Clinical Evaluation of Group A and Group C Meningococcal Polysaccharide Vaccines in Infants

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ABSTRACT Group A and group C meningococcal polysaccharide vaccines were evaluated in infants. No significant local or systemic reactions were observed with 908 doses of vaccine given to 396 infants between 3 and 12 mo of age. The antibody response varied with the age of the infant, vaccine dose, molecular weight of vaccine, prior immunization with vaccine, and prior exposure to naturally occurring cross-reactive antigens. Only 7% of 3-mo-old infants had detectable antibody responses to primary immunization with 5–200 μg of A vaccine, presumably because of suppressive effects of high concentrations of maternal anti-A. More than 90% of 7- and 12-mo-old infants responded to A vaccine, achieving geometric mean anti-A concentrations of 0.38 and 0.98 μg/ml, respectively. The dose-response curve was flat between 10 and 200 μg of A vaccine. Geometric mean anti-A concentrations of 2.51 and 4.00 μg/ml were induced in 7- and 12-mo-old infants by booster injections of A vaccine.

Approximately 90% of 3-mo-old and 100% of 7- and 12-mo-old infants had detectable antibody responses to primary immunization with C vaccine. The 100-μg dose appeared to be optimal, resulting in geometric mean anti-C concentrations of 0.49, 1.55, and 2.64 μg/ml in 3-, 7-, and 12-mo-old infants, respectively. Significant booster responses were not observed with C vaccine. Indeed, except for the 10-μg dose, booster injections of C vaccine in 7- and 12-mo-old infants resulted in lower anti-C concentrations than did primary immunizations.

INTRODUCTION

Meningococcal disease is a major cause of mortality and morbidity among children throughout the world. The peak incidence in the United States occurs in infants 4–6 mo of age (1). Under endemic conditions, 50% of cases occur in children under 2, 10% in children 2–4, and 10% in children 5–9 yr of age (1–3). During epidemics, there is a shift in the age distribution so that 20% of cases occur in children under 2 and more than 50% in children 2–14 yr of age (4). Endemic disease is caused primarily by meningococcal serogroups B and C; epidemic disease by serogroup A (with the exception of group C epidemics among military recruits and the recently described group C epidemic in Brazil) (5). Most major outbreaks of meningococcal disease during the past 10 yr have been caused by sulfonamide-resistant strains of meningococci.

Vaccines, consisting of highly purified group A and group C meningococcal capsular polysaccharides, were developed at the Walter Reed Army Institute of Research (6) and have undergone extensive evaluation. The group C vaccine has been shown to be safe and effective in U. S. Army recruits (7, 8). Field trials underway in Sao Paulo, Brazil, indicate that the group C vaccine provides significant protection against disease in children over 2 yr of age (9). The efficacy of the group A vaccine in preventing disease has been demonstrated in Egyptian school children (10) and in a mixed-age population in the Sudan (11).

Relatively little is known about the safety, immunogenicity, and efficacy of the meningococcal polysaccharide vaccines in infants. Preliminary studies with small numbers of infants have indicated that their antibody responses to the vaccines are significantly lower than those of older children (12–16).

The present study has been designed to extend the knowledge about the safety and immunogenicity of the meningococcal vaccines in infants. The responses of infants to primary and booster immunizations with different doses of group A and group C vaccines adminis-

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TABLE I

Schedule of Immunizations of Infants with Groups A and C Meningococcal Polysaccharides

	Age								
	3 mo			7 mo			12 mo		
Group	Vaccine	Dose	n*	Vaccine	Dose	n	Vaccine	Dose	n
		μg			μg			μg	
1	A	5	24	NG‡	—	—	A§	10	23
2	A	25	21	A	25	20	C	25	19
3	A	25	19	C	25	18	A	25	17
4	A§	50	22	C	10	21	NG	—	—
5	A§	50	17	C	100	16	NG	—	—
6	A	100	21	A	100	21	C	100	20
7	A	100	17	C	100	16	A§	100	16
8	A§	100	19	C	10	18	NG	—	—
9	A	200	23	NG	—	—	C	10	21
10	NG	—	—	A§	25	24	NG	—	—
11	C	5	25	NG	—	—	A§	100	21
12	C	10	18	C	10	16	NG	—	—
13	C	25	21	C	25	21	A	25	21
14	C	25	24	A	25	21	C	25	21
15	C	50	21	A§	100	19	NG	—	—
16	C	100	24	C	100	24	A	100	22
17	C	100	29	A	100	28	C	100	27
18	C	200	27	NG	—	—	A§	10	25
Total of infants			372			383			253

* Number of infants immunized.

‡ NG, no vaccine given.

§ Lot A-7. All other groups received lot A-5.

tered at 3, 7, and 12 mo of age have been determined. Although the minimal concentration of antibody required for protection against disease remains unknown, we have attempted to determine the maximal response attainable in infancy with currently available vaccines.

METHODS

This project has been a cooperative effort involving the University of Connecticut Health Center, the Rockefeller University, the Health Department of the City of Danbury, Danbury Hospital, the physicians caring for children in the Danbury area, and the people of Danbury and its neighboring communities. The greater Danbury area, located in the southwestern part of Connecticut, has a population of approximately 137,000 people.

Subjects. 396 infants, aged 2–3 mo, were recruited between June 1972 and May 1973. An informational letter explaining the vaccine program was sent to parents of all infants born at Danbury Hospital during this period. Consent forms were signed at the first visit. The sample consisted primarily of infants from white, middle-class families.

Vaccine. Two lots of A vaccine[1] were used: lot A-5, prepared by the Department of Bacterial Disease, Walter Reed Army Institute of Research, Washington, D. C., and lot A-7, prepared by E. R. Squibb & Sons, Princeton, N. J. The molecular weights of lot A-5 and lot A-7 were 85,000 and 47,000, respectively (13). The group C meningococcal polysaccharide C vaccine (lot C-10) was prepared by E. R. Squibb & Sons. All lots had been tested in adults and older children before use in infants. The vaccines, lyophilized in 0.9% NaCl and stored at −20°C, were reconstituted with pyrogen-free water to a concentration of 100 μg/ml. Additional dilution to a concentration of 50 μg/ml was made with 0.9% NaCl for use in infants receiving a dose of 5 μg. Vaccine was administered subcutaneously with needle and syringe into the deltoid region of the arm.

Schedule of immunization. The ages of the infants at the times of immunization were: 2.8±1.0; 7.0±1.1, and 11.5 ±1.0 mo (mean±2 SD). The schedule of immunizations outlined in Table I was designed with several goals in mind: (*a*) to analyze the dose response of 3-mo-old infants to each vaccine over a dose range of 5–200 μg; (*b*) to determine the effect of age upon the response to primary immunization at 3, 7, and 12 mo of age; (*c*) to determine whether immunization at 3 mo of age leads to an anamnestic response at 7 or 12 mo of age; (*d*) to determine the effect of A vaccine on subsequent immunization with C vaccine and vice versa; and (*e*) to compare the response to two lots of A vaccine.

