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BRIEF REPORT

Quantity of Vaccine Poliovirus Shed Determines the Titer of the Serum Neutralizing Antibody Response in Indian Children Who Received Oral Vaccine

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Replication of oral poliovirus vaccine (OPV) in the intestine (ie, vaccine take) is associated with seroconversion and protection against poliomyelitis. We used quantitative polymerase chain reaction analysis to measure vaccine shedding in 300 seronegative infants aged 6–11 months and in 218 children aged 1–4 years 7 days after administration of monovalent or bivalent OPV. We found that the quantity of shedding correlated with the magnitude of the serum neutralizing antibody response measured 21 or 28 days after vaccination. This suggests that the immune response to OPV is on a continuum, rather than an all-or-nothing phenomenon, that depends on efficient vaccine virus replication.

Key words. Poliovirus; seroconversion; shedding.

Oral poliovirus vaccine (OPV) contains live-attenuated (Sabin) polioviruses that can replicate at mucosal sites in the gastrointestinal tract and induce mucosal and systemic antibody. Virus replication can be detected shortly after vaccination and persists for a median time of about 2–3 weeks in stool [1]. The probability of replication (ie, vaccine 'take') following vaccine administration depends on a number of factors, including the potency of the vaccine, maternal antibodies, preexisting immunity, and infection with other enteric viruses [2, 3]. Vaccine take and seroconversion is substantially lower when administered to infants in low-income countries, compared with those in high-income countries [4].

Intestinal antibodies to poliovirus can be detected in stool beginning in the second week after vaccination and coincide with a decline in the amount of poliovirus shed [5]. The development of neutralizing antibodies in serum is usually measured 4 weeks after vaccination and is associated with detection of vaccine poliovirus shedding, such that the majority of children who seroconvert have poliovirus in their stool after vaccination [6]. Thus, poor immunogenicity and efficacy of OPV in low-income countries is typically characterized as a problem of vaccine take [6]. In this view, OPV is an all-or-nothing vaccine that either 'takes' and induces protective serum neutralizing antibodies or does not 'take'. Detection of these antibodies at a dilution of 1 in 8 or more is a mechanistic correlate of protection against poliomyelitis [7]. Virus specific $CD8^+$ T cells can also be detected after vaccination with OPV, but the contribution of cellular immunity to protection against poliomyelitis is unknown [8].

We recently conducted 2 clinical trials of oral and inactivated poliovirus vaccines in Indian infants aged 6–11 months and in children 1–4 years old [9, 10]. We used quantitative real-time polymerase chain reaction (PCR) analysis to accurately quantify poliovirus shedding in stool after vaccination with OPV and measured serum neutralizing antibody responses at a range of dilutions. Here we present an analysis of these data to determine the association between the quantity of vaccine poliovirus shed and the magnitude of the immune response.

METHODS

Study Design and Sample Collection

A total of 300 infants aged 6–11 months and 218 children aged 1–4 years were included in the study. The 300 infants were part of a randomized, placebo-controlled trial (CTRI/2014/05/004588) evaluating the effect of prophylactic azithromycin treatment on the immunogenicity of serotype 3 monovalent OPV (mOPV3) in Indian infants who lacked antibodies against this serotype [9]. The children received mOPV3 containing at least $10^{5.8}$ median cell culture infectious doses of serotype-3 poliovirus (GlaxoSmithKline Biologicals, Belgium). Serum samples were collected before vaccination and 21 days after vaccination, and stool samples were obtained 7 days after vaccination. All infants completing the study (the intention-to-treat group) were included in this study.

The 218 children aged 1–4 years (12–59 months) were part of an open-label, randomized, controlled trial (CTRI/2012/09/003005) examining the effect of 1 dose of inactivated poliovirus vaccine or no vaccine on poliovirus shedding after a subsequent dose of serotype 1 and 3 bivalent OPV

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(bOPV) in Indian children who had received OPV at least 6 months previously [10]. Here we include children from the no-vaccine arm who received bOPV 28 days after enrollment, and who provided a blood sample at the time of vaccination, a stool sample 7 days after vaccination, and a second blood sample 28 days after vaccination.

Both the studies were conducted in Vellore, India, and approved by the Institutional Review Board of Christian Medical College, Vellore, and the Drugs Controller General of India. Informed consent was obtained from the parents/legal guardians of all study subjects.

