Infant Immunization with Pneumococcal CRM$_{197}$ Vaccines: Effect of Saccharide Size on Immunogenicity and Interactions with Simultaneously Administered Vaccines

Robert S. Daum, Deborah Hogerman, Margaret B. Rennels, Kathleen Bewley, Frank Malinoski, Edward Rothstein, Keith Reisinger, Stan Block, Harry Keyserling, and Mark Steinhoff

Six pentavalent pneumococcal conjugate vaccines (Pn-CRM$_{197}$) were evaluated among 400 infants. The vaccines differed in saccharide chain length (oligosaccharide [OS] or polysaccharide [PS]) and saccharide quantity (0.5, 2, or 5 µg). Subjects were randomized into groups 1–6 (Pn-CRM$_{197}$ recipients) or 7 (controls) for immunization at 2, 4, and 6 months of age. Pn-CRM$_{197}$ were well tolerated and elicited mean antibody concentrations that exceeded those in controls for all 5 capsular serotypes. PS formulations were generally more immunogenic than their OS counterparts. For PS vaccines, a dose-response was documented (5 µg > 2 µg > 0.5 µg), but the differences between the 5- and 2-µg formulations were insignificant. The mean anti-PRP antibody concentration was significantly higher among Pn-CRM$_{197}$ recipients. It is concluded that PS vaccines are more immunogenic than OS vaccines. The improved immunogenicity from Haemophilus type b oligosaccharide conjugate (HbOC) vaccine when given with Pn-CRM$_{197}$ suggests that a decreased dose of HbOC vaccine may be sufficient to elicit protection.

There has been long-standing interest in the prevention of pneumococcal infections by vaccination. A hexavalent, unconjugated capsular polysaccharide (PS) vaccine against Streptococcus pneumoniae intended for use in children was marketed by Squibb in 1946 [1, 2] but withdrawn in 1954 when antimicrobial therapy was believed to have diminished the need for disease prevention. Subsequently, the observation by Austrian and Gold [3] that mortality in adults remained high despite early antimicrobial therapy [3] led to the licensure of 14-valent and, subsequently, 23-valent unconjugated capsular PS vaccines. While several studies have suggested these vaccines have a role in preventing pneumococcal infections in adults and older children, it is generally held that they are inconsistently immunogenic and ineffective in young children [4, 5].

The great success of recent efforts to prevent Haemophilus influenzae type b infections by vaccination with conjugate vaccines consisting of the capsular saccharide covalently joined to a protein carrier [6] has spurred development of pneumococcal saccharide conjugate vaccines. As was the case for H. influenzae type b conjugate vaccines, pneumococcal conjugate vaccines prepared for clinical trials differ in the carrier protein used, the saccharide molecular size, and the method of conjugating the saccharide to the protein.

One protein carrier used in the manufacture of an H. influenzae type b vaccine (HbOC) is a cross-reacting mutant diphtheria toxin molecule called CRM$_{197}$. This protein has been linked to pneumococcal saccharides to prepare pneumococcal conjugate vaccines (Pn-CRM$_{197}$) for clinical evaluation. Although the immunogenicity of the type 6B component was minimal, the recent demonstration that a bivalent vaccine consisting of 2 pneumococcal polysaccharides, types 6B and 23F, separately conjugated to CRM$_{197}$ was immunogenic and well tolerated in toddlers [7] prompted interest in the evaluation of the safety and immunogenicity of pneumococcal saccharide conjugate vaccines in infants. A pentavalent oligosaccharide (OS)-CRM$_{197}$ conjugate vaccine (10 µg per dose) was well tolerated and immunogenic in Finnish infants immunized at 2, 4, and 6 months of age [8]. Similar results were obtained in Gambian infants immunized at 2, 3, and 4 months of age with a PS vaccine containing 5 µg PS per dose (PS-5) [9].

These efforts have spurred further investigation. The optimal saccharide chain length for a pneumococcal conjugate vaccine is not known. For H. influenzae type b, the theoretical advantage in terms of better immunogenicity accorded to an OS with
Table 1. Occurrence of fever (≥38.4°C) in infants vaccinated with *Haemophilus* b oligosaccharide conjugate vaccine with diphtheria–tetanus toxoids–pertussis vaccine (HbOC/DTP) and those vaccinated with HbOC/DTP plus pneumococcal saccharide conjugate vaccine (Pn-CRM197).

