

Host and Viral Factors Affecting the Decreased Immunogenicity of Sabin Type 3 Vaccine after Administration of Trivalent Oral Polio Vaccine to Rural Mayan Children

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Factors affecting immunogenicity of the first 2 doses of oral poliovirus vaccine (OPV) among unimmunized Mayan infants were prospectively evaluated. The relative impact of multiple variables, including mass or routine vaccination, concurrent enteric bacterial (*salmonella*, *shigella*, and *campylobacter*) and viral (adenovirus 40/41, astrovirus, nonpolio enteroviruses, and rotavirus) infections, interference among Sabin vaccine viruses, and preexisting poliovirus antibodies were studied. Sera were available from 181 infants after 2 OPV doses. Seroresponses were 86% to Sabin type 1, 97% to Sabin type 2, and 61% to Sabin type 3 vaccines. Mass versus routine vaccination and preexisting poliovirus antibodies did not affect immunogenicity. By multiple logistic regression analysis, fecal shedding of homologous Sabin strains was associated with increased seroresponses to all Sabin types, especially to Sabin type 3. Decreased OPV immunogenicity was primarily attributable to interference of Sabin type 3 by Sabin type 2. OPV formulations with higher doses of Sabin type 3 could improve immunogenicity among infants in developing countries.

The World Health Organization (WHO) proposes global eradication of paralytic poliomyelitis by the year 2000 [1]. Paralytic poliomyelitis has been eliminated from industrialized countries, except in unusual circumstances, and was reported to be eradicated from the Western Hemisphere in 1994 [2]. Substantial decreases in poliomyelitis have been achieved elsewhere through intensive vaccine campaigns, primarily by administration of trivalent oral poliovirus vaccine (OPV). However, WHO surveillance studies continue to document wild poliovirus circulation in parts of Europe, Asia, the Middle East, and Africa [3, 4]. Persistent circulation of wild polioviruses, even in developed countries with highly immunized popula-

tions, is evident from the periodic outbreaks of paralytic poliomyelitis involving members of communities who refuse vaccination [5]. Therefore, achieving global poliomyelitis eradication will require inducing and maintaining protective immunity in all individuals.

The immunogenicity of OPV given during the first year of life remains poor among children in developing countries [6–9]. Although technical obstacles, such as poor vaccine delivery systems and vaccine instability in tropical climates, are responsible for continued poliomyelitis in some areas, these problems have been addressed effectively in many parts of the world [7, 10–14]. The primary objective of this study was to identify factors that affect the immunogenicity of OPV in children in a developing area. We studied the effect of mass versus routine vaccination on seroconversion rates to OPV in a Mayan community in Chiapas, Mexico, where infants receive OPV in mass or routine vaccination programs. We also studied interference among 3 Sabin vaccine viruses, concurrent enteric infections, and the effects of transplacentally acquired poliovirus antibodies on the immunogenicity of OPV.

Methods

Study population. The study was conducted in the Mayan village of Navenchac in the highlands of Chiapas, Mexico, 10 km east of San Cristobal de las Casas. Navenchac has 629 households with a population of 4000 and participates in all federal and provincial health care programs, including the poliovirus mass vaccine campaigns that target all children <5 years old (all children <5 years participate in the annual mass vaccination programs). The

Received 1 August 1996; revised 25 September 1996.

Presented in part: 34th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Orlando, Florida, October 1994; 36th ICAAC, New Orleans, September 1996.

Informed consent for study participation was obtained from parents during home visits.

The study was approved by the Instituto Nacional de Diagnostico y Referencia Epidemiologicos, Mexico City, and the Committee for the Protection of Human Subjects, Stanford University.

Grant support: NIH (AI-31475). M.F.C. was supported by a Daland Fellowship grant, American Philosophical Society.

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health center also provides all acute and well-child care. Of the village population, 35% is <10 years old (median, 15 years).

The field study was conducted over 3 years (1992–1995). The field team identified pregnant women and young infants eligible for the study through the village census. All healthy unimmunized infants were eligible to participate in this study. Mass vaccination occurred from January to June and consisted of 2 doses of OPV given 8 weeks apart. Infants enrolled in the mass vaccination program were 6 weeks to 6 months old at vaccination. Routine vaccination occurred from July through December and consisted of 2 doses of OPV given at ages 2 and 4 months. Routine OPV immunization was not offered during the mass vaccination period. In each phase, eligible infants received an OPV dose at study weeks 1 and 9. Stool samples were collected at weeks 0, 1, 2, 4, 6, 8, 9, 10, 12, 14, 16, and 17, and serum samples were collected at weeks 1, 9, and 17. Infants received their third OPV dose after the study period; all other routine childhood immunizations were administered according to Mexican Ministry of Health guidelines, and all infants and children not enrolled in the field study received their usual immunizations. The field team was the only source of OPV in the village. All infants in the village were exclusively breastfed through the first year of life.

Vaccination. Prior to use, OPV vaccine lots were stored at -20°C at the central laboratory, where freezer temperatures were monitored daily. Vaccine vials were transported to the village in WHO-approved cold boxes on the day of vaccination. For vaccine potency testing, one unopened vial of polio vaccine per week that had been carried into the field was stored at -20°C ; during routine vaccination, one unopened vial per month was stored. Vaccine potency was tested by R. Lundquist (US Food and Drug Administration, Rockville, MD).