Serological methods. Venous blood samples were obtained immediately before and approximately 1 mo after each immunization. Sera could not be obtained from all

[1] *Abbreviations used in this paper:* A vaccine, group A meningococcal polysaccharide; anti-A, antibody to A vaccine; C vaccine, group C meningococcal polysaccharide; anti-C, antibody to C vaccine.

infants at every visit and sufficient quantities of each serum were not always available for testing against both A and C polysaccharides. However, all available sera were tested. The number of infants from whom sera were not available for testing are indicated in each of the tables giving the results of the antibody assays. Serum was separated and stored in 1-ml portions at −70°C. All sera were analyzed by the radioactive antigen binding test previously described (13, 17). Results of the antibody responses to immunization with A and C vaccine are expressed in two ways: as the geometric mean antibody concentrations (micrograms of antibody protein per milligram of serum) of each group of infants before and 1 mo after immunization, and as the seroconversion rate. Sera that had undetectable levels of anti-A or anti-C were assigned the lower limits of the antibody assay, namely 0.13 and 0.10 μg/ml, when calculating the geometric means. A seroconversion was defined as a change from nondetectable (less than 0.10 μg/ml for anti-C and less than 0.13 μg/ml for anti-A) to detectable concentrations, or a two-fold increase in detectable antibody protein concentration.

Bacteriological methods. High posterior pharyngeal swabs were obtained at each visit and plated on Thayer-Martin medium. Isolates were identified as *Neisseria meningitidis* and serogrouped by standard methods (18). No group A or C strains were recovered from any infants. Details of the carrier data will be the subject of a separate communication.

Reaction surveillance. Infants were observed for 15–30 min after immunization for immediate reactions. 48 h after immunization, mothers were contacted by telephone by the study secretary, who inquired about local and systemic reactions. Mother were also urged to report any reactions directly to their own pediatricians.

Statistical analysis. Geometric mean antibody concentrations were calculated for each group of infants, and differences between the means were tested for significance by comparing the 95% confidence limits of the means. Differences in seroconversion rates were analyzed by chi square test (19).

RESULTS

Compliance. 95% of the 396 infants received all their scheduled immunizations. 25 infants were lost to follow-up; 10 families moved from the Danbury area; 14 failed to return and could not be reached, and 1 was dropped from the study because of a large local reaction after the first immunization (see next paragraph).

TABLE II

Reactions to Groups A and C Meningococcal Polysaccharides in 396 Infants

	Age			
	3 mo	7 mo	12 mo	Total
Number of immunizations	372	283	253	908
Local reaction only, %*	3.9	3.9	3.7	3.9
Irritability only, %‡	6.1	7.2	2.5	5.6
Both, %	0.3	0.3	0.4	0.3
Total reactions, %	10.3	11.4	6.6	9.8

* Local reaction: erythema at injection site less than 1 cm in diameter and less than 24 h in duration except in one infant (see text).
‡ Irritability: unusual irritability or fussinesss of less than 18 h duration.

TABLE III

Heterologous Antibody Responses of Infants Receiving Primary or Booster Immunizations at 12 mo of Age with Groups A or C Meningococcal Polysaccharides

			Heterologous* antibody concentration			
			Before‡		After‡	
Vaccine	Dose	1st or 2nd injection	n§	GM‖	n	GM
	μg		μg/ml		μg/ml	
A	25	1st	15	0.15	15	0.15
		2nd	14	0.30	14	0.25
	100	1st	29	0.19	29	0.27
		2nd	13	0.49	13	0.45
C	25	1st	19	1.25	19	1.18
		2nd	18	0.31	16	0.53
	100	1st	19	0.93	19	1.23
		2nd	24	0.40	22	0.41

* Heterologous, anti-C concentration in infants receiving A vaccine or anti-A concentration in infants receiving C vaccine.
‡ Before immunization and 1 mo after immunization.
§ *n* is the number of infants from whom sera were available for testing.
‖ GM, geometric mean antibody concentration.

Reactions. Telephone contact was made within 48 h after immunization with 90% of the mothers. Total reactions observed or reported after the 908 immunizations given to this group of 396 infants (Table II) were 9.8%. One infant developed a 2 × 4 cm area of erythema associated with tenderness and irritability that persisted for 18 h after 100 μg of the group C vaccine. Local reactions consisting of small areas of erythema (less than 1 cm), lasting less than 24 h, were observed after 3.9% of the immunizations. Irritability or unusual fussiness occurred after 5.6% of the immunizations. Reaction rates did not correlate significantly with type or dose of vaccine nor with primary or booster injection. There was a trend toward fewer reactions with increasing age.

Cross-reactivity of A and C vaccines. The heterologous antibody responses of 12-mo-old infants receiving primary or booster immunization with A or C vaccine are given in Table III. No significant differences between pre- and postimmunization geometric mean antibody concentrations were observed. Similar lack of cross-reactivity of the A and C vaccine was seen after immunization of infants at 3 and 7 mo of age.

Antibody response to A vaccine

Natural acquisition of anti-A. At 3 mo of age, 60% of infants had detectable antibodies to the A polysaccharide, resulting in a geometric mean anti-A concentration of 0.36 μg/ml (Table IV). The anti-A concentration decreased to 0.20 μg/ml by 7 mo of age and ranged between 0.17 and 0.30 μg/ml at 12 mo of age.

Response to primary immunization. The response of infants to primary immunization with A vaccine at 3, 7,

TABLE IV

Antibody Response of Infants to Primary Immunization with Group A Meningococcal Polysaccharide

	Anti-A concentration before and after immunization at indicated age											
	3 mo				7 mo				12 mo			
	Pre*		Post*		Pre		Post		Pre		Post	
Dose	No tested‡	GM (95% limits)§	No tested	GM (95% limits)	No tested	GM (95% limits)	No tested	GM (95% limits)	No tested	GM (95% limits)	No tested	GM (95% limits)
μg		μg/ml		μg/ml		μg/ml		μg/ml		μg/ml		μg/ml
Control‖	66	0.36 (0.29–0.44)	71	0.30 (0.25–0.35)	12	0.20 (0.17–0.25)	13	0.21 (0.17–0.26)	—	—	—	—
5	16	0.32 (0.22–0.46)	16	0.25 (0.18–0.34)	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	22	0.30 (0.24–0.37)	22	0.96 (0.74–1.24)
25	26	0.34 (0.25–0.45)	29	0.32 (0.25–0.41)	35	0.20 (0.17–0.23)	39	0.37 (0.28–0.50)	14	0.17 (0.13–0.24)	14	0.77 (0.40–1.48)
50	27	0.25 (0.20–0.32)	32	0.21 (0.18–0.24)	—	—	—	—	—	—	—	—
100	34	0.46 (0.34–0.62)	43	0.40 (0.31–0.52)	37	0.18 (0.17–0.20)	37	0.40 (0.29–0.53)	27	0.23 (0.18–0.29)	27	1.07 (0.64–1.79)
200	11	0.24 (0.19–0.33)	11	0.25 (0.19–0.34)	—	—	—	—	—	—	—	—
					—	—	—	—	—	—	—	—
Not tested¶	69	—	52	—	20	—	16	—	26	—	26	—

* Pre, before immunization; post, 1 mo after immunization.

