An Oligosaccharide-Tetanus Toxoid Conjugate Vaccine against Type III Group B Streptococcus*

(Received for publication, May 29, 1990)

Lawrence C. Paoletti‡‡, Dennis L. Kasper‡‡, Francis Michon‡‡, José DiFabio‡‡, Kevin Holme‡‡, Harold J. Jennings‡‡, and Michael R. Wessels‡‡

From the ‡‡Channing Laboratory, Brigham and Women’s Hospital and the ‡‡Division of Infectious Diseases, Beth Israel Hospital, Harvard Medical School, Boston, Massachusetts 02115 and the ‡‡Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario K1A OR6, Canada

We have developed an oligosaccharide-tetanus toxoid conjugate vaccine against type III group B Streptococcus. Purified group B streptococcal type III capsular polysaccharide was depolymerized by enzymatic digestion using endo-β-galactosidase produced by Citrobacter freundii. Following enzymatic digestion, oligosaccharides were fractionated by gel filtration chromatography on Sephadex G-75. An oligosaccharide-rich pool of average Mr = 14,500 (corresponding to 13.6 repeating units of the type III polysaccharide) was used for conjugation to tetanus toxoid. Tetanus toxoid was covalently coupled via a synthetic spacer molecule to the reducing end of the oligosaccharide by reductive amination. The oligosaccharide-tetanus toxoid conjugate elicited type III-specific anticapsular antibodies (measured in enzyme-linked immunosorbent assay) in three out of three rabbits whereas the unconjugated native type III polysaccharide was nonimmunogenic. Antiserum from rabbits vaccinated with the oligosaccharide-protein conjugate protected mice against lethal challenge with live group B streptococci (16 out of 16 mice survived) and opsonized group B streptococci for phagocytosis in vitro. No protection was conferred by preimmune serum nor by serum from rabbits vaccinated with unconjugated native type III polysaccharide. An oligosaccharide-protein conjugate vaccine of this design may prove to be an effective immunogen for protection against group B streptococcal infection in humans. In addition, the approach to vaccine design utilized in these studies will facilitate further definition of the structural parameters that determine immune response to glycoconjugate vaccines.

Infections due to group B Streptococcus cause approximately 10,000 cases of septicemia and meningitis per year in human neonates born in the United States. Neonatal sepsis and meningitis due to group B Streptococcus have a mortality rate of 10–20% despite aggressive supportive care and early antibiotic treatment. In addition, as many as 30–50% of survivors of group B Streptococcus meningitis have long term neurologic sequelae (1). One approach to prevention of neonatal group B Streptococcus infections is to immunize childbearing women with a vaccine designed to elicit protective antibodies directed against the capsular polysaccharide antigen of one or more of the important group B Streptococcus serotypes. Capsular type III strains account for approximately 60% of blood and cerebrospinal fluid isolates from affected infants overall and up to 90% of isolates associated with late onset meningitis (2, 3). Studies of mother-infant pairs from cases of neonatal sepsis due to type III group B Streptococcus have shown that infected infants have uniformly low or undetectable levels of antibodies directed against the type III capsular polysaccharide antigen (4). Naturally occurring maternal antibodies of the IgG class are transferred across the placenta after 34 weeks gestation and, in term infants, result in levels in the infant similar to those in the mother.

These observations have formed the rationale for development of a vaccine against type III group B Streptococcus which could be given to childbearing women before or during pregnancy. The feasibility of this approach is supported by the results of a study by Baker et al. (5) in which purified type III group B Streptococcus capsular polysaccharide was administered to 40 women during the third trimester of pregnancy. Among women with low (<2 µg/ml) or undetectable preexisting levels of specific antibody, 57% developed a rise in antibody titer to 2 µg/ml, a level considered to be protective (5). All vaccinated women delivered normal, healthy infants, in whom the level of group B Streptococcus III-specific IgG was highly correlated with the maternal antibody level.

