Immune Response of Healthy Women to 2 Different Group B Streptococcal Type V Capsular Polysaccharide–Protein Conjugate Vaccines

Carol J. Baker, 1, 2 Lawrence C. Paoletti, 3 Marcia A. Rench, 1 Hilde-Kari Gutormsen, 2 Morven S. Edwards, 1 and Dennis L. Kasper 3

1 Section of Infectious Diseases, Department of Pediatrics, and 2 Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas; 3 Channing Laboratory, Harvard Medical School, Department of Medicine, Brigham and Women’s Hospital, Boston, Massachusetts

Background. Infections caused by group B streptococcal (GBS) type V are increasingly common. Capsular polysaccharide (CPS)–protein conjugate GBS vaccines are immunogenic in healthy adults, but type V vaccines have not previously been tested.

Methods. Thirty-five healthy, nonpregnant women were randomized to receive an intramuscular dose of GBS type V CPS–tetanus toxoid (TT) vaccine ( ), GBS type V CPS–cross-reactive material (CRM 197) conjugate vaccine ( ), or placebo ( ) (double-masked design). Levels of serum antibodies to type V CPS were measured by ELISA, and functional activity was measured by opsonophagocytosis.

Results. The vaccines were well tolerated. Significant increases in type V CPS–specific immunoglobulin G (IgG) were elicited by both vaccines, peaking at 4–8 weeks and persisting for 26 weeks. Four-fold or greater increases in type V CPS–specific IgG concentrations were noted in postimmunization serum samples obtained from 93% of subjects in each vaccine group. These concentrations persisted in >85% of conjugate-vaccine recipients 104 weeks later. Type V CPS–specific immunoglobulin M was a dominant isotype of immune response to each conjugate. Postimmunization serum samples promoted opsonophagocytic killing of GBS type V in vitro, whereas those from placebo recipients did not.

Conclusion. GBS type V conjugate vaccines are safe and immunogenic and would be appropriate for inclusion in a candidate multivalent GBS vaccine.

Throughout the 1970s and 1980s, virtually all invasive group B streptococcal (GBS) infections were caused by serotypes Ia, Ib, II, and III. In the early 1990s, reports of infection in newborn infants caused by a new serotype, GBS type V, were published in the pediatric literature [1–3]. In 1985, Jelinkova and Motlova [4] first described this provisional new GBS serotype. The type V capsular polysaccharide (CPS) subsequently was purified and was shown by Wessels et al. [5] to have a unique repeating-unit structure consisting of a trisaccharide backbone with 2 distinct side chains. The type V CPS was antigenically distinct and exhibited no immunologic cross-reactivity with other GBS capsular serotypes.

By the mid-1990s, it had become evident that infections caused by GBS type V were increasingly common in pediatric and adult populations. Population-based surveillance of invasive GBS disease in metropolitan Atlanta revealed that 21% of 279 cases of invasive GBS disease were caused by type V [6]. GBS type V now is recognized as a common cause of infections in newborn infants and pregnant women [7], and numerous reports indicate that it has emerged as the most frequently identified serotype causing invasive GBS disease in non-pregnant adults, accounting for 24%–31% of cases [6–9]. This increase in the incidence of GBS type V disease apparently is not related to the introduction of a new, more virulent clone. In a study by Elliott et al. [10] of invasive GBS isolates sent to the Centers for Disease Control and Prevention, a single pulsed-field gel electrophoresis (PFGE) pattern accounted for 56% of GBS type V isolates, but this clone predated the increase in
disease incidence, and a limited number of different PFGE patterns were documented to cause invasive GBS type V infections [10].

The emergence of GBS type V has important implications for vaccine strategies aimed at preventing GBS disease. We envision a multivalent GBS CPS–protein conjugate vaccine that would include the CPSs from the serotypes comprising at least 95% of isolates from invasive infections. Within the past decade, CPS–tetanus toxoid (TT) conjugate vaccines for GBS serotypes Ia, Ib, II, and III have been prepared and have been found to be safe and immunogenic in healthy young adults [11–13]. Although the type V CPS is antigenically distinct, it shares with other GBS serotypes a side chain that terminates with sialic acid [5]. This property means that, as with the other serotypes, reductive amination could be used to create a type V CPS–protein conjugate vaccine. For each of the GBS conjugates evaluated to date, TT has been used as the protein carrier for the vaccine.

