Conjugate Vaccines against Group B Streptococcus Types IV and VII

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Although rarely encountered, group B Streptococcus (GBS) types IV and VII have been isolated from infants and adults with invasive disease. This study was designed to determine the immunogenicity and efficacy in animals of conjugate vaccines prepared with GBS types IV and VII capsular polysaccharide (CPS). Despite the striking similarities in structure of these 2 carbohydrate antigens, high-titer rabbit antiserum to each conjugate vaccine was serotype specific. Active vaccination of female mice with the conjugate vaccines induced type-specific IgG and resulted in survival of >90% of newborn pups challenged with viable GBS of homologous serotype. If needed, types IV and VII CPS conjugate vaccines of the design described can be added to the formulation of a multivalent GBS vaccine.

Nine serotypes of the bacterial pathogen group B Streptococcus (GBS) have been identified [1]. Conjugate vaccines have been prepared against the most prevalent GBS serotypes in the United States (types Ia, Ib, II, III, and V) and Japan (types VI and VIII), and their effectiveness in preventing disease in animals has been established (reviewed in [1]). Most of these conjugate vaccines have been administered safely to healthy adults in phase 1 and phase 2 clinical trials [1]. Because the 2 remaining GBS serotypes, types IV and VII, were rarely encountered, generation of vaccines against them was not given high priority. However, although the prevalence of GBS types IV and VII is low, they have been isolated from pediatric populations and from pregnant and nonpregnant women. For example, type IV accounted for 3.3% of the total GBS isolated from pregnant women in Zimbabwe [2], 2.1% of colonizing serotypes in pregnant Canadian women [3], 1.7% of invasive infections in southern Taiwan [4], and 1% of isolates from pregnant women in Dublin [5]. GBS type VII was isolated from 1% of Canadian adults with invasive disease [6] and from 0.7% of 129 infected neonates <7 days old in the United States [7].

We now report the generation of vaccines with purified capsular polysaccharide (CPS) antigens from GBS types IV and VII, whose repeating unit structures include a side chain terminating with sialic acid that, when modified, is a site for covalent coupling to proteins [1]. Types IV and VII CPS–tetanus toxoid (TT) conjugate vaccines were tested for their ability to induce specific antibody in mice and rabbits and for their efficacy in a maternal immunization–neonatal mouse model of GBS disease. Antiserum to each conjugate vaccine also was examined for serotype specificity against all 9 GBS serotypes.

Materials and Methods

Preparation of GBS type IV CPS-protein conjugate vaccine. GBS type IV CPS was isolated from strain 3139, as described elsewhere [8], except that cells were grown in a 2-L chemostat containing a chemically defined medium at a cell-mass doubling time of 1.8 h. Nuclear magnetic resonance was used to confirm the purity of the type IV CPS, as described elsewhere [9]. Purified type IV CPS had a relative molecular mass of 81,000.

GBS type IV CPS was oxidized with sodium periodate, dialyzed, and lyophilized, as described elsewhere for type III CPS [10]. Oxidized type IV CPS was coupled to TT monomer by using sodium cyanoborohydride to start the reductive amination coupling reaction, as described elsewhere for GBS type III–TT [10]. Protein and carbohydrate composition of the purified IV-TT conjugate was determined as described elsewhere [10], with standard curves prepared with TT and type IV CPS.

Preparation of GBS type VII CPS-protein conjugate vaccine. Purification and chemical characteristics of the GBS type VII CPS used to create the conjugate vaccine have been described elsewhere [11]. Aldehydes were created on a selected number of sialic acid residues by oxidation with sodium periodate, as described above. Analysis of the dialyzed and lyophilized material showed that 17% of the sialic acid residues on GBS type VII CPS were converted to aldehydes. The oxidized type VII CPS was coupled to monomeric TT, as described elsewhere for type III CPS [10]. Compositional analyses of the purified VII-TT vaccine were performed as...
described above, with standard curves prepared with TT and purified type VII CPS.

**Vaccination of rabbits with types IV and VII conjugate vaccines.** Vaccination of rabbits with the IV-TT and VII-TT conjugate vaccines was performed at Lampire Biological Laboratories (Pipersville, PA). Each rabbit was injected subcutaneously with a total of 200 μg of conjugate mixed 1:1 with Freund’s adjuvant, delivered at 4 sites in the back. Rabbits received 3 doses delivered at 3-week intervals. Freund’s complete adjuvant was used for the first dose and Freund’s incomplete adjuvant for subsequent doses. Rabbits were bled before vaccination and at weeks 6, 7, 9, and 11 (IV-TT) or at weeks 6, 8, and 10 (VII-TT).