One lot of standard trivalent, live attenuated OPV was obtained by the Stanford Regional Center through the Mexican Ministry of Health; the vaccine contained 10^6 , 10^5 , and $10^{5.6}$ TCID₅₀ of poliovirus types 1–3, respectively, and was the only OPV administered to study participants. Each dose was administered as 0.1 mL placed in the back of the throat with a dropper supplied by the manufacturer. Vaccinated infants had serum collected before their first and second vaccinations and 8 weeks after the second vaccination. Serum specimens were tested for neutralizing antibody titers against poliovirus types 1, 2, and 3.

All 237 infants enrolled were followed throughout the study period. Complete sera were unavailable from 56 infants because of parent refusal to complete blood collection ($n = 54$) and infant deaths ($n = 2$).

Seroconversion. Seroconversion was defined as either the presence of a detectable neutralizing antibody titer of $\geq 1:8$ in subjects with no detectable titer in prevaccination samples or a rise in antibody titer ≥ 4 -fold between pre- and postvaccination samples over the expected decay in maternal antibody, based on a 4-week half-life. In calculating geometric mean titers (GMTs), undetectable titers were assigned a value of 1:4; those $> 1:1024$ were assigned a value of 1:2048.

Laboratory methods. Stool samples were collected in paper containers at the study subject's home, taken to the field laboratory, and separated into two aliquots. One aliquot was tested immediately for *Salmonella*, *Shigella* and *Campylobacter* organisms by standard methods [15–17]; the second was placed in 1.8-mL vials (Nunc, Roskilde, Denmark) and frozen at -20°C before shipment

on dry ice to the virology research laboratory at Stanford University. Upon receipt, samples were stored at -70°C . Serum specimens were frozen at -20°C until shipment and then at -70°C .

A modified cytopathic end-point microneutralization assay was used to compare acute and convalescent sera for the presence of neutralizing antibodies to poliovirus types 1–3; all sera from each patient were tested concurrently [18, 19]. The microneutralization assay was standardized by proficiency testing against references provided by the WHO International Laboratory for Biological Standards.

Stool samples were tested for the presence of viral pathogens, including polio and nonpolio enteroviruses (NPEV), adenovirus 40/41, astrovirus, and rotavirus. Enteroviruses were identified by a cytopathic effect in tissue culture using four cell lines (PMK, BGMK, RD, MRC-5; Viomed Laboratories, Minnetonka, MN) with high enterovirus recovery rates [20–25]. The presence of enterovirus was confirmed by RNA-RNA hybridization with an enterovirus group probe [26]. Samples positive by enterovirus group probe hybridization were further tested with Sabin vaccine strain probes to identify poliovirus vaccine isolates. Because poliomyelitis epidemics in highly vaccinated populations are usually caused by wild poliovirus type 3, we tested samples positive by group probe hybridization but negative by Sabin strain probe hybridization for the presence of wild poliovirus type 3 using an RNA probe specific for wild type 3 from isolates recovered prior to eradication of poliomyelitis from southern Mexico. Samples and positive and negative controls, including known Sabin vaccine strain controls, NPEV, and herpes simplex virus type 1, were spotted in duplicate for each of four sets: one set for the group probe and the other for the Sabin strain-specific probes. Control virus samples were included in each assay for quality control. Enterovirus group probe-positive, Sabin strain-negative, and wild type 3 probe-negative samples were presumed to be NPEV.

Enterovirus group probes were constructed from nucleotide sequences at the 5'-noncoding region of the enterovirus genome. These sequences are highly conserved among most of the 68 enterovirus serotypes, except for echovirus 22 and 23. Sabin poliovirus vaccine probes were constructed from sequences coding for the VP1 surface protein of the Sabin vaccine strains. The wild poliovirus type 3 probe was constructed from sequences that code for the VP1 surface protein of the wild type 3 isolates most commonly found in southern Mexico and Central America. All probes were obtained from O. Kew (CDC, Atlanta), and published hybridization methods were used [26]. Identification of enteric adenovirus 40 and 41, astrovirus, and rotavirus was done by screening immunoassays [27–31].

Sample size. A minimum of 90 unvaccinated infants was required in each study group (total, 180), based on the following assumptions: (1) no more than 75% of infants receiving OPV under routine circumstances would seroconvert to all 3 poliovirus serotypes; (2) $\geq 90\%$ of infants would seroconvert after vaccination during mass campaigns; and (3) the α error in detecting such differences = 5%, $\beta = 20\%$ (one-sided test).

Statistical analysis. Seroconversion rates to Sabin types 1–3 were calculated and compared for several variables, including method of vaccination (mass vs. routine); presence of viral or bacterial pathogens in stool the week before, at the time of, or the week after vaccine administration; presence of homologous antibody at the time of first vaccination; and fecal shedding of

homologous and heterologous Sabin vaccine viruses after each vaccine dose. GMTs were calculated. Infants with incomplete data were excluded from analysis. χ^2 and Fisher's exact tests (two-tailed) were used in analysis of discrete variables; Student's *t* test was used for comparison of continuous variables. To control for multiple factors that may affect immunogenicity, logistic regression analysis was used to determine the magnitude of effect, odds ratios (ORs), and significance of each factor affecting OPV immunogenicity on seroresponse for all 3 Sabin types.