In the present investigation, there were 3 objectives. First, the safety and immunogenicity of type V CPS–protein conjugate vaccine was assessed in healthy, nonpregnant women. Second, we determined whether 2 different carrier proteins, TT and diphtheria mutant protein cross-reactive material (CRM197), would provide a T cell–dependent immune response in these young adults. Third, we assessed the isotype of immune response to type V conjugate vaccine. Although both type V CPS–protein conjugate vaccines were well tolerated and similarly immunogenic, the isotype of immune response was predominantly IgM rather than IgG, in contrast to other GBS conjugate vaccines [11–14].

MATERIALS AND METHODS

Preparation of vaccines. Two lots of GBS type V CPS conjugate vaccine were prepared with CPS purified from GBS type V strain CJBI111 grown in continuous culture with a chemically defined medium [15]. In brief, GBS cells were grown in a 2-L fermentor at a cell-mass-doubling time of 2 h. Collected cells were harvested by centrifugation, and the CPS was removed by base extraction. After neutralization, the mixture was treated with RNAse A, DNase I, RNAse T1, and pronase. The CPS-positive material was isolated by use of size-exclusion and ion exchange chromatography, as described elsewhere for GBS type III CPS [16]. Because base treatment causes partial de-N-acetylation, the purified CPS was reacetlyated with acetic anhydride.

Oxidation of type V CPS, conjugation reactions, and vaccine purification methods were performed as described in detail elsewhere [17]. Both lots 95-1 and 95-2 were manufactured according to good laboratory practices by reductive amination coupling of purified type V CPS to TT or CRM197, respectively [17]. The degree of sialic acid oxidation of type V CPS used to prepare type V CPS–TT conjugate vaccine (V-TT) lot 95-1 and type V CPS–CRM197 conjugate vaccine (V-CRM197) lot 95-2 conjugate vaccines was 18%. Diphtheria mutant protein CRM197 was provided by Dr. Rino Rappuoli (Biocine, S.p.A., Siena, Italy). Purified vaccine lots 95-1 and 95-2 were lyophilized in multidose vials with sucrose excipient.

The V-TT lot 95-1 included 76% (wt/wt) CPS and 24% protein; therefore, a 50-µg CPS dose contained 12 µg of TT. The V-CRM197 lot 95-2 included 73% (wt/wt) CPS and 27% (wt/wt) protein; therefore, a 50-µg CPS dose contained 13.5 µg of CRM197. Each vaccine, when reconstituted with 0.45% NaCl, contained 0.005% thimerosal. Both vaccines previously have been shown, by use of the mouse maternal immunization–neonatal pup challenge of GBS infection, to have good potency [18]. The placebo was 0.9% NaCl containing 0.01% thimerosal (provided by the National Institute of Allergy and Infectious Diseases, Bethesda, MD).

Study design. A phase 1 randomized, double-blind, placebo-controlled trial of 2 different type V polysaccharide-protein conjugate vaccines was conducted in healthy, nonpregnant women. Study subjects were women who met each of the following eligibility criteria: 18–45 years old, good health without acute or chronic illness, a negative serum pregnancy test result at study enrollment, use of an acceptable birth control method 3 months after immunization, not breast-feeding, no TT-containing immunization within the prior 12 months, no prior immunization with a GBS type V vaccine, and no allergy to the preservative thimerosal.

Informed consent was obtained from all subjects. Human experimentation guidelines of the US Department of Health and Human Services and/or those of the Baylor College of Medicine institutional review board were followed in the conduct of clinical research.

Thirty-five eligible subjects were randomized to receive either V-TT conjugate (50 µg of CPS/12 µg of TT) or V-CRM197 conjugate (50 µg of CPS/13.5 µg of CRM197) (n = 15 subjects/group) or placebo (n = 5). Each vaccine and placebo was delivered as a single intramuscular injection in the deltoid region at a volume of 0.5 mL. Of the 35 study subjects, 17 (48.6%) were white, 10 (28.6%) were Hispanic, 4 (11.4%) were black (non-Hispanic), and 4 (11.4%) were Asian.

For assessment of reactogenicity, subjects and study personnel, with the exception of the nurse administering the vaccine, were blinded to vaccine or placebo group assignment. Subjects were observed by study personnel for 15–30 min after immunization. Subjects were interviewed by telephone 1 day after immunization and were examined in the clinic 2 days after immunization. Systemic symptoms, including temperature measurement, and injection-site signs and symptoms, when present, were recorded by subjects on a vaccine diary card daily for 8 days after immunization.