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Age at vaccine administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 months</td>
</tr>
<tr>
<td>HbOC/DTP + Pn-CRM&lt;sub&gt;197&lt;/sub&gt;</td>
<td>30/343 (8.7)</td>
</tr>
<tr>
<td>HbOC/DTP</td>
<td>6/56 (11)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. with fever/no. vaccinated (%).
* P < .01 compared with first- and second-dose Pn-CRM<sub>197</sub> recipients. † P = .04 compared with HbOC/DTP controls.

the higher multiplicity of protein coupling [10, 11] led to the development of the 20-mer OS-CRM<sub>197</sub> conjugate vaccine, HbOC. Furthermore, the optimal amount of saccharide that should be present for optimal immunogenicity has not received evaluation in young infants. Also, if a pneumococcal vaccine were introduced into clinical practice, it would be convenient to administer it at visits where other vaccines are currently recommended for administration. Therefore, it would be of interest to know whether children who received a Pn-CRM<sub>197</sub> vaccine had acceptable serum antibody responses to diphtheria and tetanus toxoids and the serotype b capsule of *H. influenzae*.

Table 2. Percentage of infants with local adverse events after receiving *Haemophilus* b oligosaccharide conjugate vaccine with diphtheria–tetanus toxoids–pertussis vaccine (HbOC/DTP) alone (n = 157) or with pneumococcal conjugate vaccine (n = 970).

<table>
<thead>
<tr>
<th>Vaccine administration site</th>
<th>n</th>
<th>Erythema</th>
<th>Induration</th>
<th>Tenderness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumococcal conjugate vaccine*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS-5</td>
<td>158</td>
<td>12</td>
<td>18</td>
<td>28</td>
</tr>
<tr>
<td>OS-5</td>
<td>164</td>
<td>20</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td>PS-2</td>
<td>159</td>
<td>19</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td>OS-2</td>
<td>162</td>
<td>16</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>OS-0.5</td>
<td>160</td>
<td>20</td>
<td>11</td>
<td>28</td>
</tr>
<tr>
<td>OS-0.5</td>
<td>167</td>
<td>21</td>
<td>20</td>
<td>28</td>
</tr>
<tr>
<td>HbOC/DTP&lt;sup&gt;§&lt;/sup&gt;</td>
<td>970</td>
<td>32&lt;sup&gt;›&lt;/sup&gt;</td>
<td>33&lt;sup&gt;›&lt;/sup&gt;</td>
<td>32&lt;sup&gt;›&lt;/sup&gt;</td>
</tr>
<tr>
<td>HbOC/DTP&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>157</td>
<td>41&lt;sup&gt;†&lt;/sup&gt;</td>
<td>36&lt;sup&gt;†&lt;/sup&gt;</td>
<td>32&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* PS = polysaccharide; OS = oligosaccharide. No. after hyphen refers to saccharide dose in μg.
† % experiencing adverse event significantly (P < .002) exceeded that for any pneumococcal conjugate vaccine group.
†† In subjects not receiving simultaneous pneumococcal conjugate vaccine.

To study these issues, we evaluated the safety and immunogenicity of several pentavalent Pn-CRM<sub>197</sub> vaccines that differed in the quantity of saccharide per dose and the chain length of the saccharide moiety in a population of healthy US infants.

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**Figure 1.** Anticapsular antibody against serotype 6B *Streptococcus pneumoniae*–CRM<sub>197</sub> conjugates. Geometric mean anticapsular antibody concentration (GMC) for each of 6 type 6B conjugate formulations is plotted on y axis; age at each immunization is plotted on x axis. At 4 months, GMC for all type 6B conjugate formulations decreased significantly (P = .0001) relative to GMC at previous visit. At 6 months, GMC did not change significantly relative to GMC at previous visit for any of type 6B formulations; however, at 7 months, GMC for all formulations increased significantly (P = .0001) relative to GMC at previous visit. PS = polysaccharide; OS = oligosaccharide. Nos. following PS- and OS- refer to saccharide dose in μg.
Infants who received these vaccines also received vaccines appropriate for age; thus, as serum volumes allowed, we also evaluated the possibility of interference with relevant antigens present in these other vaccines.