Variables with significant effects on seroconversion at $P \leq .05$ in univariate analysis were included in multiple linear logistic regression analysis (SAS, Cary, NC). Regression coefficients were considered significant at $P \leq .05$. Attributable risk (AR) = $(a/a + b) - (c/c + d)$, population attributable risk percent (PAR%), and population prevented fraction (PPF), respectively, were used to identify the quantitative effect of each variable on Sabin strain-specific primary vaccine failure or success. When AR was ≥ 1.0 , PAR% was calculated as $\{(a/a + c)/(a/a + b) - (c/c + d)/(a/a + b)\} \times 100$. When AR was < 1.0 , PPF was calculated as $[(c/c + d) - (a/a + b)/(c/c + d)] \times 100$. The hypothetical number of vaccine failures or seroconversions attributable to each factor was then calculated.

Results

Immune response to OPV. In total, 237 infants were enrolled during the 3-year study: 120 in the mass vaccination program and 117 in the routine vaccination program. Seroresponses after 2 doses of OPV were evaluated for 76% (181/237) of the study infants (85, mass vaccination program; 96, routine vaccination program). Eighty-six percent developed neutralizing antibodies to Sabin type 1, 97% to Sabin type 2, and 61% to Sabin type 3. No significant differences were identified in seroresponse to any Sabin strain in infants vaccinated by mass versus routine methods after 1 or 2 doses of OPV (figure 1). The high seroresponse rate to Sabin type 2 after 1 dose of OPV administered by mass vaccination was not significantly improved by a second OPV dose (89% vs. 95%, $P = .12$, χ^2). However, seroresponses to Sabin types 1 and 3 at mass and routine vaccination and for Sabin type 2 at routine vaccination were significantly increased after a second dose of OPV. Because mass and routine vaccination programs were seasonally divided, the comparison of the two programs also tested the seasonal effects on the immunogenicity of OPV.

GMTs were generally higher among infants receiving routine vaccination (figure 1); significantly higher GMTs were obtained during routine vaccination after the second OPV dose of Sabin type 1 and after both the first and second OPV doses of Sabin type 2. A second dose of OPV significantly increased GMTs to all Sabin types for both mass and routine vaccinees. GMTs to Sabin types 1 and 2 after the second OPV dose were significantly higher among infants who had no concurrent enteric bacterial or viral infections compared with infants with concurrent enteric infections (Sabin type 1 after dose 2: GMTs, 155 and 331, respectively, for infants with and without con-

current infection, $P = .02$; Sabin type 2 after dose 2: GMTs, 468 and 776, respectively, for infants with and without concurrent infection, $P = .009$, Student's *t* test), but the prevalence of concurrent enteric infections was not significantly different between mass and routine vaccinees (prevalence of concurrent enteric infection: 56% vs. 63% at dose 1 and 69% vs. 59% at dose 2 among mass vs. routine vaccinees, respectively; $P > .1$, χ^2).

Shedding of Sabin vaccine poliovirus after OPV. Figure 2 shows fecal shedding Sabin vaccine patterns. Peak shedding of Sabin types 1 and 2 occurred 2–4 weeks after the first OPV vaccination and continued up to 8 weeks. Fecal shedding patterns were distinct for each Sabin type. Sabin type 1 was shed by 73% of infants after the first OPV dose and by only 25% after the second OPV dose. Sabin type 2 was shed by 69% of infants after the first OPV dose and by only 6% after the second dose. Thus, compared with the first OPV dose, both the peak and duration of shedding of Sabin types 1 and 2 diminished after the second OPV dose. In contrast, shedding of Sabin type 3 was similar after the first and second OPV doses (34% and 25%, respectively). Fecal shedding of Sabin types 1 and 3 was significantly associated with development of homologous poliovirus neutralizing antibodies after each OPV dose (figure 3). Because the majority of infants had seroresponses to Sabin type 2 after the first OPV dose, fecal shedding of Sabin type 2 was associated with an increased seroresponse to Sabin type 2 only after the first OPV dose. We found only one significant association between fecal shedding of a Sabin strain and diminished seroresponses to a heterologous Sabin type. Fecal shedding of Sabin type 2 was significantly associated with diminished seroresponse to Sabin type 3 ($P = .05$, Fisher's exact test).

Effect of transplacentally acquired neutralizing poliovirus antibodies on OPV immunogenicity. Preexisting antibodies to at least 1 poliovirus serotype, presumed to be transplacentally acquired, were present in only 32% of study participants at baseline. While there was no difference between the mean age at the time of first vaccination of seroresponders and nonresponders to any of the 3 Sabin types, infants with maternal antibodies at the time of first OPV vaccination were significantly younger than those without maternal antibodies (mean age, 106 vs. 138 days, $P < .001$, Student's *t* test). The presence of preexisting maternal antibodies was associated with decreased seroresponses only to Sabin type 1 (54% vs. 73% seroresponse with and without maternal antibodies, respectively, $P = .04$, χ^2). The GMTs to all 3 Sabin types after the first dose of OPV in children with and without preexisting immunity were not significantly different.

