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Safety and Immunogenicity of Meningococcal A and C Polysaccharide Conjugate Vaccine in Adults

EDWIN L. ANDERSON,[1]* TERESA BOWERS,[1] CHRISANNA M. MINK,[1] DONALD J. KENNEDY,[1] ROBERT B. BELSHE,[1] HANI HARAKEH,[2,3] LORNA PAIS,[2] PATRICIA HOLDER,[2] AND GEORGE M. CARLONE[2]

Center for Vaccine Development, Division of Infectious Diseases, Departments of Medicine and Pediatrics, Saint Louis University School of Medicine, St. Louis, Missouri[1]; Meningitis and Special Pathogens Branch, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia[2]; and Department of Biology, American University of Beirut, Beirut, Lebanon[3]

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A meningococcal vaccine containing group A and C polysaccharides conjugated to CRM_{197} was evaluated in 50 adults. Vaccinees were entered into one of five groups: 30 adults received a single dose of either 22, 11, or 5.5 μg of the conjugated A-C vaccine; 10 received an approved meningococcal vaccine; and 10 received saline injections. Local and systemic reactions to vaccines were recorded, and immune responses were determined. The experimental meningococcal vaccine was well tolerated, with the most frequent reaction being pain at the injection site. Both A and C polysaccharide components of the experimental vaccine were highly immunogenic, and total antibody concentrations 1 month postvaccination were not significantly different from the mean antibody concentrations among adults given the approved meningococcal vaccine. In addition, significant rises in immunoglobulin G, A, and M antibodies to both A and C polysaccharides occurred. Antibody concentrations measured at 6 and 12 months postvaccination had declined but remained significantly higher than prevaccination concentrations. Postvaccination meningococcal group C functional antibody activity increased more than 600-fold for both the polysaccharide and the conjugate vaccines. Further studies of this conjugated meningococcal vaccine are indicated for young children and infants.

Meningococcal meningitis is a worldwide health problem, occurring in both endemic and epidemic forms (9, 22). The most clinically important serogroups are A to C, Y, and W135. Meningococcal vaccines have been effective in interrupting outbreaks of meningococcal disease in both adults and children, but questions about persistence of antibody and lasting protection remain (5, 21, 23, 24). The meningococcal vaccine approved in the United States contains polysaccharide antigens for serogroups A, C, Y, and W135 and is highly immunogenic in children older than 2 years (9). The group B polysaccharide is not immunogenic in humans. The current vaccine is recommended for adults and children at high risk for meningococcal disease but not for those under 2 years of age because of their poor antibody response to vaccine polysaccharide (6). A vaccine that is immunogenic and that produces long-term protection for infants and young children should be developed.

Conjugation of bacterial polysaccharide to protein carriers has resulted in enhanced immunogenicity to the polysaccharide antigens in young children and infants (8). *Haemophilus influenzae* type b polysaccharide has been conjugated to tetanus toxoid, diphtheria toxoid, diphtheria CRM_{197} protein, and the outer membrane protein complex of group B meningococcus and has been highly immunogenic in young infants (8, 14). The incidence of invasive *H. influenzae* disease among young children has declined in the United States since the approval and widespread use of conjugated *H. influenzae* vaccines for 2-month-old infants (1, 3). Other experimental vaccines consisting of polysaccharide antigens conjugated to protein carriers are being investigated. An experimental pneumococcal serotype 6B conjugated to the outer membrane protein complex of *Neisseria meningitidis* group B was immunogenic in children and infants (16, 20). Application of conjugate technology for meningococcal polysaccharides may enhance the immunogenicity of these polysaccharides in children and infants. In the present study, a meningococcal A-C conjugate vaccine was evaluated for safety, immunogenicity, and antibody duration in adults.

MATERIALS AND METHODS

The conjugate meningococcal A-C vaccine was produced by Sclavo (Siena, Italy) by coupling CRM_{197} through a hydrocarbon spacer to purified meningococcal polysaccharides (7). CRM_{197} is a mutant of diphtheria toxin that is not toxic but contains the immunogenic properties of the toxin. The meningococcal polysaccharides were obtained from culture filtrates of *N. meningitidis* A1 and C11. The final vaccine preparation contained 11 μg of each oligosaccharide, 48.7 μg of CRM_{197}, 1 μg of aluminum hydroxide, and 50 μg of thimerosal per 0.5 ml.