The rate of immunogenicity in this study is similar to that observed by Baker et al. (6) and by Eisenstein et al. (7) in nonpregnant subjects. One strategy to enhance the immunogenicity of polysaccharide antigens is to couple the polysaccharide or a derivative oligosaccharide to a carrier protein (8–12). Theoretical considerations and limited experimental data suggest that oligosaccharides rather than full-length polysaccharides may have certain advantages as part of such a conjugate vaccine. A shorter chain length saccharide coupled to a protein may be better able to elicit T cell help than a polysaccharide-protein conjugate (10). Desirable T cell-dependent characteristics for a group B Streptococcus conjugate vaccine would include enhanced immunogenicity, predomi-

* This work was supported in part by National Institutes of Health Grants AI23339 and AI28040 and by a grant-in-aid from the American Heart Association.

This paper was issued as National Research Council of Canada No. 31889. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ To whom correspondence should be addressed: Channing Laboratory, 180 Longwood Ave., Boston, MA 02115.

** Supported by IAF Biochem Int., Laval, P.Q., Canada.
Citrobacter freundii (14) to depolymerize the type III polysaccharide enzymatically. Utilizing Smith-degraded hog gastric mucin as an inducer in a biphasic culture system, endo-β-galactosidase production by C. freundii cells was increased to levels sufficient to prepare adequate quantities of oligosaccharides for a conjugate vaccine. We selected an oligosaccharide size of approximately 14,500 daltons (13.6 repeating units) for conjugation. The epitope of the type III polysaccharide recognized by anticapsular antibodies raised against whole group B Streptococcus organisms is conformationally dependent on the chain length of the polysaccharide (15). A chain length of 14,500 repeating units was chosen to ensure that the epitope is preserved when grown in the presence of gastric mucin (Sigma) was subjected to Smith-degradation, as described previously to produce endo-P-galactosidase when grown in the presence of gastric mucin (Sigma). The culture medium contained 1 ml of sterile dH₂O added. A second harvest of spent cell supernatant with the nutrients in the solid agar phase. The aqueous overlay was precipitated at 4 °C by the addition of (NH₄)₂SO₄ to 75% (w/v) of saturation. Precipitate was collected by centrifugation (16,300 x g! 4 °C), dissolved in dH₂O, placed in dialysis-tubing (Spectropor 1, Spectrum Medical Industries, Los Angeles, CA), and dialyzed against dH₂O at 4 °C. Following dialysis against 50 mM sodium acetate buffer, pH 5.5, the crude enzyme preparation was loaded onto a 2.6 X 25-cm Bio-Gel P-2 column at 2 °C with water, and the mixture was incubated at 37 °C for 24 h. Proteins were precipitated from the digestion mixture by the addition of (NH₄)₂SO₄ to 75% (w/v) of saturation. Following centrifugation, the supernatant fluid was clarified by centrifugation and then loaded onto a 1.6 X 90-cm column of G-75 Sephadex equilibrated with 10 mM Tris, pH 7.0, and eluted with the same buffer. The material size of eluted oligosaccharides was estimated by calculation of Rₗ, based on elution volumes of the 1-repeating unit oligosaccharide (Mₘ = 1,064), 2-repeating unit oligosaccharide (Mₘ = 2,128), and 4-repeating unit oligosaccharide (Mₘ = 4,096). Fractions containing oligosaccharides of 9,000–19,000 Mₘ (average Mₘ, 14,500 or 13.6 repeating units) were pooled, dialyzed against water, and lyophilized. The molecular size and homogeneity of the oligosaccharide pool were confirmed by chromatography on the G-75 Sephadex column. Oligosaccharides were quantified by measurement of sialic acid content by thiobarbituric acid assay (18) and by phenol-sulfuric acid assay (19) using a purified type III polysaccharide as a standard. Proteins were not detected by silver-stained polyacrylamide gel electrophoresis (20) of the purified group B Streptococcus type III oligosaccharide.

