Invited Review

Poliovirus Studies during the Endgame of the Polio Eradication Program

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SUMMARY: Since the beginning of Global Polio Eradication Initiative in 1988, poliomyelitis cases caused by wild poliovirus (PV) have been drastically reduced, with only 74 cases reported in 2 endemic countries in 2015. The current limited PV transmission suggests that we are in the endgame of the polio eradication program. However, specific challenges have emerged in the endgame, including tight budget, switching of the vaccines, and changes in biorisk management of PV. To overcome these challenges, several PV studies have been implemented in the eradication program. Some of the responses to the emerging challenges in the polio endgame might be valuable in other infectious diseases eradication programs. Here, I will review challenges that confront the polio eradication program and current research to address these challenges.

1. History of polio virology and the polio eradication program

Poliovirus (PV) is a small non-enveloped virus with a single stranded positive genomic RNA of about 7,500 nucleotides (nt) belonging to Enterovirus C in the genus Enterovirus, family Picornaviridae. PV is the causative agent of poliomyelitis, which is caused by the destruction of motor neurons by direct infection of these cells with PV (1,2). The attack rate of paralytic poliomyelitis is 0.053–0.526% with differences among the serotypes (3); the vast majority of infections are asymptomatic, promoting continued transmission of PV. Since discovery of the virus in 1908 (4), PV studies have contributed to several breakthroughs in the fields of virology and biology, including in vitro cultivation of viruses (5), an understanding of the mechanisms of central nervous system invasion by viruses (6), and ultimately, the development of effective vaccines in the 1950s to early 1960s (7,8). In the post-vaccine era, PV serves as one of the most well-established models for positive-stranded RNA viruses, and infections with this virus have been extensively studied with modern molecular biological techniques. Enumerating all of the milestones reached in PV studies is far beyond the scope of this review, however, this list would include sequencing of the PV genome (9,10), the development of reverse genetics for the virus (11), elucidation of the crystal structure of the virion (12), identification of the internal ribosomal entry site (IRES) (13), identification of the PV receptor (PVR, CD155) (14,15), development of a transgenic mouse model of poliomyelitis (16,17), identification of attenuation determinants in oral polio vaccine (OPV) strains (18,19), identification of drug candidates (20,21), development of a cell-free replication system (22), identification of cis-acting replication elements (23,24), identification of circulating vaccine-derived PVs (cVDPVs) (25), and identification of host targets for drug candidates (26–30). PV studies in the post-vaccine era have produced valuable tools and information essential to the eradication program, including nucleotide sequences of PV and other enteroviruses (9,10,31), which have been used to design primers for current reverse transcription (RT)-PCR systems for PV detection (32,33); neurovirulence determinants of PV that are used for quality control of vaccines and evaluations of environmental isolates (18,34,35); PV-sensitive murine cell line (L20B) (14), which is a cell line that expresses the PVR and is currently used for PV surveillance because of its high specificity for PV infection, a PVR-transgenic mouse (TgPVR21) model as an alternative to monkeys used in neurovirulence tests for PV vaccines (36), and candidate compounds for antivirals, including V-073 (37).

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Following PV elimination success in some countries, the Global Polio Eradication Initiative was initiated in 1988, a year when more than 350,000 cases were reported in 125 endemic countries. The Global Polio Labora-
torv Network, which currently consists of 145 laboratories, was established, including our Laboratory of Enteroviruses of National Institute of Infectious Diseases (NIID), which has served as one of 7 specialized global laboratories. After an extensive global vaccination campaign using OPV for about 3 decades, transmission of wild PV has largely been terminated. In 2015, there were only 74 cases in 2 endemic countries. The current limited number of poliomyelitis cases, and areas where transmission is occurring, along with continuing vaccine campaign, suggests that polio eradication program is now in its endgame.

2. Challenges in the polio endgame

Budget for surrounding the polio eradication endgame: In the of the polio eradication endgame, 3 major challenges remain to be solved. The 1st and possibly most important challenge is the budget. The Polio Eradication & Endgame Strategic Plan 2013–2018 of the World Health Organization (WHO) (38), which defined the aim of global eradication and containment of wild PVs by the end of 2018, proposed a total budget of US $5.5 billion. It should be noted that about 47% of the planned budget was for immunization activities with OPV and inactivated polio vaccine (IPV). Nevertheless, US $0.45 billion of this budget remained unfunded as of 2014.