‡ No tested, number of infants from whom sera were available for testing.

§ GM (95% limits), geometric mean and 95% confidence limits of the mean.

‖ Control, 189 infants 3 mo of age and 61 infants 7 mo of age who had received C vaccine but had not received A vaccine. All such infants received A vaccine at 12 mo of age. Sufficient amounts of sera were available for testing against A polysaccharide from indicated number of infants.

¶ Not tested, the number of infants receiving primary immunization with A vaccine from whom sera were not obtained or were insufficient for testing.

and 12 mo of age is indicated in Table IV and Fig. 1. In a dose range from 5 to 200 μg, no significant increase in the geometric mean anti-A concentration was observed after immunization at 3 mo of age. Moreover, seroconversion rates of immunized infants were not significantly different from those of unimmunized infants (Table V: $\chi^2 = 0.601$, $P > 0.3$). However, primary immunization at 7 and 12 mo of age resulted in significant increases in geometric mean anti-A concentrations (Table IV). Primary immunization at 7 or 12 mo of age induced seroconversion rates of 60–75% at 7 mo and 85% at 12 mo of age, rates significantly higher than those seen at 3 mo of age (Table V: $\chi^2 = 99.81$, $P < 0.001$). In 7-mo-old infants, the seroconversion rates of immunized infants were significantly higher than that of the control infants ($\chi^2 = 11.21$, $P < 0.001$).

As illustrated in Fig. 1, for infants receiving 25 μg of A vaccine at 3, 7, or 12 mo of age, there was a trend for increasing response with age. Similar results were seen after the 100-μg dose (Table IV). Seroconversion rates were also higher at 12 than at 7 mo of age, although the differences were not statistically significant (Table V: $\chi^2 = 3.029$, $P > 0.05$).

At 3, 7, and 12 mo of age the dose-response curves were flat. There were no significant differences between the postimmunization geometric mean anti-A concen-

TABLE V

Seroconversion Rate of Infants after Primary Immunization with Group A Meningococcal Polysaccharide

Age	Vaccine dose	No infants	No tested‡	No seroconversions§
mo	μg			%
3	0	189	65	3 (4.6)
	5	24	16	0 (0.0)
	25	40	26	2 (7.7)
	50	39	27	1 (3.7)
	100	57	34	6 (17.6)
	200	23	16	0 (0.0)
7	0	16	12	2 (16.7)
	25	45	35	21 (60.0)
	100	47	36	27 (75.0)
12	10	25	13	13 (100)
	25	21	14	12 (85.7)
	100	22	13	11 (84.6)

* Age of primary immunization.

‡ No tested, number of paired pre- and post-immunization sera available for testing.

§ Seroconversion, change from nondetectable anti-A concentration (less than 0.13 μg/ml) before immunization to detectable concentration after immunization or a twofold rise in detectable anti-A concentration.

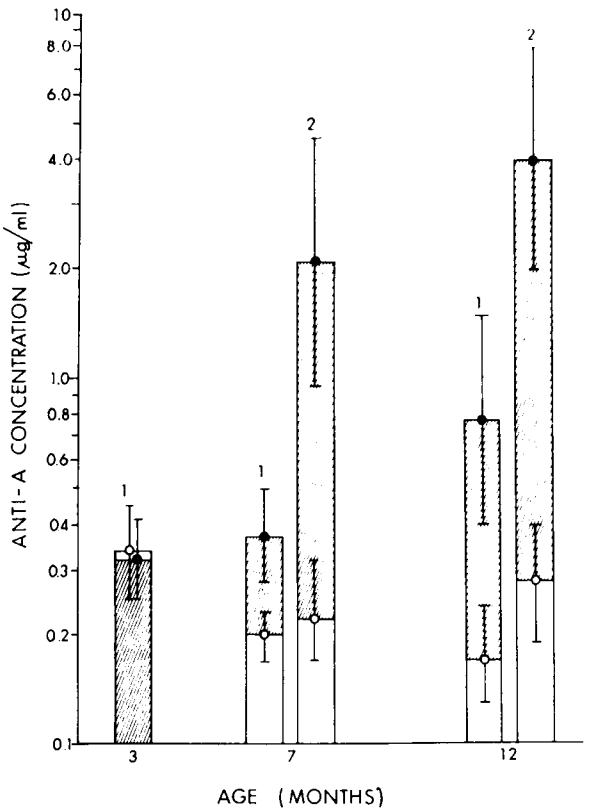

FIGURE 1 Geometric mean anti-A concentration after primary (1) and booster (2) immunization of infants with 25 μg of group A meningococcal polysaccharide. Unshaded and shaded vertical bars indicate geometric mean and 95% confidence limits of the mean, before and 1 month after immunization, respectively.

trations induced or the seroconversion rates with any of the doses of A vaccine employed within each age group.

Response to booster immunization. Significant anamnestic responses were observed when infants previously immunized at 3 mo of age were given boosters of A vaccine at 7 or 12 mo of age (Table VI and Fig. 1). Thus, for example, the geometric mean anti-A concentrations after booster immunization with 25 μg of A vaccine were 2.09 and 4.00 μg/ml at 7 and 12 mo, respectively (Table VI), significantly higher than the responses observed after primary injections at the same ages, 0.37 and 0.77 μg/ml. Furthermore, the seroconversion rates after boosters were also higher than after primary immunization (compare Tables V and VII) although differences were statistically significant only at 7 mo ($\chi^2 = 6.784$, $P < 0.02$). The magnitude of the booster response tended to increase with age, although the differences were not statistically significant.

No significant differences between responses to booster immunization with 25- or 100-μg doses were observed at 7 mo of age either in terms of geometric mean anti-A concentration (Table VI) or seroconversion rate (Table VII). However, infants boosted at 12 mo of age with 100 μg of lot A-7 (mol wt 47,000) had significantly lower responses than infants boosted with 25 μg of lot A-5 (mol wt 85,000), achieving geometric mean anti-A concentrations of 1.18 and 4.00 μg/ml, respectively. No such differences between responses to lots A-5 and A-7 were observed after primary immunization of infants: e.g. the response to 10 μg of lot A-7 was the same as that to 25 or 100 μg of A-5 at 12 mo of age (Table IV).