Materials and Methods

Vaccines. Two types of pentavalent pneumococcal saccharide conjugate vaccines were used. Both contained capsular saccharides from serotypes 6B, 14, 18C, 19F, and 23F. In one, the capsular material from each serotype was an OS of defined length. In the other, capsular PS from serotypes 6B, 14, 19F, and 23F and the OS from serotype 18C formulated the vaccine, which we designated PS conjugate vaccine.

The OS-CRM197 conjugates were synthesized by either first hydrolyzing the native PS to smaller fragments and then subjecting the fragments to limited periodate oxidation or by oxidizing the native PS and then hydrolyzing it into smaller fragments. The PS-CRM197 conjugates were synthesized by oxidizing native pneumococcal PS with sodium metaperiodate to introduce random aldehyde functional groups. Oxidized OS or PS fragments were coupled to CRM197 in the presence of sodium cyanoborohydride, a reductive amination similar to that used in the preparation of HbOC [12].

The pentavalent vaccines were a mixture of the individual serotype saccharide-protein conjugates. For each pneumococcal serotype, three dose levels (5 µg [PS-5], 2 µg [PS-2], or 0.5 µg [PS-0.5]) were formulated on the basis of the saccharide content.

Subjects and study protocol. Four hundred healthy 2-month-old infants were recruited at seven sites (Pennridge Pediatric Associates, Pittsburgh Pediatric Research, Nesset Pediatric Pavilion in suburban Chicago, the University of Maryland School of Medicine, Emory University, Johns Hopkins University, and Kentucky Pediatric Research). Subjects were randomized into 7 groups for immunization at 2, 4, and 6 months of age. Groups 1–6 received one of the six pentavalent pneumococcal conjugate vaccine formulations. Group 7 (controls) did not receive a pneumococcal vaccine. All subjects were given HbOC/diphtheria–tetanus toxoids–pertussis vaccine (HbOC/DTP, Tetramune; Lederle Laboratories, Pearl River, NY) intramuscularly in a separate syringe at a separate site (anterior thigh) and oral polio vaccine (OPV, Orimune; Lederle Laboratories) at the same visit. The parents of children receiving HbOC/DTP and a pneumococcal conjugate vaccine were blinded as to the site of administration of the two vaccines.

Blood samples were obtained prior to the three immunizations and 1 month after the third immunizations, when the infants were 7 months old. Serum was separated from blood, stored at −20°C or −70°C, and shipped to Lederle-Praxis (West Henrietta, NY) on dry ice.

Children were observed for 30 min following each immunization, and parents were requested to record, on a standardized form, information regarding the presence and magnitude of fever (≥38.4°C), erythema, and induration or tenderness at a site of immunization.
Antibody assays. IgG antibody to each pneumococcal saccharide contained in the vaccine was measured at Lederle-Praxis by ELISA after preabsorption with cell wall PS as previously described [13]. Specimens were encoded to ensure blinding of laboratory personnel. The serotype-specific antibody concentration was expressed in micrograms per milliliter relative to human anti-pneumococcal standard serum (lot 89-S; Center for Biological Evaluation and Review, US Food and Drug Administration, Rockville, MD) [10]. Antibody to the serotype b capsule of H. influenzae type b was quantitated by an ELISA previously characterized at Lederle-Praxis [14, 15]. Antibodies to tetanus and diphtheria toxoids were determined by ELISA. For both, the relevant toxoid was used to coat polystyrene microtiter plates. Bound antibody was detected with goat anti-human IgG coupled to alkaline phosphatase, and p-nitrophenyl phosphate was used to quantitate bound enzyme. Absorbance was compared with that for reference sera: H89-F for tetanus toxoid (assigned value, 55 IU/mL) and C587769 for diphtheria toxoid (assigned value, 0.3 IU/mL). The lower limit of detection was 0.001 U/mL for anti-tetanus toxoid antibodies and 0.001 IU/mL for anti-diphtheria toxoid antibodies.

