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Safety and immunogenicity of three doses of a *Neisseria meningitidis* A + C diphtheria conjugate vaccine in infants from Niger

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Background. **High rates of endemic disease and recurrent epidemics of serogroup A and C meningococcal meningitis continue to occur in sub-Saharan Africa. A meningococcal A + C polysaccharide diphtheria-toxoid-conjugated vaccine may address this issue.**

Methods. **In Niger three doses of a bivalent meningococcal A + C diphtheria-toxoid-conjugated vaccine (MenD), containing 1, 4 or 16 μg of each polysaccharide per dose, administered at 6, 10 and 14 weeks of age, were compared with *Haemophilus influenzae* type b-tetanus toxoid-conjugated (PRP-T) vaccine given with the same schedule or with a meningococcal A + C polysaccharide vaccine (MenPS) given at 10 and 14 weeks of age. One blood sample was taken at the time of enrollment (6 weeks of age) and another was taken 4 weeks after the primary series.**

Results. **All doses of MenD were well-tolerated. After the primary series a higher proportion of infants had detectable serum bactericidal activity against serogroup A for each dose of MenD (from 94% to 100%) than for MenPS (31%) or *H. influenzae* type b-tetanus toxoid-conjugated vaccine (18.9%); $P \leq 0.05$. Significant differences were also observed for serogroup C MenD 4 μg or MenD 16 μg (100%) *vs.* MenPS (69.7%) or *Haemophilus influenzae* type b-tetanus toxoid-conjugated vaccine (24.3%); $P \leq 0.05$. When MenPS vaccine was given to 11-month-old children, the immune response measured by both enzyme-linked immunosorbent assay and serum bactericidal assay was greater in those previously immunized with MenD than in those immunized with MenPS vaccine.**

Conclusion. **MenD was safe among infants in Niger, and immunization led to significantly greater functional antibody activity than with MenPS. The 4-μg dose of MenD for both the A and C serogroups has been selected for further studies.**

INTRODUCTION

In Niger, which lies within the "meningitis belt" of sub-Saharan Africa,[1,2] *Neisseria meningitidis* causes an annual average between epidemics of 30 cases of meningitis per 100 000 population; 85% of meningococcal cases are caused by serogroup A strains, with the remaining cases predominantly caused by serogroup C meningococci.[3] Although meningococcal polysaccharide vaccine is currently used to control serogroup A and C epidemics in Niger, even if the epidemic is detected early, vaccination is nearly always started too late to prevent most cases. Conjugation of polysaccharide antigens to protein carriers could potentially overcome the poor immunogenicity of polysaccharide vaccines in infancy by converting a T cell-independent response to a T cell-dependent one and result in efficacious conjugate vaccines that could be incorporated into the Expanded Program of Immunization (EPI).

This study determined the immunogenicity and safety in infants from Niamey, Niger of three formulations (containing 1, 4 or 16 μg of both polysaccharides per dose) of *N. meningitidis* A + C-diphtheria toxoid-conjugated vaccine (MenD) in comparison with two controls: a meningococcal A + C polysaccharide vaccine (MenPS) and a *Haemophilus influenzae* type b-tetanus toxoid-conjugated vaccine (PRP-T). The anamnestic response was assessed 8 months after primary immunization by vaccination with a single dose of MenPS at 11 to 12 months of age in the children already immunized with MenD or MenPS. This study followed preliminary studies conducted in adults, toddlers and infants in the US to evaluate the safety and immunogenicity of the same three formulations of MenD.

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METHODS

The study was conducted between January, 1996, and March, 1997, at Yantala Centre de Santé Maternelle et Infantile in Niamey, Niger. The parents of the infants gave their written consent before inclusion, after being informed about the nature of the trial and any potential risks. If neither parent could read or write, two witnesses had to attest, by signing the consent form, that the parents had been properly informed about the protocol and had agreed to their child's inclusion. Free medical care was provided to the infants during the course of the study. The study protocol was approved by the Ethics Committee of Niger, the WHO Ethics Committee, the Western Institutional Review Board and the CDC's Institutional Review Board. It was also submitted to the Food and Drug Administration and conducted under the IND No. 6201. The study was performed in accordance with the Declaration of Helsinki and the 1996 ICH Good Clinical Practice guideline.

Subjects. Healthy children of either sex between 5 and 9 weeks of age were recruited for the study on presentation for routine EPI administration of the first diphtheria and tetanus toxoid-whole cell pertussis (DTP) vaccine.