Correlation of anti-A concentration before and after immunization. There was a significant, positive correlation between the presence or absence of detectable anti-A in the 7-and 12-mo-old infants and the magnitude of the subsequent antibody response to primary immunization with the A vaccine (Table VIII). A similar trend was noted after booster immunizations at 7 mo, although the differences were not statistically significant.

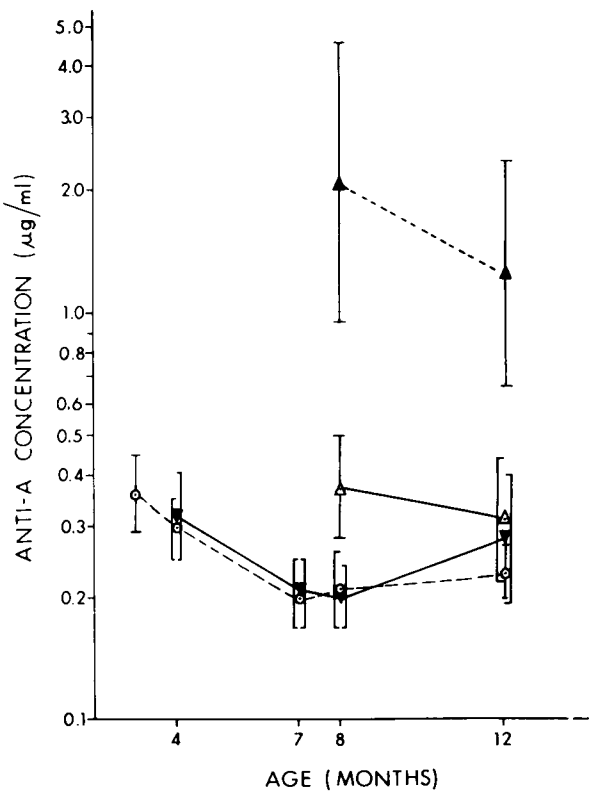

FIGURE 2 Persistence of anti-A antibody in infants after primary or booster immunization at 3 or 7 mo of age with 25 μg of group A meningococcal polysaccharide (○---○, unimmunized; ▼——▼, primary at 3 mo; △——△, primary at 7 mo; ▲---▲, booster at 7 mo; vertical lines indicate geometric mean and 95% confidence limits of the mean).

TABLE VI

Antibody Responses of Infants to Booster Immunization with Group A Meningococcal Polysaccharide at 7 or 12 mo of Age after Primary Immunization at 3 mo of Age

Vaccine dose	Anti-A concentration before and 1 mo after immunization at indicated age							
	7 mo				12 mo			
	Before		After		Before		After	
	No tested§	GM (95% limits)‖	No tested	GM (95% limits)	No tested	GM (95% limits)	No tested	GM (95% limits)
μg		μg/ml		μg/ml		μg/ml		μg/ml
10	—	—	—	—	15	0.18 (0.17–0.20)	17	0.46 (0.29–0.73)
25	16	0.22 (0.15–0.32)	16	2.09 (0.95–4.58)	14	0.28 (0.19–0.40)	14	4.00 (1.98–8.09)
100	12	0.21 (0.15–0.29)	12	3.20 (1.81–5.65)	13	0.25 (0.17–0.37)	13	1.18 (0.78–1.74)
Not tested¶	13		13		14		12	

* Vaccine dose: booster dose at 7 or 12 mo of age was the same as the primary dose at 3 mo of age for all infants except those receiving 5 μg at 3 and 10 μg at 12 mo of age (see Table I). All groups received lot A-5 except those given 10- and 100-μg boosters at 12 mo of age. These received lot A-7.

‡ No tested, number of infants from whom sera were available for testing.

§ GM (95% limits), geometric mean and 95% confidence limits of the mean.

‖ Not tested: the number of infants receiving booster immunizations of A vaccine from whom sera were not obtained or were insufficient for testing.

Persistence of anti-A response. The anti-A concentration of infants immunized at 3 mo of age was not significantly higher at 7 mo of age than that of unimmunized infants (Table IV). 85% (23 out of 27) of the immunized infants had detectable anti-A at 12 mo of age, compared to 59% (16 out of 27) of those not previously immunized ($\chi^2 = 4.53$, $P < 0.05$). Fig. 2 illustrates the persistence of anti-A antibody levels after immunization of infants at 3 or 7 mo of age with 25 μg of A vaccine. The anti-A concentration of infants receiving primary immunization at 7 mo of age showed an insignificant decline, from the peak of 0.37 μg/ml to 0.31 μg/ml by 12 mo of age. The anti-A concentration of infants given boosters at 7 mo of age declined by more than 50% from peak values by 12 mo of age (from 2.09 to 1.25 μg/ml), but this latter anti-A con-

TABLE VII

Seroconversion Rates of Infants after Booster Immunization with Group A Meningococcal Polysaccharide

Age	Vaccine dose	No infants	No tested§	No seroconversions‖
mo	μg			%
7	25	20	16	14 (87.5)
	100	21	12	12 (100)
12	10	23	15	10 (66.7)
	25	17	14	14 (100)
	100	16	13	12 (92.3)

* Age at time of booster immunization.

‡ Vaccine dose: dose of booster, which was the same as primary dose given at 3 mo of age, except for infants receiving 5 μg at 3 and 10 μg at 12 mo of age (see Table I). All groups received lot A-5 except those receiving 5 μg at 3 and 10 μg at 12 mo of age. These received lot A-7.

§ No. tested, number of paired pre- and 1 mo postimmunization sera available for testing.

‖ Seroconversion = Change from nondetectable anti-A concentration (less than 0.13 μg/ml) before immunization to detectable concentration after immunization or a twofold rise in detectable anti-A concentration.

TABLE VIII

Correlation between Preimmunization Anti-A Concentration and Response to Group A Meningococcal Vaccine

Age	Injection	Anti-A concentration at indicated preimmunization concentration			
		Under 0.13 μg/ml		Over 0.13 μg/ml	
		No tested	GM (95% limits)*	No tested	GM (95% limits)*
mo			μg/ml		μg/ml
7	1st	22	0.24 (0.18–0.31)	18	0.64 (0.37–1.11)
7	2nd	9	1.59 (0.73–3.47)	19	3.11 (1.64–5.89)
12	1st	12	0.30 (0.20–0.44)	16	1.63 (0.98–2.70)
12	2nd	2	2.48 (. . . . . .)	12	4.34 (1.98–9.51)

* GM (95% limits), geometric mean and 95% confidence limits of the mean.