Statistical analyses. Because antibody concentrations in pre- and postimmunization sera were not normally distributed, the values were transformed to their logarithms for analysis. A value equal to 0.5 times the lower limit of detection was assigned to sera with no detectable antibody. Antibody concentrations for the 7 groups were compared by use of unpaired $t$ tests. Proportions were compared by use of $\chi^2$ tests or, when appropriate, Fisher’s exact test. Statistical software (Statview; Abacus Concepts, Berkeley, CA) was used for all calculations. For comparisons, $P < .05$ was considered significant.

Results

Subjects. Four hundred infants with a mean age of 2.2 months (range, 1.4–3.3) at entry were enrolled in the study. The mean age at the second vaccination was 4.3 months (range, 2.9–8.3), and at the third vaccination it was 6.4 months (range, 4.9–10.8). Three hundred fifty patients (88%) completed the study protocol. Reasons for withdrawal included adverse events associated with vaccine administration (7 infants total: high-pitched, unusual cry [4], inconsolable cry [1], persistent crying for $>3$ h [1], hypotonic and hyporesponsive episode [1]), parental request (11 infants), noncompliance (31 infants), and culture-proven pertussis (1 infant). Ten subjects were hospitalized during the study period. Discharge diagnoses included otitis media (1 infant), reactive airway disease (2 infants; 1 had otitis media), upper respiratory tract infection (1 infant), gastroesophageal reflux with tracheomalacia (1 infant), respiratory syncytial virus pneumonia (1 infant), bronchiolitis (1 infant), croup (1 infant), paroxysmal coughing disorder (1 infant), and a presumed viral illness (1 infant).

Adverse reactions. Temperature $\geq 38.4^\circ\text{C}$ occurred in subjects after 141 (12.5%) of 1127 doses. A trend toward an in-
Figure 4. Anticapsular antibody against serotype 19F Streptococcus pneumoniae--CRM\textsubscript{197} conjugates. Geometric mean anticapsular antibody concentration (GMC) for each of 6 type 19F conjugate formulations is plotted on y axis; age at each immunization is plotted on x axis. At 4 months, GMC decreased significantly ($P \leq .0001$) relative to GMC at previous visit for all formulations except for all type 19F polysaccharides (PS) ($P \geq .14$). For PS conjugate formulations at 6 and 7 months, GMC significantly ($P = .0001$) increased. In contrast, at 6 months, oligosaccharide (OS) conjugate formulations did not change significantly relative to GMC at previous dose. However, at 7 months, GMC for all conjugate formulations uniformly increased significantly ($P \leq .01$) relative to GMC at previous dose except for 2-µg PS vaccine ($P = .05$). Nos. following PS- and OS- refer to saccharide dose in µg.

Increased rate of fever with increasing dose occurred in the pneumococcal vaccine recipients: After the third dose of HbOC/DTP + Pn-CRM\textsubscript{197} (administered to infants at 6 months of age), the rate was significantly higher than that occurring at 2 and 4 months of age and also higher compared with rates for controls, who received HbOC/DTP only (table 1). Temperature $\geq 39.1^\circ\text{C}$ occurred in subjects after 22 (2.0%) of 1127 administered doses. There was no pattern observed with respect to receipt of any of the pneumococcal vaccines, group, or dose number.

When summed across dose number and stratified by vaccine regimen (table 2), erythema was significantly more frequent at sites where HbOC/DTP was administered than at the site of any pneumococcal conjugate vaccine. The rate of erythema did not change with dose number for any of the vaccine regimens, although erythema was significantly more frequent at sites where the pneumococcal OS conjugate vaccine containing 0.5 µg of each saccharide (OS-0.5) was administered than at sites of administration of the PS-5 vaccine. Similarly, induration and tenderness were more frequent at sites where HbOC/DTP was administered, and the rate of induration at the sites of the OS-5.0 (containing 5 µg) and the OS-0.5 pneumococcal conjugates exceeded that occurring at sites where the PS-5 vaccine was administered.

Immunogenicity. Irrespective of saccharide dose and chain length, 1 month after receipt of a 3-dose regimen of a pneumococcal conjugate vaccine, the mean anticapsular antibody concentration exceeded that found in pneumococcal vaccine–naive control infants (figures 1–5).