The study was approved by the Saint Louis University Institutional Review Board and was performed in a blinded and randomized fashion. Healthy adults 18 to 50 years of age were randomized to one of five groups and were immunized with either 5.5 μg (group 1), 11 μg (group 2), or 22 μg (group 3) of conjugated polysaccharide; approved quadrivalent meningococcal vaccine (Menomune; Connaught Laboratories, Swiftwater, Pa.) (group 4); or saline (group 5). The approved meningococcal vaccine contained 50 μg each of the A and C polysaccharides. Volunteers were injected with 1 ml intramuscularly in the upper arm. Blood was drawn before vaccination and at 48 h, 30 days, and 6 and 12 months after vaccination.

* Corresponding author. Mailing address: Division of Infectious Diseases, Saint Louis University Health Sciences Center, 3635 Vista Ave., FDT-8N, P.O. Box 15250, St. Louis, MO 63110. Phone: (314) 577-8648. Fax: (314) 771-3816.

Blood drawn before vaccination and at 48 h was sent for a complete blood count, serum chemistry measurements, and studies of liver and renal function. Sera obtained before vaccination, 30 days postvaccination, and 6 and 12 months postvaccination were used for antibody determinations.

Volunteers were given a form on which to record reaction information. Vaccinees recorded oral temperature and any reactions at the injection site or systemic symptoms at 6, 24, 48, and 72 h postvaccination. Reaction report forms were returned to the vaccine center after 72 h.

Serology. Anti-total immunoglobulin (Ig) antibodies to group A and group C polysaccharides of *N. meningitidis* were measured at the Centers for Disease Control and Prevention by enzyme-linked immunosorbent assay (ELISA) as previously described (4). The reference serum (PB-2) was provided by Carl Frasch of the U.S. Food and Drug Administration.

Isotype-specific ELISA antibodies (IgG, IgM, and IgA) to group A and group C polysaccharides were measured at Saint Louis University. Antigens and reagents needed for the isotype antibody assays were provided by George Carlone. Immulon II (Dynatech, Chantilly, Va.) plates were coated with a mixture of methylated human serum albumin and individual meningococcal polysaccharide (A or C) at a final concentration of 5 μg/ml in 10 mM phosphate-buffered saline (PBS). All anti-human antibodies (total, IgG, IgM, and IgA) conjugated to alkaline phosphatase were purchased from Kirkegaard and Perry Laboratories (Gaithersburg, Md.). In addition to PB-2, an internal reference serum (SLU-2) and a Centers for Disease Control and Prevention standard (900242) were used for the class-specific ELISAs. PB-2 had assigned total ELISA units (EU) of 4,800/ml for serotype A and 2,800/ml for serotype C. Relative to the optical density readings of PB-2, 900242 was assigned 200 EU/ml for IgG, -A, and -M to serotype C, and SLU-2 was assigned 150 EU/ml for IgG, 60 EU/ml for IgA, and 100 EU/ml for IgM to serotype A. Calculations were performed by the parallel line bioassay method requiring a minimal slope of 0.9 compared with the reference curve. This program was modified from the Food and Drug Administration Laboratory of Pertussis program used for pertussis-specific ELISAs (19). ELISA values of less than 3.0 EU/ml were considered below the level of detection and reported as 1.5 EU/ml.

Serum bactericidal assay. The meningococcal serogroup C serum bactericidal assay was performed in microtiter plates with pooled sterile baby rabbit (3 to 4 weeks old; lot 15912; Pel-Freez Clinical Systems, Brown Deer, Wis.) serum as the complement source. The baby rabbit serum was tested against *N. meningitidis* group C strain 60E and shown to have essentially no bactericidal activity against this strain. A frozen aliquot of the seed lot was streaked onto a Mueller-Hinton agar–horse serum plate and incubated overnight at 35°C in 5% CO_2. Cells were harvested and suspended in 2 ml of sterile Mueller-Hinton broth to yield an A_{660} of between 0.8 and 1.4 (stock cell suspension). Approximately 0.5 to 1 ml of the stock cell suspension was added to 20 ml of Mueller-Hinton broth, preequilibrated to room temperature, in a 125-ml sidearm flask (side arm, 18 mm) (starter culture). The starter culture was incubated at 35°C with rotation (120 rpm) until the A_{660} was between 0.4 and 0.5; this normally required 2.5 to 3 h and yielded approximately 4×10^8 CFU/ml.