TABLE IX

Antibody Responses of Infants to Primary Immunization with Group C Meningococcal Polysaccharide Anti-C Concentration before and after Immunization at Indicated Age

Vaccine dose	3 mo				7 mo				12 mo			
	Pre*		Post*		Pre		Post		Pre		Post	
	No tested‡	GM (95% limits)§	No tested	GM (95% limits)	No tested	GM (95% limits)	No tested	GM (95% limits)	No tested	GM (95% limits)	No tested	GM (95% limits)
μg		μg/ml		μg/ml		μg/ml		μg/ml		μg/ml		μg/ml
Control‖	76	0.11 (<0.10–0.14)	88	0.11 (<0.10–0.12)	20	0.14 (<0.10–0.18)	20	0.19 (0.15–0.24)	15	<0.10 (— – – –)	17	0.13 (<0.10–0.16)
5	14	0.13 (<0.10–0.28)	14	0.17 (<0.10–0.33)	—	—	—	—	—	—	—	—
10	10	0.11 (<0.10–0.15)	13	0.25 (0.12–0.54)	26	0.10 (<0.10–0.11)	28	0.73 (0.56–0.96)	20	0.13 (<0.10–0.19)	20	1.08 (0.71–1.66)
25	19	0.11 (<0.10–0.13)	17	0.42 (0.28–0.62)	15	0.10 (<0.10–0.11)	14	0.80 (0.67–0.95)	19	0.12 (<0.10–0.16)	19	1.69 (1.18–2.42)
50	17	0.11 (<0.10–0.12)	19	0.28 (0.19–0.42)	—	—	—	—	—	—	—	—
100	25	0.14 (<0.10–0.18)	25	0.49 (0.34–0.71)	26	0.12 (<0.10–0.14)	30	1.41 (0.95–2.10)	10	0.10 (<0.10–0.11)	19	2.62 (1.78–3.87)
200	15	0.10 (<0.10–0.11)	15	0.26 (0.15–0.46)	—	—	—	—	—	—	—	—
Not tested¶	89		85		22		17		2		2	

* Preimmunization; Post, 1 mo after immunization.

‡ No tested, number of infants from whom sera were available for testing.

§ GM (95% limits), geometric mean and 95% confidence limits of the mean.

‖ Control, 183 3-mo-old infants, 65 7-mo-old infants and 21 12-mo-old infants who had received A vaccine but not C vaccine. Sufficient amounts of sera were available for testing against C polysaccharide from the indicated numbers of infants at each age.

¶ Not tested, the number of infants receiving primary immunization with C vaccine from whom sera were not obtained or were insufficient in amount for testing.

centration was still significantly higher than that of infants who had received primary immunization at 3 or 7 mo of age. No differences in persistence of anti-A levels were observed after doses of 25 or 100 μg of A vaccine.

Antibody response to group C polysaccharide

Natural acquisition of anti-C. Only 9.6% of infants had detectable anti-C at 3 mo of age (geometric mean anti-C at least 0.10 μg/ml). There was no significant increase in the percentage of infants with detectable anti-C nor in the geometric mean anti-C concentration between 3 and 12 mo of age (Table IX).

Response to primary immunization. There was an almost linear increase in the geometric mean anti-C concentration induced by primary immunization with C vaccine at 3, 7, and 12 mo of age (Table IX and Fig. 3). The seroconversion rates at 3, 7, and 12 mo of age are indicated in Table X. Even at 3 mo of age, over 80% of infants had significant rises in anti-C concentrations, except for those receiving the 5- or 200-μg doses of C vaccine. At each age, the seroconversion rates were significantly higher in immunized than in nonimmunized infants (3 mo, $\chi^2 = 98.27$, $P < 0.001$; 7 mo, $\chi^2 = 43.50$; $P < 0.001$; 12 mo, $\chi^2 = 21.34$, $P < 0.001$).

Although the difference between the antibody responses to primary immunization with 10, 25, or 100 μg were not significant ($P > 0.05$), there was a consistent trend toward higher anti-C concentration after larger doses of vaccines at each age. The 5-μg dose of C vaccine at 3 mo of age was inferior to larger doses; the anti-C concentration was significantly lower and the seroconversion rate was only 36%. The 200-μg dose of C vaccine elicited an anti-C response no better than that seen after lower doses.

Response to booster immunization. When infants previously immunized with C vaccine were given boosters at 7 or 12 mo of age, no anamnestic responses were seen (Compare Tables IX and XI). In fact, the mean anti-C concentrations after booster immunizations with 25- or 100-μg doses were significantly lower ($P < 0.05$) than those after primary immunization. Moreover, only 43% (12 of 28) of infants receiving the 25- and 100-μg booster at 7 mo and 76% (29 of 38) at 12 mo of age showed rises in anti-C concentration, rates significantly lower than those seen after primary immunization at these ages (compare Tables X and XII: 7 mo, $\chi^2 = 39.38$, $P < 0.001$; 12 mo, $\chi^2 = 6.19$, $P < 0.02$). This phenomenon is illustrated for the 25-μg dose of C vaccine in Fig. 3.

A notable exception to the depressed response after

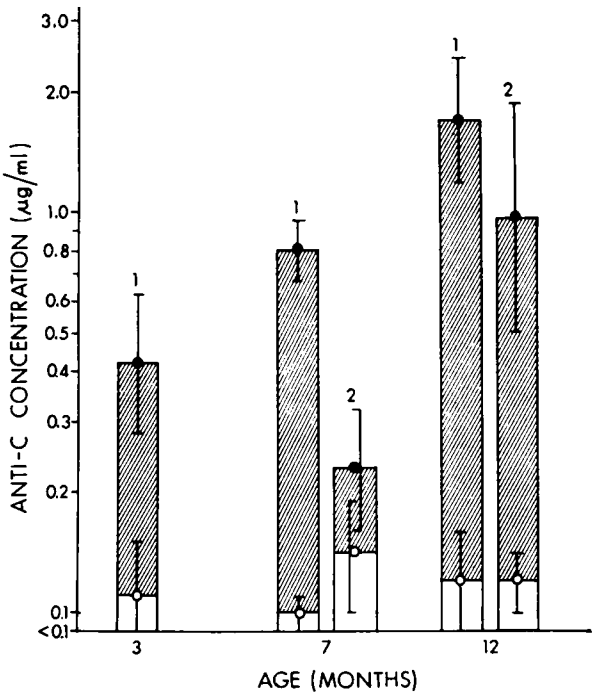

FIGURE 3 Geometric mean anti-C concentration after primary (1) and booster (2) immunization of infants with 25 μg of group C meningococcal polysaccharide. Unshaded and shaded vertical bars, geometric mean and 95% confidence limits of the mean, before and 1 mo after immunization, respectively.

boosters was observed in infants who received 10 μg of C vaccine at 3 and 7 mo of age. Neither a depression nor an elevation of the anti-C response was observed. Rather the geometric mean anti-C concentrations after primary and booster immunizations at 7 mo of age with 10 μg were similar, 0.74 and 0.77 μg/ml, respectively (Tables IX and XI), as were the seroconversion rates of 80 and 92%. (Tables X and XII).