**Conjugation of 13.6-Repeating Unit Oligosaccharide to Tetanus Toxoid: Introduction of 6-Aminohexyl-1-β-D-galactopyranoside spacer into the Oligosaccharide.** The aminoalkyl glycoside spacer was prepared according to the protocol of Lee et al. (21). The formate salt of the deprotected spacer was first applied to an Amberlite resin IRC-45 (O17) (DDHI Chemicals) column to recover the free amine. The material eluted from the column with water was lyophilized and kept at 20 °C prior to use. The spacer (50 mg) was incubated with 100 mg of purified type III polysaccharide as a standard. Proteins were not detected by silver-stained polyacrylamide gel electrophoresis (20) of the purified group B Streptococcus type III oligosaccharide.

The degree of spacer incorporation was estimated by measuring the remaining reducing activity of the oligosaccharide obtained in the pool by the Park and Johnson assay (22) in which the hydrolysis step was omitted. The degree of spacer incorporation was also deduced by examination of the “H NMR spectrum (500 MHz) in which specific resonances corresponding to the spacer incorporated were detected and their areas integrated, e.g., signals at 61.43, 1.65, and 1.74 corresponding to the methylene protons of the spacer arm and 6.30 assigned to the anomeric proton of the galactosyl moiety.

**Treatment of the Oligosaccharide-Spacer Adduct with Galactose Oxidase:** The oligosaccharide-spacer adduct was treated with 0.1 mg/ml of horseradish peroxidase (1 mg/ml) in 1 ml of 0.1 M phosphate buffer, pH 7.0, containing 100 units of galactose oxidase (EC 1.1.3.9) from Dactylium dendroides (Sigma). Catalase (1 mg of 10,000 units/mg) from bovine liver (Sigma) was added to the solution, and the mixture was incubated at 37 °C for 24 h. Proteins were then extracted from the solution with 1 ml of 80% phenol solution prepared with 0.1 M phosphate buffer, pH 7. Following the extraction, the solution was mixed to the appropriate ratio and purified by HPLC on a 2.5-μm Phenomenex Luna column with a 40,000) standards. Fractions containing oligosaccharides of 9,000–19,000 Mₘ (average Mₘ, 14,500 or 13.6 repeating units) were pooled, dialyzed against water, and lyophilized. The molecular size and homogeneity of the oligosaccharide pool were confirmed by chromatography on the G-75 Sephadex column. Oligosaccharides were quantified by measurement of sialic acid content by thiobarbituric acid assay (18) and by phenol-sulfuric acid assay (19) using a purified type III polysaccharide as a standard. Proteins were not detected by silver-stained polyacrylamide gel electrophoresis (20) of the purified group B Streptococcus type III oligosaccharide.

Conjugation of 13.6-Repeating Unit Oligosaccharide to Tetanus Toxoid: Introduction of 6-Aminohexyl-1-β-D-galactopyranoside spacer into the Oligosaccharide. The aminoalkyl glycoside spacer was prepared according to the protocol of Lee et al. (21). The formate salt of the deprotected spacer was first applied to an Amberlite resin IRC-45 (O17) (DDHI Chemicals) column to recover the free amine. The material eluted from the column with water was lyophilized and kept at 20 °C prior to use. The spacer (50 mg) was incubated with 100 mg of purified type III polysaccharide as a standard. Proteins were not detected by silver-stained polyacrylamide gel electrophoresis (20) of the purified group B Streptococcus type III oligosaccharide.
vigorous shaking at room temperature for 10 min, the sample was centrifuged and the aqueous layer applied to a Sephadex G-15 column (Pharmacia) eluted with deionized water. Eluate from the column was monitored by refractometry, and the void volume fractions were pooled and lyophilized. The degree of oxidation generated by the enzyme was measured after labeling the C-6 aldehyde function of the galactosyl spacer moieties with NaBD₄, followed by permethylolation. The gas chromatography of the methylated alditol acetates derived from the oligosaccharide. The ratio of intensities of ions at m/z 205 versus m/z 206 for the terminal 2,3,4,6-tetraacetylatedgalactosyl residue was a measure of the percentage of oxidation.