Neutralization Test for Anti–Poliovirus Antibodies

For infants aged 6–11 months, prevaccination serum samples were tested at 1:4 and 1:8 dilutions by a modified microneutralization assay according to World Health Organization guidelines, and only children seronegative to serotype 3 poliovirus (antibody titer, <1:8) were enrolled in the study [11, 12]. Postvaccination samples were tested in 2-fold serial dilutions from 1:4 to 1:512 to determine the poliovirus serotype 3 neutralizing antibody response. For children aged 1–4 years, prevaccination and postvaccination serum samples were tested for anti–poliovirus serotype 1 and 3 neutralizing antibodies in 2-fold serial dilutions from 1:8 to 1:1024. Seroconversion was defined as either (1) seronegative (antibody titer, <8) to seropositive (antibody titer, ≥8), or (2) a 4-fold rise in antibody titer for children who were seropositive before vaccination.

Quantitative Real-Time PCR for Detection of Poliovirus Serotypes 1 and 3

Quantitative real-time PCR assays were performed to determine Sabin poliovirus 1 and 3 shedding in stool samples as previously described [9, 10]. Standard curves using poliovirus plasmids ranging from 3×10^7 to 3 copies/μL were used to determine the detection limit, which was 3 copies per reaction with a cycle threshold (Ct) cutoff of <40 for both assays (Sabin poliovirus serotypes 1 and 3).

Statistical Analysis

Correlation between poliovirus shedding and serum neutralizing antibody titers was assessed as continuous (log scale) and categorical (yes or no) variables, using the Pearson correlation coefficient and the Fisher exact test, respectively. Differences in the mean quantity of poliovirus shed, by seroconversion or shedding status, were assessed using the nonparametric Wilcoxon rank sum test. The geometric mean titers of antibodies among the children at the time of vaccination were calculated by assigning a value of 1:6 and 1:1448 for the censored values below and above the limits of the dilution series, respectively. A P value of < .05 was considered statistically significant. All tests were 2-tailed.

RESULTS

Of the 300 poliovirus serotype 3–seronegative infants aged 6–11 months who were given a dose of mOPV3, 160 (53.3%) had serotype 3 Sabin poliovirus detected in stool specimens on day 7 after vaccination. Among these infants, 85% seroconverted to serotype 3 poliovirus by 21 days after vaccination (Table 1). Among those who did not have poliovirus serotype 3 detected in stool specimens, only 10% seroconverted ($P < .001$, by the Fisher exact test). The quantity of Sabin serotype 3 poliovirus shed was significantly higher in the group that seroconverted as compared to the group that did not seroconvert ($P < .001$, by the Wilcoxon rank sum test). In addition, the quantity of shedding was correlated with the titer of serum neutralizing antibodies achieved 21 days after vaccination (Pearson correlation coefficient, 0.508; $P < .001$; Figure 1A).

Of the 218 children aged 1–4 years who were given a dose of bOPV, 42 (19.3%) and 56 (25.7%) shed serotype 1 or 3 Sabin poliovirus, respectively, 7 days after vaccination. Of the

Table 1. Correlation Between Sabin Poliovirus Shedding on Day 7 After Vaccination and Subsequent Seroconversion

	Seroconversion Among Shedders		Seroconversion Among Nonshedders		
Age, Serotype, Variable	Yes	No	Yes	No	*P*
6–11 mo					
Poliovirus 3					
Subjects, no.	136	24	14	126	<.001
Virus load per 0.2 g of stool, $\log_{10}$ copies, mean ± SE	4.70 ± 0.11	2.27 ± 0.33	NA	NA	<.001
12–59 mo					
Poliovirus 1					
Subjects, no.	29	13	15	161	<.001
Virus load per 0.2 g of stool, $\log_{10}$ copies, mean ± SE	3.41 ± 0.28	2.11 ± 0.39	NA	NA	.013
Poliovirus 3					
Subjects, no.	46	10	21	141	<.001
Virus load per 0.2 g of stool, $\log_{10}$ copies, mean ± SE	3.01 ± 0.19	1.66 ± 0.45	NA	NA	.011

Abbreviation: NA, not applicable.