The pattern of appearance of antibody differed according to the serotype administered and the saccharide chain length. For the serotype 6B conjugate vaccines (figure 1), the first and second doses elicited mean antibody concentrations that did not differ from those of controls. Only after the third dose did the mean antibody concentration in the pneumococcal vaccine recipients exceed that measured in the control infants. For the serotype 14 conjugates (figure 2), the first dose elicited a mean antibody concentration that did not differ from that of controls, but the mean concentrations after the second and third doses did. For the serotype 18C OS-conjugates (figure 3), with the exception of OS-0.5, a statistically significant ($P \leq .03$) increase in anticapsular antibody occurred after each dose. For the serotype 19F conjugate vaccines (figure 4), the mean response differed when stratified according to the saccharide administered. Among those receiving the 19F PS conjugates, the mean anticapsular response after the first dose did not differ from that documented prior to immunization, but significant ($P \leq .01$) increases occurred after the second and third doses.
In contrast, among those receiving the 19F OS conjugates, the mean antibody concentration significantly declined after the first dose and increased only after the third dose. For the serotype 23F conjugates (figure 5), the pattern of response resembled that occurring among recipients of the serotype 14 conjugate vaccines.

In general, PS formulations were more immunogenic than OS formulations of the same dose level, although the difference was not always significant (figure 6). For the OS type 18C conjugates, no significant difference was noted in the mean anticapsular antibody response whether the type 18C OS was a component of the PS or the OS conjugate vaccines.

Following three doses of pneumococcal conjugate vaccine, the mean anticapsular antibody concentrations varied significantly when stratified by serotype \((P < .0001\), analysis of variance). The mean concentration of anticapsular antibody directed against the capsular PSs of serotypes 14 and 19F exceeded all others \((P < .001\), unpaired \(t\) test). Serotype 6B conjugate vaccines were the least immunogenic \((P < .001\), unpaired \(t\) test); types 18C and 23F were intermediate.

For the PS vaccines, a relationship was noted between the saccharide content (dose level) and the magnitude of the anticapsular antibody response. For 4 of the 5 serotypes, the mean antibody response for the PS-5 vaccine was the highest (figure 6), but, for any serotype, this mean did not differ significantly from that elicited by the pneumococcal conjugate PS vaccine containing 2 \(\mu\)g of each saccharide (PS-2).

A detailed analysis of anticapsular antibody responses to the PS-5 formulation is presented in table 3. The mean fold increase for pneumococcal anticapsular antibody in serum obtained from the control infants 1 month after the third immunization ranged from 0.10 to 0.15 for the 5 serotypes contained in the pneumococcal conjugate vaccines. These values reflected a net decline from antibody concentrations measured in serum obtained at the first immunization visit, presumably indicating decay of maternal antibody. For recipients of the PS-5 vaccines, the mean fold increases 1 month after the third immunization were 2.7, 18.9, 13.4, 7.7, and 15.1 for serotypes 6B, 14, 18C, 19F, and 23F, respectively. These mean fold increases were all significantly greater than those calculated for the control infants. Similar data were obtained for the PS-2 conjugate vaccines.

The proportion of children with an anticapsular antibody concentration exceeding 0.15 and 1.0 \(\mu\)g/mL in serum obtained \(\sim 1\) month after the third dose of vaccine uniformly exceeded that in the control infants (table 3).

Anti–tetanus toxoid antibody was assayed in serum obtained prior to immunization and 1 month after receipt of the third dose from subjects receiving the PS-2 vaccine and the control subjects. Prior to immunization, the geometric mean anti–tetanus toxoid antibody concentrations were 0.86 IU (95% confidence interval [CI], 0.58–1.28) and 0.77 IU (95% CI, 0.53–1.13) for PS-2 and control subjects, respectively, values that did not differ significantly. One month after the third immunization...
Figure 6. Geometric mean serum anticapsular antibody concentrations in 7-month-old infants vaccinated with various doses of pneumococcal conjugate polysaccharide (PS) or oligosaccharide (OS) vaccine. * and † indicate significant differences in magnitude of indicated means (insignificant comparisons are not shown). Both PS and OS vaccines contained serotype 18C OS (see text).
Table 3. Geometric mean serum anticapsular antibody concentrations (95% confidence intervals) elicited by pneumococcal saccharide–CRM$_{197}$ polysaccharide (5 μg) conjugate vaccines.

