

Induction of Immunologic Memory in Gambian Children by Vaccination in Infancy with a Group A plus Group C Meningococcal Polysaccharide-Protein Conjugate Vaccine

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Two hundred twenty-one Gambian children vaccinated previously with one, two, or three doses of a meningococcal conjugate vaccine or two doses of polysaccharide vaccine before the age of 6 months were revaccinated at the age of 18–24 months with either meningococcal polysaccharide, conjugate, or inactivated polio vaccines. Children who had previously received one, two, or three doses of conjugate vaccine had significantly ($P < .001$) higher anti-group C meningococcal antibody levels following revaccination than did children vaccinated with a polysaccharide vaccine for the first time. Children vaccinated previously with two doses of polysaccharide vaccine had a lower group C antibody response than did control children. Group A antibody responses following revaccination of children who had previously received polysaccharide or conjugate vaccine were not significantly higher than those in control children. Thus, immunologic memory was probably induced by the group C but not by the group A component of the conjugate vaccine.

Vaccination with group A meningococcal polysaccharide vaccines is an effective way of stopping epidemics of group A meningococcal disease. However, the protection induced by these vaccines is short-lasting, at least in children. In one study in Burkina Faso, vaccine efficacy had declined to 54% by 3 years after vaccination. This decline was particularly marked in children <4 years old, for whom vaccine efficacy was not significantly different from background levels 3 years after vaccination [1]. Serologic studies have shown rapid declines in antibody levels in African children following meningococcal polysaccharide vaccination [2]. Thus, the recent development of a group A plus group C meningococcal polysaccharide and protein conjugate vaccine [3, 4] that might give a longer duration of protective immunity than a polysaccharide vaccine is an important step forward toward the control of epidemic meningococcal disease.

Recently, we described a safety, immunogenicity, and dose-finding trial of a group A and group C meningococcal polysaccharide/CRM₁₉₇ conjugate vaccine in Gambian infants who

were vaccinated during the first 6 months of life [5]. Groups of ~50 infants were vaccinated with one dose of conjugate vaccine at the age of 6 months; two doses of conjugate vaccine at the ages of 2 and 6 months; three doses of conjugate vaccine at the ages of 2, 3, and 4 months; or two doses of polysaccharide vaccine at the ages of 3 and 6 months. Blood samples were collected for antibody determinations before and 1 and 3 months after completion of vaccination. Infants were observed carefully for any local or systemic side effects; these were few.

After vaccination, group A meningococcal polysaccharide antibody levels measured by ELISA increased progressively after one, two, or three doses of conjugate vaccine. There was no significant difference in postvaccination group A antibody concentrations between children given two doses of polysaccharide vaccine and those given two doses of conjugate vaccine. In contrast, two doses of conjugate vaccine induced higher concentrations of group C antibody than did two doses of polysaccharide vaccine, although the response to two doses of conjugate vaccine was less than that to one dose of conjugate vaccine. Three months after vaccination with conjugate vaccine, antibody concentrations had declined to about one-quarter of their peak value.

A potentially important advantage of a conjugate vaccine over a polysaccharide vaccine is that it should induce immunologic memory, facilitating a brisk and enhanced response on subsequent exposure to the vaccine antigen. Because the peak age of occurrence of meningococcal disease during African epidemics is ~10 years [6], it is essential that if meningococcal conjugate vaccines are to be given as part of an infant program of immunization, they should induce immunologic memory that persists for many years. As a first step toward determining

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Table 1. Characteristics of children in the study of revaccination against meningococcal disease and the reasons for exclusion.

