Induction of Immunologic Refractoriness in Adults by Meningococcal C Polysaccharide Vaccination

Dan M. Granoff, Rajesh K. Gupta, Robert B. Belshe, and Edwin L. Anderson

Thirty-four adults were vaccinated with 1/50 of the usual dose of meningococcal polysaccharide vaccine (1 μg of A, C, Y, and W135 polysaccharides, given intramuscularly). This dose was selected as a probe to assess B cell memory. The probe elicited meningococcal C bactericidal antibody responses in all 18 adults who had been vaccinated 4 years earlier with an investigational meningococcal A and C oligosaccharide–protein conjugate vaccine and in the majority of the 11 subjects vaccinated for the first time. In contrast, the responses of the 5 adults given a full dose of licensed polysaccharide vaccine 4 years earlier were <1/10 of those of the other 2 groups. Thus, adults previously given a full dose of meningococcal polysaccharide vaccine show evidence of immunologic refractoriness to group C polysaccharide, whereas refractoriness is not observed after conjugate vaccination. These findings have implications for the use of meningococcal polysaccharide vaccine when the risk of disease is low.

Neisseria meningitidis remains an important cause of invasive bacterial disease [1]. In most industrialized countries, the vast majority of isolates causing meningococcal disease are serogroups B or C [1, 2]. Effective meningococcal polysaccharide vaccines have been available for >20 years but are used infrequently in industrialized societies [1]. The principal reasons are their poor immunogenicity in infants and toddlers <2 years of age, the age groups at greatest risk of developing meningococcal disease [1]. Further, currently available vaccines provide no protection against disease caused by serogroup B strains, which account for ~40% of cases in the United States and the United Kingdom [1, 2].

More effective polysaccharide-protein conjugate vaccines for prevention of disease caused by meningococcal A and C strains are currently under development. Being thymic-dependent antigens, these conjugate vaccines are more immunogenic in infants and toddlers than are the plain meningococcal polysaccharide vaccines [3, 4]. Additionally, conjugate vaccines elicit long-term immunologic memory to unconjugated meningococcal C polysaccharide [5], which is not observed after plain meningococcal polysaccharide vaccine [5]. Induction of immunologic memory could be an important second mechanism of protection, by permitting a vaccinated person with low serum concentrations of antibody to develop booster antipodal antibody responses on encountering encapsulated meningococci.

Meningococcal conjugate vaccines also are highly immunogenic in adults [6]. However, the magnitude of the serum antibody responses appears to be similar to that elicited by the corresponding plain polysaccharide vaccine [6, 7]. Thus, the advantage of using a conjugate vaccine for protection against meningococcal disease in adults, compared with unconjugated polysaccharide vaccine, is uncertain. One potential advantage of a meningococcal conjugate vaccine in adults could be its ability to induce polysaccharide-responsive memory B cells and resulting long-term immunologic memory. However, to date, evidence of induction of memory B cells by conjugate vaccination is limited to infants and toddlers and has not been observed in adults [7]. The purpose of the present study was to assess whether previous vaccination of adults with meningococcal conjugate vaccine or plain meningococcal polysaccharide vaccine induces memory B cells capable of responding to unconjugated meningococcal C polysaccharide.

Methods

Subjects. Thirty-four healthy adults, ages 20–53 years, were divided into 3 groups on the basis of their previous meningococcal vaccination histories. Group 1 consisted of 11 control adults who had not previously received meningococcal vaccine. Group 2 consisted of 5 adults, each of whom had received previously a full dose of a US-licensed tetravalent meningococcal polysaccharide vaccine (Menomune; Connaught Laboratories, Swiftwater, PA; 50 μg of A, C, Y, and W135 polysaccharides/0.5-mL dose). This dose was given 4 years earlier as part of a randomized study comparing the safety and immunogenicity of Menomune (n = 10).
to that of different doses of an investigational meningococcal A and 
C oligosaccharide–protein conjugate vaccine \( n = 40 \) or saline \( n = 10 \) [6]. Group 3 consisted of 18 subjects who had previously 
received a dose of an investigational meningococcal A and C 
oligosaccharide–CRM\(_{197}\) protein conjugate [6]. In this group, the 
antibody responses to the booster vaccination in the subjects pre-
viously given different doses of conjugate vaccine were similar. 
Therefore, in the present study, the data from the booster immuni-
zation in subjects previously given different doses of the conjugate 
vaccine were combined.