**ELISAs.** Type-specific IgG titers were measured by ELISA in microtiter plates coated with IV-poly-L-lysine (PLL) or VII-PLL (100 ng/well), as described elsewhere for type IV [8]. The absorbance at 405 nm was measured, and titers were recorded as the reciprocal dilution giving an absorbance of ≥0.3.

**Adsorption of rabbit antiserum.** Strains representing each of the 9 known GBS serotypes (Ia, Ib, II, III, IV, V, VI, VII, and VIII) were grown at 37°C overnight on blood agar plates. Growth from each plate was collected and incubated with 200 μL of IV-TT antiserum (diluted 1:100) or VII-TT antiserum (diluted 1:10) on ice for 30 min. The bacteria were pelleted by centrifugation, and serum samples were collected and tested for specific reactivity by direct ELISA with plates coated with type IV or VII CPS.

**Efficacy of GBS conjugate vaccines in mice.** The efficacy of the IV-TT and VII-TT conjugate vaccines was tested in mice, using the maternal vaccination–neonatal challenge model [12]. Adult female mice were immunized at weeks 1 and 3 with 2 μg (based on CPS) of VII-TT or IV-TT, 2 μg of uncoupled VII CPS, 2 μg of IV CPS, or 0.9% saline. Each vaccine was mixed 1:1 with alum (Al-hydrogel 1.3%; Superfos Biosector), and 0.5 mL was delivered to each mouse intraperitoneally. Mice were mated immediately after receiving the second dose. Newborn pups were challenged with GBS of the homologous strain within 48 h of birth, and survival of pups was assessed 48 h later. The LD₅₀ for unvaccinated newborn mouse pups was 1.1 x 10⁴ cfu/pup for GBS type IV strain 3139 and 3.8 x 10⁴ cfu/pup for type VII strain 7271.

**Statistics.** Efficacy of the GBS vaccines was compared by Fisher’s exact test (Instat version 2.0; Graphpad Software). P values <.05 were considered to be significant.

### Results

**Composition of the conjugate vaccines.** The VI-TT vaccine was 46% (w/w) carbohydrate and 62% (w/w) protein. The VII-TT vaccine was 44% (w/w) carbohydrate and 47% (w/w) protein.

**Immunogenicity of conjugate vaccines in rabbits.** Type IV CPS–specific IgG in rabbit serum increased from a prevaccination titer of <100 to a titer of 256,000 after 3 doses of IV-TT vaccine. Similarly, type VII CPS–specific IgG in rabbit serum increased from a prevaccination titer of <100 to a titer of 330,000 after 3 doses of VII-TT vaccine.

**Serotype specificity of IV-TT and VII-TT antiserum.** Type-specific antibody was adsorbed only with the homologous GBS serotype (figure 1), indicating that the polyclonal rabbit antibody was monospecific with respect to its reactivity with GBS CPS.

**Efficacy and immunogenicity of IV-TT and VII-TT vaccines in mice.** Most (93%) of the neonatal mice born to GBS IV-TT–immunized dams were protected against challenge with an ordinarily lethal dose of GBS type IV strain 3139 (table 1). In contrast, none of the neonatal mice born to dams immunized with IV CPS or saline survived the GBS challenge ($P < .0001$).

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**Figure 1.** Binding of type IV–tetanus toxoid (TT) rabbit antiserum to type IV capsular polysaccharide (CPS)–coated ELISA plates (left) and binding of type VII–TT rabbit antiserum to type VII CPS–coated ELISA plates after adsorption with whole group B Streptococcus (GBS; right). GBS serotypes (strains) used to adsorb antiserum were Ia (315, □), Ib (H36B, ■), II (18RS21, ○), III (M781, ●), IV (3139, △), V (CJB111, ▲), VI (SS1214, ◇), VII (7271, ◽), and VIII (JM9-130013, ▼).
backbone sugar that links the side chain saccharides, is specific, which, given that these 2 antigens differ only in the induced high-titer IgG in rabbits. The antiserum was serotype GBS type VII [17].