Characteristic	Previous vaccination				Total
	Conjugate vaccine			Polysaccharide vaccine, 2 doses	
	1 dose	2 dose	3 doses*		
Initial recruitment	66	62	114	62	304
Completed primary course of vaccination [†]	59	58	109	53	279
Recruitment to booster study	45	50	79	47	221
Reasons for exclusion					
Death [‡]	0	0	10	0	10
Severe illness	0	0	1	0	1
Refusal	4	1	4	3	12
Traveling	10	7	15	3	35

NOTE. Data are no. of children.
* Twice as many children were included in this group as in other groups because 2 different blood sampling schedules were used [5].
† These figures differ from those reported previously [5], because all children who were vaccinated were approached to join this study (in earlier report, children who refused blood testing were excluded).
‡ See text for details of deaths during whole course of primary and revaccination studies.

whether this might be the case, we revaccinated children in the Gambian conjugate vaccine trial and a group of control children when they reached the age of 18–24 months with a further dose of either conjugate or polysaccharide vaccine and compared group A and group C meningococcal antibody concentrations in children with different previous vaccinations.

Subjects and Methods

Subjects. The study area, the eastern part of the Gambia, and the study population have been described [5]. When children who had been recruited into the conjugate vaccine trial reached the age of ~18 months, as many as possible were traced, and their families were asked if the child could join a further study. Of the 304 children originally entered into the trial, 221 (73%) were recruited into the follow-up study. Their mean age was 19.7 months (1.3 SD). Reasons for exclusion are shown in table 1. When it became apparent that a high proportion of deaths had occurred among children who had received three doses of conjugate vaccine, a further exhaustive survey was undertaken to determine the status of all children who had entered the original trial. Information was obtained on the status of 289 children (95%). A further group of children from the study area who were age-matched with children in the main trial and who had received no previous meningococcal vaccination were recruited as controls.

Children from the main trial were randomized according to a random numbers list to receive an injection of (1) a 0.5-mL dose of group A plus group C meningococcal polysaccharide/CRM₁₉₇ conjugate vaccine (lot 090991; Biocine, Siena, Italy) containing ~11 µg of each polysaccharide, 49 µg of CRM₁₉₇, and 1 mg of aluminum hydroxide per 0.5-mL dose; (2) a 0.5-mL dose of meningococcal polysaccharide vaccine (Menpovax A plus C; Biocine) containing 50 µg of each meningococcal polysaccharide per dose; or (3) a 0.5-mL dose of inactivated poliomyelitis vaccine (IPV; Pasteur Mérieux, Lyon, France). Further details on the development of the conjugate vaccine are given elsewhere [3]. No hypersensitivity reactions were recorded following revaccination. At

10–14 days (mean, 12.3) after vaccination, a finger-prick blood sample was collected for antibody determinations. Children in the control group were vaccinated with a single dose of the same batch of polysaccharide vaccine ~6 months after children in the booster cohort; blood samples were obtained from these children before and 10–14 days after vaccination.

Laboratory methods. Group A and group C meningococcal antibody titers were measured at the Centers for Disease Control and Prevention (CDC, Atlanta) by ELISA as described [7, 8]. Results are expressed in relation to a standard reference serum that has been ascribed total and class-specific antibody concentrations for group A and C meningococcal antibodies [9]. Sera from groups of children, selected to give the most useful comparisons, were assayed for serum bactericidal antibodies using a method described previously [4]. The group A isolate used in this assay (CDC strain F8238) was isolated during an epidemic in Kenya in 1989, and the group C isolate used (strain C-11; provided by C. Frasch, Center for Biologics Evaluation and Research, Bethesda, MD) was isolated in Germany in 1965. Serum bactericidal antibody titers are expressed as the reciprocal of the highest serum dilution that gave ≥50% killing.

Statistical methods. Antibody concentrations were log-transformed. The lower limits of detectability of the polysaccharide antibodies were 0.3 µg/mL (group A) and 0.1 µg/mL (group C). Sera that contained concentrations of antibody lower than these were assigned these figures for the purpose of statistical evaluations. Comparisons of the effects of revaccination with different vaccines were made after adjustment for prior vaccinations by an analysis of variance. Comparisons between initial vaccine groups were made after adjustment for the type of vaccine used for revaccination. No interaction was found between initial vaccination and revaccinations, justifying a stratified analysis. All analyses were done using SAS System for Windows (SAS Institute, Cary, NC).

