieu. The virus-containing vesicles undergo an unusually rapid actin-dependent movement. Capsid-binding drugs prevent both RNA release and movement to the pH-insensitive compartment. It is unclear whether factors present in the vesicles are required for RNA release and whether entry is constitutive or induced. It is also still unclear whether the viral genome exits through a pore or whether the above vesicles become disrupted. Viral capsids are rapidly transferred to the perinuclear region after RNA release, supporting the notion that the vesicles remain intact [2, 46]. In brain endothelial cells, virus has been shown to enter via clathrin-mediated endocytosis. Thus, entry pathways among picornaviruses can vary and depend on virus type, passage history, receptor usage and the particular cell type.

Currently, the resolution of the 3D structure of a complex of receptor and virus is at 6–7 Å. At physiological temperatures, the receptor induces conformational changes resulting in subviral particles. Concomitantly, VP4 and N-terminal sequences of VP1 are externalized and insert into cellular membranes. Electrophysiology has demonstrated that peptide insertion results in formation of channels, and genetic experiments have indicated that channel formation correlates with the ability to release RNA into the cytoplasm and initiation of infection. Extension of the resolution of cryo-EM reconstruction of subviral 80S particles to $\sim$17 Å and average classification into 'early' (80Se) and 'late' (80Sl) particles demonstrated variable RNA content and holes close to the twofold axes. In a few cases, intermediate structures were caught in the act of releasing their RNA. Particles with variable RNA content and no visible RNA outside may indicate hydrolysis of externalized RNA. According to the textbooks, the RNA leaves the virion at the fivefold axis. However, a low fraction of the subviral particles showed density close to a twofold axis that most probably corresponds to exiting RNA [25]. Currently, Hogle is attempting to extend the

resolution of the intermediate structures, including those with RNA inside and outside, with a data set including 500,000 particles. Using liposomes decorated with receptor, membrane-attached virus was also visualized either by conventional cryo-electron microscopy or by tomography. Currently, more data are being acquired to push the resolution and to attempt to visualize virus attached to the endosomal membrane within cells by use of electron tomography.

Poliovirus: a 100-year pathfinder

Eckard Wimmer started his presentation by listing the large number of scientific discoveries that were first made using PV. These discoveries started in the 1950s with the development of cell culture systems to grow cells and animal viruses on a large scale [10, 41] as well as the quantification of animal viruses by plaque assay [8]. During this decade, PV became the first animal virus to be examined by X-ray crystallography [44]. In the 1960s and 1970s, two major discoveries were the discovery of recombination in an RNA virus and genetic expression as a polyprotein [17, 27]. The 1980s began with the generation of the complete sequence of PV on both the genomic RNA [18] and cDNA levels [39]. The availability of the cDNA of PV allowed the generation of the virus by transfection of the DNA into susceptible cells, thus opening the way for the investigation of PV by reverse genetics [40]. A novel mechanism of translational control through IRES elements was also described during the 1980s [15, 36]. At the beginning of the 1990s, infectious virus was produced from full-length viral RNA in a cell-free system made from HeLa cells [29].

The main part of Wimmer's talk was, however, dedicated to two recent achievements in PV research that greatly impinge on the eradication program. These were the synthesis of the PV cDNAs using modern oligonucleotide synthesis techniques and the development of attenuated PV strains by altering the codon usage in the PV genome. Thus, in 2002, Wimmer and colleagues showed that PV could be generated in the absence of both the natural RNA template and live cells [3]. Specifically, a cDNA copy of the PV genome was generated by the ligation of large in vitro-synthesised oligonucleotides. T7 RNA polymerase was used to transcribe the DNA into RNA, which was then added to a cell-free extract of HeLa cells. Infectious PV was produced in the extracts that was immunogenically and pathogenically identical to wild-type PV. In itself, this experiment represents a tour de force of modern virology. However, in terms of the PV eradication program, the experiments mean that it will always be possible to synthesise the virus and that the possibility of reintroduction of the virus cannot be excluded. Wimmer stressed that the world had to be ready for an event such as the template-free synthesis of a virus and that this is true for all viruses whose genome sequences are known [48].