Switching from OPV to IPV: Second is a switch of the vaccines from OPV to IPV. This was, in part, related to costs of the vaccines. Currently, there is an approximately 4 to 23-fold difference in the costs of OPV and IPV, with a cost of about 0.12–0.21 US $ for a single dose of OPV, and US $0.83–2.8 (or 0.75–2.5 euro based on 1 US $ = 0.90 euro) for a single dose of IPV (39). The critical biological differences between OPV and IPV are that only OPV is able to suppress regional circulation of wild PVs (40–43), and the side effects with vaccine-associated paralysis and the emergence of cVDPVs following immunization with OPV vs. no serious side effects with IPV. Confirmation that no OPV strains are circulating after complete changing to IPV is a primary challenge for eradicating PV, because cVDPVs are currently emerging/circulating throughout the world and were the cause of a recent outbreak of type 1 cVDPV in the Lao Republic (44). Currently, 2 types of IPV, in terms of seed virus, are available: wild PV strain or Sabin strain-based IPV (the background/history of Sabin strain-based IPV development is in (45)). These IPVVs have different antigenicities especially for type 1 wild (Mahoney) and Sabin 1 PV (46), possibly because of a VP3-T60K mutation in the Sabin 1 (47,48).

Biosecurity: The third challenge follows reclassification of the biosafety level (BSL) of PV strains. In 2015, a revised edition of the WHO Global Action Plan (GAP) to minimize poliovirus facility-associated risks after type-specific eradication of wild PVs and sequential cessation of OPV use (GAP III) was implemented (49), raising the BSL for type 2 PV strains (biosafety level of Annex 2 and 3 in GAP III, which corresponds to BSL3 plus additional conditions). Implementation of GAP III restricted the use of the Sabin 2 strain in conventional biological tests and for laboratory serosurveillance. This change also affects the production system for Sabin-based IPV, which was originally planned for BSL-2 containment, and plans for acute flaccid paralysis (AFP) surveillance. Once the materials are identified as Sabin 2 in the laboratory, its containment needs to comply with GAP III Annex 2 and 3.

3. PV study supported by WHO and the Bill & Melinda Gates Foundation

PV studies complementing the polio eradication program have been supported mainly by WHO and the Bill & Melinda Gates Foundation, which has been serving as a major external contributor for the eradication program, with more than 25% of external budget contribution in 2014.

WHO is calling for collaborative studies, mainly for effective implementation of the vaccine campaign, including biological studies that can support polio eradication (50), including regional serosurveillance, environmental surveillance (51), direct detection of PV (33), and OPV/IPV implementation (52). However, the overall picture of projects supported might not be clear, because not all these studies have been made public as of 2016.

The Gates Foundation provided a grant opportunity specific to polio eradication in the 2011 Global Grand Challenges in 2011 (the Poliovirus Endgame: Creating ways to Accelerate, Sustain and Monitor Eradication) (53). Specific examples of topics that were proposed for effective polio eradication, and thus not necessarily exclusively for the polio experts; e.g., rigorous short-term studies to directly test the ability of practical and low-cost water and/or sanitation interventions to reduce the transmission of poliovirus in populations where the force of infection is high. This specific challenge has public health importance beyond PV eradication, and it continues as topics independent of PV eradication (e.g., ‘reinvent the toilet’) that is supported by the Foundation (54). In Phase I, a total of 23 proposals were accepted, and 3 projects were selected for Phase II. The projects selected for Phase II included intense continuous polio surveillance in China (Dr. Yong Zhang) (55), establishment of new high-producing cell lines for a PV vaccine (Dr. Ralph Tripp) (56), and increasing production capacity of empty PV capsids (Dr. Ian Jones) for affordable IPV. We might see a down-to-earth way of the Foundation in the selection of PV studies, but innovation, in terms of drastic breakthroughs for the eradication program endgame, has been limited.