1104 • JID 2004:189 (15 March) • Baker et al.
Redness or swelling followed by scores: 0, none; 1, mild, injection site is tender to touch; 2, moderate, arm is sore with movement; and 3, severe, unable to move arm.

Redness or swelling was scored as follows: 0, none to <1 cm; 1, 1–3 cm; 2, >3–5 cm; and 3, >5 cm.

The placebo administered was 0.9% NaCl.

**Table 1. Reactogenicity of group B streptococcal (GBS) type V–tetanus toxoid (TT) and type V–cross-reactive material (CRM) conjugate vaccines in healthy women.**

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>No. of recipients</th>
<th>Pain&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Redness or swelling&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Systemic reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>V-TT</td>
<td>15</td>
<td>26.7</td>
<td>20.0</td>
<td>53.3</td>
</tr>
<tr>
<td>V-CRM&lt;sub&gt;197&lt;/sub&gt;</td>
<td>15</td>
<td>26.7</td>
<td>46.7</td>
<td>26.7</td>
</tr>
<tr>
<td>Placebo</td>
<td>5</td>
<td>60.0</td>
<td>40.0</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE.** Data are percentage of women with the indicated reaction. Both vaccines were administered at a 50-μg capsular polysaccharide dose.

<sup>a</sup> Pain was scored as follows: 0, no pain; 1, mild, injection site is tender to touch; 2, moderate, arm is sore with movement; and 3, severe, unable to move arm.

<sup>b</sup> Redness or swelling was scored as follows: 0, none to <1 cm; 1, 1–3 cm; 2, >3–5 cm; and 3, >5 cm.

**Serologic methods.** Blood samples were obtained before and 2, 4, 8, and 26 weeks after immunization. Thirteen subjects who received GBS V-TT conjugate and 14 subjects who received V-CRM<sub>197</sub> also had blood samples obtained 104 weeks after immunization. Type V CPS–specific IgG, IgA, and IgM in serum samples were measured by use of ELISA using CPS covalently linked to human serum albumin as the coating antigen. Methods for these ELISAs for the quantitative determination of type III CPS–specific IgG and of type II CPS–specific IgG, IgA, and IgM have been described elsewhere [11, 19]. In each assay, a standard human reference serum known to contain 25.1 μg/mL type V CPS–specific IgG, 25.1 μg/mL CPS-specific IgA, and 10.5 μg/mL CPS-specific IgA was included as a control. The lower limits of detection were 0.012 μg/mL for the IgG ELISA, 0.05 μg/mL for the IgM ELISA, and 0.021 μg/mL for the IgA ELISA. No serum specimen had values less than these lower limits of detection. A radioactive antigen–binding assay (RABA) using purified type V CPS extrinsically labeled (but not structurally altered) with tritium was used to quantify type V CPS–specific antibodies in serum by use of a method described elsewhere [19]. The results were expressed as geometric mean concentrations (GMCs) of type V CPS–specific IgG, IgM, or IgA, 95% confidence intervals, and ranges. The immune response to the tetanus component of the V-TT conjugate vaccine was determined by use of the IgG ELISA (performed by Dr. Donna Ambrosino, Massachusetts Public Health Laboratories, Jamaica Plains).

**Opsonophagocytosis assay.** Preimmunization (week 0) and postimmunization (week 4) serum samples from selected recipients of V-TT conjugate (n = 8), V-CRM<sub>197</sub> conjugate (n = 8), and placebo (n = 3) were tested for their ability to promote opsonization, phagocytosis by human polymorphonuclear neutrophils (PMNs), and killing of GBS type V strain G106. Those placebo and vaccine recipients with the lowest type V CPS–specific IgG concentrations in their preimmunization serum samples (each had <0.20 μg/mL) were selected for study. We used an opsonophagocytosis assay described elsewhere [20] but modified it for GBS type V [21]. Reaction mixtures for opsonization consisted of 50 μL of bacteria (2 × 10<sup>6</sup> cfu), 100 μL of heat-inactivated serum, and 150 μL of PBS that were incubated for 30 min at 4°C and then centrifuged at 4°C. The supernatant was decanted, and 15 μL of infant rabbit complement (Serotec), 50 μL of PMNs (1 × 10<sup>8</sup>) from healthy adults, and 50 μL of PBS were added. Results were expressed as the mean log<sub>10</sub> decrease in colony-forming units of GBS before and after incubation for 40 min at 37°C and represented the mean of 2 to 3 experiments.