The demographic characteristics of the 3 vaccine groups were 
similar with respect to median age at the time of the booster 
vaccination (40, 38, and 36 years for groups 1, 2, and 3, respec-
tively), sex (female: 92%, 100%, and 89%, respectively), and race 
distribution (white: 91%, 100%, and 94%, respectively).

**Vaccination.** All 34 healthy adults in groups 1, 2, and 3 were 
vaccinated with 1/50 of the usual dose of tetravalent meningococ-
cal A, C, Y, W135 polysaccharide vaccine (1 mL, containing 1 
\( \mu g \) of each polysaccharide, given intramuscularly in the deltoid). 
Since adults normally respond well to a full dose of meningococcal 
polysaccharide vaccine, this low dose was selected on theoretical 
grounds as a probe to assess whether immunologic B cell memory 
was elicited by the previous conjugate vaccination (a memory 
response being defined as a higher and/or more rapid response to 
the low-dose probe in the previously vaccinated subjects, com-
pared with that observed in subjects immunized for the first time 
with the low-dose probe). To prepare this dose, the lyophilized 
meningococcal polysaccharide vaccine (Menomune) was pur-
chased from Connaught Laboratories and was reconstituted with 
0.6 mL of diluent provided by the manufacturer. The resulting 
solution contained 100 \( \mu g/mL \) each of the polysaccharides. From 
this solution, 0.5 mL was diluted into 49.5 mL of preservative-
free saline for injection, to yield the 1-mL dose.

**Immunoaassays.** Serum samples were obtained immediately be-
fore vaccination (time 0) and 3, 7, and 28 days later, for measure-
ment of antibody responses to the meningococcal A and C compo-
nents of the vaccine. All assays were done under blinded conditions 
on coded serum samples. Serum IgG antibody concentrations to 
the polysaccharide antigens were measured by an ELISA done as 
previously described [8–10]. The buffer used for diluting the se-
rum samples in the anti-C assay contained 75 mM ammonium 
thiocyanate, which favored the detection of high-avidity antcapsu-
lar antibodies over low-avidity antibodies [10]. In both the anti-A 
and anti-C assays, bound antibody was detected by mouse mono-
clonal antibody specific for human IgG (clone HP6043 [9], pro-
vided by G. Carlone, CDC, Atlanta).

Complement-mediated bactericidal antibody to *N. meningitidis* 
group C was assayed as previously described [9, 11], with the 
following modifications: The test organism was *N. meningitidis* 
group C, strain 60E (obtained from W. Zollinger, Walter Reed 
Institute for Medical Research, Washington, DC), and it was grown 
for \( \sim 2 \) h in Mueller-Hinton broth containing 0.25% glucose. 
The complement source for the bactericidal assay was pooled sera ob-
tained from 3 healthy adults who had no detectable antcapsular 
antibody to meningococcal C and whose sera lacked intrinsic bac-
tericidal activity when tested at 20% or 40%. When assaying test 
sera, the complement source was used at a final concentration of 
20%.

**Statistical analysis.** Antibody concentrations were trans-
formed \( \log_{10} \). For these calculations, bactericidal titers \(< 1:8 \) were 
assigned a value of 1:4, and IgG antibody concentrations \(< 0.6 \U/mL \) 
were assigned a value of 0.3 \( \U/mL \). Geometric means and 
95% confidence intervals were calculated using the log-trans-
formed means, and SEs were computed from a one-way analysis 
of variance (ANOVA) model. Differences between each pair of 
groups with respect to geometric means were tested by using the 
\( P \) values from the ANOVA model.

**Results**

**Clinical tolerability.** Vaccination with 1/50 of the usual 
doese of polysaccharide vaccine was well tolerated, irrespective 
of previous meningococcal vaccination status. During the 28 
days of follow-up, there were no clinically significant injection-
site reactions or systemic reactions, such as fever, rash, or 
myalgia, in any of the 34 subjects.