Vaccines. The study vaccine was a polysaccharide-protein conjugate vaccine manufactured by Aventis Pasteur (Swiftwater, PA) prepared with serogroup A and C meningococcal polysaccharides, each covalently bound to diphtheria toxoid (MenD). Three vaccine lots were prepared (Lots 950213, 950214 and 950215) that contained, respectively, per 0.5-ml dose, 1, 4 or 16 μg of each polysaccharide and 5.8, 32.2 or 161.0 μg of diphtheria toxoid. The investigational vaccine was presented in a prefilled (0.5-ml) ready to use glass syringe.

Infants in the control groups received either meningococcal polysaccharide A + C vaccine (MenPS, Vaccin Polyosidique Meningococcique A + C, Batches L0878 and L0991, containing 50 μg of group A and 50 μg of group C meningococcal polysaccharides) or one 0.5-ml dose of *H. influenzae* type b conjugate vaccine (PRP-T, Act-HIB, Batch L0670), both manufactured by Aventis Pasteur (Lyon, France). The placebo was 0.5 ml of a 0.4% normal saline solution. The study vaccine or control vaccines were administered intramuscularly in the anterolateral aspect of the right thigh.

Infants recruited into the study already had received Calmette-Guérin bacillus and oral polio vaccine at birth according to Niger's EPI schedule. At the ages of 6, 10 and 14 weeks, they received DTP vaccine subcutaneously in the left deltoid region and oral polio vaccine. At 9 months of age measles and yellow fever vaccines were administered. All vaccines were obtained from EPI-approved vaccine manufacturers.

Study design. This was a randomized dose escalation study. A total of 180 children were randomly allocated to 1 of 5 groups (Table 1). The 108 infants allocated to the MenD group received 1 of the 3 formulations of the meningococcal conjugate vaccine at 6, 10 and 14 weeks of age. Dose escalation was sequential. A first subgroup of 36 infants received the 1-μg polysaccharide dose; if no reactogenicity was observed exceeding that commonly expected for DTP vaccine, then a second group of 36 infants received the 4-μg polysaccharide dose; a third group of 36 infants received the 16-μg polysaccharide dose. Two other groups of infants in this joint randomization, ~36 subjects each, enrolled as 3 series of 12 subjects, served as controls at each step. In the preceding year, 1995, a massive serogroup A meningococcal outbreak in Niger had caused more than 40 000 reported cases,[4] so the study was designed such that all participating infants would be offered a meningococcal vaccine by the end of the study. Thus one control group received the MenPS vaccine (1 dose of saline placebo at 6 weeks and 2 doses of MenPS at 10 and 14 weeks of age), and the other group, the PRP-T vaccine control group (3 doses at 6, 10 and 14 weeks of age), received a dose of meningococcal A + C polysaccharide vaccine at 18 weeks of age, after participation in the initial study was complete.

TABLE 1. Design of a randomized trial in Niamey, Niger, assessing the safety and immunogenicity of three doses of MenD, MenPS or PRP-T

Study Groups		Primary Series					Amendment MenPS Vaccination at 11–12 mo of Age		
		Preinjection blood sample	Injections			Postinjection blood sample	Preinjection blood sample	Injection	Postinjection blood sample
Vaccine	*N*	6 wk	6 wk	10 wk	14 wk	18 wk	11–12 mo	11–12 mo	+4 wk after MenPS
MenD 1 μg	36	◆*	MenD 1 μg	MenD 1 μg	MenD 1 μg	◆	◆	MenPS	◆
MenD 4 μg	36	◆	MenD 4 μg	MenD 4 μg	MenD 4 μg	◆	◆	MenPS	◆
MenD 16 μg	36	◆	MenD 16 μg	MenD 16 μg	MenD 16 μg	◆	◆	MenPS	◆
MenPS	35	◆	Placebo	MenPS	MenPS	◆	◆	MenPS	◆
PRP-T†	37	◆	PRP-T	PRP-T	PRP-T	◆			

* ◆, blood sample.

† Infants in the PRP-T control group were offered a dose of meningococcal A + C polysaccharide vaccine at 18 weeks of age, but antibodies were not measured subsequently.

To evaluate the impact of MenD vaccine on memory priming, as compared with the plain polysaccharide vaccine, an amendment to this study was proposed in December, 1996, such that all infants of the MenD and MenPS groups received one dose of MenPS vaccine between 11 and 12 months of age. (Infants of the PRP-T control group were not tested for memory priming.)