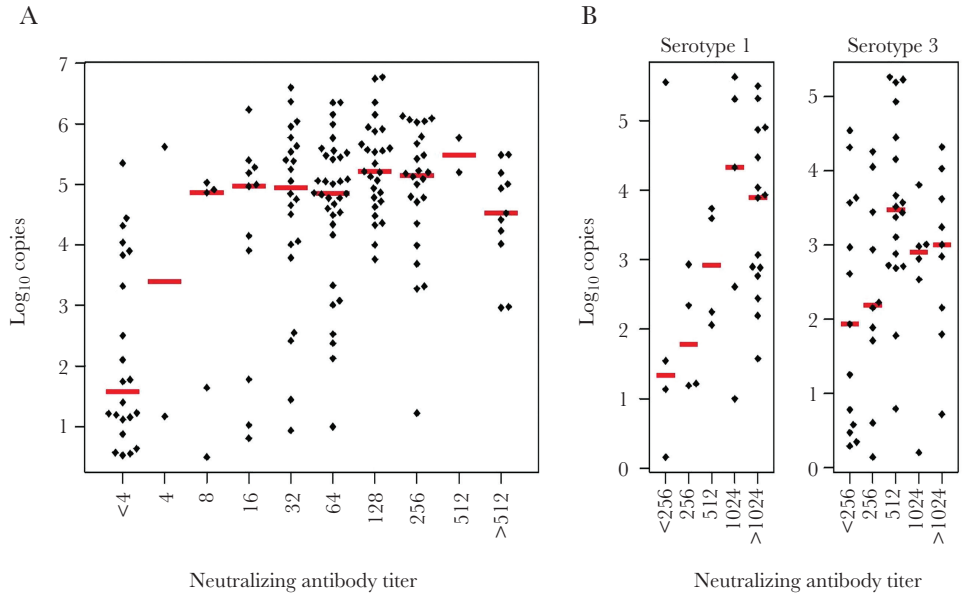

Figure 1. Quantity of poliovirus shed, compared with serum neutralizing antibody titer achieved after vaccination with serotype 3 monovalent oral poliovirus vaccine (OPV) in infants (*A*) and serotypes 1 and 3 bivalent OPV in children aged 1–4 years (*B*). Poliovirus shedding was measured using quantitative polymerase chain reaction analysis. Antibody titers are shown as the reciprocal of the dilution at which neutralization was detected in half of the wells, calculated using the Spearman-Karber method.

children who shed serotype 1 Sabin poliovirus, 29 (69.0%) had seroconverted 28 days after vaccination, whilst only 15 (8.5%) of non-shedders seroconverted ($P < .001$ by the Fisher exact test; Table 1). Similarly, 46 (82.1%) children who shed Sabin serotype 3 poliovirus seroconverted, and just 21 of nonshedders (13%; $P < .001$ by the Fisher exact test). Among children shedding vaccine polioviruses, the quantities of poliovirus serotypes 1 and 3 shed by children who seroconverted were significantly higher than by children who did not seroconvert ($P = .013$ and .011, respectively, by the Wilcoxon rank sum test; Table 1). In addition, the quantity of poliovirus shedding correlated with the titer of serum neutralizing antibodies achieved 28 days after vaccination, although this was not significant (Pearson correlation coefficients, 0.298 and 0.212 for serotypes 1 and 3, respectively; $P = .0552$ and .118, respectively; Figure 1B).

Infants enrolled in the study lacked serum neutralizing antibodies to serotype 3 poliovirus as per the study protocol, which involved screening for these antibodies before enrollment. Among children aged 1–4 years, 215 (98.6%) and 205 (94.0%) had detectable serum neutralizing antibodies against serotypes 1 and 3, respectively, at the time of vaccination, with geometric mean titers (±standard errors) of 221 ± 24.6 and 111 ± 15.8, respectively. Children who shed vaccine poliovirus had significantly lower baseline antibody titers than those who did not shed (142 vs 236 and 72.2 vs 115 for serotypes 1 and 3, respectively; $P = .017$ and .033, respectively, by the Wilcoxon rank sum test).

DISCUSSION

Whether a child sheds vaccine poliovirus in stool after immunization with OPV (ie, whether the vaccine 'take') is known to predict seroconversion [6]. In addition, the appearance of poliovirus-specific immunoglobulin A in stool and blood specimens from about 2 weeks after administration of OPV typically correlates with a decline in the amount of poliovirus shed [5, 13]. Previous studies have speculated that the amount of virus replication could determine the magnitude of the antibody response, but they have been limited to observations from very few individuals or to comparisons between children immunized with inactivated or oral poliovirus vaccines [5, 14]. Using quantitative PCR analysis, we were able to address this question in >500 individuals and found that the quantity of poliovirus shed 7 days after vaccination was positively correlated with the magnitude of the subsequent serum neutralizing antibody response. A stool virus titer of about 4 log copies was associated with a higher antibody response in infants aged 6–11 months (Figure 1A), whereas for children aged 12–59 months, a substantial antibody response was observed even with a stool virus titer of about 3 log copies (Figure 1B). This suggests that the amount of poliovirus replication in the intestine in the first weeks following immunization determines the amount of neutralizing antibody produced during the initial response.