Influence of concurrent enteric infection on OPV immunogenicity. In total, 1865 stool specimens from 181 study participants for whom complete serologic data were available were tested for bacterial and viral pathogens. Bacterial pathogens were identified in 71 samples from 52 children. Four specimens were positive for *Salmonella*, 19 for *Shigella*, and 48 for

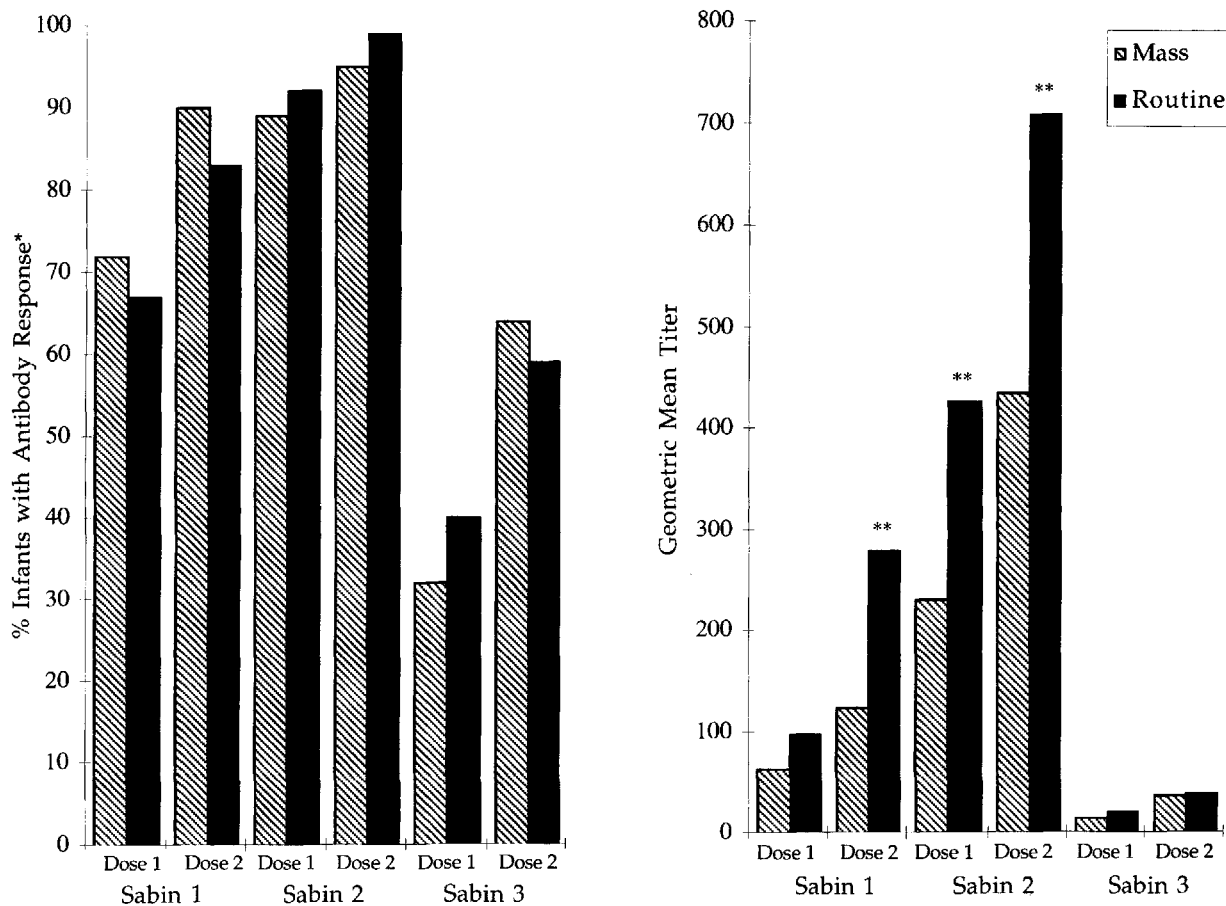


Figure 1. Seroconversion rates and geometric mean poliovirus neutralizing antibody titers (GMTs) after mass or routine vaccination with 1 and 2 OPV doses. * $P > .05$, χ^2 , difference in seroresponse rates among infants receiving mass vs. routine vaccination for all 3 Sabin types. ** $P \leq .02$, Student's t test, difference in GMTs among infants receiving mass vs. routine vaccination.

Campylobacter organisms. Nine samples were positive for rotavirus, 33 for adenovirus 40/41, 104 for astrovirus, and 848 for enteroviruses. Of the enterovirus isolates, 481 were Sabin vaccine strains. None of the remaining 367 enterovirus isolates were wild poliovirus type 3. Seroresponses to poliovirus types 1–3 were compared for the presence of concurrent enteric bacterial and viral infections, defined as isolation of a pathogen from a stool sample obtained the week before, at vaccination, or 1 week after vaccination (figure 4).