Each test well of the microtiter plate contained four components. The four components in order of addition are 25 μl of Gey's balanced salt solution buffer (GIBCO BRL, Gaithersburg, Md.) with 0.2% added gelatin, 25 μl of heat-inactivated (56°C for 30 min) human serum (final starting dilution, 1:20), 25 μl of baby rabbit serum (complement source), and 25 μl of bacteria (diluted to 500 CFU per well); the final volume in each well was 100 μl. A known positive serum sample was included on each plate; the acceptable limit of variability was ±1 well dilution. Bacteria were sampled at time zero and after 60 min of incubation to determine the number of organisms in each microtiter well.

After all components were added to the microtiter wells, the plates were covered and incubated with rotation (150 rpm) for 60 min at 37°C on a microplate shaker (Dynatech). By using a multichannel pipette, 10 μl from each well in a row was pipetted onto two dry GC agar plates (15 by 150 mm) containing 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.) and allowed to run in lanes down the plate (tilt plate method). Each separate lane was approximately 10 cm in length. The plates were then incubated overnight at 35°C in 5% CO_2. The following morning, the colonies on the time zero and 60-min-incubation plates were counted, and the serum bactericidal titer was reported as the reciprocal of the serum dilution yielding ≥50% killing of bacteria.

Statistical analysis. A chi-square test was used to analyze differences in reaction rates between vaccination groups. Between-group comparisons were made by using analysis of variance when three or more groups were compared. Analysis of variance was performed with the SPSS statistical data analysis package (SPSS, Inc., Chicago, Ill.). A paired *t* test was used for comparing two groups. All data were converted to logarithms before analysis.

TABLE 1. Adverse reactions[a] among adults vaccinated with the meningococcal A-C conjugate vaccine

Group (*n*)	Treatment (μg)	No. of subjects				
		Temp ≥ 37.8°C	Pain[b]	Redness	Swelling	Pain medication
1 (10)	A-C (5.5)	0	7	3	2	3
2 (10)	A-C (11)	0	8	1	1	3
3 (10)	A-C (22)	0	9	2	4	3
4 (8)[c]	Menomune	1	8	3	3	6
5 (10)	Placebo	0	2	2	1	2

[a] Within 72 h of vaccination.
[b] The results for groups 2 to 4 were significantly different from that for group 5: group 1, $P = 0.069$; group 2, $P = 0.02$; group 3, $P = 0.005$; group 4, $P = 0.001$.
[c] Two vaccinees did not return reaction data forms.

RESULTS

Clinical reactions. Adverse reactions among the vaccinees are shown in Table 1. The experimental vaccine was well tolerated. There were no severe reactions. The most common reaction was pain at the injection site. There were no significant differences in reaction rates among vaccinees injected with the approved meningococcal vaccine and those injected with the experimental vaccine. All local and systemic reactions had resolved by 72 h.

No changes occurred in renal function, liver function, or hematologic values among the vaccinees at 48 h postvaccination.

Serologic responses. Total Ig antibody responses to meningococcal A and C polysaccharides for the five vaccine groups are shown in Fig. 1. Postimmunization meningococcal antibodies at 1 month were significantly different ($P < 0.0001$) for both groups A and C because of the rise in antibody among vaccine groups 1 to 4. No statistically significant differences in the concentrations of total antibody to either meningococcal polysaccharide occurred among the groups receiving different doses of the experimental vaccine (groups 1 to 3) and the