**Purification of Tetanus Toxoid**—Tetanus toxoid (Institute Armand Frénay, Munster, Switzerland) was purified by gel filtration chromatography prior to use. Fifteen ml of a liquid preparation containing 1 mg/ml monomer (8 mg) were dissolved in 1 ml of 0.1 M sodium bicarbonate, pH 8.5, and 0.5 ml of sodium cyanoborohydride (20 mg/ml) was added, and the mixture was incubated at 37°C for 4 days. The progress of the conjugation was monitored by removing aliquots at various time points for analysis by fast protein liquid chromatography using a Superose 12, HR 10/30 column (Pharmacia) with PBS as eluant at a flow rate of 1 ml/min. The void volume fractions were designated peak 1 and peak 2, respectively. The column fractions corresponding to each peak were pooled separately and dialyzed overnight at 4°C against PBS. Following dialysis each pool was adjusted to a total volume of 5 ml with PBS, tested for group B Streptococcus III-specific antibodies by enzyme-linked immunosorbent assay and for opsonic activity using an in vitro opsonophagocytic assay.

**Opsonophagocytic Assay**—Opsonic activity of vaccine-induced antibodies was tested using an in vitro opsonophagocytic assay, as described by Baltimore et al. (26). Briefly, 3 × 10⁵ human peripheral blood leukocytes were mixed with group B Streptococcus strain M781 (1.5 × 10⁶ cfu), human serum absorbed previously with organisms of strain M781 as a complement source (50 μl), and 50 μl of diluted antisera. Surviving group B Streptococcus cells were enumerated by quantitative cultures at t = 0 min and at t = 60 min.

**RESULTS**

**Production of Endo-β-galactosidase from Biphase Cultures of C. freundii**—Endo-β-galactosidase from *F. kerolyticus* has been used by us previously to depolymerize enzymatically the type III group B *Streptococcus* polysaccharide into oligosaccharides that retain the native repeating unit structure (13, 15). *C. freundii* produces an enzyme of similar specificity when grown in the presence of an inducer substance such as keratan sulfate or Smith-degraded hog gastric mucin, but the amount of enzyme produced per culture volume by *C. freundii* is 13-fold greater than that produced by *F. kerolyticus* (14, 27). To augment enzyme yield further, *C. freundii* cells were grown in biphasic cultures. The yield of cells in the liquid phase of biphasic cultures reached 2.5 × 10⁹ cfu/ml at 48 h whereas cells grown in batch cultures with the same amounts of nutrients reached 7 × 10⁹ cfu/ml. This represented a 3.6-fold increase in cell yield/volume of culture fluid. The reduction in culture fluids allowed for practical quantities of spent culture fluids to be processed for endo-β-galactosidase recovery.

**Production and Purification of an Oligosaccharide from Group B Streptococcus III Polysaccharide**—Purified group B *Streptococcus* type III polysaccharide was depolymerized by incubation with endo-β-galactosidase for 2–7 days. The course of enzymatic digestion was followed by removing small aliquots from the incubation and analyzing these by TLC. The smallest fragment produced by endo-β-galactosidase digestion of the type III polysaccharide is the pentasaccharide single repeating unit oligosaccharide, which is easily identified by TLC (13). Digestion was stopped when the density of the stained single repeating unit band was approximately equal to the band remaining at the origin.

Oligosaccharides having an average M₉ of approximately 14,500 were isolated from the digestion mixture by fractionation over a column of G-75 Sephadex. Oligosaccharides of this molecular size purified from several batches of enzymatically digested type III capsular polysaccharide were combined and rechromatographed on the same column (Fig. 1). This oligosaccharide pool had a M₉ of 0.25, which corresponded to an M₉ of 14,500 (range 9,000-19,000) or 13.6 repeating units (range 8.5-17.9).