**Statistical analysis.** GMCs of CPS-specific antibodies before and after immunization were compared by a modification of the Wilcoxon matched pairs test [13]. Comparisons between vaccine groups were assessed by use of the Mann-Whitney U test because of the nonparametric distribution of the data. Differences between proportions of vaccine recipients exhibiting certain response characteristics, such as reactogenicity or ≥4-fold increases in antibody concentrations at intervals after immunization, were evaluated by use of Fisher’s exact test. Immune response also was illustrated by use of reverse cumulative distribution plots [22].

**RESULTS**

**Reactogenicity of GBS V-TT and V-CRM<sub>197</sub> conjugate vaccines.**

The V-TT and V-CRM<sub>197</sub> conjugate vaccines were well tolerated when administered to 30 healthy women of childbearing age. No serious adverse effects were reported. Only 1 subject, a recipient of V-TT conjugate, had probable vaccine-associated systemic symptoms, with onset of headache, malaise, myalgia, and nausea a few hours after immunization. These symptoms persisted for ~48 h and were not accompanied by fever. This woman remembered that she “always experienced” these symptoms after receiving a TT vaccine. The most frequent local reaction in all vaccine recipients was subjective pain at the injection site (table 1). Soreness with arm movement (grade 2 pain) was noted somewhat more often among recipients of V-TT conjugate (53.3%) than among recipients of either the V-CRM<sub>197</sub> conjugate (26.7%) or placebo (40%), but these differ-
Table 2. Immune response of healthy women to group B streptococcal (GBS) type V conjugate vaccines.

<table>
<thead>
<tr>
<th>Vaccine (no. of recipients), ELISA</th>
<th>Week after immunization, GMC of type V CPS–specific antibodies (range) [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>V-TT (n = 15)</strong></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>0.2 (0.02–2.9) [0.09–0.5]</td>
</tr>
<tr>
<td>IgM</td>
<td>1.0 (0.2–8.6) [0.6–1.7]</td>
</tr>
<tr>
<td>IgA</td>
<td>0.1 (0.03–1.0) [0.01–0.2]</td>
</tr>
<tr>
<td><strong>V-CRM197 (n = 15)</strong></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>0.09 (0.02–2.8) [0.05–0.2]</td>
</tr>
<tr>
<td>IgM</td>
<td>0.8 (0.2–6.0) [0.5–1.4]</td>
</tr>
<tr>
<td>IgA</td>
<td>0.2 (0.06–0.5) [0.1–0.2]</td>
</tr>
<tr>
<td><strong>Placebo (n = 5)</strong></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>0.2 (0.1–4.0) [0.01–5.3]</td>
</tr>
<tr>
<td>IgM</td>
<td>1.2 (0.3–8.8) [0.2–7.4]</td>
</tr>
<tr>
<td>IgA</td>
<td>0.1 (0.04–1.2) [0.02–0.8]</td>
</tr>
</tbody>
</table>

**NOTE.** Both vaccines were administered at a 50 μg capsular polysaccharide (CPS) dose. CI, confidence interval; CRM, cross-reactive material; GMC, geometric mean concentration; TT, tetanus toxoid.

- GMC values are given as microgram per milliliter.
- P < .005, antibody levels before immunization vs. antibody levels after immunization (Wilcoxon matched pairs).
- P < .04, vs. placebo group (Mann-Whitney U test).
- n = 13 subjects.
- n = 14 subjects.
- P < .05, vs. GBS type V–TT group (Mann-Whitney U test).
Figure 1. Reverse cumulative distribution plot of group B streptococcal (GBS) type V capsular polysaccharide (CPS)–specific IgG concentrations in serum samples obtained from women before (dashed gray lines) and 8 weeks after (solid black lines) immunization with a single 50-μg CPS dose of GBS type V–tetanus toxoid conjugate (squares) or GBS type V–cross-reactive material (CRM197) conjugate vaccine (triangles).

ences were not significant (P > .05). Mild redness or swelling at the injection site was noted in 13.3% of recipients of each conjugate vaccine but not in placebo recipients.