Using this ability to synthesise entire PV genomes in vitro, Wimmer and colleagues subsequently proceeded to generate a PV in which the natural pairs of codons were replaced with over- or underrepresented codons across the entire genome [4]. Infectious PV could be produced from such genomes; in most cases, such viruses had an attenuated phenotype. Wimmer suggested strongly that such viruses could represent a novel approach to producing PV vaccine strains that could not revert to wild-type, thus vastly reducing the risk of vaccine-associated poliomyelitis.

Stability, instability and organisation of the poliovirus genome

Vadim Agol looked back at the contribution of PV research to our understanding of variability in RNA viruses and commented on how this variability affected the efficacy of vaccines, the usefulness of anti-viral drugs and the eradication program in general.

It is nowadays text book knowledge that RNA viruses comprise a set of closely related genomic sequences termed a quasispecies. These genomic sequences vary in their fitness and thus in their ability to replicate in different environments and respond to environmental pressure. These sequences may compete with each other or, in contrast, they may, under certain circumstances, cooperate with each other [47]. Agol reminded the audience that the basis for this knowledge was laid by early research on PV [1, 5].

The variability of PVs results from the error-prone nature of the PV RNA-dependent RNA polymerase [37] and from the possibility of recombination amongst RNA viral genomes [43]. Recombination, again now seen as a common property of many RNA viruses, was first described by examining the properties of PV [24]. PV recombination has been described in the cell-free system, in cell culture and in vivo [16, 43]. The in vivo recombination of attenuated vaccine strains with other enteroviruses was constantly referred to in the symposium, as it may lead to cases of vaccine-associated paralytic poliomyelitis (VAPP). Together, the mutation and recombination observed in PV serve to restore the infectivity of debilitated or disabled genomes, thus greatly contributing to the robustness of PV (i.e. fitness and viability despite a number of changes in the genome or in the environment) [12, 38].

Given the potential of the PV genome to mutate and to recombine, Agol emphasised that, under certain conditions, any live attenuated vaccine was likely to undergo conversion into a more virulent form and that PV was likely to readily mutate in the presence of an anti-viral drug [9], thus

reducing the efficacy of the drug. In his view, there was thus a great need to continue research on PV to find solutions to these problems, rather than to reduce it as required by the WHO.

The poliovirus eradication program

Epidemiology and genetics of the eradication program

Rudolf Tangermann, a long-term member of the WHO's Global Polio Eradication Initiative (GPEI) team, and Olen Kew, from the CDC's Division of Viral Diseases, reported on the present state of the eradication campaign. Data on the number of cases reported by the three speakers are available on the websites of the WHO (http://apps.who.int/immunization_monitoring/en/diseases/poliomyelitis/case_count.cfm), the CDC (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5918a1.htm) and the GPEI: (http://www.polioeradication.org/casecount.asp).

Of the three wild-type PV serotypes, type 2 has not been detected worldwide since 1999. It can consequently be regarded as having been eradicated, thus demonstrating proof of principle for the eradication program. Furthermore, three WHO regions have been certified as free of all three wild-type PVs: the Americas (1994), the Western Pacific Region (2000) and the European Region (2002). These achievements of the eradication program were referred to several times during the meeting as representing the paradoxes of eradication. Why is it possible to eradicate serotype 2 but not types 1 or 3? Why is it possible to eradicate all three PV serotypes from three WHO regions, but not from other parts of the world?

The resolutions to the two paradoxes were shown during the meeting to lie in the properties of both wild-type and vaccine strains of each of the serotypes as well as in the particular situation in each of the four countries in which the transmission of endemic wild-type PV has never been interrupted. These are, namely, Afghanistan, India, Nigeria and Pakistan. A further contributing factor is that wild-type virus from these so-called endemic countries has been reintroduced into countries that had previously been certified as PV-free; at the time of the meeting, about 15 previously PV-free countries in Africa had reported cases of poliomyelitis.