<table>
<thead>
<tr>
<th>Pentavalent vaccine serotype</th>
<th>Before vaccination</th>
<th>After vaccination 1</th>
<th>After vaccination 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaccine</td>
<td>Control</td>
<td>Vaccine</td>
</tr>
<tr>
<td></td>
<td>(95% CI)</td>
<td>(95% CI)</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>6B</td>
<td>0.46 (0.30–0.69)</td>
<td>0.12 (0.09–0.18)</td>
<td>0.19* (0.12–0.30)</td>
</tr>
<tr>
<td>23F</td>
<td>0.16 (0.10–0.24)</td>
<td>0.07 (0.05–0.10)</td>
<td>0.51* (0.34–0.76)</td>
</tr>
<tr>
<td>14</td>
<td>0.21 (0.12–0.35)</td>
<td>0.19* (0.13–0.29)</td>
<td>1.69* (1.15–2.47)</td>
</tr>
<tr>
<td>18C§</td>
<td>0.17 (0.12–0.25)</td>
<td>0.32* (0.21–0.47)</td>
<td>1.36* (1.01–1.83)</td>
</tr>
<tr>
<td>19F</td>
<td>0.49 (0.33–0.75)</td>
<td>0.66* (0.48–0.91)</td>
<td>2.62* (1.97–3.48)</td>
</tr>
</tbody>
</table>

* $P \leq .02$ compared with control.
$^1$ $P \leq .03$ compared with serotypes 14, 19F, and 18C.
$^2$ $P \leq .01$ compared with all other serotypes.
$^3$ Oligosaccharide.

visit, when infants were 7 months old, the values were 8.99 IU (95% CI, 6.38–12.68) and 8.49 IU (95% CI, 6.17–11.69), respectively, a difference that was also not significant.

For anti–diphtheria toxoid antibody, the geometric means prior to immunization were 0.07 IU (95% CI, 0.05–0.09) and 0.05 IU (95% CI, 0.03–0.07) for PS-2 and control subjects, respectively, values that did not differ significantly. One month after the third immunization visit, when infants were 7 months old, the means were 1.70 IU (95% CI, 1.30–2.33) and 1.07 IU (95% CI, 0.80–1.43), respectively, a significant ($P = .02$) difference; all subjects had values $>0.05$ and 0.01 U/mL, antibody concentrations said to be protective against diphtheria [16] and tetanus [17], respectively.

The geometric mean serum antibody directed at the capsular serotype of type b $H$. influenzae (anti-PRP) was higher for each group of pneumococcal vaccine recipients than for control infants who received HibOC/DTP alone (table 4); this was an unexpected finding. The mean for each group significantly ($P \leq .03$) exceeded that for the control children with the single exception of PS-5, for which the mean was 3.80 μg/mL and the same trend was observed, but the difference was not significant ($P = .07$).

Discussion

Our data demonstrate that the pentavalent pneumococcal saccharide conjugate vaccines elicited mean anticapsular antibody concentrations that exceeded those occurring in unimmunized controls, irrespective of saccharide length or quantity of antigen. As expected, the vaccines were safe; no major untoward effects were documented. Minor differences in rates of adverse reactions between groups likely reflected α errors.

The PS conjugate vaccines were more immunogenic than their OS counterparts. For unconjugated capsular PS vaccines, this result would have been expected since a relationship between immunogenicity and saccharide molecular mass established for the dextran elaborated by $Leuconostoc mesenteroides$ has been presumed to extend to other PSs. Indeed, PSs of presumably sufficiently large size to function as effective immunogens from $Salmonella typhi$ [20], $H$. influenzae type b, $S$. pneumoniae [22], and $Neisseria meningitidis$ [23] have provided protection, at least in certain populations. The relationship between chain length and immunogenicity is undoubtedly more complex for conjugate vaccines, with which as few as two repeating units of carbohydrate can evoke an immune response. Conformation of the saccharide moiety, availability of antigenic structural components, and site(s) of saccharide attachment to protein carriers are among the factors, in addition to chain length, that may influence the immune response [9].