**Anticapsular antibody response.** Figure 1 shows the serum 
IgG meningococcal C anticapsular antibody concentrations of 
the individual subjects at different time points and the respec-
tive geometric mean antibody concentrations of each group. 
Before vaccination, there were no significant differences be-
tween the geometric mean antibody concentrations of the 3 
groups (geometric means of 0.73, 0.30, and 0.78 \( \U/mL \), respec-
tively). At 3 days after vaccination, there was no evidence of 
a significant increase in serum IgG antibody concentrations to 
the C polysaccharide in any of the groups. However, by 7 days, 
subjects in group 1, vaccinated for the first time and subjects in 
group 3 (previously given the conjugate vaccine) showed 
no IgG response to meningococcal C polysaccharide vaccine, 
this low dose was selected on theoretical 
grounds as a probe to assess whether immunologic B cell memory 
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Antibody concentrations were transformed \( \log_{10} \). For these calculations, bactericidal titers \(< 1:8 \) were assigned a value of 1:4, and IgG antibody concentrations \(< 0.6 \U/mL \) were assigned a value of 0.3 \( \U/mL \). Geometric means and 95% confidence intervals were calculated using the log-transformed means, and SEs were computed from a one-way analysis of variance (ANOVA) model. Differences between each pair of groups with respect to geometric means were tested by using the \( P \) values from the ANOVA model.

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**Anticapsular antibody response.** Figure 1 shows the serum IgG meningococcal C anticapsular antibody concentrations of the individual subjects at different time points and the respective geometric mean antibody concentrations of each group. Before vaccination, there were no significant differences between the geometric mean antibody concentrations of the 3 groups (geometric means of 0.73, 0.30, and 0.78 \( \U/mL \), respectively). At 3 days after vaccination, there was no evidence of a significant increase in serum IgG antibody concentrations to the C polysaccharide in any of the groups. However, by 7 days, subjects in group 1 (vaccinated for the first time) and subjects in group 3 (previously given the conjugate vaccine) showed significant IgG responses compared with their respective IgG serum antibody concentrations at time 0 (\( P < .05 \) for each group). In contrast, at 7 days, the adults in group 2, who previously had received a full dose of licensed meningococcal A and C polysaccharide vaccine, showed no IgG response to meningococcal C polysaccharide (geometric mean IgG antibody concentration of 0.38 \( \U/mL \) at 7 days vs. 0.42 \( \U/mL \) at time 0). Similarly, there was no significant increase in geometric mean antibody concentration in this group when measured at 28 days (0.68 \( \U/mL \); \( P > .5 \)). At 28 days, only 1 subject in group 2 showed a \( \geq 2 \)-fold increase in IgG antibody concentration, and the geometric mean IgG antibody response of group 2 was 1/6 to 1/10 lower than those of groups 1 or 3 (\( P < .01 \)). Although the 1-\( \mu g \) dose of polysaccharide vaccine was capable of eliciting significant increases in serum IgG antibody concentrations to meningococcal C polysaccharide in most subjects in groups 1 and 3, this low dose was less effective in eliciting IgG responses to the A polysaccharide. Only 2 of 11 subjects in group 1, and 5 of 18 in group 3, showed \( \geq 2 \)-fold IgG responses to meningococcal A polysaccharide (comparing the respective serum antibody concentrations at day 28 with those at time 0). For group 2, previously given the polysaccharide vaccine, there were no responders (0 of 5). The respective differences were not significant (\( P > .05 \)).

**Bactericidal antibody response.** Table 1 summarizes the meningococcal C bactericidal antibody responses of the 3 groups to the low-dose booster immunization, as measured in
Figure 1. Serum IgG anticapsular antibody responses to meningococcal C polysaccharide booster immunization in relation to previous priming with conjugated or unconjugated meningococcal vaccine. For booster injection, all subjects were given 1/50 of usual dose of meningococcal polysaccharide vaccine (1 µg of each polysaccharide intramuscularly). Respective geometric mean antibody responses of group 2 (given full dose of meningococcal polysaccharide vaccine 4 years earlier) were significantly lower than those of group 1 (vaccinated for first time) or of group 3 (given conjugate vaccine 4 years earlier) \( (P < .003\) at 7 days and \( P < .01\) at 28 days). Respective differences between groups 1 and 3 were not significantly different \( (P > .05\) at both time points).