Correlation of anti-C concentration before and after immunization. There was no correlation between the presence or absence of detectable anti-C at 7 or 12 mo of age and the magnitude of the antibody response to primary or booster immunization with C vaccine.

Persistence of anti-C response. The persistence of anti-C concentration of infants immunized with 25 μg of C vaccine is illustrated in Fig. 4. Similar results were observed with doses of 100 μg. The geometric mean anti-C concentration of infants immunized at 3 mo of age declined from a peak of 0.42 μg/ml 1 mo after immunization to 0.17 μg/ml by 7 mo and to 0.12 μg/ml by 12 mo of age. Moreover, 69% (31 or 45) of the infants immunized at 3 mo of age had detectable anti-C at 12 mo of age, compared to only 21% (8 of 37) of the unimmunized infants ($\chi^2 = 18.88$; $P < 0.001$). The rate of decline of anti-C concentration from the post-immunization peak in infants receiving primary immunization at 7 mo of age was similar to that of infants immunized at 3 mo of age. There was no significant difference in the anti-C concentration at 12 mo of age between the infants who had received a single dose of C vaccine at 3 mo of age and those given two doses at 3 and 7 mo of age.

DISCUSSION

Results of preliminary studies of the groups A and C meningococcal polysaccharides in infants (15) have been confirmed by the present study. The antibody responses of infants to immunization with A and C vaccines depend on a number of factors including the age of the infant, the dose of antigen, the molecular weight of the antigen, the number of doses of antigen, and the prior experience of the infant with naturally occurring antigens cross-reactive with the meningococcal polysaccharides. Each of these factors will be discussed briefly.

Age. No response to A vaccine was detected in infants immunized at 3 mo of age. This lack of response was most likely due to the suppression of endogenous

TABLE X

Seroconversion Rate of Infants after Primary Immunization with Group C Meningococcal Polysaccharide

Age	Vaccine dose	No infants	No tested‡	No seroconversion§
mo	μg			%
3	0	183	85	6 (7.1)
	5	25	14	5 (35.7)
	10	18	10	8 (80.0)
	25	45	19	18 (94.7)
	50	21	17	15 (88.2)
	100	53	25	23 (92.0)
	200	27	15	11 (73.3)
7	0	24	20	7 (35.0)
	10	39	25	25 (100)
	25	18	13	13 (100)
	100	32	24	24 (100)
12	0	21	15	5 (33.3)
	10	21	20	17 (85.0)
	25	19	13	11 (84.6)
	100	20	18	18 (100)

* Age of primary immunization.

‡ Number of paired before and 1 mo after immunization sera available for testing.

§ Seroconversion, change from nondetectable anti-C concentration (less than 0.10 μg/ml) before immunization to detectable concentration after immunization or a twofold rise in detectable anti-C concentration,

TABLE XI

Antibody Responses of Infants to Booster Immunization with Group C Meningococcal Polysaccharide at 7 or 12 mo of Age after Primary Immunization at 3 mo of Age

Vaccine dose*	Anti-C concentration before and after immunization at indicated age							
	7 mo				12 mo			
	Before		After		Before		After	
	No tested§	GM (95% limits)‖	No tested	GM (95% limits)	No tested	GM (95% limits)	No tested	GM (95% limits)
μg		μg/ml		μg/ml		μg/ml		μg/ml
10	12	0.18 (0.11–0.29)	13	0.77 (0.53–1.11)	—	—	—	—
25	16	0.14 (0.10–0.19)	18	0.23 (0.16–0.32)	19	0.12 (0.10–0.14)	17	0.96 (0.50–1.86)
100	16	0.15 (0.11–0.20)	20	0.32 (0.22–0.48)	25	0.20 (0.14–0.29)	23	0.76 (0.50–1.14)
Not tested¶	17	—	10	—	4	—	8	—

* Vaccine dose, booster dose at 7 or 12 mo of age was the same as primary dose at 3 mo of age.
‡ No tested, number of infants from whom sera were available for testing.
§ GM (95%), geometric mean and 95% confidence limits of the mean.
‖ Not tested, number of infants receiving booster immunizations of C vaccine from whom sera were not obtained or were insufficient in amount for testing.

antibody production by the high levels of maternal anti-A present in most infants. The origin of the maternal anti-A is not likely the result of exposure to group A meningococci, because such strains have rarely been found in healthy carriers or in cases of meningococcal disease since the last major group A epidemic in the United States in 1945. The source of maternal antigenic stimulus may be organisms with polysaccharide capsules cross-reactive with group A meningococcal polysaccharide (see below). After 3 mo of age, there was a trend of increasing antibody responses to A vaccine with increasing age throughout infancy. This trend has been found to extend through childhood (12, 14) and into early adulthood (20, 21). A similar age-

TABLE XII

Seroconversion Rates of Infants after Booster Immunization with Group C Meningococcal Polysaccharide

Age	Vaccine dose‡	No infants	No tested§	Seroconversions‖	
mo	μg			n	%
7	10	16	12	11	(91.7)
	25	21	14	5	(53.7)
	100	24	14	7	(50.0)
12	25	21	17	15	(88.2)
	100	27	21	14	(66.7)

* Age at time of booster immunization.
‡ Dose of booster, the same as primary dose at 3 mo of age.
§ Number of paired before and 1 mo after immunization sera available for testing.
‖ Change from nondetectable anti-C concentration (less than 0.10 μg/ml) before immunization to detectable concentration after immunization or a twofold rise in anti-C concentration.

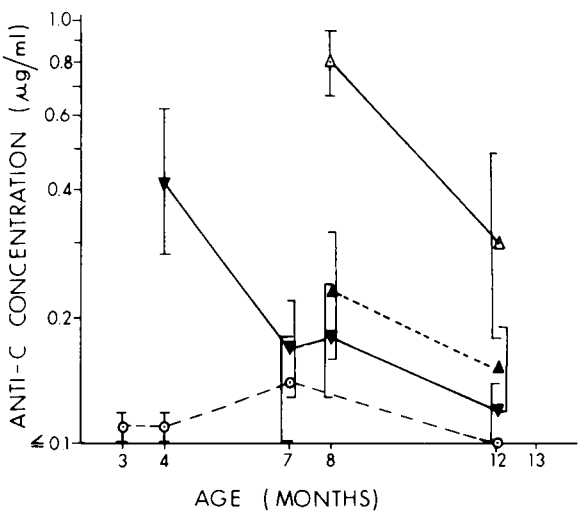

FIGURE 4 Persistence of anti-C antibody in infants after primary or booster immunization at 3 or 7 mo of age with 25 μg of group C meningococcal polysaccharide (○- - -○, unimmunized; ▼——▼, primary at 3 mo; △——△, primary at 7 mo; ▲- - -▲, booster at 7 mo; vertical lines are geometric mean and 95% confidence limits of the mean).

dependent relationship was seen with antibody production to the C vaccine in this and other studies (12, 15, 16). Unlike the situation with the A vaccine, 3-mo-old infants had very low levels of maternal anti-C and produced a detectable antibody response to the C vaccine.