The mean concentration of anticapsular antibody elicited by the PS vaccines in infants at 7 months of age varied according to the serotype; the geometric mean ranged from 1.24 μg/mL to 3.87 μg/mL for type 6B to 3.87 μg/mL for type 14. The implication of these data for protection from invasive disease is uncertain because the antibody concentration necessary to infer protection is not known with certainty. Using a model of experimental type 6B pneumococcal bacteremia in mice and rats, Rubin et al. [24] suggested that 0.06–0.09 μg/mL anticapsular antibody was associated with protection. Saladino et al. [25] calculated a minimum protective serum antibody concentration for type 6B that was somewhat higher (0.12–0.54 μg/mL).

The mean anticapsular antibody concentration produced by PS-5 and PS-2 exceeded these estimates of protective efficacy by several fold. For serotypes 18C, 19F, and 23F, the protective concentration estimates of Saladino et al. [25] were 0.9, 0.55–2.9, and 0.27 μg/mL, respectively. Geometric mean antibody concentrations well in excess of these estimates were also produced by the corresponding PS-5 and PS-2 serotype–specific vaccine components. The mean estimates of 2.6–13.0
Table 3. Continued.

<table>
<thead>
<tr>
<th>After vaccination 3</th>
<th>% &gt;0.15 after vaccination 3</th>
<th>% &gt;1.0 after vaccination 3</th>
<th>Mean fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine Control</td>
<td>Vaccine Control</td>
<td>Vaccine Control</td>
<td>Vaccine Control</td>
</tr>
<tr>
<td>1.24* (0.70–2.21)</td>
<td>0.07 (0.05–0.11)</td>
<td>85* 27</td>
<td>60* 4.5</td>
</tr>
<tr>
<td>2.34* (1.56–3.51)</td>
<td>0.03 (0.02–0.04)</td>
<td>96* 9.1</td>
<td>76* 0</td>
</tr>
<tr>
<td>3.87* (2.92–5.15)</td>
<td>0.04 (0.02–0.05)</td>
<td>100* 18</td>
<td>89* 2.3</td>
</tr>
<tr>
<td>2.29* (1.79–2.92)</td>
<td>0.03 (0.02–0.04)</td>
<td>98* 6.7</td>
<td>89* 0</td>
</tr>
<tr>
<td>3.78* (2.98–4.81)</td>
<td>0.06 (0.04–0.10)</td>
<td>100* 25</td>
<td>98* 6.8</td>
</tr>
</tbody>
</table>

$\mu g/mL$ required for protection from type 14 challenge by these authors [25] (which are substantially higher than those estimated for the other serotypes) suggest that a higher concentration of circulating antibody may be required for protection from challenge by *S. pneumoniae* from this serotype. This observation will require clarification.

Relating the concentration of circulating antibody to protective surrogates for pneumococcal conjugate vaccines may be a complex task. For saccharide-protein conjugate vaccines against *H. influenzae* type b, it has been appreciated that protection against disease probably occurs by three mechanisms: (1) provision of circulating anticapsular antibody to the vaccinee, (2) decrease in pharyngeal, asymptomatic carriage among vaccinees with implied decreased circulation of the bacterium, and (3) the apparent ability of these vaccines to prime for an anamnestic response to unconjugated capsular PS vaccine and, by inference, to native capsular PS when presented on the surface of the bacterium. Thus, consideration of the anticapsular response is important but may be limited in terms of direct extrapolation to protection of vaccinated hosts. With parental consent, it is our intention to offer a dose of unconjugated pneumococcal PS vaccine to the recipients of the pneumococcal conjugate vaccines, the hypothesis being that they may have an anamnestic response to the serotypes contained in the conjugate vaccines since they have been primed by the regimens described in this trial.

