

Oral Poliovaccine: Will it Help Eradicate Polio or Cause the Next Epidemic?

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Abstract

Background: Poliovirus rapidly evolves by nucleic acid substitutions and genetic recombination with other polioviruses and non-polio enteroviruses. Evolving oral poliovaccine can rapidly revert to neurovirulence and undergo antigenic alterations.

Objectives: To evaluate the threat of vaccine-derived poliovirus (1–15% divergence from the respective Sabin strain) for a poliomyelitis-free population in a country with a long-standing routine vaccination program.

Methods: We characterized genetic and antigenic changes in OPV (Sabin) strains isolated from sewage in Israel and evaluated intestinal immunity by measuring fecal excretion after OPV challenge of vaccinated children.

Results: Characterization of poliovirus from sewage revealed eight type 2 and three type 3 vaccine polioviruses that had replicated and started to evolve (vaccine that replicated and diverged by 0.5 to ≤ 1.0%) and nine highly diverged type 2 vaccine-derived polioviruses (1–15% divergence from the respective Sabin strain) with 8–14% divergence between the years 1998 and 2005. Six of the eleven VRPV uniquely recombined with OPV and/or NPEV. The nine VDPV were epidemically related, genotypically neurovirulent, and had 10–15 amino acid substitutions in antigenic sites altering their antigenicity, but shared a single recombination. Type 2 OPV was excreted by 23% and 17% of infants challenged with OPV 3 months after partial immunization (two doses each of OPV and enhanced inactivated poliovirus) or full immunization (three doses of each) respectively, despite high humoral antibody titers.

Conclusions: Our findings, which show that OPV is excreted for a significant period by children with high humoral immunity, emphasize the long-term potential threat from VDPV in highly vaccinated populations. An adequate immunization program, combined with environmental surveillance, is necessary to prevent poliomyelitis and community transmission of poliovirus.

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Between 1990 and 2004, Israeli infants received three doses each of enhanced inactivated Salk vaccine and attenuated oral

OPV = oral poliovaccine (Sabin strains)

VRPV = vaccine poliovirus that has replicated and started to evolve but is < 1 % but at least 0.5% diverged from the respective Sabin strain

NPEV = non-polio enterovirus

VDPV = vaccine-derived poliovirus 1–15% divergence from the respective Sabin strain

Sabin vaccine during their first year and a half of life [1], with the goal of preventing disease and spread of polio by inducing high humoral and intestinal immunity in individuals and herd immunity in the community. Annual national coverage since 1999 ranged between 92% and 95% and annual sero-surveys documented > 95% immunity to all three strains [1]. As a consequence, Israel has been free from poliomyelitis since 1988 [2,3]. Monthly monitoring of sewage from sentinel communities in Israel was initiated in 1988 for the surveillance of poliovirus activity in the absence of poliomyelitis [3,4]. Poliovirus in sewage was recovered as individual plaques which were then characterized by serologic and molecular methods [5]. Most (> 98%) of these poliovirus isolates were excreted by recently immunized individuals or contacts and had diverged by < 1%. Divergence was minimal since only 11 isolates (eight type 2 and three type 3) within this group had accumulated ≥ 0.5% substitutions [6]. Unexpectedly, nine type 2 VDPV isolates were obtained between 1998 and 2005, which had diverged by 8–14% and were genotypic neurovirulent revertants [7,8]. Finally, in 1995, one wild-type was identified from sewage in Israel. This strain was one of the 123 wild-type 1 polioviruses isolated from 21 sewage samples collected in a common sewage-monitoring program conducted in Israel and the Palestinian Authority between 1990 and 2005. Presumably the Israeli isolate was imported from the Gaza District at a time when there was increased poliovirus activity [3,4] [Shulman LM et al., unpublished].

Poliovirus evolves rapidly (1% per year) by accumulation of nucleotide substitutions [9,10]. It also evolves by recombination with other polioviruses and non-poliovirus enteroviruses. These recombinations serve as markers for a common ancestry [6,11,12]. Type 1, 2 and 3 OPV differ from the respective wild-types from which they were derived by 55, 23 and 11 nucleotides; however, attenuation of neurovirulence is attributed to only two or three of these differences [13].

This report analyzes the pattern of recombination during initial and advanced stages of vaccine evolution (VRPV and VDPV, respectively) and relates changes in amino acids in and adjacent to neutralizing antigenic sites with changes in antigenicity. It also assesses the intestinal immunity to transmission of type 2

poliovirus in infants immunized with the combined vaccination program [1].

Materials and Methods

Isolation of polioviruses from sewage samples, and molecular sequencing and serologic characterization were as previously described [7]. Sabin and Salk reference strains were used as before [7]. Recombination was assessed by reverse transcriptase-polymerase chain reaction [14]. Altered antigenicity was analyzed by comparing geometric mean neutralizing antibody titers [15] to isolates with those for type 2 OPV and IPV in different groups aged between 1.5 and 14 years with known vaccination coverage, and in those aged 20–50 years from convenient samples from the Israel Center for Disease Control serum collection. Intestinal immunity was measured by challenging infants with OPV 3 months after they completed a partial (two doses each of OPV and eIPV) or full immunization program (three doses each of OPV and eIPV) and determining the number of infants excreting type 2 virus between 7 and 28 days and the titer per gram of stool of the virus excreted.