Both wild-type serotypes 1 and 3 are found in all four endemic countries. Wild-type serotype 1 has been the culprit in most reinfected countries, although wild-type serotype 3 has reinfected Chad, the Central African Republic and Cameroon. The serotypes 1 and 3 that remain, however, have a much reduced diversity. This loss of diversity was reported by Kew to be a direct result of the eradication program. Thus, for serotype 1, 17 out of 20 lineages that were circulating in 1985 have been eliminated; indeed, only one distinct lineage is circulating now in India. For serotype 3, 14 out of 17 lineages from 1985 have now been eliminated.

Why has the transmission of wild-type PV types 1 and 3 not been interrupted in the four endemic countries? Tangermann and Kew reported that each of the countries faced different problems and challenges. For instance, in India, the states of Uttar Pradesh and Bihar have very high birth rates (approximately 500,000 newborns per month) and contain pockets of under-vaccinated children in areas that are highly prone to flooding and hard to reach by vaccination teams. In addition, nomadic migrant labour is thought to be one cause of spread of the virus, whilst re-infection from asymptomatic carriers is postulated to be another contributing factor that keeps serotypes 1 and 3 circulating. One piece of data from Kew also showed that wild-type PV isolated from sewage in Mumbai was genetically related to virus isolated from an acute flaccid paralysis (AFP) patient in Bihar, suggesting that reservoirs of wild-type virus may exist in parts of the country that have been free of wild-type PV for many years. This supports the view that vaccination against PV will have to continue for many years after eradication has been achieved.

India has also observed alternating outbreaks of type 1 and type 3. These have arisen by the use of monovalent OPV (mOPV) to combat type 1, leaving the population with a lower immunity against serotype 3. Bivalent OPV is now being used to combat this effect.

Nevertheless, despite all these difficulties, the number of children vaccinated has remained relatively high. In contrast to other parts of the world, in the Indian situation, trivalent OPV (tOPV) fails to provide sufficient immunity against all three serotypes. Both Tangermann and Kew spoke of limited efficacy of tOPV in the Indian setting.

The challenges in Pakistan and Afghanistan are more of an operational and security nature. In Afghanistan, the vast majority of cases are in the Southern Region, around Kandahar, close to the Pakistan border. Districts in these areas, controlled by the Taliban, have had a low rate of immunisation over the last few years, due to the vaccinators' inability to access children in conflict areas. Political intervention by the WHO with NATO and the Taliban has led to the establishment of days of tranquillity on which vaccination campaigns can be carried out. It is hoped that these should lead to higher vaccine rates in these regions. In Pakistan, although cases of poliomyelitis have been noted throughout the country, the majority have occurred in the northwest, close to the border with Afghanistan. Similar to Afghanistan, limited access to children in conflict areas had led to immunisation levels under 80% in parts of this region. However, both the President and Prime

Minister of Pakistan have lent their weight to support national immunisation days. As a consequence, access to regions such as the previously inaccessible Swat river valley has improved to such an extent that over 95% of the area was accessible to vaccination days in October 2009.

In Nigeria, especially in the northern regions, a failure to vaccinate several years ago resulted from false rumors, spread for religious and political reasons, about vaccine side effects. The subsequent low levels of immunity led to an increased number of cases of poliomyelitis; PV excreted by these patients then spread to cause poliomyelitis in a number of countries in the surrounding regions. The resulting political pressure from re-infected countries and the WHO led to an increase in vaccination levels in 2008, such that by the first quarter of 2009, much higher levels of vaccination coverage were being noted. One of the best sources of information on the level of coverage is the number of vaccine doses received by patients suffering from non-PV-induced AFP. During 2008, the percentage of such patients who had received 3 doses of vaccine rose from 35% to over 50%, whilst the number who had received no PV vaccine fell from 45% to just over 10%.