**Preparation of an Oligosaccharide-Tetanus Toxoid Conjugate Vaccine**—Because the oligosaccharides were generated by the specific enzymatic hydrolysis of galactose β1→4 glucose bonds in the backbone of the group B *Streptococcus* III polysaccharide, each oligosaccharide termined in a -0-
lactosyl residue at its reducing end. The heterobifunctional spacer molecule, 6-aminohexyl-1-β-D-galactopyranoside, was coupled to the reducing galactosyl end of the oligosaccharide by reductive amination using sodium cyanoborohydride as the reducing agent (Fig. 2). Analysis of the oligosaccharide-spacer adduct for the presence of free reducing sugars revealed <5% of the reducing activity present in the native oligosaccharide preparation, indicating that 95% of the oligosaccharide chains were linked to spacer molecules. This result was confirmed by examination of the 1H NMR spectrum of the oligosaccharide-spacer adduct. Areas of resonances at δ1.43, 1.65, and 1.74 corresponding to the methylene protons of the spacer arm and at δ4.39 assigned to the anomeric proton of the galactosyl moiety relative to those assigned to anomeric protons of the oligosaccharide indicated incorporation of spacer molecules into 90–100% of the oligosaccharide chains. Oxidation of the oligosaccharide-spacer adduct was accomplished by treatment with galactose oxidase resulting in the generation of an aldehyde group at C-6 of the spacer β-D-galactopyranosyl residues. The oxidized oligosaccharide-spacer adduct was conjugated to tetanus toxoid monomer using standard reductive amination conditions (Fig. 2). The OS-TT conjugate was purified from the reaction mixture by gel filtration chromatography on a column of Bio-Gel A, 0.5 M. The purified conjugate contained 43% carbohydrate by weight, corresponding to a molar ratio of oligosaccharide to protein of 8:1 (Table I).

**Immunogenicity of OS-TT Conjugate**—Groups of three rabbits were vaccinated with TT, OS-TT, or with unconjugated group B Streptococcus III polysaccharide (III). A sharp rise in type III polysaccharide-specific IgG class antibodies was seen in rabbits (three of three) that received a priming and booster dose of OS-TT (Table II). Antibody titers increased upon a second boost in these animals with titers ranging from 8,000 to 64,000 (Table II). In contrast, rabbits that received native type III group B Streptococcus polysaccharide or tetanus toxoid did not attain an antibody titer greater than 200 throughout the course of the study (Table II). We chose to use the native full-length polysaccharide as a comparison immunogen rather than unconjugated oligosaccharide for two reasons. The native polysaccharide, but not a derivative oligosaccharide, has been used previously in human subjects as an experimental vaccine, so an improved preparation should have demonstrably superior immunogenicity to the unconjugated native antigen. Second, virtually all unconjugated polysaccharides smaller than about 90,000 molecular weight are poor immunogens (28). Rabbits immunized with the conjugate also produced tetanus toxoid-specific IgG class antibodies. Following the initial immunization, anti-tetanus toxoid titer on

**Table I**

<table>
<thead>
<tr>
<th>Biochemical composition of OS-TT conjugate vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average no. of pentasaccharide repeating units (range)</td>
</tr>
<tr>
<td>Average molecular weight (range)</td>
</tr>
<tr>
<td>Amount of vaccine as tetanus toxoid (%) of dry weight</td>
</tr>
<tr>
<td>Amount of vaccine as carbohydrate (%) of dry weight</td>
</tr>
<tr>
<td>Mol of oligosaccharide/mol of tetanus toxoid</td>
</tr>
</tbody>
</table>

* Molecular size estimated by Kav on Sephadex G-75, as described in Fig. 1.
* Protein content as measured using the Lowry et al. (23) method.
* Carbohydrate content determined by phenol-sulfuric acid method (19) using native type III polysaccharide as a standard.
* Based on molecular weight of 14,500 and 160,000 for type III oligosaccharide and monomeric tetanus toxoid, respectively.
Group B Streptococcus Oligosaccharide Conjugate Vaccine

**TABLE II**

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Day 0</th>
<th>Day 20</th>
<th>Day 34</th>
<th>Day 55</th>
<th>Day 75</th>
</tr>
</thead>
<tbody>
<tr>
<td>III native</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 1</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Rabbit 2</td>
<td>100</td>
<td>100</td>
<td>200</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Rabbit 3</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>OS-TT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 1</td>
<td>100</td>
<td>100</td>
<td>9,000</td>
<td>8,000</td>
<td>8,000</td>
</tr>
<tr>
<td>Rabbit 2</td>
<td>100</td>
<td>800</td>
<td>8,000</td>
<td>32,000</td>
<td>32,000</td>
</tr>
<tr>
<td>Rabbit 3</td>
<td>100</td>
<td>800</td>
<td>16,000</td>
<td>64,000</td>
<td>64,000</td>
</tr>
<tr>
<td>TT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 1</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>200</td>
<td>-</td>
</tr>
<tr>
<td>Rabbit 2</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Rabbit 3</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

*Titers of 100 or less are reported as 100.