4. Overview of ongoing PV studies in the polio endgame

There are 4 major ongoing projects that are relevant to the polio endgame (Table 1). For development of affordable and safer PV vaccines, i) reducing costs; ii) evaluating the effects of changing the immunization schedule; and iii) developing hyper-attenuated strains. The cost of IPVVs are expensive (about 4 to 23-fold more than OPV) (39). To reduce the IPV costs, the effectiveness of a fractional dose (one fifth of a full dose) of IPV has been evaluated (52). The effects of changing the immunization (including IPV) schedule have also been evaluated (57). The results suggested that reducing the IPV dose and changing the immunization schedule
can slightly decrease or increase the resulting neutralizing antibody titer, depending on the PV serotypes, but its actual effectiveness in the suppression of wild PV circulation remained to be verified. Development of engineered knock-out cell lines might increase the PV yield of for IPV production (56). Hyper-attenuated strains have been developed by adaptation of PV to cold temperatures (58), or by modification of the RNA stem-loop in the PV-IREs (59). The major expected use of these hyper-attenuated strains would be as seed strains for IPV production at less restricted biosecurity levels than needed for Sabin strains after the implementation of GAP III. Hyper-attenuated strains also have the potential to serve as alternative OPVs for outbreak response, instead of Sabin strains.

In the polio endgame, efficient PV surveillance is crucial. There are 2 types of surveillance that are distinct in their targets: one is surveillance of suspected polio cases based on AFP, and the other is environmental surveillance. This project could solve the 2nd (changing the immunization schedule) and 3rd challenges (reducing BSL needed for the production of PV strains) discussed above. The targets of AFP surveillance and environmental surveillance are different; poliomyelitis cases caused by wild PVs and cVDPVs, and PV, respectively. The target countries for AFP surveillance and environmental surveillance are also different; potentially low vaccine coverage countries and high vaccine coverage countries, respectively. Both methods have been implemented, considering the balance of effectiveness, efficiency, and cost. WHO has provided a manual and guidelines for these types of surveillance (60,61). Polio case surveillance is based on the isolation of PV from stool samples of AFP cases by using cell culture. The major challenges for this type of surveillance are timeliness (currently, final results are obtained >100.5 CCID50 (50% cell culture infectious dose) or >850–1,300 copies of PV genomes in 50 μL of stool extracts (62,63). The stool extracts may contain PV genomes derived from ‘non-viable’ PVs (33,63). For detection, intratypic differentiation, nucleotide sequencing of VP1-coding region of PV, amplification of PV genomes is essential by using cell culture method (i.e., virus isolation) or by RT-PCR. The most sensitive direct detection method for PV to date utilizes PVR-sensitized beads (partial purification of PV from stool extract) and an efficient whole capsid-coding region amplification method (amplification of the entire capsid-coding region of Enterovirus C species, including PV), which allow detection of PV with sensitivity equal to or higher than that of the cell culture method, along with VPI sequencing for intratypic differentiation (33,63). The challenges remained in the implementation include optimization of the condition of the method in terms of the effectiveness and cost, and also the discussion on the target cost of the surveillance. In addition, the current real-time RT-PCR system for intratypic differentiation of PV needs to be improved so that cVDPVs are detected (64). The major challenges in environmental surveillance are i) the need to validate the surveillance method; ii) the inefficiency of the PV identification procedure; and iii) the implementation of direct detection method for PV. Direct detection of PVs in environmental surveillance is important due to the increased biosecurity rules after implementation of GAP III. Environmental surveillances currently performed is not necessarily specific to PVs, but targets enteric viruses more generally.

The development of biological tests and serosurveillance method that do not require live PV could solve the 3rd challenge (BSL requirements for PV strains) discussed above. Type 2 Sabin strain cannot be handled in BSL-2 laboratories since the implementation of GAP III. PV pseudoviruses and hyper-attenuated strains might serve the alternatives to live PV, including the type 2 Sabin strain, in the biological tests that currently require live PV (e.g., neutralization test) (46,58,59,65).