A further complication for the eradication campaign are circulating vaccine-derived PVs (cVDPV) that arise by recombination between any of the three PV vaccines and the closely related human C-cluster enteroviruses. Such neurovirulent viruses occur with any of the three vaccine strains when some of the attenuating mutations are lost and/or recombination takes place with another enterovirus. Small cVDPV epidemics can occur every year in different parts of the world [34, 49]; however, Nigeria stands out as special breeding ground of cVDPV from serotype 2, with over 120 cases of paralysis being observed in 2009 alone [42]. Why cVDPVs from the serotype 2 vaccine predominate in Africa is not known (http://www.polioeradication.org/content/general/cvdpv_count.pdf). The use of mOPV to combat either type 1 or type 3, coupled with the low level of vaccination in certain countries with tOPV, has led to a cohort of children with low immunity to a particular serotype and its related cVPDV. The sudden cVDPV-caused outbreaks in the absence of vaccination indicate that cVPDVs can circulate for some time in a suboptimally immunized population without causing disease.

Nigeria has combated this problem of cVDPVs by carrying out two very well organised rounds of vaccination with tOPV. Consequently, only a handful of cases of type 2 cVDPV were documented between September and November 2009. Given this knowledge, India, following its use of mOPV, is also planning to carry out one or two rounds of vaccination with tOPV to prevent cVDPVs arising from serotype 2.

Two further pieces of positive news from Nigeria were the agreement of the governors of the 36 states of Nigeria in February 2009 to provide their full support for the vaccination program and the absence of any cases of type 1 poliomyelitis in Nigeria in the 3 months up to November 2009. According to Tangermann, Nigeria appears to have turned a corner, and the situation is brighter than for many years.

Kew reported on the nature of the PV that had been reintroduced into countries that were previously free of PV. Countries to the west of Nigeria were, on the whole, infected by wild-type serotype 1 circulating in Nigeria, whereas countries to the east were infected by Nigerian serotype 3. In addition, Angola was reinfected by transmission of a serotype 3 strain from northern India, with the strain subsequently being transmitted further to the north and east. Several other factors also contributed to the re-emergence of PV in African countries. These included poor infrastructure, armed conflicts and widening gaps in the immunisation programs after the first successful control of PV. Kew remarked that one indication of the lack of infrastructure for surveillance was the reappearance of virus lineages (in Kew's terminology, "orphan viruses") that had not been observed for several years. For example, a lineage detected only in the Sudan between 2004 and 2005 suddenly reappeared in the same area in 2008. Thus, the transmission of this lineage escaped observation, clearly indicating that the coverage of surveillance with AFP was not 100%.

Tangermann and Kew both stressed the achievement of the tOPV in the eradication program. However, Tangermann observed that certain properties of tOPV meant that its use in a post-eradication era would compromise a polio-free world. Specifically, the number of VAPP-associated cases (2–4 per million vaccinated), the number of iVDVP cases [neurovirulent immunodeficiency-associated vaccine-derived PV (iVDPV), detailed by Almond below] and the emergence of cVDPV, especially at lower levels of vaccination coverage, would mean the continued occurrence of paralytic poliomyelitis. Thus, following eradication, it would be necessary for the world to cease the use of OPV and possibly switch, at least for some time, to vaccination with inactivated poliovirus vaccine (IPV). Tangermann then discussed this issue in terms of prerequisites and unknown quantities.

Two of the most important factors that he considered were cost and efficacy of IPV, especially when used in the developing world, themes that were also discussed at length in the presentations of Chumakov and Almond.

Obstacles to the elimination and eradication of poliovirus

Konstantin Chumakov, using data available from the WHO, GPEI and CDC (see sources above), summarised

the obstacles that have to be overcome before PV can be eradicated. Regarding the failure to interrupt transmission in India, Afghanistan, Pakistan and Nigeria, he agreed with other speakers that there had been a significant failure to vaccinate in northern Nigeria and vaccine failure in northern India. Specifically, the level of protection afforded per dose of tOPV in Uttar Pradesh in northern India was only about 9%. Indeed, 80% of AFP cases occurred in children that had had ten or more doses of tOPV; 96% of cases occurred in children that had received four or more doses [13]. Thus, in northern India, the tOPV is, for unknown reasons, less efficacious than in other parts of the world or even in other parts of India.

Chumakov also emphasised that the problems of VAPP, cVDPV and iVDPV associated with OPV cannot be solved. For VAPP and cVDPV, these problems derive from the properties of the live PV used, as outlined by Vadim Agol. The reason why iVDPV arises in certain immunodeficient patients remains unknown, so it is difficult to know whether OPV can be modified to prevent iVDPV.