**TABLE III**

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS-TT</td>
<td>10/16</td>
<td>10/16</td>
<td>10/16</td>
<td>100%</td>
</tr>
<tr>
<td>III native</td>
<td>1/17</td>
<td>0/17</td>
<td>0/17</td>
<td>0%</td>
</tr>
<tr>
<td>Preimmune</td>
<td>9/17</td>
<td>1/17</td>
<td>0/17</td>
<td>0%</td>
</tr>
</tbody>
</table>

**TABLE IV**

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>1/100</th>
<th>1/1400</th>
<th>1/1600</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS-TT, rabbit 1</td>
<td>0.65*</td>
<td>0.58</td>
<td>0.31</td>
</tr>
<tr>
<td>OS-TT, rabbit 2</td>
<td>1.90</td>
<td>1.47</td>
<td>0.29</td>
</tr>
<tr>
<td>OS-TT, rabbit 3</td>
<td>1.99</td>
<td>1.88</td>
<td>0.24</td>
</tr>
<tr>
<td>Preimmune</td>
<td>0.20</td>
<td>ND*</td>
<td>ND</td>
</tr>
<tr>
<td>III native</td>
<td>0.04</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Log10 cfu at 60 min minus log10 cfu at 0 min.

Pooled rabbit sera was 3.200, which increased to and remained at 25,600 after the first and second boosts. Preimmune rabbit serum had an anti-tetanus toxoid titer of less than 100.

**DISCUSSION**

The age-dependent susceptibility of human infants to invasive infections with group B *Streptococcus* is not understood fully. Immaturity of the complement system, of other serum factors, or of phagocytic function may contribute although none has been proved to play a role in susceptibility of neonates to group B *Streptococcus* infection. However, the presence of adequate levels of maternal antibody directed against the type-specific capsular polysaccharide is highly effective in preventing group B *Streptococcus* disease in the neonate (4). Detailed immunochemical studies of native and chemically modified forms of the type III group B *Streptococcus* polysaccharide have elucidated the importance of certain structural features of the polysaccharide in relation to the epitope recognized by protective anticapsular antibodies. Kasper et al. (29) showed that removal of the sialic acid residues from the polysaccharide side chains produced an antigenically incomplete core polysaccharide. Although a subpopulation of antibodies that recognized the native sialylated polysaccharide also reacted with the desialylated core, only those antibodies that recognized the sialylated native antigen were protective. The structural features of the native epitope were defined further by studies of anticapsular antibody binding to oligosaccharides produced by enzymatic digestion with endo-β-galactosidase from *F. keratolyticus* (13). These experiments showed that the affinity of binding of anticapsular antibody was highly dependent on saccharide chain length. These data suggested that the epitope of the type III polysaccharide was conformationally influenced by the polymeric state of the antigen.

Development of a biphasic culture system utilizing an enzyme inducer permitted the production of increased amounts of endo-β-galactosidase as compared with the methods we have used previously. The availability of adequate amounts of enzyme made it possible to depolymerize 100-mg amounts of native type III polysaccharide and to isolate sufficient quantities of oligosaccharides of defined molecular size for use in an oligosaccharide-protein conjugate vaccine. In designing an oligosaccharide-protein conjugate vaccine against type III group B *Streptococcus*, we chose a saccharide chain length approximately 1/40 of that of the native polysaccharide. The choice of chain length was based on several considerations. First, short chain length saccharides may be better T-dependent immunogens than conjugates using a higher molecular weight saccharide. Seppälä and Mäkelä (10) examined the immunogenicity of conjugate vaccine prepared from dextrans of various chain lengths coupled to albumin. Conjugates...
of dextran oligosaccharides of 1,000–10,000 daltons produced a higher secondary IgG response in mice than conjugates of higher molecular mass saccharides (10). Second, coupling by reductive amination via the reducing end of the saccharide chain is more efficient for shorter chain lengths. Third, the epitope of the native polysaccharide is expressed more fully in longer chain length saccharides. An oligosaccharide of 14,500 daltons (13.6 repeating units) is short enough for efficient coupling and represents a chain length intermediate between the suggested optimum for T cell dependence (4,000–10,000) and that for expression of the conformational epitope of the native polysaccharide (100,000).