Antivirals against PV are anticipated to play an important role in the post-eradication era of PV in controlling cVDPV transmission, in conjunction with IPV, and in treating patients chronically infected with PV or individuals exposed to PV (66,67). This project could, in part, solve the 2nd challenge (switching of the vaccines) discussed above, because currently PV outbreaks could only be suppressed with OPV. There is currently no antiviral available to treat PV infection. As a candidate direct-acting antiviral, capsid-binding inhibitor V-073 (also known as pocapavir) has been intensively studied in terms of the resistance mutations, pathogenicity of resistance mutants, and effects on immunization with IPV (37,68,69). The effectiveness V-073 in treating neonatal enteroviral sepsis was recently reported (70), and V-073 is currently in a clinical trial as an anti-PV antiviral (EudraCT Number: 2011-004804-38). Viral 2A protease, 2C helicase, 3C protease, and 3D polymerase have also been identified as potential targets of direct-acting antiviral agents, including elastase inhibitors (2A inhibitor) (71), guanidine hydrochloride and related compounds (2C inhibitor) (72–75), rupintrivir (AG-7088) or AG-7404 (3C inhibitor) (76–78), and ribavirin (79,80), respectively. However, the efficacy of these candidates against PV infection in vivo remains to be clarified. Host-targeting antivirals have the advantage of limiting emergence of resistance mutants (81,82), which arise from modulation of the viral/host proteins interaction (for brefeldin A) or from changing the ratio of viral protein expression (for PI4KB/OSBP inhibitors) (83,84), and its broad antiviral spectrum on picornavirus. However, the utility of a promising candidate, P14KB inhibitor (27,28,85–91), is currently limited because of its in vivo toxicity (92,93). In addition to chemical compounds, monoclonal antibodies that can neutralize PVs of different serotypes might be useful as anti-PV therapeutics.

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Table 1. Major basic studies of PV in the endgame of polio-eradication

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<tr>
<th>No.</th>
<th>Study Description</th>
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<tbody>
<tr>
<td>1.</td>
<td>Development of affordable and safer PV vaccines</td>
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<tr>
<td>2.</td>
<td>Establishment of efficient PV surveillance</td>
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<tr>
<td>3.</td>
<td>Development of biological tests/serosurveillance without live PV</td>
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<tr>
<td>4.</td>
<td>Development of effective anti-PV drugs</td>
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relevant to the eradication program (97). In addition, have provoked novel discussions on biosecurity issues. For host-targeting antivirals, circumvention of endogenous toxicity is the major challenge. Examination of a novel administration schedule might provide a solution that both increases efficacy and reduces toxicity of host-targeting antivirals (95).

5. Perspectives

How much will current polio studies contribute to polio eradication? In terms of technology for PV surveillance, differences between current (2016) and initial (1988) strategies only include the introduction of real-time RT-PCR for PV intratypic differentiation, detailed classification of PV isolates, and slight modification of virus isolation procedures in terms of the timeliness. Wild PV eliminations in WHO regions of the Americas, Europe, and the Western Pacific were certified before 2003 (96); thus, without further technical advances, wild PV strains can be eradicated only if the OPV coverage is sufficiently high. The primary challenge for PV studies might be whether technical advances or other innovations can overcome the low vaccine coverage rate resulting from ‘polio fatigue’, public safety in certain countries, and any other implementation issues.

Polio eradication in the early stage and in the endgame is different. For example, the sensitivity of PV detection required when PV circulated in more than 135 countries is different from that required with limited PV circulation in just 2 countries. In 2015, cVDPV cases accounted for about one-third (32/106) of total PV cases in the world. Therefore, discrimination of OPV-associated poliomyelitis cases from cases of poliomyelitis caused by cVDPV is essential in the endgame of the vaccine campaign, but not in the early stage of the eradication.

Scientific advances have altered our views on the eradication program in terms of technology. Generation of PVs from ‘non-infectious’ oligo nucleotides have provoked novel discussions on biosecurity issues relevant to the eradication program (97). In addition, analysis of picornavirus genomes has revealed that the non-structural protein-coding region of the PV genome is largely equivalent to those of PV-like Enterovirus species C (coxsackie A virus types 11, 17, and 20) in terms of amino acid sequence identity (more than 95% identity with PV sequences) (31) and compatibility with the PV capsid (i.e., some coxsackie A viruses can use the PV capsid for the production of infectious virus) (25,98–100). Thus, the PV-like non-structural protein-coding region is circulating in the field with coxsackie A virus capsid, which could not be the target for the eradication program until severe clinical symptoms like poliomyelitis with high attack rate are identified. This suggests that the target for PV surveillance might be the PV capsid protein-coding region, rather than other parts of the PV genome, including the 5’ and 3’ non-translated regions and non-structural protein-coding region. These points raise profound questions about the PV eradication and containment, including what we should eradicate, and how we can ensure eradication.

PV studies that complement polio eradication are required to solve emerging challenges in the endgame. The future direction of PV study depends on the changing world in the eradication program. PV will remain an exciting research subject, and many important public health and molecular biology questions remain to be answered even after PV eradication.

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