**Immunogenicity of GBS V-TT and V-CRM197 vaccines.** The immune responses of young women to GBS V-TT and V-CRM197 conjugate vaccines are summarized in table 2. Volunteers in each group had low GMCs of type V CPS–specific IgG, IgM, and IgA in their preimmunization serum samples. The 2 groups of conjugate vaccine-recipients developed significant increases in type V CPS–specific IgG concentrations at each interval tested after immunization (P < .001). As expected, there were no changes noted for the placebo group, who were monitored only through week 26. The V-TT recipients had somewhat higher IgG concentrations than did the V-CRM197 recipients (week-4 GMC, 8.9 vs. 6.5 μg/mL), but these differences were not statistically significant. When the 2 groups of vaccine recipients were compared with the placebo recipients, each had significantly higher concentrations of type V CPS–specific IgG through week 26 after immunization (P < .04).

Each of the GBS type V conjugate vaccines induced ≥4-fold increases in type V CPS–specific IgG concentration in 93%–100% of serum samples obtained from vaccine recipients through week 26 after immunization. Two years after immunization, these increases persisted in 85%–93% of subjects, which indicates that the IgG response to the type V vaccines is long lived. There was a significant and spontaneous increase in type V CPS–specific IgG in 1 placebo recipient that occurred after the week-8 study visit.

Figure 1 is a reverse cumulative distribution plot that shows the percentage of V-TT or V-CRM197 vaccine recipients who had varying concentrations of GBS type V CPS–specific IgG before and 8 weeks after immunization. Before immunization, 13 (86.7%) of 15 GBS V-TT recipients and 14 (93.3%) V-CRM197 recipients had type V CPS–specific IgG serum concentrations <1 μg/mL. When immune responses 8 weeks after immunization were compared, no difference in the magnitude of the response to V-CPS coupled to either protein carrier was noted. Nearly 95% of women who received 1 of these type V conjugate vaccines had ≥1.0 μg/mL type V CPS–specific IgG concentrations after immunization. Thus, TT and CRM197 appeared to be comparable as protein carriers in achieving significant immune response to the type V CPS.

A substantial proportion of the antibodies elicited in response to each GBS type V conjugate vaccine belonged to an immunoglobulin isotype other than IgG. The type V CPS–specific IgM and IgA concentrations in serum samples from vaccine and placebo recipients are summarized in table 2. Each study group had similarly low IgM and IgA GMCs to type V CPS before immunization, but the type V CPS–specific IgM GMCs exceeded those of IgG. Both groups of type V conjugate-vaccine recipients had significant increases in type V CPS–specific IgM and IgA concentrations in their serum through
week 26 after immunization (P < .001), and, in a subgroup of vaccine recipients, these levels remained significantly elevated for 104 weeks. V-TT conjugate recipients had significantly higher type V CPS–specific IgM and IgA concentrations than did placebo recipients through week 8 after immunization, and, for V-CRM197 recipients, this difference persisted through week 26 after immunization (P < .04). Furthermore, V-CRM197 recipients had substantially greater increases in type V CPS–specific IgA concentrations than did V-TT conjugate recipients, but these apparent differences were statistically significant only at the 2-week interval (P < .05).

Figure 2 is a reverse cumulative distribution plot that shows the percentage of V-TT or V-CRM197 vaccine recipients who had varying concentrations of GBS type V CPS–specific IgM before and 8 weeks after immunization. Before immunization, the antibody distributions were almost identical, with nearly 50% of subjects with serum type V CPS–specific IgM concentrations ≥ 1 μg/mL. Eight weeks after immunization, both V-TT and V-CRM197 recipients experienced a substantial shift to the right, indicating a robust immune response that exceeded that for type V CPS–specific IgG, especially among V-CRM197 recipients.

The GMCs determined by use of type V CPS–specific IgG, IgM, and IgA ELISA or by use of RABA in serum samples from recipients of the V-TT and V-CRM197 conjugate vaccines are summarized in figure 3. The type V CPS–specific antibody concentrations quantified by RABA, which measures all antibody isotypes, was highly correlated when type V CPS–specific IgG, IgM, and IgA concentrations determined by ELISA were combined. The correlation coefficients for the type V–specific antibodies measured by RABA or ELISA in serum samples from V-TT or V-CRM197 recipients at each interval range from 0.83 (V-CRM197 at week 0) to 0.99 (V-TT at week 26). All postimmunization correlation coefficients for each vaccine were > .923.