Serum samples obtained at times 0, 7, and 28 days (meningococcal A bactericidal assays were not done because of the low IgG responses to the booster). The geometric mean meningococcal C bactericidal antibody responses at 0, 7, and 28 days paralleled the respective serum IgG antibody responses. Specifically, at time 0, before the booster, there were no significant differences in the titers of the 3 groups \( (P = .5)\). At day 7, the geometric mean titer of group 2 (previously given the polysaccharide vaccine) was 1/10 lower than the respective titer of group 1 (previously unvaccinated subjects) and 1/25 that of group 3 (subjects previously given the conjugate vaccine) \( (P < .001)\). At 28 days, similar respective differences in geometric mean titers were present among the 3 groups \( (P < .006)\). The 1 subject in group 2 showing a bactericidal titer \( \geq 1:8\) after

Table 1. Complement-mediated bactericidal antibody responses of adults to meningococcal polysaccharide booster immunization.

<table>
<thead>
<tr>
<th>Group</th>
<th>Meningococcal priming vaccine</th>
<th>No. tested</th>
<th>Reciprocal GMT (95% confidence interval)</th>
<th>% with titer ( \geq 1:8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Unvaccinated</td>
<td>11</td>
<td>Pre 9.2 (3.4–25) 43 (11–174) 88 (17–463)</td>
<td>36 64 73</td>
</tr>
<tr>
<td>2</td>
<td>Polysaccharide</td>
<td>5</td>
<td>Pre 4.0 (4–4) 4.9 (2.8–8.6) 8.5 (1.1–68)</td>
<td>0 20 20</td>
</tr>
<tr>
<td>3</td>
<td>Conjugate</td>
<td>18</td>
<td>Pre 9.3 (4–21) 136 (69–268) 200 (108–371)</td>
<td>22 100 100</td>
</tr>
</tbody>
</table>

Note. For booster injection, all subjects were given 1/50 of usual dose of Menomune (1 µg of each polysaccharide intramuscularly). Group previously given polysaccharide vaccine showed no significant antibody response to booster. Comparing respective geometric mean titers (GMTs) of 3 groups at each time point: Pre, \( P = .5\); 7 days, \( P < .001\); 28 days, \( P = .005\). Pairwise comparisons of GMTs in group previously vaccinated with polysaccharide vs. previously unvaccinated group: 7 days, \( P = .01\); 28 days, \( P = .02\). Pairwise comparisons of GMTs in group previously vaccinated with conjugate vaccine vs. previously unvaccinated group: 7 days, \( P = .06\); 28 days, \( P = .24\). Pairwise comparisons of % with titers \( \geq 1:8\) in group previously vaccinated with conjugate vs. previously unvaccinated group: 7 days, \( P < .02\); 28 days, \( P < .05\) (by \( \chi^2 \) analysis).
vaccination (table 1) was the same subject with an IgG response (figure 1). Note also, there was a trend at 7 days for a higher geometric mean titer in group 3 than in group 1 \( (P = .06) \) and a higher respective percentage of subjects with titers \( \geq 1:8 \) (presumed to be protective) \( (100\% \text{ vs. } 64\% ; \ P < .02) \).