Results

Mortality in vaccinated children. When study children were called for revaccination at the age of 18 months, we

found that 10 had died since completing their primary course of vaccination; all of these deaths occurred among the 38% of the children who received three doses of conjugate vaccine. However, children in the three-dose group finished their primary course of vaccination by the age of 4 months, while children in the other groups did not finish until age 6 months. Two of the 10 deaths in the three-dose conjugate vaccine group were of children between the ages of 4 and 6 months, leaving only 8 deaths for comparison with data from other groups. None of these comparisons were statistically significant ($P > .05$). Nevertheless, concern over these deaths led to an intensive search for all children in the original trial, even if they had not been recruited to the follow-up study. Information was obtained about 295 (95%) of the 304 children. Twenty-three had died between the time of recruitment at the age of 2 months and final follow-up at a mean age of 24 months. Seven deaths occurred between the ages of 2 and 11 months, 13 between the ages of 12 and 23 months, and 3 after the age of 24 months. None of the deaths could be related directly to vaccination.

Interviews with the families of children who had died and review of any health records available suggested the following causes of death: malnutrition (6), acute gastroenteritis (5), pneumonia (3), septicemia (2), pericarditis caused by *Haemophilus influenzae* type b (Hib; 1), meningitis of unknown etiology (1), and measles (1). The cause of 4 deaths was uncertain. Fourteen children in the three-dose conjugate vaccine group died (mortality rate per 1000 child-months at risk, 5.3), 1 child in the two-dose conjugate vaccine group (mortality rate, 0.6), 5 children in the one-dose conjugate vaccine group, 3 of whom died before vaccine was given (mortality rate, 3.0), and 3 children in the two-dose polysaccharide vaccine group (mortality rate, 1.9).

Comparisons between groups showed only one statistically significant difference: that between the three-dose and two-dose conjugate vaccine groups ($\chi^2 = 4.3$; 1 df; $P = .04$). However, when only deaths after vaccination had been completed were considered this difference disappeared and no significant differences between groups were found.

The pattern of deaths among children in the three-dose conjugate vaccine group (8 deaths from infections [pneumonia, 2; acute gastroenteritis, 2; Hib pericarditis, 1; septicemia, 1; measles, 1; meningitis, 1], 3 from malnutrition, and 3 from unknown causes) did not differ from that seen for the remaining children. Data collected previously on child mortality in Upper River Division [10] suggested that ~18 deaths would have been expected in a cohort of this size, a statistically nonsignificant difference. Eighty percent of these deaths would have been expected to be due to infections and 10% to malnutrition. Thus, the pattern of deaths among the children in the three-dose conjugate vaccine group was not different from the expected.

ELISA antibodies. Group A and C meningococcal ELISA antibody concentrations in vaccinated children are shown in table 2. Both group A and C antibody concentrations had fallen to nearly background levels by the age of 18–24 months in

children who had been vaccinated during the first few months of life with either polysaccharide or conjugate vaccines.

Control children showed group A and C antibody responses to a primary vaccination with a single dose of group A plus group C polysaccharide vaccine (for comparisons between pre- and postvaccination antibody concentrations, $P < .001$ in each case).

All groups of children revaccinated with polysaccharide or conjugate vaccine had significantly higher group A antibody concentrations ($P < .001$) than did control children who received IPV. However, the group A antibody responses to a single dose of polysaccharide vaccine in children vaccinated previously with two doses of polysaccharide vaccine or with one, two, or three doses of conjugate vaccine did not differ significantly from the response in children of the same age vaccinated with polysaccharide vaccine for the first time. There was, however, a small and statistically significant difference ($P < .01$) between two-dose polysaccharide and three-dose conjugate vaccine groups. There was no significant difference in group A antibody responses following revaccination with polysaccharide or conjugate vaccine when previous vaccinations were taken into account ($P = .29$).