Two blood samples were collected by venipuncture from each infant at 6 and 18 weeks of age. For infants in the MenD and MenPS groups included in the study amendment, two additional blood samples were collected, one before the MenPS vaccination dose at 11 months of age and one 4 weeks later.

The parents, the home visitors who assessed local and systemic reactions and the laboratory personnel were blinded to the subjects' study groups.

Reactogenicity. Infants in the study were observed in the clinic for 15 min after each vaccination for immediate reactions. During the dose escalation stage of this study, local and systemic reactions were monitored by home visitors during each of the 5 days after each injection; after MenPS vaccination at 11 months of age, local and systemic events were monitored at 24 and 72 h after injection, respectively.

Laboratory methods. Meningococcal A and C polysaccharide total antibody concentrations (measured as IgG) were determined for all serum samples using a standardized enzyme-linked immunosorbent assay (ELISA) at Aventis Pasteur (US) and expressed as micrograms/ml.[5, 6] Serum bactericidal activity (SBA) was measured for all specimens at the Centers for Disease Control and Prevention Laboratory (Atlanta, GA) by adding group-specific meningococcal bacteria in the presence of baby rabbit origin complement to serial dilutions of a serum sample.[7] Bactericidal activity was defined as the reciprocal serum dilution yielding ≥50% killing as compared with the controls. Seroprotection levels were defined as the percentage of subjects with postimmunization ELISA antibody concentrations ≥2 μg/ml and reciprocal SBA titers ≥8.[8, 9]

Data analysis. Safety data were described for each group and each vaccine dose by the percentage of infants with at least one local or systemic reaction. Geometric mean titer (GMT) values of ELISA antibody concentrations and SBA activities, as well as the seroprotection rates, were calculated with their 95% confidence intervals. Groups were compared by analysis of covariance, including the log10-transformed 18-week baseline titer as the dependent variable, the $\log_{10}$-transformed 6-week baseline titer as the independent factor and the group-by-baseline interaction as the covariate. As a result of multiple endpoints (i.e. two serogroups) and multiple comparisons, statistical significance was declared for the global comparison at an alpha risk of 0.025 (two-sided) and of the linear contrasts at an alpha risk of 0.00625 (two-sided). Statistical analysis was carried out by the Aventis Pasteur Biometry Department (Lyon, France) on SAS Version 6.08 (SAS Inc., Cary, NC)

RESULTS

A total of 180 infants were enrolled; the male-to-female ratio was 0.97 and the mean age at inclusion was 44.8 ± 5.8 days, with no difference between the study groups for either characteristic. Eight months later 116 infants were recontacted and included in the 11-month MenPS vaccination.

Local and systemic reactions after primary series. No child suffered an immediate reaction during the 15 min after any injection. The most frequently observed local reactions were tenderness and redness at the site of injection (Table 2), which were mild and short lived. The cumulative frequency of tenderness after three injections at the injection site in the three MenD groups combined was 37.6%; for MenPS, it was 30.8%, and for PRP-T, 19.6%. The cumulative frequency of redness after three injections was 18.1% for children in the three MenD groups, 7.5% for MenPS recipients and 11.2% for PRP-T recipients. There was no increase in the incidence of local reactions with subsequent injections during the primary immunization series.

The systemic reactions in the MenD 16-μg vaccine group, ranging from 38.9 to 57.1%, appeared to be more frequent than with either one of the lower doses, which were never more than 37% (Table 2). The rates of systemic reactions during the primary immunization series also did not increase with successive injections in any group.

Local and systemic reactions after MenPS vaccination of the 11-month-old-children. The reactogenicity profile after the MenPS vaccination at 11 months of age in infants who had previously received the MenD vaccine was similar to that observed (Table 2) during the primary series. There appeared to be, once again, a more pronounced local reactogenicity observed among the infants receiving MenD at the lower doses as compared with the MenD 16-μg group. This was particularly noteworthy for local tenderness (Data not shown).

Serious adverse events. Eight serious adverse events requiring hospitalization were reported during the trial, all of which were considered by the investigator either to be unrelated to or have an improbable relationship with the study vaccine. In the 1-μg MenD group, there was one case of diarrhea and dehydration associated with fever, and one infant in the MenPS group developed an intercurrent infection and severe dehydration; both led to the death of the child. For all other events, by the end of documented study follow-up, the infant had at least recovered partially.