This viewpoint was also shared by Jeffrey Almond. In his talk, he considered in some detail the problem of the long-term excretion of neurovirulent iVDPV [28]. Almond presented data on the most well-known case of this disease, in a patient from Birmingham (UK) studied over several years by Minor and colleagues [35]. The patient, suffering from hypogammaglobulineamia, became persistently infected with what was originally the serotype 2 vaccine at least as early as 1996; however, the more probable date is around 1987, the last time that he was vaccinated. Since the initial infection, the virus has evolved to become de-attenuated and has been excreted constantly since its detection in 1996 (over 150 sequential isolates have been collected). Furthermore, all efforts (such as the administration of the antiviral drug ribavirin or the oral administration of IgA) to eliminate the virus from the patient have failed [26]. At present, the patient continues to shed the virus, and there is no obvious strategy to prevent this or eliminate the virus. Chumakov showed that long-term excretors of all three serotypes have been detected in many parts of the world. In total, between 20 and 34 such people are known across the world [28].

Both Chumakov and Almond also were united in stating that, despite the success of OPV, the problems associated with OPV will almost certainly prevent the attainment of the goal of PV eradication using OPV alone. In addition, should eradication be achieved, vaccination against PV will still be necessary, given the presence of patients with iVDPV as well as the orphan PV documented by Kew. The use of OPV after eradication will lead to continuous introduction of live PV into the environment. This may result in emergence of newly virulent PV and therefore defeat the purpose of the eradication campaign. The only solution, according to both speakers, to overcome the problems of VAPP, cVDPV and iVDPV as well as to continue safe vaccination is to introduce IPV. As a positive example for the feasibility of this, Chumakov reported that the recent decision by Russia to introduce IPV had proceeded smoothly and had reduced the number of VAPP cases to almost zero. There are now over 50 countries using IPV in their national immunisation programs (http://en.sanofi-aventis.com/binaries/20081112_ipv_russie_en_en_tcm28-22721.pdf).

The role of IPV in achieving and maintaining the eradication of poliovirus

Several of the above speakers had stated clearly that the goal of the eradication program would be difficult to achieve if vaccination with OPV were to continue and that the use of OPV after eradication would compromise the goal by constantly reintroducing the very virus that had been eradicated. The alternative would be to use IPV instead of OPV, but would this be feasible in terms of efficacy, production and cost? Both Almond and Tangermann addressed the prerequisites for OPV cessation and replacement with IPV in both the pre- and post-eradication eras, with Tangermann placing special emphasis on the affordability of IPV.

Almond presented data in the literature from studies in the Ivory Coast [30] and Puerto Rico [7] that documented the efficacy of IPV. The Puerto Rico study showed that three doses of IPV were sufficient to provide 100% seroconversion against all three serotypes. Furthermore, the study in the Ivory Coast revealed that the IPV gave better rates of seroconversion in 9-month-old infants who had failed to seroconvert after three doses of OPV than did a further dose of OPV. Tangermann, using data from the strategic advisory group of experts (SAGE) working group on IPV, estimated that, in low-income countries, three doses of IPV would be sufficient to generate over 95% protection at the individual level and 70% at the population level.

Both Almond and Tangermann then addressed the questions of feasibility and cost of producing the around 425 million doses of IPV needed if this vaccine is to be used to replace OPV. Speaking from the manufacturer's viewpoint, Almond first stressed that developing a novel IPV using the Sabin vaccine strains was not a viable option, as both the time-frame (around 10 years) due to the necessary clinical data and the cost would be prohibitive. For instance, no clinical data on the efficiency of a Sabin-based IPV have ever been obtained. Almond then cited the Wyman report (http://www.polioeradication.org/content/general/March%202009%20OW%20IPV%20Effort%20Report.pdf) as stating that global capacity for the production of such

amounts of IPV from wild-type PV strains would be possible, provided that health authorities made a clear commitment to the use of this form of the vaccine. Clearly, this may require further investment to construct the necessary production plants using the latest technology at biosafety level three. Nevertheless, Almond was of the opinion that the additional cost of including IPV would not be prohibitive. Indeed, it could be rather small if the IPV were to be included in presently available pentavalent vaccine combinations that are used in current immunological schedules.

