Induction of Immunologic Memory by Conjugated vs Plain Meningococcal C Polysaccharide Vaccine in Toddlers

A Randomized Controlled Trial

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Context.—Meningococcal polysaccharide vaccines are not used routinely in infants and toddlers, the groups at highest risk of invasive disease, because of poor immunologic responses to the Neisseria meningitidis serogroup C polysaccharide in these age groups. Meningococcal C conjugate vaccines offer the prospect of circumventing this problem.

Objective.—To assess the immunogenicity and the induction of immunologic memory in toddlers by meningococcal C conjugate vaccine.

Design.—A multicenter, randomized, observer-blinded controlled trial.

Setting.—Urban and suburban family medicine or pediatric practices.

Participants.—Two hundred eleven healthy toddlers aged 15 to 23 months.

Intervention.—Two injections at 2 months apart of meningococcal C conjugate (group 1, n = 69), plain meningococcal polysaccharide (group 2, n = 72), or hepatitis B virus vaccine (group 3, n = 70). All toddlers received a follow-up dose of plain meningococcal polysaccharide vaccine 12 months later.

Main Outcome Measures.—IgG meningococcal C anticapsular antibody concentrations determined by enzyme-linked immunosorbent assay and complement-mediated bactericidal antibody.

Results.—In group 1, the magnitude of the IgG response to meningococcal C conjugate vaccine was more than 4-fold higher after dose 1 and more than 10-fold higher after dose 2 compared with meningococcal polysaccharide vaccine (group 2) (P < .001). Higher titers persisted in the meningococcal C conjugate group for at least 12 months (P < .001). Group 1, primed with meningococcal C conjugate, had 25-fold higher IgG responses to the meningococcal polysaccharide 1-year booster dose than the controls who had received hepatitis B virus vaccine initially and were given meningococcal polysaccharide vaccine 1 year later for the first time (P < .001). In contrast, group 2, primed with meningococcal polysaccharide, had a 2-fold lower response to the 1-year booster meningococcal polysaccharide vaccine dose than the hepatitis B virus control group (P = .006). Serum bactericidal responses paralleled the enzyme-linked immunosorbent assay responses.

Conclusions.—Immunization of toddlers with meningococcal C conjugate vaccine induces high titers of anticapsular and bactericidal antibody. Furthermore, this vaccine induces immunologic memory to meningococcal C polysaccharide. In contrast, meningococcal polysaccharide vaccine is less immunogenic than the conjugate vaccine and also induces a hyporesponsive state that persists for at least 12 months.

WITH THE CONTROL of invasive Haemophilus influenzae type b disease by routine immunization, Neisseria meningitidis has become a major cause of bacterial meningitis in North America and Europe, with 45% of meningococcal cases caused by N meningitidis serogroup C. Although meningococcal vaccines containing N meningitidis serogroup C polysaccharide have been available for more than 20 years, their routine use is not currently recommended because the vaccines are poorly immunogenic in the age group at highest risk (ie, infants and toddlers), and the serum antibody response is short-lived in young children. Furthermore, data suggest that administration of plain meningococcal polysaccharide vaccine to infants may induce a hyporesponsive state to meningococcal C polysaccharide (ie, impaired serum antibody responses to a second injection given 6 to 12 months later) compared with responses of controls of similar age immunized for the first time. In contrast, a hyporesponsive state is not observed after vaccination with the N meningitidis serogroup C polysaccharide.

Meningococcal polysaccharide-protein conjugate vaccines are currently being studied in infants and toddlers for the prevention of disease caused by meningococcal C strains. Preliminary work suggests that these thymic-dependent antigens are more immunogenic in infants and toddlers than plain meningococcal C polysaccharide, which is thought to evoke serum antibody responses by a thymic-independent mechanism. Furthermore, the conjugate vaccines, but not the plain polysaccharide vaccines, elicit long-term memory to plain meningococcal C polysaccharide. The present study was a phase-2, observer-blinded, randomized controlled trial to evaluate the comparative safety and im-

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munogenicity in toddlers of meningococcal C conjugate vaccine compared with meningococcal polysaccharide vaccine. We also assessed whether vaccination induces immunologic memory or a hyporesponsive state to meningococcal C polysaccharide.