Use of a short spacer increased the efficiency of conjugation over that achievable by direct coupling. Oxidation of the terminal galactose residue of the spacer adduct introduced an aldehyde function, permitting efficient coupling to tetanus toxoid by reductive amination. Because of the conformational nature of the native epitope, we avoided coupling procedures that introduce activated groups as sites for coupling at random sites along the saccharide chain. By coupling through the reducing end of the saccharide, a conjugate was created which retained the repeating unit structure of the native polysaccharide, thereby ensuring preservation of the native epitope.

An important criterion for vaccine immunogenicity is the ability of the vaccine to elicit a specific antibody response against the target antigen. However, demonstration of vaccine efficacy also requires that the immune response be protective in vivo. Lagergard et al. (9) and Wessels et al. (10) have reported that coupling of the full length type III group B Streptococcus polysaccharide to tetanus toxoid, either through random (9) or selected (10) sites along the saccharide chain, resulted in conjugates with enhanced immunogenicity in animals as compared with the unconjugated polysaccharide. Wessels et al. (10) demonstrated protective activity in vivo of antibodies elicited by the polysaccharide-protein conjugate; the paper by Lagergard et al. (9) did not include data on in vivo protection. Studies presented in the current report provide evidence for immunogenicity in rabbits of the type III group B Streptococcus oligosaccharide-protein conjugate vaccine as well as evidence that the antibodies elicited by the vaccine are protective against group B Streptococcus infection in vivo. The structural features of the OS-TT vaccine are well defined, both in molecular size and homogeneity of the components and in a uniform site of coupling. The method we have used here has the potential advantage over the previously described group B Streptococcus conjugates of allowing a systematic approach to defining saccharide chain length as a critical structural parameter in the design of a glycoconjugate vaccine.

The OS-TT conjugate was highly effective in eliciting antibodies directed against the type III group B Streptococcus capsular polysaccharide whereas unconjugated native group B Streptococcus III polysaccharide was nonimmunogenic in rabbits. Antcapsular antibodies were predominantly of the IgG isotype and were opsonically active against type III group B Streptococcus in vitro. Passive transfer experiments confirmed the capacity of vaccine induced antibodies to protect mice against lethal challenge with group B Streptococcus organisms. These results indicate that conjugation of an oligosaccharide derivative to tetanus toxoid is a useful means of enhancing the antibody response to the group D Streptococcus type III polysaccharide. That vaccine-induced antibodies recognize the native polysaccharide and are functionally active against live type III group B Streptococcus organisms suggests that the essential structural features of the native polysaccharide epitope have been retained in the conjugate. Conjugates of this type, having well defined components coupled in a uniform fashion, will be useful tools for elucidating the structural determinants of immunogenicity and T cell dependence of glycoconjugate vaccines.

Acknowledgments—We thank April Blodgett, Tom DiCesare, and Jason Berry for expert technical assistance and Fred Cooper for the gas chromatography-mass spectrometry analysis. We thank Manabu Kitamikado and Michiko Fukuda for providing us with the strain of C. freundii, samples of enzyme inducers, and helpful advice.

REFERENCES
An oligosaccharide-tetanus toxoid conjugate vaccine against type III group B Streptococcus.

L C Paoletti, D L Kasper, F Michon, J DiFabio, K Holme, H J Jennings and M R Wessels


Access the most updated version of this article at http://www.jbc.org/content/265/30/18278

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/265/30/18278.full.html#ref-list-1