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 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.
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 (J35021L1) 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. 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-

Figure 1.—Trial profile.
mately 2 hours in Mueller-Hinton broth containing 0.25% glucose. The comple-
ment source was pooled serum samples obtained from 3 healthy adults who had
no detectable anticapsular 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 re-
action mixture.

Antibody concentrations were trans-
formed (logarithm to base 10) for calculation of geometric means. IgG antibody
centrations of less than 0.4 U/mL were assigned a value of 0.2 U/mL and bac-
tericidal titer 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 geo-
metric means were tested by using the
P values from the analysis of variance
type A. There were 1 primary and 3 sec-
ondary planned comparisons. The pri-
mary 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 men-
ingococcal polysaccharide C as measured by
ELISA or serum 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 meningococcal serogroup C as measured by ELISA and bac-
tericidal assay 2 months after the first
injection; (2) there was no difference be-
tween the 2 meningococcal vaccines
(group 1 and group 2) in the antibody
response to N meningococcal serogroup C
as measured by ELISA and bactericidal
assay 12 months after the second in-
jection; and (3) there was no difference
among all 3 groups in the antibody
response to N meningococcal 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 vac-
cine-related serious adverse events were
observed during the study and all vac-
cines were well tolerated. Table 1 pre-
sents data on reactogenicity within 48
hours following the first and second
doses of vaccine.

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

The IgG anticapsular antibody re-
response figures 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
dose 2 than the corresponding
response to the meningococcal polysac-
charide vaccine (group 2). Twelve
months after the last primary dose, the
IgG antibody concentrations in the me-
ningococcal C conjugate vaccine
(group 1) were 2-fold higher than those in
the meningococcal polysaccharide vaccine
(group 2) ($P<.001$). Following the
plain meningococcal C polysaccharide
booster dose 12 months after the last
injection of the priming vaccine, group 1
did not show evidence of induction of immu-
nologic B-cell memory by the conjugate
toxin (25-fold higher anticapsular
antibody responses than control tod-

dlars [group 3] vaccinated with the poly-
saccharide 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

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 $\geq$38°C</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&gt;.99</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>0</td>
<td>0</td>
<td>0</td>
<td>&gt;.99</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 $\geq$38°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>0</td>
<td>0</td>
<td>0</td>
<td>&gt;.99</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>31</td>
<td>29</td>
<td>15</td>
<td>.08</td>
</tr>
</tbody>
</table>

†P value is from the Pearson $\chi^2$ 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>
</tr>
</thead>
<tbody>
<tr>
<td>Priming Vaccine</td>
</tr>
<tr>
<td>Prior to Vaccine†</td>
</tr>
<tr>
<td>2 mo After Dose 1†</td>
</tr>
<tr>
<td>1 mo After Dose 2†</td>
</tr>
<tr>
<td>Prior to Booster §</td>
</tr>
<tr>
<td>1 mo After Booster §</td>
</tr>
<tr>
<td>Group 1, meningococcal C conjugate (control)</td>
</tr>
<tr>
<td>0.20 (0.19-0.21) (n = 69)</td>
</tr>
<tr>
<td>3.0 (2.8-3.4) (n = 66)</td>
</tr>
<tr>
<td>20.0 (16.0-26.0) (n = 65)</td>
</tr>
<tr>
<td>1.6 (1.2-2.0) (n = 58)</td>
</tr>
<tr>
<td>69.0 (50.9-96.0) (n = 60)</td>
</tr>
<tr>
<td>Group 2, meningococcal polysaccharide (n = 70)</td>
</tr>
<tr>
<td>0.21 (0.20-0.22) (n = 70)</td>
</tr>
<tr>
<td>1.2 (0.96-1.6) (n = 69)</td>
</tr>
<tr>
<td>1.5 (1.1-1.9) (n = 67)</td>
</tr>
<tr>
<td>0.93 (0.65-1.1) (n = 61)</td>
</tr>
<tr>
<td>1.3 (0.96-1.8) (n = 65)</td>
</tr>
<tr>
<td>Group 3, hepatitis B virus (control)</td>
</tr>
<tr>
<td>0.21 (0.19-0.22) (n = 70)</td>
</tr>
<tr>
<td>0.20 (0.15-0.26) (n = 66)</td>
</tr>
<tr>
<td>0.20 (0.16-0.26) (n = 65)</td>
</tr>
<tr>
<td>0.21 (0.16-0.28) (n = 62)</td>
</tr>
<tr>
<td>2.5 (1.8-3.5) (n = 61)</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 booster 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.
Table 3.—Percentage of Toddlers in Each Group With Serum Bactericidal Antibody Response of at Least 1:8

<table>
<thead>
<tr>
<th>Priming Vaccine</th>
<th>Subjects, % (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prior to Vaccine†</td>
</tr>
<tr>
<td></td>
<td>2 mo After Dose †</td>
</tr>
<tr>
<td></td>
<td>1 mo After Dose †</td>
</tr>
<tr>
<td></td>
<td>Prior to Booster§</td>
</tr>
<tr>
<td></td>
<td>1 mo After Booster</td>
</tr>
<tr>
<td>Group 1, meningococcal C conjugate</td>
<td>2 (0-12) (n = 43)</td>
</tr>
<tr>
<td></td>
<td>90 (79-97) (n = 52)</td>
</tr>
<tr>
<td></td>
<td>98 (88-100) (n = 45)</td>
</tr>
<tr>
<td></td>
<td>88 (72-97) (n = 33)</td>
</tr>
<tr>
<td>Group 2, meningococcal polysaccharide</td>
<td>2 (0-12) (n = 45)</td>
</tr>
<tr>
<td></td>
<td>32 (19-47) (n = 47)</td>
</tr>
<tr>
<td></td>
<td>32 (19-47) (n = 47)</td>
</tr>
<tr>
<td></td>
<td>22 (9-40) (n = 32)</td>
</tr>
<tr>
<td>Group 3, hepatitis B virus (control)</td>
<td>0 (0-13) (n = 26)</td>
</tr>
<tr>
<td></td>
<td>0 (0-13) (n = 27)</td>
</tr>
<tr>
<td></td>
<td>0 (0-13) (n = 27)</td>
</tr>
<tr>
<td></td>
<td>15 (4-34) (n = 27)</td>
</tr>
<tr>
<td></td>
<td>54 (34-72) (n = 28)</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, $P = .002$ for all comparisons.

‡For 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 vaccine. 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.8,9 Since meningococcal C polysaccharide is highly immunogenic in toddlers,8 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 al18 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 vaccinates 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 the quadrivalent meningococcal C 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 increased immunogenicity in toddlers vs those marked 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.

Extrapolation 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 Leung, MS, for statistical analysis; George Santos 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