Group C antibody responses to revaccination differed in several respects from those seen for antibodies to group A polysaccharide. All groups of previously vaccinated children revaccinated with polysaccharide or conjugate vaccine had significantly higher ($P < .001$) group C antibody concentrations than did control children who received IPV. Children who had previously received one, two, or three doses of conjugate vaccine had significantly higher group C antibody concentrations ($P < .001$) when boosted with polysaccharide vaccine than did age-matched children given polysaccharide for the first time. In contrast, the group C antibody response of children who had previously received two doses of polysaccharide vaccine and who were revaccinated with polysaccharide was significantly less ($P = .02$) than that of children who received polysaccharide for the first time. This was not the case when conjugate was used as the second vaccine.

After adjustment for the type of vaccine used for revaccination, the postbooster group C antibody concentration in children who had received two doses of conjugate vaccine at age 2 and 6 months was lower than that of children who had received only one dose of conjugate vaccine at age 6 months, but the difference between groups was not statistically significant. After adjustment for previous vaccinations, the conjugate vaccine was found to give a significantly higher group C booster antibody response than the polysaccharide vaccine ($P < .001$).

Bactericidal antibodies. Measurement of bactericidal antibody titers in sera from selected groups of children showed a pattern similar to that obtained for ELISA antibodies (table 2). Children who received polysaccharide vaccine for the first time showed a significant rise in group A and C antibody titers ($P < .001$). For group A antibodies, this response was not significantly different from that of children vaccinated pre-

Table 2. Meningococcal antibody concentrations in Gambian children (mean age, 19.7 months) with different previous meningococcal vaccine experiences 10–14 days after revaccination with polysaccharide vaccine or conjugate vaccine.

Initial vaccination	Reimmunization	ELISA antibodies		Bactericidal antibodies	
		Group A	Group C	Group A	Group C
None	None*	1.2 [0.9–1.5] (34)	0.2 [0.1–0.3] (34)	9 [7–10] (34)	8 [7–9] (34)
None	Polysaccharide	12.2 [8.8–16.8] (34)	19.0 [13.7–26.3] (34)	338 [181–630] (34)	239 [147–388] (34)
Polysaccharide, 2 doses	None†	2.4 [1.9–3.0] (14)	0.7 [0.4–1.4] (14)		
	Polysaccharide	22.8 [14.5–35.8] (18)	9.4 [6.2–14.3] (18)	1783 [1097–2896] (17)	26 [14–49] (16)
	Conjugate	12.7 [8.9–18.0] (15)	38.6 [26.2–56.8] (15)		
Conjugate 1 dose	None†	1.4 [0.9–2.1] (17)	0.4 [0.2–0.9] (17)		
	Polysaccharide	22.8 [12.4–42.0] (12)	77.8 [50.0–121.0] (12)		
	Conjugate	8.3 [5.7–11.6] (16)	86.7 [58.1–129.3] (16)		
2 doses	None†	2.0 [1.5–2.6] (15)	0.4 [0.2–0.8] (15)		
	Polysaccharide	14.5 [8.1–26.1] (17)	55.7 [33.5–87.3] (17)	549 [194–1048] (15)	4390 [1261–14,263] (12)
	Conjugate	17.9 [10.4–30.6] (18)	57.9 [41.4–80.8] (18)		
3 doses	None†	2.2 [1.5–3.4] (27)	0.6 [0.3–1.0] (27)		
	Polysaccharide	10.1 [7.2–14.2] (27)	46.9 [28.2–77.9] (27)		
	Conjugate	13.4 [9.5–18.9] (25)	117.0 [80.2–170.5] (25)		

NOTE. ELISA antibody data are geometric mean concentrations in $\mu\text{g/mL}$; bactericidal antibody data are geometric mean reciprocal titer [95% confidence interval] (n).