METHODS

The study design is outlined in Figure 1. Institutional ethics review was obtained at the 3 study sites (Ottawa, Ontario; Halifax, Nova Scotia; and Winnipeg, Manitoba). Healthy toddlers 15 to 23 months of age with no underlying serious disease or previous meningococcal disease were recruited through family or pediatric suburban and urban practices. The study was introduced either by letter or by the primary care physician and followed up by formal discussions with a research assistant. Of the 297 parents formally approached by the research assistants, 211 (71%) met the eligibility criteria and agreed to participate. After parental informed consent, 211 healthy toddlers aged 15 to 23 months on enrollment were randomized centrally according to a prearranged, computer-generated randomization schedule for each study site into 3 vaccine groups. Each group received 2 doses of the designated vaccine 2 months apart. Group 1 received meningococcal C conjugate vaccine from a single lot (J35021LI) containing 10 µg of meningococcal C oligosaccharide conjugated to the protein carrier, CRM197 (Chiron Vaccines, Chiron Corp, Emeryville, Calif). Group 2 received a licensed quadrivalent plain polysaccharide vaccine (Menomune, Connaught Laboratories Ltd, Willowdale, Ontario) containing 50 µg each of the A, C, Y, and W135 meningococcal polysaccharides. Group 3 (control) received a licensed hepatitis B virus vaccine (Recombivax HB, Merek, Sharp and Dohme, Kirkland, Quebec). No other vaccines were administered concurrently. The selected sample size of 70 subjects per group had greater than 98% power to detect a 2-fold pairwise difference among the 3 groups with respect to geometric mean antibody concentrations at 1 month following the second immunization. All 3 vaccines were prepared for administration by research assistants who were not involved in the assessment of the vaccines, the assessment of adverse events, or serum collection to ensure observer blinding. To assess induction of immunologic B-cell memory or a state of hyporesponsiveness to meningococcal C polysaccharide, children in all 3 groups received a follow-up dose of the plain meningococcal polysaccharide vaccine 12 months after their second study vaccine dose (ie, 14 months after study entry).

Local and systemic reactions were noted by the subject’s parent or guardian daily for 7 days in the subject diary following each immunization and reviewed during the follow-up and clinic visits. Serum samples were obtained immediately before the first vaccine dose and at 2 and 3 months after study entry (just before and 1 month after the second dose of vaccine). Additional samples were obtained at 14 and 15 months after study entry (just before and 1 month after the plain booster polysaccharide vaccine injection). The following assays were performed on coded serum samples: (1) Serum IgG anti-N. meningitidis serogroup C polysaccharide antibody concentrations were measured by an enzyme-linked immunosorbent assay (ELISA) using an alkaline-phosphatase conjugated mouse monoclonal antibody specific for human IgG (clone HP 6043). The buffer used to dilute the serum samples contained 75 mmol/L of ammonium thiocyanate, which favored detection of high-avidity anticapsular antibodies. (2) Complement-mediated bactericidal antibody titers to N. meningitidis serogroup C were measured on a convenience sample of approximately 50% of the serum samples, performed as previously described. For the present study, the test strain, N. meningitidis serogroup C 60E (obtained from Wendell Zollinger, PhD, Walter Reed Institute for Medical Research, Washington, DC), was grown for approxi-
mately 2 hours in Mueller-Hinton broth containing 0.25% glucose. The complement source was pooled serum samples obtained from 3 healthy adults who had no detectable antcapsular antibody to meningococcal C and whose sera lacked intrinsic bactericidal activity when tested at concentrations of up to 40%. When carrying out the bactericidal assays, the complement source was used at a final concentration of 20% in the reaction mixture.

Antibody concentrations were transformed (logarithm to base 10) for calculation of geometric means. IgG antibody concentrations of less than 0.4 U/mL were assigned a value of 0.2 U/mL and bactericidal titers of less than 1:8 were assigned a value of 1:4. Geometric means and 95% confidence intervals were calculated using the least squares means and SEs were computed from a 2-way analysis of variance model. Differences in terms of group, center, and group by center interaction with respect to geometric means were tested by using the P values from the analysis of variance model. There were 1 primary and 3 secondary planned comparisons. The primary comparison was to test the null hypothesis that there was no difference between the 2 meningococcal vaccine groups (group 1 and group 2) in the antibody response of toddlers to N meningitidis serogroup C as measured by ELISA and bactericidal assay 1 month after the second immunization.

Secondary comparisons were to test the null hypothesis that (1) there was no difference between the 2 meningococcal vaccines (group 1 and group 2) in the antibody response to N meningitidis serogroup C as measured by ELISA and bactericidal assay 2 months after the first injection; (2) there was no difference between the 2 meningococcal vaccines (group 1 and group 2) in the antibody response to N meningitidis serogroup C as measured by ELISA and bactericidal assay 12 months after the second injection; and (3) there was no difference among all 3 groups in the antibody response to N meningitidis serogroup C as measured by ELISA and bactericidal assay 1 month after the booster dose that was given 12 months after the second injection. If the null hypothesis for this objective was rejected, then all pairwise comparisons would be performed.