Which of the 90 pneumococcal serotypes should be represented in a pneumococcal vaccine suitable for use in young infants? The distribution of serotypes that cause invasive disease in young children [26–30] and otitis media [31–35] differ. Moreover, differences in the serotypes of invasive disease isolates in developed and developing countries suggest that it may be necessary to formulate different vaccines for use in different populations. The 5 serotypes of *S. pneumoniae* that comprised the vaccines we evaluated were chosen with the help of data from surveys of invasive disease isolates in the United States. The 5 serotypes in the vaccines that we evaluated were responsible for 67.9% of isolates from patients with invasive disease in California in 1992–1994 [36] and for 68.5% in Connecticut in 1984–1993 [26]. If protection could have been extended to serotypes that were immunologically related to the 5 serotypes contained in the vaccine studied herein, then 77.3% (California) and 74.0% (Connecticut) of the invasive infections could have been prevented. Similar considerations will undoubtedly apply to the prevention of otitis media. For example, the 5 serotypes contained in the pneumococcal conjugate vaccines we studied caused 46% of the cases of symptomatic otitis media in children [35]. If protection had been accorded to immunologically related serotypes, then 56% of cases could have been prevented.

While the importance of conjugate vaccine priming in protecting a vaccinated host is uncertain, it is of interest to speculate whether a three-dose schedule (e.g., at 2, 4, and 6 months of age) is necessary to achieve this effect or whether fewer doses might suffice. The implications of this question relate to decreasing the number of needle sticks for a child to be "up-to-date," to the development of combination vaccines that minimize the likelihood of antigenic interference and to decreasing the cost of a vaccination program, an issue particularly important in some developing countries. Our study was not designed to address whether fewer than three doses are sufficient to render a subject "primed." However, from the data in figures 1–5, one might infer that the number of doses of conjugate vaccine required for priming may vary by serotype in the pentavalent vaccines we studied. A study designed to address this question a priori could clarify this interesting and important issue.

The finding of a higher mean anti-PRP antibody response in the children who received the Pn-CRM$_{197}$ conjugates is of obvious interest but will require further study. These differences are unlikely to reflect $\alpha$ error since they were observed
Table 4. Geometric mean anticapsular antibody concentrations elicited by Haemophilus b oligosaccharide conjugate vaccine with diphtheria–tetanus toxoids–pertussis vaccine given simultaneously with but in a syringe separate from Streptococcus pneumoniae conjugate vaccines.

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>S. pneumoniae conjugate vaccine</th>
<th>Geometric mean Haemophilus influenzae type b anticapsular antibody (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>PS-5</td>
<td>3.8</td>
</tr>
<tr>
<td>38</td>
<td>PS-2</td>
<td>12.0*</td>
</tr>
<tr>
<td>38</td>
<td>PS-0.5</td>
<td>6.4*</td>
</tr>
<tr>
<td>34</td>
<td>OS-5</td>
<td>4.1*</td>
</tr>
<tr>
<td>38</td>
<td>OS-2</td>
<td>5.2*</td>
</tr>
<tr>
<td>39</td>
<td>OS-0.5</td>
<td>8.7*</td>
</tr>
<tr>
<td>32</td>
<td>None (control)</td>
<td>1.7</td>
</tr>
</tbody>
</table>

NOTE. PS = polysaccharide; OS = oligosaccharide. No. after hyphen refers to saccharide dose in µg.

* P ≤ .02 compared with control group.
1 P ≤ .03 compared with PS-5, OS-5, and OS-2.
1 P = .03 compared with PS-5 and OS-5.

...for each of the pneumococcal conjugate vaccine formulations. It is possible that the larger dose of the CRM197 carrier presented to the immune system when HbOC and the pneumococcal conjugate were given at the same time recruited additional T cell help and the resultant greater B cell response.

Antibodies to tetanus toxoid did not differ when the antibody responses of recipients of pneumococcal conjugate vaccines were compared with those of controls. However, anti-diphtheria toxoid antibodies among the PS-2 pneumococcal conjugate vaccines we studied were higher than those for the controls. We speculate that this difference reflects a greater antitoxin antibody response to the higher dose of the carrier protein CRM197 received by the pneumococcal vaccinees, although α error cannot be excluded.

Because the mean pneumococcal anticapsular antibody responses for all 5 serotypes for the infants immunized with the PS-2 vaccine did not differ significantly from those associated with the PS-5 vaccine, additional infant immunization trials will be performed with the vaccine with the lower saccharide content.

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References