In 60% of the infants (108/111), concurrent enteric bacterial and viral infections were identified at the first OPV dose and in 64% (116/181) at the second OPV dose. There were no differences in overall concurrent enteric infections between mass and routine vaccination periods. Concurrent enteric infections at the first OPV dose were not associated with decreased seroresponses to any Sabin type. However, concurrent infections at the second OPV dose interfered with seroresponses to Sabin types 1 and 2. Although the seroresponses to Sabin type 2 were high in all infants, infants with concurrent NPEV infection were significantly less likely to respond to Sabin type 1 after 2 OPV doses (42%, 61/146) than were infants without concurrent

NPEV infection (74%, 17/23) ($P = .004$, χ^2). Infants with concurrent astrovirus and enteric bacterial infections were also significantly less likely to develop seroresponses to Sabin type 2 after 2 OPV doses (astrovirus infection: 80%, 4/5; enteric bacterial infection: 60%, 3/5) compared with infants without these enteric infections (astrovirus: 28%, 47/168, $P = .03$, Fisher's exact test; enteric bacterial: 6%, 10/168, $P = .0003$, Fisher's exact test).

AR and multiple linear logistic regression analysis of factors affecting the immunogenicity of OPV in rural Mayan children. Using multiple logistic regression models and AR determination, we quantitated the impact of factors found to influence OPV immunogenicity by univariate analysis (table 1). In a stepwise regression model, the dependent variable was the seroresponse profile to each Sabin type after each OPV dose; the independent variables included only the variables that showed a significant association in univariate analysis at $P \leq .05$. Therefore, mass versus routine vaccination was not included in regression analysis. The presence of homologous maternal antibodies had no significant effect on immunogenicity of any Sabin type. Infants who shed a vaccine strain were more likely

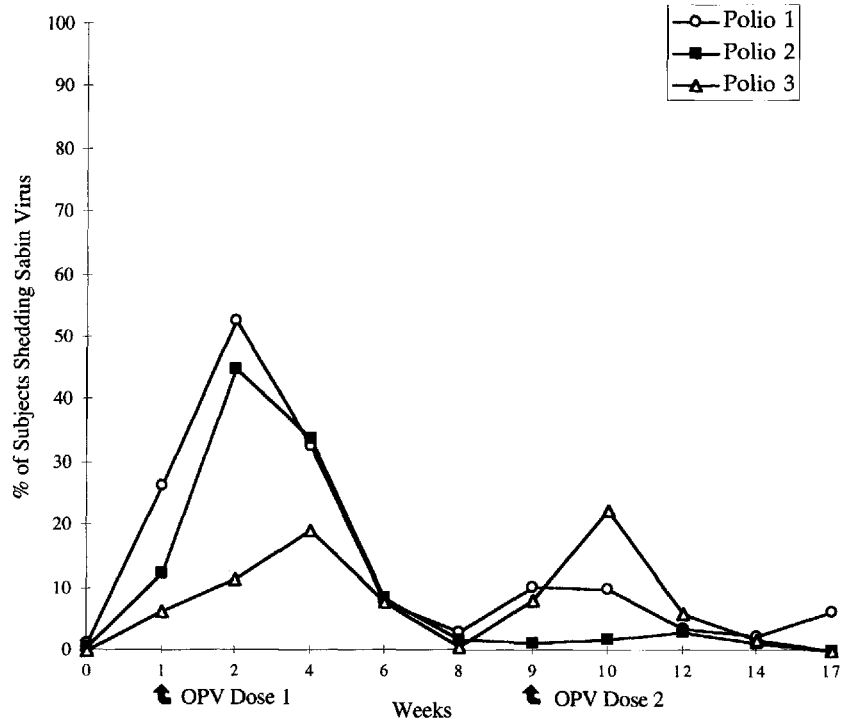


Figure 2. Fecal shedding of Sabin vaccine virus types 1–3 after vaccination with 2 OPV doses in 181 study subjects.

to have a serologic response to that strain, particularly infants who shed Sabin type 3 (OR; 11.0, $P = .0001$). In addition, infants who shed Sabin types 1 or 2 after the first vaccine dose were more likely to have a seroresponse to Sabin type 1. Other factors affecting the immunogenicity of OPV varied for each

Sabin type. Infants with concurrent enteric bacterial or viral infections at the time of the second OPV dose were significantly less likely to develop a seroresponse to Sabin type 1, and infants who shed Sabin type 2 were significantly less likely to have a seroresponse to Sabin type 3 (OR, 0.32; $P = .006$).