TABLE 2. Percentage of infants experiencing local or systemic reactions during the 5 days after primary immunization at 6, 10 and 14 weeks of age with MenD, MenPS or PRP-T

	N*	Local Reaction (%)				Systemic Reaction (%)					
		>1	Redness	Tenderness	Other†	>1	Fever		Vomiting	Diarrhea	Irritability
							>38°C	>39°C			
Dose 1											
MenD											
1 μg	36	66.7	36.1	55.6	13.9	33.3	5.6	0	8.3	8.3	16.7
4 μg	36	41.7	22.2	41.7	0	27.8	2.8	2.8	8.3	11.1	8.3
16 μg	36	22.2	8.3	22.2	0	38.9	13.9	2.8	8.3	11.1	13.9
MenPS	35	25.7	11.4	25.7	5.7	31.4	5.7	0	17.1	11.4	17.1
PRP-T	37	29.7	16.2	29.7	2.7	21.6	5.4	0	10.8	2.7	10.8
Dose 2											
MenD											
1 μg	35	54.3	25.7	51.4	0	37.1	8.6	0	5.7	17.1	8.6
4 μg	35	42.9	20.0	42.9	0	28.6	2.9	0	0	22.9	2.9
16 μg	35	31.4	8.6	31.4	0	57.1	17.1	0	8.6	11.4	34.3
MenPS	35	28.6	8.6	28.6	5.7	45.7	14.3	0	14.3	14.3	25.7
PRP-T	37	24.3	8.1	24.3	0	37.8	8.1	0	8.1	10.8	24.3
Dose 3											
MenD											
1 μg	35	48.6	20.0	48.6	0	25.7	14.3	2.9	2.9	11.4	5.7
4 μg	34	32.4	11.8	32.4	0	11.8	0	0	0	5.9	5.9
16 μg	32	9.4	9.4	9.4	0	40.6	3.1	0	3.1	15.6	25.0
MenPS	33	9.1	3.0	9.1	0	36.4	6.1	0	0	30.3	9.1
PRP-T	36	25.0	8.3	25.0	0	38.9	5.6	0	5.6	13.9	19.4

* Total number of infants, 180.
‡ Other local reactions: edema, induration, crying on movement of injected limb.

Immunogenicity: antibody response measured by ELISA. Table 3 shows the immune response expressed in terms of GMT values of antibody measured by ELISA. (In the text that follows, the proportion of infants achieving an antibody titer ≥ 2 μg/ml is given in parentheses.)

For serogroup A, the post-Dose 3 immune responses by ELISA observed with the MenD vaccine were 4.6 μg/ml (88.6%), 5.8 μg/ml (88.2%) and 9.9 μg/ml (96.9%) for the 1-, 4- and 16-μg vaccine doses, respectively. There was a significant difference in dose effect between the 16-μg and the 1-μg dose ($P = 0.0002$). For serogroup C the post-Dose 3 ELISA immune responses measured with the MenD vaccine were 1.5 μg/ml (34.0%), 2.8 μg/ml (64.5%) and 4.8 μg/ml (87.5%), respectively, for the three dose levels. The MenPS vaccine induced comparable ELISA results for serogroups A, 5.5 μg/ml (87.9%), and C, 5.3 μg/ml (81.8%). After the 11-month MenPS vaccination we observed serum antibody titers ≥2 μg/ml in 91.7, 83.3 and 69.2% against serogroup A and 83.3, 83.3 and 92.3% against serogroup C, for the 1-, 4- and 16-μg MenD dose groups, respectively. The same proportion of infants in the MenPS group (58.6%) had seroprotective levels against serogroups A and C after this 11-month MenPS vaccination.

TABLE 3. ELISA antibody titers (micrograms/ml) against meningococcal serogroups A + C before and after both the primary series immunization (6, 10 and 14 weeks of age) and the vaccination with the plain meningococcal A + C polysaccharide vaccine given at 11 to 12 months of age

