

Safety and Immunogenicity of Capsular Polysaccharide–Tetanus Toxoid Conjugate Vaccines for Group B Streptococcal Types Ia and Ib

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About 40% of invasive group B streptococcal (GBS) isolates are capsular polysaccharide (CPS) types Ia or Ib. Because infant and maternal GBS infections may be preventable by maternal vaccination, individual GBS CPS have been coupled to tetanus toxoid (TT) to prepare vaccines with enhanced immunogenicity. Immunogenicity in rabbits and protective capacity in mice of a series of type Ia- and Ib-TT conjugates increased with the degree of polysaccharide-to-protein cross-linking. In total, 190 healthy, nonpregnant women aged 18–40 years were randomized in four trials to receive Ia- or Ib-TT conjugate (dose range, 3.75–63 μ g of CPS component), uncoupled Ia or Ib CPS, or saline. All vaccines were well-tolerated. CPS-specific IgG serum concentrations peaked 4–8 weeks after vaccination and were significantly higher in recipients of conjugated than of uncoupled CPS. Immune responses to the conjugates were dose-dependent and correlated in vitro with opsonophagocytosis. These results support inclusion of Ia- and Ib-TT conjugates when formulating a multivalent GBS vaccine.

For nearly 30 years, group B *Streptococcus* (GBS) has been the major life-threatening bacterial infection in neonates and young infants [1]. GBS also causes serious infections in pregnant women [2] and in nonpregnant adults with underlying medical conditions, such as diabetes mellitus, malignancy, or liver disease [3, 4]. Studies in the 1970s established a relationship between a deficiency of antibodies to GBS capsular polysaccharides (CPS) in maternal sera at delivery and susceptibility of infants to invasive GBS infections [5, 6]. These observations suggested that vaccination of women might evoke IgG class antibodies that would be placentally transported, thereby passively protecting infants from GBS disease during the relatively short period of their susceptibility.

Although the purified CPS from the major GBS serotypes

were appealing as potential vaccine candidates, their immunogenicity in adult volunteers was disappointing. Immune response rates (arbitrarily defined as >1 μ g/mL rise in CPS-specific antibody 4 weeks after vaccination) were 40%, 88%, and 60%, respectively, for Ia, II, and III CPS vaccines [7]. GBS CPS-protein conjugate vaccines for serotypes Ia, Ib, II, III, and V have been constructed by coupling each CPS to tetanus toxoid (TT). Each CPS-TT conjugate vaccine was more immunogenic in animals than was uncoupled CPS [8–13]. A recent clinical trial in young women demonstrated significantly better CPS-specific antibody responses to type III CPS coupled to TT (III-TT) than to uncoupled III CPS (90% vs. 50%) [14].

Subsequent studies designed to optimize the immunogenicity of GBS conjugates with type III CPS revealed the importance of two physiochemical parameters in vaccine design, the molecular mass of the CPS used in the vaccine and the degree of polysaccharide-to-protein cross-linking [15]. In the present investigation, we examined the influence of these parameters in designing conjugate vaccines for GBS types Ia and Ib, serotypes that together account for 35%–40% of infant and maternal infections in the United States [16, 17]. Results with these experimental CPS conjugates guided the design of type Ia-TT and type Ib-TT conjugate vaccines that were then evaluated for safety, immunogenicity, and optimal dose in healthy women.

Materials and Methods

Synthesis of experimental Ia and Ib conjugate vaccines. CPS types Ia and Ib (GBS strains 090 and H36B, respectively) were purified by methods described previously for the purification of

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These studies were approved by the Baylor Institutional Review Board for Human Subject Research and the US Food and Drug Administration. Informed consent was obtained from each participating subject, and human experimentation guidelines of the US Department of Health and Human Services (DHHS) were followed in the conduct of the clinical research.