We also found an inverse relationship between the preexisting neutralizing antibody titer in children and the probability of shedding homotypic virus in stool after vaccination. This

has previously been shown in several studies of OPV recipients mainly from high-income countries [3]. This same relationship is not observed among individuals who received inactivated poliovirus vaccine, which induces limited mucosal protection despite high titers of serum neutralizing antibodies [15].

Our study had some limitations. We only quantified poliovirus in stool specimens on day 7 after vaccination, and we therefore may have missed early shedding and were unable to estimate the duration of shedding or the dynamics of shedding over time. Also, we did not directly measure fecal antibody levels or the mucosal tissue immune response. This could perhaps be the focus of a smaller study, in which pediatric endoscopy would allow collection of intestinal tissue.

In conclusion, we found that both seronegative infants and seropositive children aged 1–4 years who received OPV made poliovirus-specific antibody in proportion to the amount of vaccine poliovirus detected in stool specimens. This indicates that the response to OPV is on a continuum rather than an all-or-nothing (ie, vaccine-take-based) phenomenon. Overcoming the poor immunogenicity of OPV in low-to-middle-income countries may therefore require strategies to promote vaccine poliovirus replication. These could include the development of new, genetically stable and replication-efficient vaccine strains or complementary therapies such as probiotics.

Notes

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References

1. Alexander J Jr, Gary H Jr, Pallansch MA. Duration of poliovirus excretion and its implications for acute flaccid paralysis surveillance: A review of the literature. J Infect Dis **1997**; 175(suppl 1):S176–S182.
2. Parker EP, Kampmann B, Kang G, Grassly NC. Influence of enteric infections on response to oral poliovirus vaccine: a systematic review and meta-analysis. J Infect Dis **2014**; 210:853–64.
3. Hird TR, Grassly NC. Systematic review of mucosal immunity induced by oral and inactivated poliovirus vaccines against virus shedding following oral poliovirus challenge. PLoS Pathog **2012**; 8:e1002599.
4. World health organization collaborative study group on oral poliovirus vaccine. Factors affecting the immunogenicity of oral poliovirus vaccine: a prospective evaluation in Brazil and the Gambia. J Infect Dis **1995**; 171:1097–106.
5. Morimoto N. The relationship between poliovirus multiplication, the sIgA antibody response and the serum neutralizing antibody titers after trivalent oral polio vaccination. Kansenshogaku Zasshi **2001**; 75:1030–9.
6. John TJ, Christopher S. Oral polio vaccination of children in the tropics. III. Intercurrent enterovirus infections, vaccine virus take and antibody response. Am J Epidemiol **1975**; 102:422–8.
7. Hammon WM, Coriell LL, Wehrle PF, Stokes J Jr. Evaluation of Red Cross gamma globulin as a prophylactic agent for poliomyelitis. IV. Final report of results based on clinical diagnoses. J Am Med Assoc **1953**; 151:1272–85.
8. Wahid R, Cannon MJ, Chow M. Virus-specific CD4+ and CD8+ cytotoxic T-cell responses and long-term T-cell memory in individuals vaccinated against polio. J Virol **2005**; 79:5988–95.
9. Grassly NC, Praharaj I, Babji S, et al. The effect of azithromycin on the immunogenicity of oral poliovirus vaccine: a double-blind randomised placebo-controlled trial in seronegative Indian infants. Lancet Infect Dis **2016**; 16:905–14.
10. John J, Giri S, Karthikeyan AS, et al. Effect of a single inactivated poliovirus vaccine dose on intestinal immunity against poliovirus in children previously given oral vaccine: an open-label, randomised controlled trial. Lancet **2014**; 384:1505–12.
11. World Health Organization (WHO). Manual of laboratory methods for testing of vaccines used in the WHO Expanded Programme on Immunization. Document WHO/VSQ/97.04. Geneva: WHO, **1997**.
12. Kaliappan SP, Venugopal S, Giri S, et al. Factors determining anti-poliovirus type 3 antibodies among orally immunised Indian infants. Vaccine **2016**; 34:4979–84.
13. Ogra PL, Karzon DT. Formation and function of poliovirus antibody in different tissues. Progress in Medical Virology **1971**; 13:156–93.
14. Ghendon YZ, Sanakoyeva II. Comparison of the resistance of the intestinal tract to poliomyelitis virus (Sabin's strains) in persons after naturally and experimentally acquired immunity. Acta Virol **1961**; 5:265–73.
15. Cuba IPV Study Collaborative Group. Randomized, placebo-controlled trial of inactivated poliovirus vaccine in Cuba. N Engl J Med **2007**; 356:1536–44.