Results

Eleven VRPV poliovirus sewage isolates, replicating vaccine virus that had VP1 sequences that diverged from their respective Sabin strain by more than 0.5%, were analyzed for recombination with other polioviruses or NPEV in genomic regions encoding non-structural genes as described. Three had undergone at least one recombination with poliovirus and four had recombined with NPEV [Table 1]. Each recombination pattern was unique by sequencing (not shown).

Sequence analysis of the nine VDPV indicated that despite a maximum of 15% inter-isolate divergence with at least four separate phylogenetic lineages, they were all related to the same epidemiologic event [Shulman LM, et al., submitted]. Evolution from the type 2 OPV component started sometime between 1988 and 1992 and all nine VDPV had genetically reverted to neurovirulence [8] [Shulman LM, et al., submitted]. Four representative VDPV were tested in an animal model system and shown to be phenotypically neurovirulent [8]. There were 12, 13, 15 and 10 amino acid substitutions in and adjacent to neutralizing antibody sites out of a total combined length of 57 amino acids for all antigenic sites for the representative 1998, 1999, 2004 and 2005 isolates [Table 2]. All isolates shared a single recombination [8] [Shulman LM, et al., submitted], further indicating a common ancestry.

The geometric mean titer of neutralizing antibody to VDPV isolates from 1998, 1999, 2004 and 2005 were compared with those against type 2 vaccinal strains (attenuated type 2 Sabin and live wild MEF, the type Salk strain) in cohorts of different ages [Table 2] to measure differences in antigenicity. There was an average 3.3-fold decrease in GMT, indicating that these VDPV were in fact antigenically diverged. Furthermore, while all of the

eIPV = enhanced inactivated poliovirus

GMT = geometric mean titer

RT-PCR = reverse transcriptase polymerase chain reaction

Table 1. Recombination in OPV during early stages in evolution

Poliovirus Isolate	%VP1		
	homology	2C	3D
Type 2			
PV2_5799-1T_ISR02	99.4	–	–
PV2_5807-1_ISR02	99.5	–	–
PV2_5807-4T_ISR02	99.3	–	S1-like
PV2_5853-2T_ISR02	99.4	–	–
PV2_5909-1T_ISR03	99.4	–	NPEV
PV2_5909-5T_ISR03	99.3	NPEV	NPEV
PV2_6042-30T_ISR04	99.5	NPEV	NPEV
PV2_6077-1T_ISR04	99.6	–	S3-like
Type 3			
PV3_5859-2T_ISR02	99.4	NPEV	S2-like
PV3_5957-1T_ISR03	99.5	–	–
PV3_6158-12T_ISR04	99.4	–	–

% homology is the % homology of VP1 sequence from isolates compared with the VP1 of their respective OPV strain. 2C and 3D are poliovirus genes located at nt positions 4224 to 4412 and 6425 to 6649, respectively, for type 2 and 4284 to 4462 and 6423 to 6650, respectively, for Sabin 3 [14].

S1-like, S-2-like or S3-like indicate that an RT-PCR fragment was amplified with non-homologous Sabin 1, 2, or 3 specific primer pairs, respectively.

NPEV indicates that no fragment was obtained when testing with RT-PCR with any of the three Sabin specific primer pairs, while a '-' indicates amplification with the homologous primer pair, i.e., that no recombination occurred.

Table 2. Geometric mean titers to VDPV in Israeli cohorts of different ages

Cohort age in years ^a	1.25 (n=28)	6 (n=22)	14 (n=20)	20 to 34 (n=75)	35 to 50 (n=75)	20 to 24 (n=15)
Sabin 2	9992	3829	2962	419	457	561
MEF ^b	8949	4363	2788	334*	357**	268**
VDPV-1998	2758*	1082*	913*	114*	164*	ND
VDPV-1999	2743*	1399*	1209*	110*	127*	ND
VDPV-2004	4074*	1643*	840*	137*	180*	ND
VDPV-2005	ND	ND	ND	ND	ND	61*

^aThe full vaccination history for all individuals aged between 1.5 and 14 years was documented and all received the full vaccination schedule of three OPV and three eIPV by 1.5 years and an OPV booster at age 6 prior to obtaining their serum. Serum from individuals ≥ 20 years old was obtained from convenient samples obtained from the Israel Center for Disease Control.

^bWild poliovirus type 2 control

ND indicates that the test was not done.

* Significantly different from Sabin 2 and MEF; $P < 0.05$ by Wilcoxon signed rank test for paired data.

** Significantly different from Sabin 2; $P < 0.05$ by Wilcoxon signed rank test for paired data.

individuals tested for this study had protective titers $> 1:8$ for OPV and MEF, 10 of 150 (7%) of people aged between 20 and 50 had titers below the minimum protective level of 1:8 for one or more VDPV strains.