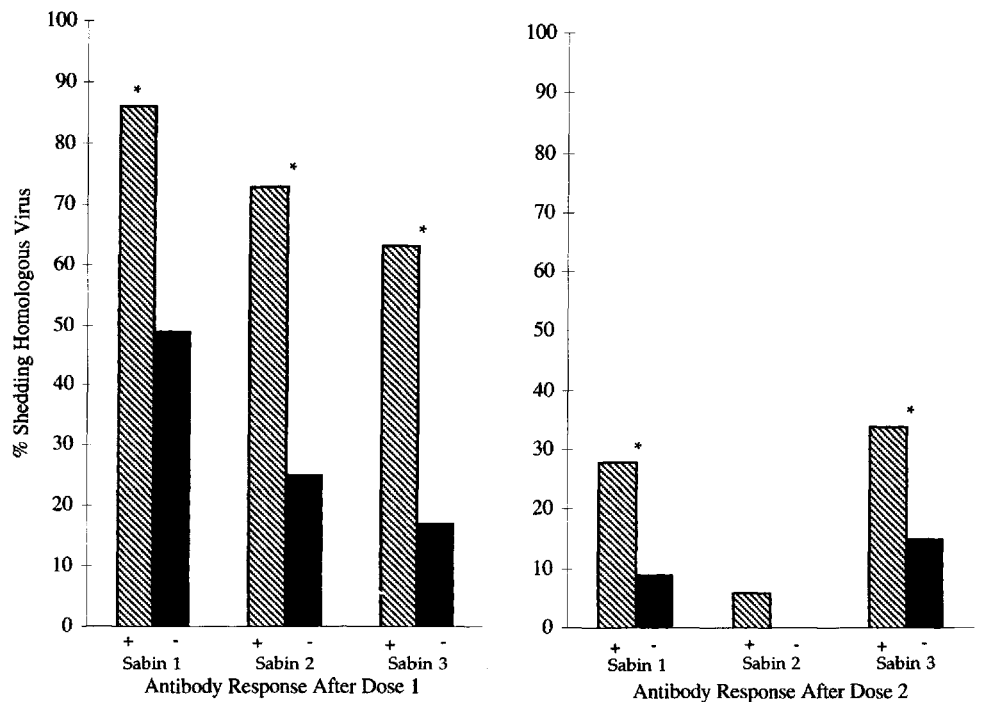


Figure 3. Shedding of Sabin vaccine viruses in relation to development of homologous Sabin neutralizing antibodies for 181 study subjects. * $P \leq .05$, χ^2 , difference in seroresponse rates among infants with and without shedding of homologous Sabin viruses.

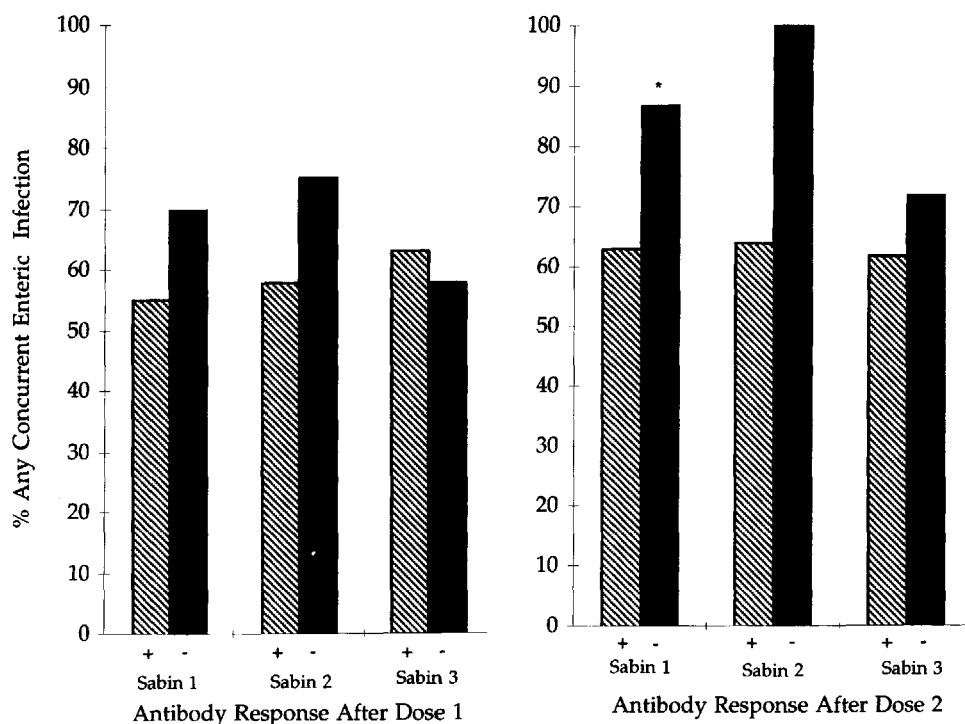


Figure 4. Seroresponse rates among 181 study subjects in relation to presence of any concurrent enteric infection. * $P = .02$, Fisher's exact test, difference in concurrent enteric infection rates among seroresponders and nonseroresponders.

Table 1. Factors associated with immunogenicity of OPV assessed by multiple logistic regression analysis, population attributable risk percent, and population prevented fraction.

Variable, vaccine	Effect	Odds ratio*	P	Population	
				Prevented fraction [†]	Attributable risk [‡]
Fecal shedding of homologous Sabin strain					
Sabin 1, dose 1	+	6.09	.0001	81	
Sabin 1, dose 2	+	5.50	.03	105	
Sabin 2, dose 1	+	6.80	.01	134	
Sabin 3, dose 1	+	11.00	.006	38	
Sabin 3, dose 2	+	2.80	.01	55	
Fecal shedding of heterologous Sabin strain					
Sabin 2, dose 1	+	4.50	.002	125	
Sabin 3, dose 1	-	0.32	.0001		34
Concurrent enteric bacterial or viral infection					
Sabin 1, dose 2	-	0.23	.02		14

* Multiple logistic regression odds ratio associated with effect of variable on immunogenicity.