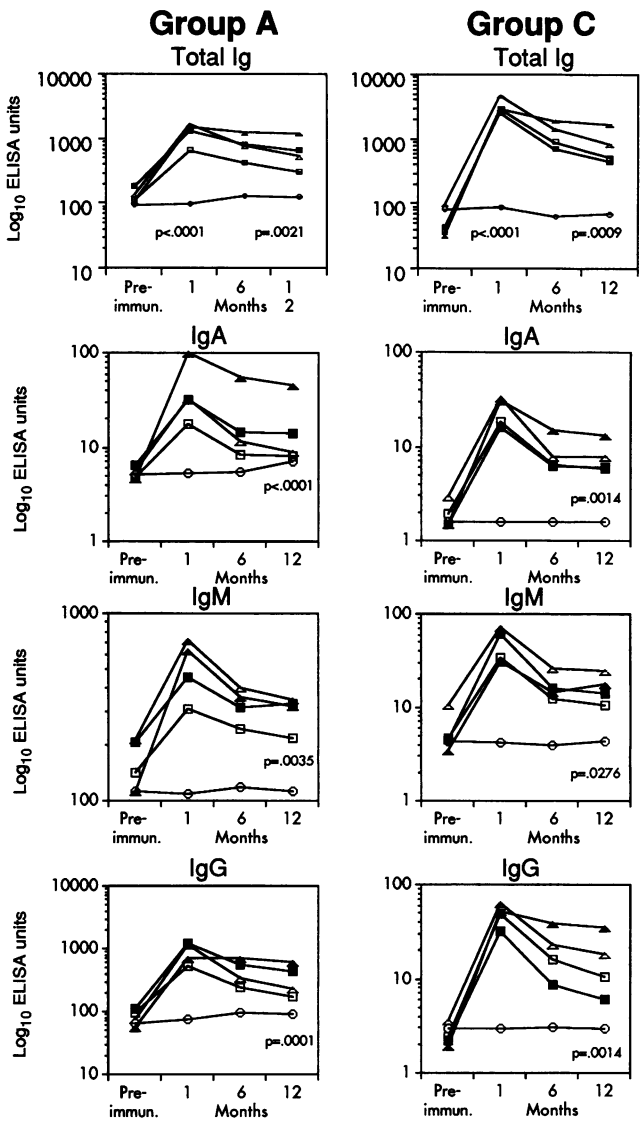

FIG. 1. Meningococcal group A and C antibody levels measured by ELISA before and 1 month, 6 months, and 12 months after vaccination with either an approved meningococcal vaccine or a conjugated meningococcal A-C polysaccharide vaccine by amount of antigen injected. Although statistically significant differences occurred at each time point postvaccination, P values are shown only at selected times for clarity. ■, □, and △, 5.5, 11, and 22 μg of antigen, respectively; ○, saline; ▲, approved vaccine.

volunteers who received the approved vaccine (group 4) (meningococcal group A, $P = 0.1358$; meningococcal group C, $P = 0.6097$). Antibody concentrations declined but remained significantly different at 12 months for both groups A ($P = 0.0021$) and C ($P = 0.0009$), primarily because of the antibody concentrations of groups 1 to 4. No dose response occurred among groups 1 to 4 for both A and C antibodies, as there were no significant differences in geometric means at 1, 6, or 12 months after vaccination.

Isotype-specific antibody results are shown in Fig. 1. At 1 month postvaccination significant differences in IgG, IgA, and IgM to both meningococcal A and C polysaccharides occurred among all vaccine groups. Group A postimmunization IgA antibody at 12 months was significantly different ($P < 0.0001$), largely because of the geometric mean of group 4 vaccinees. IgM antibody to group A remained significantly different at 12 months ($P = 0.0035$) because of the higher antibody concentrations of groups 1, 3, and 4. IgG antibody to group A remained significantly different ($P = 0.0001$), because of the variation in geometric means of groups 1 to 4. Meningococcal group C IgA and IgM were significantly different at 12 months (IgA, $P = 0.0014$; IgM, $P = 0.0276$). IgG was variable at 12 months but was significantly different ($P = 0.0014$), primarily because of the persistently higher concentration of group 4.

TABLE 2. Titers of bactericidal activity in sera of adults vaccinated with the conjugated meningococcal A-C vaccine

Treatment[a]	GMT[b]	
	Preimmunization	Postimmunization
A-C		
5.5 μg	5.0	10,233[c]
11 μg	5.0	7,761[c]
22 μg	21.4	13,489[c]
Menomune	5.8	4,169[c]
Saline	10.7	10.7

[a] $n = 10$.
[b] GMT, geometric mean titer (reciprocal serum dilution yielding ≥50% killing in the serum bactericidal assay). Strain C11 was used in the serum bactericidal assay with baby (3- to 4-week-old) rabbit serum as the complement source.
[c] Significantly different from the preimmunization titer ($P < 0.001$; paired t test).

Serum bactericidal activity against group C meningococcus was determined at the Centers for Disease Control and Prevention (Table 2). All vaccinees, regardless of which vaccine they received, had statistically significant rises in their postimmunization functional antibody activity titers. No rise occurred among vaccinees who received the saline placebo. There were no significant differences between the mean antibody activity of the experimental vaccine groups and that of the unconjugated vaccine group.

DISCUSSION

The experimental vaccine did not cause significant local or systemic adverse reactions. The most common reaction was pain at the injection site. Low adverse reaction rates were reported among adults and children in previous studies of unconjugated meningococcal vaccines (2, 10, 17, 18). Eight adults given two injections of the conjugated vaccine evaluated in this study reported only local pain and a few minor systemic reactions (7).