**RESULTS**

Of the 211 toddlers enrolled, 69, 72, and 70 were randomized to groups 1, 2, and 3, and 87%, 93%, and 89% completed the study, respectively. The primary reason for not completing the study was withdrawal of consent between doses 1 and 2 or doses 2 and 3 (7%, 6%, and 11% of toddlers assigned to groups 1, 2, and 3, respectively). The 3 groups did not differ significantly with respect to mean age at enrollment (20.9 months, 20.8 months, and 21.2 months); male-female ratio (0.97, 1.25, and 0.94); or ethnic background (86%, 90%, and 94% were white). No vaccine-related serious adverse events were observed during the study and all 3 vaccines were well tolerated. Table 1 presents data on reactogenicity within 48 hours following the first and second doses of vaccine.

Table 2 and Table 3 summarize the vaccine antibody response data. Since no significant differences were noted among the 3 study sites and the vaccine-by-site interactions were not significant, only aggregated data are presented.

The IgG anticapsular antibody responses expressed as geometric mean titer are presented in Table 2. Before vaccination, the geometric mean was 0.20 to 0.21 U/mL in all groups. The magnitude of the antibody response to the meningococcal C conjugate vaccine (group 1) was more than 4-fold higher after dose 1 and more than 10-fold higher after dose 2 than the corresponding responses to the meningococcal polysaccharide vaccine (group 2). Twelve months after the last primary dose, the IgG antibody concentrations in the meningococcal C conjugate vaccine group (group 1) were 2-fold higher than those in the meningococcal polysaccharide conjugate vaccine group (group 2) (P<.001). Following the plain meningococcal C polysaccharide booster dose 12 months after the last dose of the priming vaccine, group 1 showed evidence of induction of immunologic B-cell memory by the conjugate vaccination (25-fold higher anticapsular antibody responses than control toddlers [group 3] vaccinated with the polysaccharide vaccine for the first time). In contrast, group 2 primed with 2 doses of plain meningococcal polysaccharide showed evidence of a hyporesponsive state (2-fold lower responses to the 1-year follow-up injection than control toddlers given 2 injections separated by 2 months of either meningococcal C conjugate vaccine, plain meningococcal polysaccharide vaccine, or hepatitis B virus vaccine (control). Twelve months after dose 2, all subjects received a booster dose of plain meningococcal polysaccharide vaccine.

**Table 1.—Reactogenicity Within 48 Hours Following First and Second Doses of Vaccine**

<table>
<thead>
<tr>
<th>Subjects With Reaction, %</th>
<th>Meningococcal C Conjugate (n = 69)</th>
<th>Meningococcal Polysaccharide (n = 72)</th>
<th>Hepatitis B Virus (n = 70)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature &gt;3°C</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>.009</td>
</tr>
<tr>
<td>Induration &gt;25 mm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&gt; .99</td>
</tr>
<tr>
<td>Persistent crying</td>
<td>14</td>
<td>19</td>
<td>11</td>
<td>.40</td>
</tr>
<tr>
<td>Urticarial rash</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&gt; .99</td>
</tr>
<tr>
<td>Irritability</td>
<td>35</td>
<td>29</td>
<td>26</td>
<td>.50</td>
</tr>
<tr>
<td>Dose 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature &gt;3°C</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td>.91</td>
</tr>
<tr>
<td>Induration &gt;25 mm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&gt; .99</td>
</tr>
<tr>
<td>Persistent crying</td>
<td>18</td>
<td>13</td>
<td>14</td>
<td>.62</td>
</tr>
<tr>
<td>Urticarial rash</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&gt; .99</td>
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<tr>
<td>Irritability</td>
<td>31</td>
<td>29</td>
<td>15</td>
<td>.08</td>
</tr>
</tbody>
</table>

*The numbers of subjects studied following the second dose were for meningococcal C conjugate, n = 65; meningococcal polysaccharide, n = 70; and hepatitis B virus, n = 66.
†P value is from the Pearson χ² test for vaccine group differences. If 50% or more of the expected cell counts were less than 5, the P value of the Fisher exact test is presented.