Dose of antigen. Our results in infants confirm the observation in children and adults that 5 μg of C vaccine is inadequate and that 100 μg is superior to 25 μg (14, 21). The 200 μg of C vaccine was no more effective than lower doses at 3 mo of age. No clear-cut difference was noted between the responses to 25- and 100-μg doses of A vaccine at 7 and 12 mo of age. Further work is needed to determine the optimal dose of A vaccine in infancy.

Molecular weight of antigen. The immunogenicity of the A vaccine has been shown to be directly related to the average molecular weight of the polysaccharide. Gotschlich et al. found that lot A-5 (mol wt 84,000) was more immunogenic than lot A-7 (mol wt 47,000) in adults and that the molecular weight of other lots of A vaccine was a major determinant of the response in infants and children (13). In the present study, the difference in molecular weights of lots A-5 and A-7 may account for the significantly smaller response of 12-mo-old infants who received 100-μg boosters of lot A-7 compared to those who received 25 μg of lot A-5. No differences in the response to primary immunization with the two lots of A vaccine were noted.

Number of doses of antigen. There was a significant and unexpected difference in the antibody responses of infants to booster immunization with the A and C vaccines. Results of experiments with purified polysaccharide antigens in laboratory animals and in adult human beings (22) suggested that true anamnestic responses would not occur with the meningococcal polysaccharides. Yet 7- and 12-mo-old infants showed significant booster responses to the A vaccine. In contrast, booster injections of 25 or 100 μg of C vaccine at 7 and 12 mo of age resulted in *decreased* antibody responses as compared to primary immunizations at these ages. By 18 mo of age, there was no difference in the anti-C responses to primary and booster injections of C vaccine (12, 14).

The mechanism underlying the reduced antibody response of young infants to booster immunization with the C vaccine is not known. One possibility is that low levels of persisting antibody formed during the primary immune response interfere with the booster response by complexing with the second dose of antigen. However, this hypothesis is weakened by the observations that depressed antibody responses did no occur in infants who received two 10-μg doses of C vaccine and that there was no correlation between preexisting anti-C concentration and subsequent antibody responses to booster immunizations. Whatever the explanation for this phenomenon, it is reassuring to note that primary immunization of infants with the C vaccine during epidemic conditions, while not yet demonstrated to be protective, did not render such infants more susceptible to meningococcal disease (9).

Role of cross-reactive antigens. A possible explanation for the age-related variation in response to primary and booster immunizations with the A and C vaccines may be found in the age-related experiences of infants and children with naturally occurring antigens that cross-react with the meningococcal polysaccharides. Thus, the response to primary and booster immunizations with the A vaccine at 7 and 12 mo of age showed significant correlation with preimmunization levels of anti-A, suggesting that natural immunization had "primed" the immune system. Such priming is generally delayed with respect to the C polysaccharide until 2 yr of age, after which booster responses to the C vaccine occur. These findings are consistent with current immunological theory (22), which suggests that "thymus-independent" antigens (e.g. high molecular weight polysaccharides) do not elicit true booster responses unless "memory" B-cells have first been formed as a result of cooperative interaction of T-cells (thymus-derived lymphocytes) and B-cells (antibody-forming cell precursors). Such T- and B-cell cooperation usually occurs in response to "thymus-dependent" antigens (e.g. protein-polysaccharide complexes). Our hypothesis is, therefore, that naturally occurring antigens that cross-react with the A or C meningococcal antigens and that give rise to the age-related increases in naturally acquired anti-A and anti-C antibodies are thymus-dependent antigens, whereas the purified meningococcal polysaccharides are thymus-independent antigens.

The mechanism of natural immunization of infants to A polysaccharide remains to be determined. Enteric and genus *Bacillus* organisms have been found with capsular polysaccharides immunochemically identical or very closely related to the capsular polysaccharide of serogroups A, B, and C meningococci (23–26). Differences in prevalence and age of acquisition of such organisms may be responsible for the observed differences in age of naturally acquired anti-A and anti-C antibodies.

Efficacy of meningococcal vaccines. It remains to be determined whether the antibody levels achieved by infants in response to the meningococcal vaccines will be sufficient to protect them against meningococcal disease. Both naturally acquired and vaccine-induced antibody to the A and C vaccines have been shown to protect adults against disease (8, 9). In addition, the A vaccine has been demonstrated to be 100% protective in Egyptian school children 6–15 yr of age, and such

protection has lasted for at least 2 yr (10). The mol wt of the A vaccine used in Egypt was 170,000, approximately twice as large as lot A-5 (10). While the minimal protective concentration of anti-A is not yet established, geometric mean anti-A concentrations of 9–15 μg/ml were induced in the Egyptian children with a single dose of 50 μg of vaccine. Similar anti-A concentrations have been achieved after primary immunization with lot A-5 of children over 2 yr of age in the United States (12, 14). In the present study, anti-A concentrations of 2–4 μg/ml were induced by booster immunization of 7- and 12-mo-old infants. A field trial to determine the efficacy of these levels of anti-A in protecting infants and young children against epidemic group A meningococcal disease is currently in progress in Finland. In addition, we are evaluating the antibody response of infants to three doses of A vaccine given sequentially at 2, 4, and 6 mo of age.

The C vaccine has been demonstrated to be 75% protective in Brazilian children 24–35 mo of age under epidemic conditions (9). However, there was no protection apparent in infants 6–23 mo of age. Although the minimal protective level of anti-C has not yet been determined, the antibody responses of the Brazilian infants were considerably smaller than those of Danbury infants. Thus, it is possible that protective anti-C concentrations may be achieved at an earlier age in infants from white, middle-class American families. Nonetheless, it is clear that the presently available C vaccines are not likely to be protective in very young infants and that further research is required to learn how to enhance the anti-C response at these ages. This is not to say that the presently available vaccine is without merit, since it should be effective in controlling epidemics of serogroup C meningococcal disease, during which approximately 75% of cases occur in children more than 2 yr of age and in adults.[2]

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[2] The meningococcal group C vaccine has been licensed by the Bureau of Biologics, U. S. Food and Drug Administration, for use in adults during epidemic conditions.