Before immunization, subjects in all 3 groups had similarly low GMCs of tetanus-specific IgG (range, 28–40 μg/mL) in their serum samples. As expected, 4 weeks after immunization, there was no change in tetanus-specific IgG serum levels for recipients of either V-CRM197 conjugate vaccine or placebo. For V-TT conjugate vaccine recipients, the GMC 4 weeks after immunization (194 μg/mL) was significantly increased (P < .001) and was higher than those for V-CRM197 and placebo recipients (P < .02).

Functional activity of type V conjugate vaccine–induced antibodies. Before and 4 weeks after immunization, serum samples from all subjects were tested for functional activity against GBS type V by use of an opsonophagocytosis assay. The postimmunization serum samples from both groups of conjugate-vaccine recipients promoted significant mean log10 reductions in functional activity (0.81 for V-TT and 0.79 for V-CRM; P < .005, paired t tests) (figure 4). There were no significant differ-
Figure 3. Geometric mean concentrations (GMCs) of antibodies to group B streptococcal (GBS) type V capsular polysaccharide (CPS) measured by ELISAs (IgG plus IgM plus IgA) or a radioactive antigen-binding assay (RABA) in serum samples obtained from women administered GBS type V–cross-reactive material (CRM197) or GBS type V–tetanus toxoid (TT) conjugate vaccines, each at a 50-μg CPS dose, before and at each interval after immunization. WK, week.

**DISCUSSION**

In each of the previous clinical trials evaluating GBS CPS–protein conjugate vaccines in human subjects, the carrier protein used was TT [11–14]. The present phase I study has provided the first documentation that a GBS CPS conjugated to a different protein carrier, CRM197, also provides the T cell help associated with a reliable immune response. Both the GBS V-TT and the V-CRM197 conjugate vaccines induced ≥4-fold increases in CPS-specific IgG concentrations in at least 93% of subjects through week 26 after immunization. This information has important implications for the development of GBS conjugate vaccines. For licensed conjugate vaccines, such as those to the *Haemophilus influenzae* type b (Hib) polysaccharide, the carrier protein, the molecular size of the polysaccharide, and the method of conjugation result in different immunologic properties [23]. When 3 different conjugate vaccines were compared, the Hib polysaccharide (PRP) conjugated to outer membrane protein (OMP) of *Neisseria meningitidis* was the only vaccine that induced a response to PRP in infants after the first dose [24]. However, with PRP-OMP, there was no significant booster response in infants after the second and third doses. Infants administered the PRP-TT vaccine responded well after 2 doses, but 3 doses of Hib polysaccharide conjugated to CRM197 were needed for an equivalent antibody response. Although the anticipated target population for GBS conjugate vaccines does not include infants, it does include adults ≥65 years old. More than two-thirds of the cases of invasive GBS disease now occur in nonpregnant adults [25]. A majority of these cases occur in elderly adults who may have age-related immune dysfunction [26]. Compared with younger adults, elderly adults have significantly lower opsonophagocytosis of *Streptococcus pneu-
moniae after immunization with 23-valent pneumococcal polysaccharide vaccine [27]. This functional impairment was even more pronounced in adults ≥80 years old. Routine administration of a 23-valent pneumococcal polysaccharide vaccine is recommended at age 65 years and thereafter for all adults in the United States, as a means of decreasing the incidence of invasive pneumococcal disease in this high-risk population [28]. If GBS conjugate vaccine becomes available to elderly adults, information such as that presented here will guide the choice of the carrier protein that will optimize immunogenicity.

The CPS-specific IgG response to the 2 GBS conjugate vaccines was rapid and reached a maximum GMC 2–4 weeks after immunization. Significant CPS-specific IgG GMCs persisted in serum samples obtained from recipients of either conjugate vaccine for up to 104 weeks after immunization. In concept, the CPS-specific IgG response to these GBS type V conjugate vaccines is similar to that observed after GBS serotypes Ia, Ib, II, and III CPS-TT conjugates were administered as monovalent or bivalent preparations to healthy men and to women of childbearing age [11–14]. Our findings with these 2 different type V CPS–protein conjugate vaccines affirm that an adequate concentration of CPS IgG specific to each of the 5 major disease-causing GBS serotypes could be elicited if a pentavalent GBS conjugate vaccine were available.