Vaccine Group	6 wk (Prevaccination)			18 wk (Post-primary Series)		Before the 11–12-mo MenPS Vaccination			1 mo after the 11–12-mo MenPS Vaccination	
	n	GMT	95% CI	GMT	95% CI	*n*	GMT	95% CI	GMT	95% CI
Serogroup A										
MenD (1 μg)	35	2.7	1.7–4.2	4.6	3.6–5.8	25	0.4	0.2–0.6	10	5.5–18.3
MenD (4 μg)	34	3.4	2.2–5.4	5.8	4.3–8.0	31	0.2	0.1–0.3	7.0	4.0–12.0
MenD (16 μg)	32	3.6	2.4–5.4	9.9*	7.6–12.9	27	0.3	0.2–0.5	6.0	3.3–11.0
MenPS	35	3.0	1.7–5.3	5.5	4.1–7.3	30	0.9	0.5–1.4	3.1	2.0–4.7
PRP-T	36	3.2	2.2–4.5	0.6†	0.4–0.8	NA	NA	NA	NA	NA
Serogroup C										
MenD (1 μg)	35	1.9	1.2–3.0	1.5‡	1.1–2.0	25	0.2	0.1–0.3	8.9	5.3–15.0
MenD (4 μg)	31	1.6	0.9–2.8	2.8	2.0–3.9	31	0.1	0.1–0.2	8.1	4.5–14.6
MenD (16 μg)	32	2.4	1.5–4.0	4.8	3.7–6.3	27	0.3	0.2–0.5	8.3	5.4–12.9
MenPS	35	2.3	1.4–3.9	5.3	3.8–7.4	30	0.6	0.3–1.0	2.8	1.7–4.7
PRP-T	36	2.0	1.2–3.5	0.5†	0.3–0.7	NA	NA	NA	NA	NA

* MenD 1 μg *vs.* MenD 16 μg: P = 0.0002 (ANOVA).
† PRP-T *vs.* all MenD doses and MenPS: P = 0.0001 (ANOVA).
‡ MenD 1 μg *vs.* MenD 16 μg and MenD 4 μg: P = 0.001 (ANOVA).
NA, not applicable; ANOVA, analysis of variance.

Immunogenicity: SBA. Table 4 shows the SBA expressed as GMTs, the reciprocal of the dilution. (In the following text the proportion of infants achieving reciprocal titers ≥8 and the *P* value of the 95% confidence intervals (CIs) comparison are given in parentheses.)

For serogroup A the post-Dose 3 immune responses observed with the MenD vaccine were 261 (97.1%), 171 (94%) and 370 (100%) for the three levels of doses, with no statistically significant difference between them. In contrast the SBA values in the MenPS and the PRP-T groups were 7.0 (31%) and 6.7 (18.9%), respectively, each significantly less than for the MenD groups: $P = 0.0001$ ($P \leq 0.05$ when comparing the 95% CIs). For serogroup C the SBA values were 72.1 (94.3%), 189 (100%) and 325 (100%) for the three doses, with a statistically significant dose effect: $P = 0.0001$. The SBA values in the MenPS and the PRP-T groups were 25.4 (69.7%) and 7.3 (24.3%), respectively, both significantly less than for the MenD groups: $P = 0.0001$ ($P \leq 0.01$ when comparing MenD 1 μg *vs.* both the 4- and 16-μg MenD groups). One month after the 11-month MenPS vaccination, for both serogroups we observed good SBA GMT values and seroprotection rates at least equal to 90% in the MenD groups (Table 4). These immune responses appeared to be inversely correlated with the dose and better than that noted for the MenPS group. The same proportion of infants in the MenPS group appeared to be seroprotected (59.3%) against serogroups A and C after this 11-month MenPS vaccination.

DISCUSSION

The objectives of this study were to investigate the safety and immunogenicity in infants in Africa of a new meningococcal conjugate vaccine and to select a dose for future studies that might allow inclusion of this vaccine in the EPI.

All doses of MenD vaccines were well-tolerated. This is consistent with previous studies indicating that polysaccharide-protein conjugate vaccines, including those directed against *N. meningitidis*, are moderately reactogenic.[10–12] The local reactogenicity profiles of the different doses of MenD vaccines appear comparable with those of MenPS and PRP-T vaccines, although there appeared to be a slight decrease in the local reaction rates when the dose was increased. This may have been the result of either detection bias[13] introduced by the sequential approach or a true immunologic effect of the low doses. No difference was observed between the incidences of the systemic reactions observed for the conjugate vaccine and the licensed meningococcal polysaccharide vaccines, with the exception of an apparent increase after three injections of the MenD 16-μg dose, which might be attributable to the greater quantity of diphtheria toxoid.