**Discussion**

Vaccination of older children or adults with plain meningococcal polysaccharide vaccine is highly effective in conferring protection against meningococcal disease caused by serogroups A, C, Y, and W135. However, when this vaccine is used in infants or toddlers, the immunogenicity of the meningococcal C component is poor. Further, vaccination at this early age induces a refractory state to meningococcal C polysaccharide as defined by impaired serum antibody responses to revaccination 6–12 months later. This finding was first shown in immunized infants by Goldschneider et al. [12] in 1973, and subsequently by Gold et al. [13] and Leach et al. [5]. The study by Leach et al., which examined functional serum bactericidal antibody responses of toddlers in The Gambia boosted with plain polysaccharide vaccine, was particularly striking: Toddlers previously vaccinated at 3–6 months of age with meningococcal polysaccharide vaccine had, after receipt of a full dose of meningococcal polysaccharide vaccine 1 year later, meningococcal C bactericidal responses that were 10-fold lower than those of neighborhood control toddlers vaccinated for the first time.

In a more recent multicenter study in Canada, toddlers immunized at 15–23 months of age with meningococcal polysaccharide vaccine also showed evidence ofnergy to meningococcal C polysaccharide when given a full dose of vaccine 12 months later [14]. The present results extend these observations to healthy immunized adults. In the adults, the hyporesponsiveness was detected after revaccination with 1/50 of the usual dose of meningococcal polysaccharide vaccine, a dose chosen as an “immunologic probe.” Although the conclusion that the earlier vaccination with a full dose of unconjugated meningococcal C polysaccharide vaccine had induced immunologic refractoriness is based on the booster responses of only 5 adults, the magnitude of the impairment found was marked (10-fold) and was unlikely to have resulted from chance alone \( (P \leq .02) \).

The clinical importance of the presence of immunologic refractoriness to meningococcal C polysaccharide is difficult to ascertain. Conceivably, such adults would have responded normally if they had been given a full dose of polysaccharide vaccine. However, the presence of a refractory state raises the theoretical concern that a previously vaccinated person exposed to encapsulated group C organisms could be at increased risk of developing meningococcal disease, compared with that of an unvaccinated person, because of impaired serum antcapsular antibody responses. In this regard, the data obtained with the low dose of polysaccharide might be more clinically relevant than if a full dose of vaccine had been used. Early in infection, patients are likely exposed to low levels of polysaccharide capsule, and their ability to mount protective serum antibody responses at this stage might be decisive in affecting the outcome of disease. Of note, vaccinated mice rendered refractory to certain pneumococcal polysaccharides show increased mortality after experimental challenge with pneumococci of the homologous capsular serotype (reviewed in [15]), and this increased mortality may be the result of impaired ability to mount serum antcapsular antibody responses on exposure to the encapsulated bacteria.

Given these concerns, until further data are available, use of meningococcal polysaccharide vaccine in low-risk groups needs to take into consideration that any short- or mid-term benefit of vaccination may be offset by the presence of longer-term immunologic refractoriness, which could have adverse clinical consequences once serum antibody concentrations have declined to sub-protective levels.

Finally, the present results indicate that, in contrast to adults vaccinated only with the plain polysaccharide vaccine, adults previously vaccinated with meningococcal C conjugate vaccine do not show any evidence of immunologic refractoriness to plain meningococcal C polysaccharide. Indeed, there was an opposite trend: At 7 days, 100% of adults primed with the conjugate and boosted with the 1/50th dose of polysaccharide developed protective meningococcal C bactericidal titers \( \geq 1:8 \), versus 64% of adults vaccinated with this dose for the first time \( (P < .05) \). A similar difference was present at 28 days \( (P = .05) \). Thus, despite similar immunogenicity of the meningococcal conjugate vaccine and polysaccharide vaccine after primary vaccination of adults [6], the conjugate vaccine appears to have induced immunologic memory to meningococcal C polysaccharide, which persisted for at least 4 years. Conceivably, the presence of immunologic memory may contribute to protection against disease.

**Acknowledgments**

David Salisbury, Immunisation and Communicable Disease Branch, Department of Health, United Kingdom, and Carl Frasch, Center for Biologic Evaluation and Research, US Food and Drug Administration, provided helpful critical comments. At Chiron Vaccines, George Santos performed the meningococcal C serology studies, Ahmad Mokatrin performed the statistical analysis, and Linda Phillips and Carol Suennen provided expert editorial assistance. At St. Louis University, Carolyn DeRousse provided clinical assistance, and Kathleen Lottenbach performed the meningococcal A ELISA.

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