The quantity of vaccine poliovirus shedding determines the titer of the serum neutralizing antibody response in Indian children vaccinated with oral vaccine

Sidhartha Giri[1], Nirmal Kumar[1], Pavithra Dhanapal[1], Jayalakshmi Venkatesan[1], Anand Kasirajan[2], Miren Iturriza-Gomara[3], Jacob John[4], Asha Mary Abraham[2], Nicholas C Grassly[5], Gagandeep Kang[1]

Running title: Poliovirus replication and seroresponse

Affiliations

[1]*Division of Gastrointestinal Sciences, Christian Medical College, Vellore 632004, India.*

[2]*Department of Clinical Virology, Christian Medical College, Vellore 632004, India.*

[3]*Institute of Infection and Global Health, University of Liverpool, Liverpool L69 7BE, UK.*

[4]*Department of Community Health, Christian Medical College, Vellore 632004, India.*

[5]*Department of Infectious Disease Epidemiology, Imperial College London, London W2 1PG, UK.*

Footnote page

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Corresponding author: Sidhartha Giri (sidharthagiri@cmcvellore.ac.in). Phone: +914162282052

Brief summary: This study involving more than 500 children <5 years of age from India showed that the quantity of vaccine virus shedding after administration of oral poliovirus vaccine is positively correlated with the magnitude of serum neutralizing antibodies developed subsequently.

Abstract

Replication of oral poliovirus vaccine (OPV) in the intestine (vaccine 'take') is associated with seroconversion and protection against poliomyelitis. We used quantitative PCR 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 the quantity of shedding correlated with the magnitude of the serum neutralizing antibody response measured at 21 or 28 days respectively. This suggests 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

Background

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 (vaccine 'take') following vaccine administration depends on a number of factors, including the potency of the vaccine, maternal antibodies, pre-existing immunity and infection with other enteric viruses [2;3]. Vaccine take and seroconversion is substantially lower when administered to infants in low compared with 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 not. 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 two 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

PCR 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 shedding 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 were vaccinated with mOPV3 containing atleast $10^{5.8}$ $CCID_{50}$ (median cell culture infectious dose) of serotype-3 poliovirus (GlaxoSmithKline Biologicals, Belgium). Serum samples were collected pre-vaccination and 21 days post-vaccination, and stool samples 7 days post-vaccination. All infants completing the study (intention-to-treat) 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 one dose of IPV or no vaccine on poliovirus shedding after a subsequent dose of serotype 1 and 3 bivalent OPV (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 enrolment, and who provided a blood sample at the time of vaccination, a stool sample 7 days later and a second blood sample after 28 days.

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

Quantitative real-time PCR for PV1 and PV3

Quantitative real-time polymerase chain reaction (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 $3x10^7$ to 3 copies/µl were used to determine the detection limit, which was 3 copies per reaction with a cycle threshold (Ct) cutoff value of <40 for both assays (Sabin 1 and 3).

Statistical Analysis

Correlation between poliovirus shedding and serum neutralizing antibody titres was assessed as continuous (log-scale) and categorical (yes/no) variables using Pearson's correlation coefficient and Fisher's exact test respectively. Differences in the mean quantity of poliovirus shed by seroconversion or shedding status were assessed using the non-parametric Wilcoxon rank sum

test. The geometric mean titres (GMTs) 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 <0.05 was considered statistically significant. All tests were two-tailed.

Results

Of the 300 PV3 seronegative infants aged 6-11 months who were given a dose of mOPV3, 160 (53.3%) had serotype 3 Sabin poliovirus (PV3) detected in stool on day 7 after vaccination. Among these infants, 85% seroconverted to serotype 3 poliovirus at 21 days post-vaccination (Table 1). Among those who did not have PV3 detected in stool, only 10% seroconverted (Fisher's exact test P<0.001). The quantity of Sabin PV3 shed was significantly higher in the group that seroconverted compared to those who did not seroconvert (Wilcoxon Rank Sum (WRS) test, P-value<0.001). In addition, the quantity of shedding was correlated with the titer of serum neutralizing antibodies achieved 21 days post-vaccination (Pearson's correlation coefficient 0.508, P-value <0.001; 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 7 days after vaccination respectively. Of the children who shed serotype 1 Sabin poliovirus (PV1), 29 (69.0%) had seroconverted 28 days after vaccination, whilst only 15 (8.5%) of non-shedders seroconverted (Fisher's exact test P-value <0.001; Table 1). Similarly, 46 (82.1%) children who shed Sabin PV3 seroconverted, and just 21 (13%) of non-shedders (Fisher's exact test P-value <0.001). Among children shedding vaccine polioviruses, the quantities of both PV1 and PV3 shed in children who seroconverted was significantly higher than in children who did not seroconvert (WRS P-values 0.013 and 0.011 respectively; Table 1). In addition, the quantity of poliovirus shedding correlated with the titer of serum neutralizing antibodies achieved 28 days post-vaccination, although this was not significant (Pearson's correlation coefficients 0.298 and 0.212 for serotypes 1 and 3 respectively, P-values = 0.0552 and 0.118; Figure 1B).