**Table 2.—IgG Anticapsular Antibody Responses**

<table>
<thead>
<tr>
<th>Geometric Mean Antibody Concentration, U/mL (95% Confidence Interval)</th>
<th>Priming Vaccine</th>
<th>Prior to Vaccine†</th>
<th>2 mo After Dose ‡</th>
<th>1 mo After Dose §</th>
<th>Prior to Booster ‡</th>
<th>1 mo After Booster ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1, meningococcal C conjugate</td>
<td>0.20 (0.19-0.21) (n = 69)</td>
<td>3.0 (3.8-6.4) (n = 66)</td>
<td>20.0 (16.0-26.5) (n = 65)</td>
<td>1.5 (1.2-2.0) (n = 65)</td>
<td>69.0 (50.9-96) (n = 65)</td>
<td></td>
</tr>
<tr>
<td>Group 2, meningococcal C polysaccharide</td>
<td>0.21 (0.20-0.22) (n = 70)</td>
<td>1.2 (0.96-1.6) (n = 69)</td>
<td>1.5 (1.1-1.9) (n = 67)</td>
<td>0.83 (0.65-1.1) (n = 65)</td>
<td>1.3 (0.96-1.8) (n = 65)</td>
<td></td>
</tr>
<tr>
<td>Group 3, hepatitis B virus (control)</td>
<td>0.21 (0.19-0.22) (n = 70)</td>
<td>0.20 (0.15-0.26) (n = 66)</td>
<td>0.20 (0.16-0.26) (n = 65)</td>
<td>0.21 (0.16-0.28) (n = 62)</td>
<td>2.5 (1.8-3.5) (n = 61)</td>
<td></td>
</tr>
</tbody>
</table>

*Toddlers were given 2 injections separated by 2 months of either meningococcal C conjugate vaccine, plain meningococcal polysaccharide vaccine, or hepatitis B virus vaccine (control). Twelve months after dose 2, all subjects received a booster dose of plain meningococcal polysaccharide vaccine.‡Prior to vaccination, P = .54 for group 1 vs group 2; P = .78 for group 1 vs group 3; and P = .73 for group 2 vs group 3. §For 2 months after dose 1, 1 month after dose 2, and prior to the booster vaccination, P < .001 for all comparisons. ||One month after the booster vaccination, P < .001 for group 1 vs group 2 and for group 1 vs group 3; P = .006 for group 2 vs group 3.

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Table 3.—Percentage of Toddlers in Each Group With Serum Bactericidal Antibody Response of at Least 1:8*

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccine</th>
<th>Prior to Vaccine†</th>
<th>2 mo After Dose †</th>
<th>1 mo After Dose ‡</th>
<th>Prior to Booster§</th>
<th>1 mo After Booster</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1, meningococcal C conjugate</td>
<td>2 (0-12) (n = 43)</td>
<td>90 (79-97) (n = 52)</td>
<td>98 (88-100) (n = 45)</td>
<td>88 (72-97) (n = 33)</td>
<td>100 (89-100) (n = 33)</td>
<td></td>
</tr>
<tr>
<td>Group 2, meningococcal polysaccharide</td>
<td>2 (0-12) (n = 45)</td>
<td>32 (19-47) (n = 47)</td>
<td>32 (19-47) (n = 47)</td>
<td>22 (9-40) (n = 32)</td>
<td>19 (7-36) (n = 32)</td>
<td></td>
</tr>
<tr>
<td>Group 3, hepatitis B virus (control)</td>
<td>0 (0-13) (n = 26)</td>
<td>0 (0-13) (n = 27)</td>
<td>0 (0-13) (n = 27)</td>
<td>15 (4-34) (n = 27)</td>
<td>54 (34-72) (n = 28)</td>
<td></td>
</tr>
</tbody>
</table>

* Toddlers were given 2 injections separated by 2 months of either meningococcal C conjugate vaccine, plain meningococcal polysaccharide vaccine, or hepatitis B virus vaccine (control). Twelve months after dose 2, all subjects received a booster dose of plain meningococcal polysaccharide vaccine. For vaccination schedule see Table 2.
† Prior to vaccination, P = .36 for group 1 vs group 2; P = .69 for group 1 vs group 3; and P = .31 for group 2 vs group 3.
‡ For 2 months after dose 1 and 1 month after dose 2, P = .001 for all comparisons.
§ Prior to the booster vaccination, P = .001 for group 1 vs group 2 and for group 1 vs group 3; P = .57 for group 2 vs group 3.
§ One month after the booster vaccination, P = .001 for group 1 vs group 2 and for group 1 vs group 3; P = .002 for group 2 vs group 3.