In short, both Almond and Tangermann were cautiously optimistic about the potential for IPV to replace OPV in and after the end-game of PV eradication. However, Tangermann stressed the need for modelling studies to help stakeholders understand the value of changing from OPV to IPV and for more studies to provide information on protection of the population by IPV and on how efficient IPV will be in reducing VDPV circulation.

Funding of the eradication project

The achievements of the campaign to eradicate PV were only possible because of the efforts of several funding agencies, above all, those of Rotary International, the Bill and Melinda Gates Foundation and UNICEF. One of the highlights of the meeting was the presence of Bob Scott, chairman of the PolioPlus campaign of Rotary International (http://www.rotary.org/en/EndPolio/Pages/polio.aspx), and Tim Petersen from the Bill and Melinda Gates Foundation (http://www.gatesfoundation.org/polio/Pages/overview.aspx).

Bob Scott reported on the history of the support of his organisation in fighting PV. Rotary International began its activity 30 years ago when 6 million children in the Philippines were immunised using vaccines donated by Italian Rotarians. In 1985, 3 years before the WHO voted to eradicate poliomyelitis, Rotary International started its PolioPlus campaign to provide vaccines to immunise children in all parts of the world. In two corporate fundraising campaigns, the organisation raised 383 million USD. A third campaign, known as the Gates Challenge, is now underway with the goal of raising 200 million USD by 2012 to match the 355 million USD support promised by the Gates Foundation. In total, Scott estimates that, by 2012, Rotary International will have provided 1.2 billion USD of funding for its PolioPlus campaign. The contribution of the voluntary services provided by its members cannot be measured in financial terms.

According to Scott, about 5 million cases of poliomyelitis have been prevented through the GPEI, of which PolioPlus is a part. In addition, several other benefits can be noted, such as much improved systems for surveillance of cases of poliomyelitis, networks of certified laboratories that were initially organised to diagnose PV but that are used to detect a whole gamut of pathogens, support for the production of bivalent vaccines that are now in use in Afghanistan, and the logistical expertise to be able to vaccinate as many as 175 million children per week in India or China.

Summing up, Scott stated that the goal of the PolioPlus campaign is to prevent cases of poliomyelitis that are caused by wild-type PV. During the subsequent discussion, Wimmer posed the question whether, given the nature of OPV, Rotary International would be willing to support a switch from OPV to IPV. Scott answered that Rotary International would not be willing to do this at the present time and needed to be more convinced of the efficacy of vaccination with IPV in the setting of National Immunization Days regarding cost and logistics.

In turn, Tim Petersen also outlined the contribution of the Gates Foundation to the GPEI, which, in total, is more than 680 million USD over the last 10 years. Indeed, in the 12 months from July 2008, the Gates Foundation has been estimated to have provided about 30% of the total GPEI. As mentioned above, there is important synergy in the efforts of the Gates Foundation and Rotary International, with the Gates Foundation challenging Rotary to meet its pledges, resulting in a massive overall increase in funding. Petersen also noted the vital effect of the support of Bill Gates in dealing with governments and political institutions. The importance of Bill Gates' presence was clearly illustrated when he met with the governors of the 36 federal states of Nigeria. Following the meeting, the governors committed themselves, amongst others, to providing leadership and resources to ensure that all children under 5 years are reached and vaccinated during all eradication campaigns (http://www.polioeradication.org/content/publications/AbujaCommitments_04Feb2009.pdf). As mentioned by Tangermann, Kew and Chumakov at the meeting, the failure to vaccinate in Nigeria is one of the major obstacles in the eradication campaign. The commitment of the governors should be an important milestone in efforts to reduce the transmission of both wild-type and vaccine-derived PV.

For the future, Tangermann noted that despite the great efforts of all the funding agencies, funding gaps for future years existed and that it was also not clear whether the present level of funding could be maintained.