When the isotype of the immune response was examined, both type V conjugate vaccines elicited a response that was unique, compared with the 4 previously tested Ia, Ib, II, and III CPS-TT conjugate vaccines [11–13]. The 2 type V conjugate vaccines elicited type V CPS–specific IgM and IgA in substantial concentrations, unlike serotype Ia, Ib, and III CPS-TT conjugates, for which the predominant antibody class elicited in response to immunization is IgG, and unlike type II conjugate, for which the type II CPS–specific IgM and IgA concentrations following immunization were much lower than those of IgG and declined more rapidly than those following immunization with type V conjugate (table 2 and figure 3). Although the magnitude of total type V CPS–specific antibody response was somewhat higher in the V-CRM197 conjugate vaccine recipients, this result was not caused by type V CPS–specific IgG but by the higher concentrations of type V CPS–specific IgM and IgA in serum samples from this latter group.

One possible explanation for the dominance of IgM among the type V CPS–specific antibodies elicited after immunization with V CPS protein conjugates is that it represents a primary immune response to the antigen, whereas the immune response to other GBS conjugate vaccines, such as type III, is consistent with a memory response. Immunogenetic analysis of the immune response to pneumococcal polysaccharide supports this
interpretation [29]. A sampling of the B cell repertoire induced by polysaccharide or conjugate vaccine in adult volunteers suggests that wild-type infection or nasopharyngeal carriage of S. pneumoniae may induce memory and that the response to subsequent immunization, with either a polysaccharide vaccine or a polysaccharide-protein conjugate vaccine, may have the characteristics of a secondary response. Another potential basis for the isotype of immune response lies in the structure of the type-specific CPS. The repeating units of the CPS for GBS serotypes Ia, Ib, and III each consist of a backbone with a 3-sugar side chain terminating in sialic acid [30–32]. The sialic acid residues in the type III repeating unit have been shown to exert conformational control over the immunodominant epitope [33]. The terminal sialic acid residues control the orientation of the penultimate galactose residues, which is critical to the determinant. In contrast, the repeating units of both the type II and the type V CPS have a backbone with 2 side chains [5, 34]. For type V, 1 of these side chains is a glucose linked directly to the backbone. Thus, differences in the epitope recognized by antibodies evoked to the CPS of GBS type V, compared with GBS type III, may modulate the isotype response after immunization with GBS conjugate vaccines. Finally, Kamboj et al. [35] recently studied heptavalent pneumococcal polysaccharide-CRM<sub>197</sub> conjugate vaccine in 24 healthy young adults and observed notable differences in the isotype of response to the different pneumococcal CPSs (PnPSs), despite use of the same carrier protein. Type 4 CRM<sub>197</sub> elicited significantly higher levels of IgA-specific PnPS antibodies than did most other pneumococcal types, and types 9V and 19F CRM<sub>197</sub> elicited more IgM- than IgG-specific antibodies, unlike other types. Additional testing revealed induction of comparable CRM<sub>197</sub>-specific memory T cell responses, leading Kamboj et al. [35] to speculate that B cell repertoire may be an important factor in the adult response to pneumococcal polysaccharides, even when presented in a T cell–dependent form, such as a glycoconjugate. These hypotheses are the subject of ongoing investigations and are important to the design of additional candidate pentavalent conjugate vaccines.

A pentavalent GBS conjugate vaccine containing CPS from serotypes Ia, Ib, II, III, and V could, theoretically, prevent >95% of invasive GBS infections in pregnant women and their infants and in elderly adults [7, 9]. The findings from the present investigation document the safety and immunogenicity of a CPS-protein conjugate from the last of these 5 serotypes, type V, as a monovalent preparation in healthy young women. Although the present study has evaluated safety and immunogenicity only in women, prior studies with type II and III CPS-TT vaccines suggest no difference in immunogenicity between men and women [14, 36]. As with the other 4 types, the type V CPS, administered with either TT or CRM<sub>197</sub> as a carrier protein, was well tolerated and elicited antibodies that were functional in vitro. Despite the economic and political hurdles that exist, the ongoing disease burden caused by GBS, especially in adults, suggests a need for a licensed pentavalent GBS conjugate vaccine.

Acknowledgments

We thank Karen Peeler, Claire Skeeter, and Melissa Ward (Baylor College of Medicine) and April Blodgett, Julieanne Pinel, and Barbara G. Rienap (Channing Laboratory), for invaluable technical assistance on many aspects of this study, and Robin D. Schroeder, for excellent assistance in preparation of the manuscript.

References

17. Wessels MR, Paoletti LC, Pinel J, Kasper DL. Immunogenicity and pro-