Significant rises in total antibody to both *N. meningitidis* group A and C polysaccharides occurred 1 month after vaccination among adults who received the experimental conjugate vaccine. The conjugate vaccine also stimulated significant rises in IgG, IgA, and IgM antibodies to both polysaccharides. A significant rise in serum bactericidal titer occurred among all groups of vaccinees. Other investigators have reported rises in total antibody, isotype-specific antibody, and bactericidal antibody following both meningococcal disease and meningococcal polysaccharide vaccination (12, 13). Costantino et al. (7) reported that the conjugated meningococcal vaccine induced specific antibodies to group A and group C polysaccharides and that a booster effect was seen in response to group A polysaccharide after the second injection but not in response to group C polysaccharide. Although the type of antibody measurement that best predicts protective efficacy is not known,

original studies employed bactericidal assays (5). The lack of differences in antibody response between the conjugate meningococcal vaccine and the approved polysaccharide vaccine may be due to prior exposure of the vaccinees to meningococcal species or cross-reacting bacteria (12). The adults in this study may be exhibiting a booster response to polysaccharide antigen. Studies with toddlers and infants may help clarify this point.

Susceptibility to meningococcal disease is due primarily to a lack of circulating antibodies to the infecting strain (11). The amount of antibody necessary for protection is not known. Meningococcal vaccines containing purified capsular polysaccharides of groups A and C have been shown to be protective in adults, especially those living in close quarters, and school-age children (2, 18, 21, 23). Short-term protection after vaccination with meningococcal group A vaccine was achieved in children under 4 years of age (23). The conjugated meningococcal vaccine offers the possibility of higher antibody concentrations in infants and young children following primary immunization and booster responses following repeat vaccination or natural exposure. In this study, vaccinees given the approved vaccine maintained the highest concentrations at 12 months, regardless of antibody type.

Meningococcal polysaccharide vaccines have been effective in epidemics of meningococcal disease, but their long-term efficacy and persistence of antibody need further study. Duration of protection induced by polysaccharide antibody against meningococcal disease begins to decline after 2 to 3 years in children (15, 17). In the present study total antibody at 12 months after vaccination remained significantly above prevaccination concentrations among vaccinees given the approved vaccine. Meningococcal antibody at 10 years after vaccination among U.S. Air Force personnel persisted at concentrations significantly higher than those before vaccination (24). These results are in contrast to a report from The Gambia, where antibody among 6- to 10-year-old children had declined after 5 years to prevaccination concentrations (5). In addition, a booster injection 2 years after vaccination did not influence this decline.

In meningococcal infection nasopharyngeal carriage is necessary prior to bloodstream infection (12, 13). Immunization with meningococcal C polysaccharide resulted in a smaller number of military recruits becoming colonized with group C meningococci (13). The mechanism of this decreased rate of carriage is unclear, but high levels of IgA antibody may block this early stage of infection. Secretory antibody or systemic antibody may leak across mucosal membranes and prevent colonization. Whether secretory antibody develops following immunization has yet to be investigated. High levels of serum antibody may provide an additional barrier to bloodstream invasion among vaccinated persons who do acquire nasopharyngeal infection. Military recruits may have benefited from the short-term protection afforded by the approved meningococcal vaccine until they acquired natural immunity through colonization that occurred under close living conditions (2, 12). Enhancing the immunogenicity of meningococcal vaccine through conjugation may benefit both children and adults by reducing colonization, blocking invasion, eliciting a rapid antibody response upon exposure, and providing long-term protection.

Epidemics of meningococcal groups A and C continue to occur both in the meningitis belt and outside the meningitis belt in sub-Saharan Africa, as well as in other parts of the world (9, 22). Currently approved meningococcal vaccines are not efficacious in children under 2 years of age, in whom the peak incidence of disease occurs (6). The success of polysaccharide conjugated to carrier protein in inducing protective antibody in young infants, as has occurred with the *H. influenzae* type b conjugated vaccine (1, 3), suggests that similar success could be achieved with a meningococcal polysaccharide-protein conjugate. Immunization with conjugated meningococcal vaccines in childhood may stimulate immunologic memory that responds to booster injections or may be protective because of priming for an anamnestic response later in life. Studies with the meningococcal group A and C polysaccharides conjugated to CRM_{197} protein are indicated for young children and infants.

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