* Same children as in next group but blood samples were obtained immediately before vaccination.

† Received inactivated polio vaccine.

viously with two doses of polysaccharide or conjugate vaccine. In contrast, the group C antibody response of children who had previously received two doses of conjugate vaccine was significantly higher ($P < .001$) than that of previously nonvaccinated children, and the response of children who had previously received two doses of polysaccharide vaccine was significantly lower ($P < .001$).

ELISA and bactericidal antibody levels generally correlated well in individual samples. Overall correlation coefficients were 0.81 and 0.88 for group A and C antibodies, respectively.

Discussion

When a search for children in the original conjugate vaccine trial was made to recruit them into a booster study, we found that 10 children had died since completing their primary course of meningococcal vaccination. Because of the high mortality rate among children in this rural community (infant mortality rate, 84/1000 live births) [10] (unpublished data for 1988–1993), this number of deaths was not unexpected. However, a matter of concern was the fact that all 10 deaths had occurred among the children who had received three doses of conjugate vaccine. Although the mortality rate after the age of 6 months was not significantly higher among children in this group than among children in other groups, a full review, involving home visits, of all children initially entered into the trial was undertaken. This revealed that although more deaths had occurred in the three-dose vaccination group than in other groups, these had occurred throughout the surveillance period and at different

ages and that they were due to a variety of causes, mainly infectious. The presumed causes of death in children who had received the conjugate vaccine did not differ from those expected on the basis of previous mortality surveys in the study community. Thus, it seems likely that the apparent clustering of deaths in children in this vaccine group was a chance finding.

The group C component of the conjugate vaccine induced immunologic memory; both ELISA and bactericidal antibody levels 10 days after revaccination were significantly higher in children who had received two doses of conjugate vaccine previously than in those who had received two doses of polysaccharide vaccine or in those who had not been vaccinated before. Protective levels of meningococcal antibodies are not known for certain but are likely to be $\sim 1\text{--}2 \mu\text{g/mL}$ [11]. All children who had received two doses of conjugate vaccine had antibody titers well above this level, and it seems likely that they would have had a high degree of protection against group C meningococcal disease, had they been exposed to a virulent strain of group C meningococcus. Both group A polysaccharide and conjugate vaccines induced antibody responses on primary vaccination, as has been noted in previous studies in infants, but no evidence was found that either the group A polysaccharide vaccine or the group A component of the conjugate vaccine induced immunologic memory. Why the group C component of the conjugate vaccine, but not the group A component, was able to induce immunologic memory is unclear.

It has been reported that group C polysaccharide vaccines can, under certain circumstances, induce immunologic tolerance in infants [12]. This phenomenon was observed again in our study; children who had received two doses of group C

polysaccharide vaccine during the first 6 months of life had lower group C ELISA and bacterial antibody levels after challenge with a group C polysaccharide vaccine than did age-matched children who received this vaccine for the first time. These children will be studied again in 1997, when they reach the age of 5 years, to see if this phenomenon persists. This was not the case for children vaccinated previously with two doses of conjugate vaccine. Although their antibody level after revaccination was lower than that of children who had previously received one dose of conjugate vaccine, as noted in the initial trial [5], the difference between the groups was not statistically different, and children in the two-dose conjugate group had a better response to revaccination than control children vaccinated for the first time. Children revaccinated with conjugate vaccine had a higher mean group C antibody concentration than did children given polysaccharide vaccine, after adjustment for previous vaccinations. This effect was especially marked among the children vaccinated previously with two doses of polysaccharide vaccine. A similar phenomenon has been observed during some studies with Hib conjugate vaccines [13], although in others, polyribosylribitol phosphate polysaccharide has been as effective a booster vaccine as conjugate [14, 15]. In any case, an excellent response was seen to the group C meningococcal polysaccharide alone, and this will be satisfactory for subsequent revaccination if needed.

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