We used the 2-μg/ml cutoff in ELISA measurements and set the limit of detection of the SBA assay (i.e. 1:8) to define seroprotection; however, the seroprotective level of meningococcal antibodies measured by ELISA is not known for certain, but the evidence suggests it to be 1 to 2 μg/ml.[14–16] Likewise the functional antibody concentration indicative of protection has not been established[7, 14] for the SBA assay used in this study.

After primary immunization MenD elicited ELISA antibody concentrations against serogroup A that were comparable with those obtained after immunization with MenPS, whereas concentrations of antibodies against serogroup C tended to be lower after immunization with the MenD vaccine, especially at the 1-μg dose. The ELISA antibody concentration induced by the 1-μg MenD vaccine was significantly lower than

TABLE 4. Serum bactericidal activity titers (reciprocal of dilution) against meningococcal serogroups A + C polysaccharides before and after both the primary series immunization (6, 10 and 14 weeks of age) and the vaccination with the plain meningococcal A + C polysaccharide vaccine given at 11 to 12 months of age

Vaccine Group	6 wk (Prevaccination)			18 wk (Post-primary Series)		Before the 11–12-mo MenPS Vaccination			1 mo after the 11–12-mo MenPS Vaccination	
	n	GMT	95% CI	GMT	95% CI	n	GMT	95% CI	GMT	95% CI
Serogroup A										
MenD (1 μg)	32	7.6	4.9–11.8	261	152–450	22	17.9	8.1–39.4	747	393–1419
MenD (4 μg)	34	11.8	7.2–19.3	177	101–312	24	10.1	5.6–18	373	163–853
MenD (16 μg)	32	10.9	6.7–17.5	370	264–518	21	16.6	8.5–32.4	184	78.6–431
MenPS	32	14.7	8.5–25.4	7.0*	4.7–10.5	26	6.1	3.9–9.5	24.1	11–53
PRP-T	35	11.2	6.8–18.3	6.7*	4.3–10.5	NA	NA	NA	NA	NA
Serogroup C										
MenD (1 μg)	35	37.2	24–107	72.1†	45.5–114	25	5.4	3.7–3.8	264	104–677
MenD (4 μg)	34	50.8	24–107	189	128–278	27	4.6	3.6–5.6	287	96.2–858
MenD (16 μg)	32	61.6	29–131	325	204–517	20	6.9	4.7–11.4	178	63.6–499
MenPS	32	62.7	29–131	25.4*	14.4–44.6	26	4.1	3.9–4.3	14.4	7.9–26.1
PRP-T	36	45.3	21.9–133	7.3*	7.7–11.3	NA	NA	NA	NA	NA

* All MenD doses *vs.* the two control groups: P = 0.0001 (ANOVA).

† MenD 1 μg statistically different from both the 4- and 16-μg MenD groups: $P = 0.01$ (SNK).

NA, not applicable; ANOVA, analysis of variance, SNK, Student-Newman-Keuls test.

that for the MenPS vaccine. In contrast to the response to the primary series, concentrations of antibodies measured by ELISA against serogroup A or C after MenPS vaccination at 11 months of age were higher in infants initially immunized with MenD as compared with MenPS.

Goldschneider[17] and others[18] demonstrated that serum bactericidal assays measure functional antibody activity, whereas the standard ELISA[5,6] determines serum antibody concentrations even in the absence of detectable bactericidal activity.[11,19–21] The results of bactericidal assays reveal major differences between the responses to conjugate and polysaccharide vaccines. SBA values measured against both serogroup A and serogroup C after primary immunization are substantially greater among children who received MenD than among those who had initially received MenPS. After the injection at 11 months of age of a dose of polysaccharide vaccine, the GMT values for the SBA were 10 to 25 times greater for those infants previously immunized with MenD vaccine than for those who had received MenPS vaccine.

The 4-μg dose for both the A and C serogroups has been selected for further studies of MenD vaccine based on a balance of several factors, including the post-primary and post-11-month-MenPS immunogenicity results (ELISA and SBA) as well as the amount of diphtheria protein carrier that would be administered. For the response to serogroup C the observed difference in SBA, after the primary vaccination series, between the 1-μg dose and larger doses suggested that at least 4 μg would be needed for subsequent serogroup C formulations. Either a 1-μg or a 4-μg dose of serogroup A seemed reasonable based on SBA activity after the primary series and after polysaccharide vaccination at 11 months of age.