REFERENCES

1. Fraser, D. W., C. P. Darby, R. E. Koehler, C. F. Jacobs, and R. A. Feldman. 1973. Risk factors in bacterial meningitis: Charleston County, South Carolina. *J. Infect. Dis.* **127**: 271–277.
2. Fraser, D. W., C. E. Henke, and R. A. Feldman. 1973. Changing patterns of bacterial meningitis in Olmstead County, Minnesota, 1935–1970. *J. Infect. Dis.* **128**: 300–307.
3. Floyd, R. F., C. F. Federspiel, and W. Schaffner. 1974. Bacterial meningitis in urban and rural Tennessee. *Am. J. Epidemiol.* **99**: 395–407.
4. McCormick, J. B., R. E. Weaver, C. Thornsberry, and R. A. Feldman. 1974. Trends in disease caused by *Neisseria meningitidis:* 1972 and 1973. *J. Infect. Dis.* **130**: 212–214.
5. de Morais, J. S., R. S. Munford, J. B. Risi, E. Antezana, and R. A. Feldman. 1974. Epidemic disease due to serogroup C *Neisseria meningitidis* in Sao Paulo, Brazil. *J. Infect. Dis.* **129**: 568–571.
6. Gotschlich, E. C., T. Y. Liu. and M. S. Artenstein. 1969. Human immunity to the meningococcus. III. Preparation and immunochemical properties of the group A, group B., and group C meningococcal polysaccharides. *J. Exp. Med.* **129**: 1349–1365.
7. Artenstein, M. S., R. Gold, J. G. Zimmerly, F. A. Wyle, H. Schneider, and C. Harkins. 1970. Prevention of meningococcal disease by group C polysaccharide vaccine. *N. Engl. J. Med.* **282**: 417–420.
8. Gold, R., and M. S. Artenstein. 1971. Meningococcal infections. 2. Field trial of group C meningococcal polysaccharide vaccine in 1969–70. *Bull. W.H.O.* **45**: 279–282.
9. Taunay, A. de E., P. A. Galvao, J. S. de Morais, E. C. Gotschlich, and R. A. Feldman. 1974. Disease prevention by meningococcal serogroup C polysaccharide vaccine in preschool children: results after eleven months in Sao Paulo, Brazil. *Pediatr. Res.* **8**: 429. (Abstr.).
10. Wahdan, M. H., F. Rizh, A. M. El-Akkad, A. A. El Ghoroury, R. Hablas, N. I. Girgis, A. Amer, W. Boctar, J. E. Sippel, E. C. Gotschlich, R. Triau, W. R. Sanborn, and B. Cvjetanović. 1973. A controlled field trial of a serogroup A meningococcal polysaccharide vaccine. *Bull. W.H.O.* **48**: 667–673.
11. Erwa, H. H., M. A. Haseeb, A. A. Idris, L. Lapeyssonnie, W. R. Sanborn, and J. E. Sippel. 1973. A serogroup A meningococcal polysaccharide vaccine. Studies in the Sudan to combat cerebrospinal meningitis caused by *Neisseria meningitidis* group A. *Bull. W.H.O.* **49**: 301–305.
12. Goldschneider, I., M. L. Lepow, and E. C. Gotschlich. 1972. Immunogenicity of the group A and group C meningococcal polysaccharides in children. *J. Infect. Dis.* **125**: 509–519.
13. Gotschlich, E. C., M. Rey, R. Triau, and K. J. Sparks. 1972. Quantitative determination of the human immune response to immunization with meningococcal vaccines. *J. Clin. Invest.* **51**: 89–96.
14. Monto, A. S., B. L. Brandt, and M. S. Artenstein. 1973. Response of children to *Neisseria meningitidis* polysaccharide vaccines. *J. Infect. Dis.* **127**: 394–400.
15. Goldschneider, I., M. L. Lepow, E. C. Gotschlich, F. T. Mauch, F. Bachl, and M. Randolph. 1973. Immunogenicity of group A and group C meningococcal poly-

saccharides in human infants. *J. Infect. Dis.* **128**: 769–776.
16. Amato Neto, V., E. Finger, E. C. Gotschlich, R. A. Feldman, C. A. DeAvilo, S. R. Konichi, and W. C. Lacis. 1974. Serologic response to serogroup C meningococcal polysaccharide in Brazilian preschool children. *Rev. Inst. Med. Trop. Sao, Paulo.* **16**: 149–153.
17. Gotschlich, E. C. 1971. A simplification of the radioactive antigen-binding test by a double label technique. *J. Immunol.* **107**: 910–911.
18. Goldschneider, I., E. C. Gotschlich, and M. S. Artenstein. 1969. Human immunity to the meningococcus. I. The role of humoral antibodies. *J. Exp. Med.* **129**: 1307–1326.
19. Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Company, Publishers, San Francisco, Calif. 145–150 and 585–593.
20. Gotschlich, E. C., I. Goldschneider, and M. S. Artenstein. 1969. Human immunity to the meningococcus. IV. Immunogenicity of group A and group C meningococcal polysaccharides in human volunteers. *J. Exp. Med.* **129**: 1367–1384.
21. Artenstein, M. S., R. Gold, J. G. Zimmerly, F. A. Wyle, W. C. Branche, Jr., and C. Harkins. 1970. Cutaneous reactions and antibody response to meningococcal group C polysaccharide vaccines in man. *J. Infect. Dis.* **121**: 372–377.
22. Katz, D. H., and B. Benacerraf. 1972. The regulatory influence of activated T cells on B cell responses to antigen. *Adv. Immunol.* **15**: 1–94.
23. Myerowitz, R. L., R. Schneerson. J. B. Robbins, and M. Turck. 1972 Urinary tract Escherichia coli with cross-reactive antigens to encapsulated pyogenic bacteria. *Lancet.* **2**: 250–253.
24. Robbins, J. B., L. Myerowitz, J. K. Whisnant, M. Argaman, R. Schneerson, Z. T. Handzel, and E. C. Gotschlich. 1972. Enteric bacteria cross-reactive with *Neisseria meningitidis* groups A and C *Diplococcus pneumonia* Types I and III. *Infect. Immun.*, **6**:651–656.
25. Kasper, D. L., J. L. Winkelhake, W. D. Zollinger, B. L. Brandt, and M. S. Artenstein. 1973. Immunochemical similarity between polysaccharide antigens of Escherichia coli (07: K1(L): NM and group B Neisseria meningitidis. *J. Immunol.* **110**: 262–268.
26. Myerowitz, R. L., R. E. Gordon, and J. B. Robbins. 1973. Polysaccharides of the genus *Bacillus* cross-reactive with the capsular polysaccharides of *Diplococcus Pneumonia* Type III, *Haemophilus influenza* Type b, and *Neisseria meningitidis* group A. *Infect. Immun.* **8**: 896–900.