[†] No. of vaccine failures prevented among 181 study subjects in association with this variable.

[‡] No. of vaccine failures among 181 study subjects in association with this variable.

A separate linear regression model that assessed the impact of several variables on GMTs to each Sabin type showed that mass vaccinees had significantly lower GMTs to Sabin type 1 at the second dose and to Sabin type 2 at both doses than did routine vaccinees. Concurrent enteric infections were also associated with significantly lower GMTs for both Sabin types 1 and 2 at the first dose (not significant, $P = .08$) and for Sabin type 2 at the second dose. However, the effect of mass versus routine vaccination on decreased GMTs was independent of concurrent enteric infections, and no other variables were associated with the differential GMTs observed for mass versus routine vaccinees.

Factors affecting OPV immunogenicity were quantitated using AR, PAR (to determine the proportion of Sabin-specific vaccine failures within the vaccinated population that were directly associated with each negative risk factor), and PPF (to determine the proportion of Sabin-specific vaccine failures that were prevented by association with each positive risk factor). In this study, the largest number of vaccine successes was attributable to fecal shedding of homologous strains for all Sabin types, and the largest number of vaccine failures was directly attributable to Sabin type 2 interference with Sabin type 3.

Discussion

After the first 2 OPV doses, 86% of the infants developed neutralizing antibodies to Sabin type 1, 97% to Sabin type 2, and 61% to Sabin type 3. After 2 OPV doses, the reduced seroresponses, particularly to Sabin type 3, were similar to

those reported in other developing areas where seroconversion rates after administration of 3 doses of various OPV formulations average 73%, 90%, and 70% for poliovirus types 1–3, respectively [32]. Our study confirmed the poor immunogenicity of Sabin type 3 in the standard OPV formulation. Based upon these observations, immunity to Sabin type 3 will be low even in communities with 100% vaccination.

To define specific factors contributing to diminished OPV immunogenicity in developing areas, we prospectively compared the effect of multiple variables on the immune responses to OPV among infants in a rural Mayan village. We assessed only the first 2 OPV doses, because a primary study objective was to evaluate the immunogenicity of OPV when administered through mass vaccination programs, which usually use only 2 OPV doses per campaign. Variables evaluated with a potential effect on OPV immunogenicity included mass versus routine administration of OPV, Sabin vaccine virus shedding, interference by other enteric pathogens, and the presence of transplacentally acquired poliovirus antibodies. The capacity to correlate immunogenicity with interference by other Sabin vaccine viruses and with concurrent enteric bacterial and viral pathogens was unique to this study. The use of molecular probe-based techniques, which permit efficient and sensitive detection of fecal shedding of OPV strains and of other enteric pathogens, enabled this analysis.

Overall, we found that seroresponses to all 3 Sabin types were similar whether vaccine was given by mass or routine vaccination programs. By multiple logistic regression PAR analyses, shedding of homologous Sabin strains was the most important variable associated with immunogenicity of all 3 Sabin strains. Interference by Sabin type 2 accounted for the majority of primary OPV failures by decreasing seroresponses to Sabin type 3. Another factor that affected immunogenicity was interference by concurrent enteric infection with seroresponses to Sabin types 1 and 2, but the impact was less significant because seroresponses were 86% to Sabin type 1 and 97% to Sabin type 2.

While seroresponse rates were identical among mass and routine vaccinees, mass vaccinees had significantly lower GMTs to Sabin types 1 and 2 than did routine vaccinees. These results were partially attributable to concurrent enteric infections but did not fully account for differences in GMTs based on vaccination method. We were unable to identify any other factors that accounted for this difference.

Previous attempts to identify risk factors for primary OPV vaccine failure have had conflicting results for interference from maternal antibody, other circulating enteroviruses, and nonspecific causes [7–14, 32]. The role of enterovirus interference has been investigated most intensively, in part, because of known interference between Sabin types 1–3. Although the effect of concurrent enteric infection with NPEV on the immune response to OPV has been studied extensively, no published studies investigated the impact of other enteric viruses that may be prevalent in young children in developing coun-

tries. The 4 enteric virus types we studied account for most cases of viral gastroenteritis in children worldwide [33–39]. Testing for common bacterial enteropathogens was included to identify any interference by these infections on the immunogenicity of OPV. As expected, rates of concurrent enteric infection in this population were high. However, evidence of interference by enteric viral and bacterial pathogens was found only with the second dose of Sabin types 1 and 2. These pathogens did not affect the immunogenicity of the first OPV dose, and the overall effect was minimal, because the seroresponses to types 1 and 2 were high after the first dose. Simultaneous testing for multiple viral and bacterial organisms allowed us to demonstrate that interference with Sabin vaccine virus replication is not related to specific pathogens and is associated with the presence of 1 or more enteric pathogens. The finding that this interference occurs only after the second dose of Sabin types 1 and 2 suggests that decreased shedding of the vaccine strains after the second OPV dose allows the replication of other enteric pathogens to alter immunogenicity.