The dramatic antibody increase observed in this study after polysaccharide vaccination at 11 months in the MenD groups, in comparison with the MenPS group, supports the hypothesis that conjugate vaccines are able to induce immune memory at a young age. To evaluate this hypothesis further, an additional study is assessing, in children already primed with one to three doses of MenD vaccine during infancy, the immune response 7 to 10 days after MenPS vaccination at 24 months of age. Both total IgG and immunoglobulin classes, as well as SBA, elicited by this conjugate vaccine, in comparison with the plain polysaccharide vaccine, will be measured.

Some authors, like Goldschneider,[17] Gold,[22,23] Twumasi[10] and Leach,[24] have called attention to the possibility that multiple doses of serogroup C polysaccharide antigen administered in infancy may lead to immunologic hyporesponsiveness against *N. meningitidis* serogroup C. In these studies the post-second year of life polysaccharide vaccination titer measured for the serogroup C in the polysaccharide vaccine arm was not as elevated as might have been anticipated if the children had not been vaccinated. Others have since reported similar results both in children[25] and in adults.[26] On the other hand Peltola,[27] in a trial conducted in 1985 in Finland, called into question any suppressive effect when the first dose of group C polysaccharide is given at 3 months of age. The relevance of these observations is unknown.

In our study in Niger we observed a decline in SBA against serogroup C in the MenPS vaccine group from the pre- (62.7) to the post-primary series serum level (25.4); however, we have noted a higher SBA GMT value 1 month after the primary series of MenPS vaccine (25.4) in comparison with the PRP-T vaccine group (7.3). After boosting the SBA increased from 4 (pre-) to 14 (post-) in the MenPS vaccine group.

We have also observed in the MenPS vaccine group, with either immunogenicity assay, that 1 month after the 11-month MenPS vaccination the same proportion of children were seroprotected against both serogroups (58.6% against A and C by ELISA, 59.3% against A and C by SBA), suggesting that this MenPS-induced hyporesponsiveness is comparable for both serogroups. It would be useful to evaluate the meningococcal serogroup A and C immune status of the children who participated in this study several years later.

This study shows that the MenD vaccine is safe and that it is superior to currently licensed polysaccharide vaccines in terms of induction of functional antibodies in infants. The greater antibody GMT values, measured by ELISA or SBA, after MenPS vaccination in 11-month-old children already primed with the MenD vaccine, as compared with those primed with MenPS vaccine, suggest that the polysaccharide-protein conjugate vaccine is capable of inducing a measurable degree of immunologic memory at least up to 11 months of age.

Additional data on the number of MenD vaccine doses needed, as well as the duration of protective immunity, will help to determine a schedule by which infant immunization with meningococcal A + C conjugate vaccine can best provide long term protection against recurrent meningococcal meningitis epidemics in Africa.