Infants enrolled to the study lacked serum neutralizing antibodies to serotype 3 poliovirus as per the study protocol, which involved screening for these antibodies before enrolment. Among the children aged 1-4 years, 215 (98.6%) and 205 (94.0%) had detectable serum neutralizing antibodies against serotypes 1 and 3 at the time of vaccination, with a geometric mean titer among these children of 221 (standard error 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, WRS P-values 0.017 and 0.033).

Discussion

Whether a child sheds vaccine poliovirus in stool after immunization with OPV (vaccine 'take') is known to predict seroconversion [6]. In addition, the appearance of poliovirus-specific IgA in stool and blood 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 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, we were able to address this question in over 500 individuals and found that the quantity of poliovirus shed 7 days after vaccination is positively correlated with the magnitude of the subsequent serum neutralizing antibody response. A stool virus titer of about 4 log copy number was associated with a higher antibody response in the 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 titre of about 3 log copy number (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 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 immunized individuals mainly from high-income countries [3]. This same relationship is not observed among individuals immunized with 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 on day 7 after vaccination and we therefore may have missed early shedding and were unable to estimate the duration or dynamics of shedding over time. Also, we did not directly measure fecal antibody or mucosal tissue immune response. This could perhaps be the focus of a smaller study where pediatric endoscopy would allow collection of intestinal tissue.

In conclusion, we found that both seronegative infants and seropositive children aged 1-4 years immunized with OPV made poliovirus-specific antibody in proportion to the amount of vaccine poliovirus detected in stool. This indicates the response to OPV is on a continuum rather than an all-or-nothing ('take' based) phenomenon. Overcoming the poor immunogenicity of OPV in LMICs 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.

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Reference List

(1) Alexander JrJ, Gary JrH, 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 EPK, 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(6):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(4):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(5):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(12):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(5):422-8.

(7) Hammon WD, Coriell LL, Wehrle PF, Stokes J. Evaluation of Red Cross gammaglobulin as a prophylactic agent for poliomyelitis. 4. Final report of results based on clinical diagnosis. J Am Med Assoc 1953;151(15):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(10):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(8):905-14.

(10) John J, GiriS, 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(9953):p1505-12.

(11) World Health Organization. Manual of laboratory methods for testing of vaccines used in the WHO Expanded Programme on Immunization. WHO/VSQ/97.04. 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(41):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(15):1536-44.

Table 1: Correlation between Sabin poliovirus shedding (on day 7 after vaccination), and subsequent seroconversion

Age, Serotype		Shedding day 7		Not shedding day 7		P-values
		Seroconversion		Seroconversion		
		Yes	No	Yes	No	
6-11 months, PV3	Number	136	24	14	126	<0.001
	log_{10} virus copy number per 0.2 g stool, mean (SE)	4.70 (0.11)	2.27 (0.33)	NA	NA	<0.001
12-59 months, PV1	Number	29	13	15	161	<0.001
	log_{10} virus copy number per 0.2 g stool, mean (SE)	3.41 (0.28)	2.11 (0.39)	NA	NA	0.013
12-59 months, PV3	Number	46	10	21	141	<0.001
	log_{10} virus copy number per 0.2 g stool, mean (SE)	3.01 (0.19)	1.66 (0.45)	NA	NA	0.011

Figure legends

Figure 1: Quantity of poliovirus shed compared with serum neutralizing antibody titer achieved after vaccination with A) serotype 3 monovalent OPV in infants and B) serotypes 1 and 3 bivalent OPV in children aged 1-4 years. Poliovirus shedding was measured using quantitative PCR and is shown on a log base 10 scale. Antibody titers are shown as the reciprocal of the dilution at which neutralization was detected in half the wells calculated using the Spearman-Karber method.