Our observations that the baseline rate of concurrent enteric infections at the time of OPV administration was high, that a variety of pathogens affected the immunogenicity of OPV, and that concurrent infections occurred throughout the study period suggest that timing the administration of OPV to particular seasons will not likely result in improved responses to OPV. However, our analysis supports the WHO recommendation that an additional dose of OPV be given to infants who have diarrhea at the time of their primary OPV vaccination [40].

Because of the poor immune response to poliovirus type 3 observed in developing countries, poliomyelitis epidemics in highly vaccinated populations may be caused by wild poliovirus type 3, whereas epidemic and endemic disease in unvaccinated populations is due primarily to wild poliovirus type 1 [41–43]. Improving seroconversion to poliovirus type 3 should result in decreased breakthrough poliomyelitis in highly vaccinated populations. Data from this and other recent studies demonstrate that the most important goal of vaccine programs for infants in developing areas is to increase seroresponse to Sabin type 3 [41, 44]. Our study indicates that this objective can best be accomplished by increasing the Sabin type 3 component of the OPV formulation. Fecal shedding of Sabin type 3 increased after the second OPV dose and was associated with significant improvement in seroresponse to Sabin type 3. Fecal shedding of Sabin type 3 was inversely proportional to shedding of Sabin types 1 and 2, suggesting that interference by Sabin types 1 and 2 accounted for decreased seroresponses to Sabin type 3 after the first OPV dose. Improvement in the Sabin type 3 response after the second dose was related to decreased shedding of Sabin types 1 and 2 after the second OPV dose.

The standard OPV formulation contains 10^6 , 10^5 , and 3×10^5 TCID₅₀ (10:1:3 ratio) of Sabin types 1–3, respectively. In developing countries, OPV is usually administered in 4 doses at intervals of 4–6 weeks, beginning at birth. Previous evaluations of OPV have assessed variables that might affect immuno-

genicity, especially of Sabin type 3: absolute potency of Sabin types 1–3, relative proportions of the 3 strains in the vaccine preparation, age at first vaccine dose, and the interval between doses and the number of doses in the primary series [32]. The enhanced OPV formulation containing Sabin types 1–3 in ratios of 10:1:6 is the only revised formulation that has been associated with improved immunogenicity. The Sabin type 3 dose in the enhanced OPV formulation is twice that of the standard OPV formulation and is given in a preparation with 10^6 , 10^5 , and 6×10^5 TCID₅₀ of Sabin types 1–3.

The initial experience with enhanced OPV, reported by Patriarca et al. [41] in 1988 among Brazilian infants, demonstrated a significant increase in seroconversion rates for Sabin type 3 compared with standard OPV. In 1990, the Global Advisory Group of the Expanded Programme on Immunization recommended the use of the enhanced OPV formulation in developing countries [45]. However, a recent study in Brazil and Gambia that compared 4 OPV formulations (including the standard 10:1:3 and enhanced 10:1:6) administered to infants beginning at birth or before age 6 weeks showed no improvement in the immunogenicity of Sabin type 3; lower seroconversion rates to both standard and enhanced formulations were associated with the presence of passively acquired antibodies to all 3 vaccine serotypes [44]. The infants were younger than participants in the first Brazilian study and in our study and were more likely to have preexisting poliovirus antibodies. The fact that preexisting poliovirus antibodies did not account for poor vaccine responses in our study population can be attributed to beginning vaccination at ages 6 weeks to 6 months. Since the efficacy of both standard and enhanced OPV may depend in part upon administration after the decline of passively acquired antibodies, improved OPV immunogenicity might be achieved if infants are given their first dose after age 6 weeks.

In summary, our study demonstrates that the poor immunogenicity of Sabin type 3 accounts for the majority of primary OPV vaccine failures and is related primarily to interference with Sabin type 3 by Sabin type 2. Intestinal interference by concurrent enteric infections has a smaller but measurable impact upon the immunogenicity of OPV, particularly after the second dose of Sabin types 1 and 2, among infants in developing countries. These concurrent enteric infections are highly prevalent regardless of season. Vaccination of infants at older ages reduced the effect of passively acquired poliovirus antibodies. Factors amenable to alteration in local polio vaccination programs that could improve the immunogenicity of OPV include giving enhanced Sabin type 3 formulations of OPV and increasing the age of vaccination to ≥ 6 weeks. In young infants in developing countries, mass and routine programs for polio vaccination are equally effective.

Acknowledgments

We are indebted to the families of the village of Navenchaur for participation in this study; the field and laboratory staff, Stanford

Research Center, Chiapas, and the Public Health Laboratory staff in San Cristobal de las Casas, Chiapas, and at Instituto de Diagnostico y Referencia Epidemiologicos, Mexico City, for dedicated work; Lina De and Olen Kew, Centers for Disease Control, for assistance with probe hybridization methods; David Schnurr, Viral and Rickettsial Diseases Laboratory, California Department of Health Services, for assistance in identifying the echovirus 22 isolates; John E. Herrmann, University of Massachusetts Medical Center, for antibody reagents for the astrovirus ELISA; and Nancy Ginzton and Lucinda Smith for technical assistance.

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