Figure 2.—Serum bactericidal antibody responses to vaccination. Each child received 2 doses of the respective vaccine, separated by 2 months. Serum samples were obtained prior to vaccination, 2 months after first injection, and 1 month after second injection. All children were boosted 14 months after study entry with plain meningococcal polysaccharide. Serum samples were obtained immediately before the booster and 1 month later. Compared with the polysaccharide priming group, subjects in the conjugate vaccine priming group had higher geometric means at all points after priming or booster vaccination (P < .001). Compared with the hepatitis B virus vaccine control group, subjects assigned to the meningococcal polysaccharide priming group had higher responses 2 months after first injection and 1 month after second injection (P = .02), and lower responses after the booster vaccination (P = .02).

However, this study does not allow an assessment of the adequacy of a single dose of conjugate vaccine with respect to duration of protection because all toddlers in group 1 received 2 doses. Further studies are needed to evaluate this question.

In direct contrast with the response to the conjugate vaccine, 1 or 2 doses of plain meningococcal C polysaccharide resulted in much lower primary antibody responses than the conjugate vaccine. Furthermore, group 2, when given polysaccharide for the priming vaccination, showed evidence of a hyporesponsive state 12 months later. Previous suggestions that the plain meningococcal C polysaccharide could induce a state of hyporesponsiveness were based on small numbers of infants immunized in the first 6 months of life. Due to the poor immunogenicity of plain meningococcal polysaccharide in this age group and the question of induction of an immunologic hyporesponsive state, the plain meningococcal polysaccharide vaccine is not used routinely in this age group. The present study demonstrates that toddlers are also susceptible to the induction of hyporesponsiveness by immunization with meningococcal polysaccharide vaccine. A recent small study by Granoff et al also suggests that induction of hyporesponsiveness to plain meningococcal C polysaccharide may extend to adults. The duration of the hyporesponsive state following immunization with plain meningococcal C polysaccharide vaccine is unknown. In this toddler study, it was present for at least 1 year after 2 doses of plain meningococcal C polysaccharide, whereas in the adult study, evidence of hyporesponsiveness was observed 4 years after receipt of 1 dose of the meningococcal polysaccharide vaccine.

The clinical importance in toddlers of the development of hyporesponsiveness to meningococcal C polysaccharide after vaccination with plain meningococcal polysaccharide vaccine is unknown. However, the data are consistent with impaired serum anticapsular antibody response when encountering meningococcal C organisms, which might lead to an
increase in the risk of developing invasive disease. Although there are no epidemiological data supporting an increased risk of disease in previously vaccinated toddlers, the present results suggest caution in administering plain meningococcal C polysaccharide vaccine to toddlers, especially if the risk of meningococcal C disease is low. The results of this study also emphasize the importance of maintaining surveillance for meningococcal C disease in the large cohort of infants and toddlers who received I dose of the quadrivalent meningococcal vaccine in Canada during the meningococcal C outbreaks in 1991 and 1992. Further work also is needed to determine if toddlers who have been rendered hyporesponsive to plain meningococcal C polysaccharide by previous polysaccharide vaccination can be boosted with conjugated meningococcal C vaccine. In a previous study of Gambian infants who showed evidence of hyporesponsiveness to plain meningococcal C polysaccharide vaccine, the conjugate vaccine appeared to be effective. 

In contrast with the plain meningococcal polysaccharide vaccine, the meningococcal C conjugate vaccine appears to have very similar properties to those of the conjugated H influenzae type b vaccines, which have been highly effective in controlling H influenzae type b invasive disease. These properties include increased immunogenicity in toddlers vs plain polysaccharide and the induction of robust immunologic memory as shown by the IgG and bactericidal booster response to the plain meningococcal polysaccharide vaccine 12 months after priming. Similar results have recently been shown in infants given this meningococcal C conjugate vaccine. Although immunization with H influenzae type b vaccine also leads to decreased pharyngeal carriage, no data are yet available on the impact of meningococcal C conjugate vaccine on carriage of N meningitidis serogroup C or other serogroups. To examine this question, a very large sample size would be necessary since even in an outbreak situation, carriage of N meningitidis regardless of serogroup is a rare event in infants and young children. Extrapola-

from the H influenzae type b conjugate vaccine experience, together with the data from this study and the recent infant meningococcal C study, suggest that a universal meningococcal C conjugate vaccine program in infants and toddlers may be effective in controlling invasive meningococcal C disease and may be particularly useful in control of outbreak situations.

This study was supported by a grant from Chiron Vaccines, Inc, Emeryville, Calif.

We acknowledge the important contributions of the following individuals to this study: Wai Ping Leong, MS, for statistical analysis; George Santos and Bill Wacker for performing the laboratory assays; Howard Raff, PhD, for review of the manuscript and project management; and Helen Etherington, RN, Patricia Pottie, RN, and Joyce Good, RN, the research coordinators at the study sites.

References