Protected mice against death and arthritis due to infection with induce multifocal septic arthritis in mice [16, 17]. Rabbit an-

serotypes—a process called “serotype replacement” [15].

Clearly, nonprevalent serotypes of colonizing GBS (e.g., alent in the United States [7], whereas types VI and VIII were new GBS serotypes [14], with the latter becoming more prev-


dam was excluded from the study because it met established exclusion criteria [12].

d One dam in this group was not pregnant. The litter from a second dam was excluded from the study because it met established exclusion criteria [12].

d One dam in this group was not pregnant.

Table 1. Immunogenicity and efficacy in mice of group B
Streptococcus (GBS) types IV and VII conjugate vaccines.

<table>
<thead>
<tr>
<th>Vaccine challenge</th>
<th>No. of dams</th>
<th>No. of pups survived/ no. of pups challenged (% survival)</th>
<th>GMT of type-specific IgG in dams at delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBS type IVa</td>
<td>3</td>
<td>25/27 (93)</td>
<td>3200</td>
</tr>
<tr>
<td>IV-TT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV CPS</td>
<td>3</td>
<td>0/25 (0)</td>
<td>100</td>
</tr>
<tr>
<td>Saline</td>
<td>2</td>
<td>0/24 (0)</td>
<td>100</td>
</tr>
<tr>
<td>GBS type VIIb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII-TT</td>
<td>4d</td>
<td>18/18 (100)</td>
<td>9050</td>
</tr>
<tr>
<td>VII CPS</td>
<td>4d</td>
<td>3/33 (9)</td>
<td>100</td>
</tr>
<tr>
<td>Saline</td>
<td>2</td>
<td>0/23 (0)</td>
<td>100</td>
</tr>
</tbody>
</table>

NOTE. CPS, capsular polysaccharide; GMT, geometric mean titer; TT, tetanus toxoid.

a Challenge dose was 76–145-fold greater than the determined LD50.
b Challenge dose was 25-fold greater than the determined LD50.
c One dam in this group was not pregnant, and the litter from a second dam was excluded from the study because it met established exclusion criteria [12].
d One dam in this group was not pregnant.

Similarly, all (100%) neonatal mice born to VII-TT–immunized dams survived challenge with GBS type VII strain 7271, whereas no pups born to control dams survived the challenge (P < .0001) (table 1). Protection of the newborn pups correlated directly with the geometric mean titer of IgG of the dam at delivery (table 1), which suggests that the pups were protected via transplacental transfer of maternal antibody.

Discussion

From the 1960s through the mid-1980s, the GBS isolated from humans was predominantly serotypes Ia, Ib, II, and III [13]. Over the next decade, types IV and V were recognized as new GBS serotypes [14], with the latter becoming more prevalent in the United States [7], whereas types VI and VIII were the serotypes isolated most frequently from pregnant women in Japan (reviewed in [1]). The shift in serotype patterns in the population provides the rationale for ensuring that effective vaccines are produced with all GBS serotypes and for not limiting experimentation to the serotypes that are currently prevalent. Indeed, nonprevalent serotypes of colonizing GBS (e.g., types IV and VII) may emerge in a population vaccinated with a multivalent vaccine against currently predominant colonizing serotypes—a process called “serotype replacement” [15].

Much less is known about the pathogenesis of GBS types IV and VII than about the pathogenesis of other GBS serotypes. However, GBS types IV and VII, injected intravenously, can induce multifocal septic arthritis in mice [16, 17]. Rabbit antiserum to formalin-killed whole GBS type VII significantly protected mice against death and arthritis due to infection with GBS type VII [17].

Conjugate vaccines generated with types IV and VII CPSs induced high-titer IgG in rabbits. The antiserum was serotype specific, which, given that these 2 antigens differ only in the backbone sugar that links the side chain saccharides, is remarkable [9, 11]. The CPS-specific antiserum can be used in experiments, such as those described by von Hunolstein et al. [17], without adsorbing antibody to other GBS surface antigens. Moreover, IV-TT and VII-TT vaccines were superior to uncoupled homologous CPS in protecting newborn mice born to actively vaccinated dams. Survival rates correlated directly with maternal-specific IgG levels, suggesting transplacental transfer of antibody to the pups, as seen with other GBS conjugate vaccines [1].

Acknowledgments

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References