Following OPV challenge, type 2 poliovirus replicated in highly immune infants with documented pre-challenge GMT values of 1849 and 4506, respectively, for cohorts primed with a partial or

full combined immunization schedule. Twenty-three percent and 17% excreted type 2 poliovirus on or after the first week, respectively, with 3/87 and 2/83 excreting for as long as 3 weeks. Titers per gram stool for the three who received partial immunization ranged between 3×10^3 TCID₅₀ to 3.9×10^7 TCID₅₀, and were at 1.8×10^4 in those excreting at week 3.

Discussion

Six of nine VRPV, minimally evolved OPV, had already recombined with different poliovirus virus types or NPEV and each recombination was unique. This high frequency of unique recombinations characterizes very early steps in evolution of circulating VDPV. These minimally diverged VRPV vaccine viruses were presumably excreted by a healthy individual or after a chain of transmission through a limited number of immunocompetent individuals. Supporting this observation is the high frequency of recombination in type 1 VDPV isolated during the outbreak of poliomyelitis in Haiti and the Dominican Republic in 2000-2001 [16,17].

The nine VDPV were epidemiologically linked [8] [Shulman LM, et al., submitted] but evolved along at least four separate lineages. We have been unable to locate any significant pockets of under-immunized individuals in the Israeli population that could provide the high number of naïve individuals necessary for maintaining continual chains of transmission of multiple lineages over at least the 7 year interval during which the VDPV were isolated. Furthermore, the laboratory-measured high immune profiles [18] of documented and undocumented foreign workers eliminated this group as a potential source of naive individuals.

This pattern of multiple lineages in the highly divergent (8–14%) environmental VDPV isolates, as well as many amino acid substitutions in antigenic sites, and minimal recombination (in contrast to the VRPV and type 1 VDPV described above) is more characteristic of immune VDPV excreted by chronically immunodeficient individuals [16]. This would imply that it is possible that one individual may have continuously excreted them into the environment between 1998 and 2005. Arguing against such a single chronic excreter is the statistical enormity of repeatedly finding excreted virus from 1 of the 800,000 people served by the sewage-sampling site.

We confirmed that the predicted amino acid substitutions in neutralizing antigenic sites resulted in decreased antigenic recognition of the VDPV. The absence of individual protective titers (< 1:8) against the VDPV in 7% of individuals aged 20–50 suggests the need for further evaluation of the immune status of the adult and older segments in the general population in relation to non-vaccine strains as well as VDPV since the minimum recommended herd immunity to polio varies between 80% and 97% in developed and developing countries respectively [19,20].

The average daily pediatric stool mass is ≤ 150 g [University of Michigan, Department of Pediatric Surgery, http://pediatric.um-surgery.org/new_070198/new/Library/Nutrition/]. The high percent of immunized infants excreting high titers of type 2 poliovirus in or after the first week and the even higher percent, 59.8–56.0%, excreting at least one poliovirus type in or after the first week [Swartz T, et al., submitted] are risk factors for virus spread in the

community, in spite of its high immune profile. Older individuals with significantly less humoral protection [Table 2] and whose intestinal mucosal immunologic barriers have not been assessed may be even better able to continue the chain of transmission of antigenically altered VDPV.

Our findings on the risk of VDPV circulation should also be considered in the context of the Global Eradication Initiative in countries with decreased vaccine coverage. Since 1980 there have been a number of outbreaks of poliomyelitis, which were caused by VDPV, in communities with sub-optimal vaccine coverage [Reviewed in 16]. In addition, approximately 25 B cell-deficient individuals have been identified who chronically excrete VDPV [reviewed in 21]. Additional reports describe VDPV circulation and isolation from environmental samples in regions with low and high vaccine coverage [reviewed in 22]. As the World Health Organization Global Poliovirus Eradication Initiative approached its goal, many financially strapped polio-free countries decreased vaccine coverage, while in others religious and political conflicts have interfered with immunization programs. This has allowed the rapid re-introduction in 2004-05 of wild poliovirus into more than ten poliomyelitis-free countries [Iris Tetford, Vaccine Assessment & Monitoring, Family & Community Health, World Health Organization, Polio weekly global update, 7 September 2005]. Finding highly diverged VDPV over-extended periods in communities with very high documented immunity raises serious concern about the contribution of OPV to the circulating pool of neurovirulent polioviruses [23]. The rapid spread of wild poliovirus in 2004-2005 can serve as the model for a potential explosive re-emergence of poliomyelitis caused by phenotypically wild VDPV with decreased immunogenicity.

Conclusion

It is clear that VDPV have the potential to circulate or be excreted for extended periods, even in very intensively vaccinated populations with high documented immunity. It is also clear that viral transmission is not completely abrogated in OPV-immunized individuals with documented protective humoral titers against disease. This, together with the fact that it may be very difficult to cure chronically infected individuals [24], means that we must carefully reassess plans for cessation of poliovirus vaccination after poliomyelitis caused by wild poliovirus disappears. In fact, vaccination might have to be continued until all individuals infected with VDPV cease excreting virus. Finally, supplementary environmental sampling is a powerful tool for surveillance of poliovirus circulation and re-emergence of virulent poliovirus from attenuated vaccines in the absence of paralytic poliomyelitis, especially in populations with high vaccine coverage.

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