Open discussion: the endgame of poliovirus eradication

The discussion, with Neal Nathanson acting as chair, centred essentially on how and when the transition between OPV and IPV should take place. The first question raised was why countries in South America had not changed to IPV. Given that these countries have not seen wild-type PV for over 10 years, it was postulated that the only cases of

PV in these areas would be VAPP coupled with the appearance of cVDPV through the use of OPV. Kew reported that the health authorities in these countries were concerned about the effect of having an IPV vaccination program in a low-hygiene country neighbouring another one vaccinating with OPV. If there are gaps in the IPV program, there may be a higher chance of the production of cVDPV. To prevent such a possibility, the health authorities, along with Pan American Health Authority (PAHO), wish to make synchronised migration to vaccination with IPV.

Francois Delpeyroux (Institut Pasteur, Paris) reported data from a study in Cordoba City, Argentina. Up to the end of 2002, the city authorities performed routine vaccination with OPV. However, for the 3 years from the start of 2003 to the end of 2005, vaccination was performed with IPV [31]. During this period, only 19% of wastewater samples were positive for PV. In contrast, when the city switched back to OPV, the number of positive water samples rose to 100% within 2 weeks. Thus, according to these data, the use of IPV can reduce the transmission of OPV vaccine strains in a South American setting.

During the discussion, several participants mentioned that vaccinating with OPV in a population with a low immunity is likely to generate many cVDPVs. Chumakov stated that this had happened at least twice, once recently in Nigeria, when the vaccination program was halted for political reasons, and once in Belorussia in the 1960s [19]. Given these observations, Chumakov stated that control of outbreaks during the endgame and in the post-eradication period must be performed with IPV. In addition, it would be important to support the IPV control program with antiviral compounds against PV. In contrast, Tangermann pointed out that IPV had never been used to contain an outbreak and that this should be borne in mind when planning the switch from IPV to OPV.

Both Almond and Ellie Ehrenfeld (NIAID, Bethesda, USA) stressed that it was vital to maintain the immunity of the population through vaccination. In their view, the use of OPV would be counterproductive because this would permanently introduce live PV into the community. In Almond's view, the most sensible option was to cease the use of OPV as soon as possible and move to IPV. Ehrenfeld hoped that new vaccines might soon be available. However, Almond pointed out that the high development costs would be unlikely to be recouped by manufacturers and that there would be no direct way of testing the efficacy of a new vaccine. The eradication of PV and the maintenance of a PV-free world will have to be achieved with the tools presently available to us.

Discussion also centred on how effective the current surveillance systems are, whether wild-type PV could still linger without the appearance of cases of AFP and whether present surveillance levels can be maintained, should the eradication program be successful. Chumakov warned that surveillance was almost certain to drop in the next 10 years and that AFP cases, although very useful indicators, may not be a reflection of the amount of wild-type virus in the circulation. He pointed out that 99.9% of PV infections do not result in illness. In contrast, Kew felt that the AFP was an excellent reflection of the amount of circulating wild-type virus. Furthermore, Kew felt that the state of the surveillance efforts could be assessed by the lengths of the branches of phylogenetic trees observed for different lineages. Thus, the shorter the lineages detected, the better the surveillance. Kew also reported on environmental surveillance of waste water for the presence of PV. In Cairo, examination of waste water over the last 4 years confirmed the AFP surveillance results, indicating that wild-type virus was absent from the city. However, Kew also admitted that wild-type virus had been detected in waste water in the Gaza area but that clinical cases of AFP had not been concomitantly detected. Nevertheless, Kew stated that, although AFP surveillance is not the whole story, there is still a tight correlation between the disappearance of PV from the population as measured by AFP and its disappearance from the waste water.

In conclusion, the symposium clearly documented the successes of the global program to eradicate PVs in terms of the number of cases of poliomyelitis prevented, the reduction in the number of countries in which the transmission of wild-type PV is still continuing and in the complete eradication of wild-type PV serotype 2. However, there was a general feeling at the end of the meeting that several obstacles still lie in the path towards eradication and that IPV will continue to replace OPV in still more countries.

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