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REFERENCES

1. Lapeyssonie L. La méningite cérébro-spinale en Afrique. Bull World Health Organ 1963;28(Suppl.):1–100.
2. Tikhomirov E, Santamaria M, Esteves K, Meningococcal disease: public health burden and control. World Health Stat Q 1997;50:170–7.
3. Campagne G, Schuchat A, Djibo S, Ousseini A, Cisse L, Chippaux JP. Epidemiology of bacterial meningitis in Niamey, Niger: 1981–1996. Bull World Health Organ 1999;77: 499–508.
4. Chippaux JP, Mounkaila A, Mounkeila N, Chaibou I, Djibo SL'épidémie de méningite cérébro-spinale du Niger de 1995. OCCGE Informations 1996;105:9–12.
5. Carlone GM, Frasch CE, Siber GR, et al. Multicenter comparison of levels of antibody to the *N. meningitidis* group A capsular polysaccharide measured by using an enzyme-linked immunosorbent assay. J Clin Microbiol 1992;30: 154–9.
6. Gheesling LL, Carlone GM, Pais LB, et al. Multicenter comparison of *N. meningitidis* serogroup C anti-capsular polysaccharide antibody levels measured by a standardized enzyme-linked immunosorbent assay. J Clin Microbiol 1994; 32:1475–82.
7. Maslanka SE, Gheesling LL, Libutti DE, et al. Standardization and a multilaboratory comparison of *N. meningitidis* serogroup A and C serum bactericidal assays: the Multilaboratory Study Group. Clin Diagn Lab Immunol 1997;4:156–67.
8. Lepow ML, Goldschneider I, Gold R, Randolph M, Gotschlich EC. Persistence of antibody following immunization of children with groups A and C meningococcal polysaccharide vaccines. Pediatrics 1977;60:673–80.
9. Amato Neto V, Finger H, Gotschlich EC, Feldman RA. Serologic response to serogroup C meningococcal vaccine in Brazilian preschool children. Rev Inst Med Trop Sao Paulo 1974;16:149–53.
10. Twumasi PA Jr., Kumah S, Leach A, et al. A trial of a group A plus group C meningococcal polysaccharide-protein conjugate vaccine in African infants. J Infect Dis 1995;171:632–8.
11. Leiberman JM, Chiu SS, Wong VK, et al. Safety and immunogenicity of a serogroups A/C *N. meningitidis* oligosaccharide-protein conjugate vaccine in young children: a randomized controlled trial. JAMA 1996;275:1499–503.
12. Fairley CK, Begg N, Borrow R, Fox AJ, Jones DM, Cartwright K. Conjugate meningococcal serogroup A and C vaccine: reactogenicity and immunogenicity in United Kingdom infants. J Infect. Dis 1996;174:1360–3.
13. Sackett DL. Bias in analytic research. J Chron Dis 1979;32: 51–63.
14. Gotschlich EC, Goldschneider I, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. J Exp Med 1969;129:1307–26.
15. Peltola H, Makela H, Kayhty H, et al. Clinical efficacy of meningococcus group A capsular polysaccharide vaccine in children three months to five years of age. N Engl J Med 1977;297:686–91.
16. Kayhty H, Jousimies-Somer H, Peltola H, Maketa PH. Antibody response to capsular polysacharides of groups A and C *N. meningitidis* and *H. influenzae* type b during bacteremic disease. J Infect Dis 1981;143:32–41.
17. Goldschneider I, Lepow ML, Gotschlich EC, Mauck FT, Bachl F, Randolph M. Immunogenicity of group A and group C meningococcal polysaccharides in human infants. J Infect Dis 1973;128:769–76.
18. Frasch CE, Cartwright K, eds. Meningococcal disease. In: Meningococcal vaccines: past, present, and future. Chichester, UK: Wiley, 1995:245–83.
19. Maslanka SE, Tappero JW, Plikaytis BD, et al. Age-dependent *N. meningitidis* serogroup C class-specific antibody concentrations and bactericidal titers in sera from young children from Montana immunized with a licensed polysaccharide vaccine. Infect Immun 1998;66:2453–9.
20. Schlesinger Y, Granoff DM, Avidity and bactericidal activity of antibody elicited by different *H. influenzae* type b conjugate vaccines: The Vaccine Study Group. JAMA 1992;267: 1489–94.
21. Granoff DM, Maslanka SE, Carlone GM, et al. A modified enzyme-linked immunosorbent assay for measurement antibody responses to meningococcal C polysaccharide that correlate with bactericidal responses. Clin Diagn Lab Immunol 1998;5:479–85.
22. Gold R, Lepow ML, Goldschneider I, Gotschlich EC. Immune response of human infants of polysaccharide vaccines of group A and C *N. meningitidis*. J Infect Dis 1977;136(Suppl): S31–5.
23. Gold R, Lepow ML, Goldschneider I, Draper TF, Gotschlich EC. Kinetics of antibody production to group A and group C meningococcal polysaccharide vaccines administered during the first six years of life: prospects for routine immunization of infants and children. J Infect Dis 1979;140:690–7.
24. Leach A, Twumasi PA, Kumah S, et al. Induction of immunologic memory in Gambian children by vaccination in infancy with a group A plus group C meningococcal polysaccharide-protein conjugate vaccine. J Infect Dis 1997;175:200–4.
25. MacDonald NE, Halperin SA, Law BJ, Forrest B, Danzig LE, Granoff DM. Induction of immunologic memory by conjugated *vs.* plain meningococcal C polysaccharide vaccine in toddlers: a randomized controlled trial. JAMA 1998;280: 1685–9.
26. Granoff DM, Gupta RK, Belshe RB, Anderson EL. Induction of immunologic refractoriness in adults by meningococcal C polysaccharide vaccination. J Infect Dis 1998;178:870–4.
27. Peltola H, Safary A, Kayhty HM, Karanko V, Andre FE. Evaluation of two tetravalent (ACYW135) meningococcal vaccines in infants and small children: a clinical study comparing immunogenicity of *O*-acetyl-negative and *O*-acetyl-positive group C polysaccharides. Pediatrics 1985;76: 91–6.