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type III CPS [9]. Sodium meta-periodate was used to form aldehydes on about 5%, 10%, 25%, 50%, and 100% of total sialic acid residues as described for type III CPS [9]. The actual percentage of sialic acid residues oxidized was determined using gas chromatography/mass spectrometry on trimethylsilyl derivatives of coded samples (0.5 mg) [9]. For conjugation to TT, each of the oxidized Ia or Ib CPS (~9 mg) was combined with an equal amount of monomeric TT in 0.5 mL of 0.1 M sodium bicarbonate, pH 8.3. Sodium cyanoborohydride (30–34 mg) was added to each vial, and the mixtures were incubated at 37°C static. The progress of the conjugation reactions and subsequent isolation and purification of conjugates was conducted as detailed for the preparation of the type III conjugate vaccine [9].

Immunogenicity of experimental vaccines in rabbits. Rabbits ($n = 3/\text{vaccine}$) were vaccinated subcutaneously with 50 μg of Ia-TT or Ib-TT on days 0, 19, and 41. Vaccines were mixed immediately prior to vaccination with equal amounts of aluminum hydroxide gel (1.3% Alhydrogel; Superfos Biosector, Vedbek, Denmark). Sera were collected before and 2 weeks after administration of the third dose of vaccine. Antibody responses to the experimental Ia and Ib conjugate vaccines were determined by ELISA using alkaline phosphatase-conjugated goat anti-rabbit IgG (H + L chains) as detailed previously for type III CPS [18]. The assay was standardized using rabbit reference sera in which the concentrations of GBS CPS-specific antibodies were determined by quantitative precipitin analyses to be 2.1 mg/mL for type Ia and 0.9 mg/mL for type Ib-specific rabbit antisera.

Vaccination of female mice with experimental GBS vaccines. The protective efficacy of Ia-TT and Ib-TT vaccines was evaluated in a neonatal mouse model of GBS infection [13]. In brief, female CD-1 mice were vaccinated intraperitoneally with 2 μg of type Ia_{60%}-TT, Ia_{12%}-TT, Ia_{27%}-TT, Ib_{1%}-TT, Ib_{2%}-TT, Ib_{15%}-TT, Ib CPS, or 0.9% saline in a total volume of 0.5 mL. A booster dose of vaccine was given 3 weeks after the primary dose. All vaccines were mixed 1:1 with 2% alum (Alhydrogel). Mice were bred after the second vaccine dose. Mouse pups were challenged within 48 h of delivery with a single 0.05-mL intraperitoneal injection containing type Ia strain 515 (4×10^6 – 1×10^7 cfu per pup or 12- to 31-fold LD₅₀) or type Ib strain H36B (3.1 – 5.7×10^5 cfu per pup or 200- to 368-fold LD₅₀). Survival of mouse pups was assessed 48 h later.

Preparation of vaccines for clinical trials. Types Ia and Ib CPS were purified from 100-L fermenter cultures of strains 090 and H36B, respectively. Growth parameters and purification methods were similar to those described previously [9] except that Todd-Hewitt instead of Columbia broth was used in the cultivation of type Ia strain 090. Component sugar analysis was done by gas-liquid chromatography analysis of alditol acetate derivatives of acid-hydrolyzed polysaccharides. Sialic acid content was determined by the thiobarbituric acid assay on separate samples after hydrolysis with 6% acetic acid. Purified Ia CPS (lot C) was composed of 45% (wt/wt) galactose, 19% (wt/wt) glucose, 19% (wt/wt) *N*-acetylglucosamine, and 17% (wt/wt) sialic acid; it contained <0.5% (wt/wt) protein, <6 $\mu\text{g}/\text{mg}$ nucleic acid, and had an M_r of $>1 \times 10^6$. Purified Ib CPS (lot 3S) was composed of 41% (wt/wt) galactose, 22% (wt/wt) glucose, 19% (wt/wt) *N*-acetylglucosamine, and 19% (wt/wt) sialic acid; it contained <1% (wt/wt) protein, <6 $\mu\text{g}/\text{mg}$ nucleic acid, and had an M_r of $>1 \times 10^6$. Type Ia and type Ib CPS were periodate-oxidized prior to coupling, resulting in the

formation of aldehydes on 25% and 9%, respectively, of total sialic acid residues.

Bulk TT (lot XXXVI; Statens Seruminstitut, Copenhagen) was provided by AMVAX (Beltsville, MD). Monomeric TT with an M_r of 150,000 was isolated on an S-300 HR gel filtration column. Periodate-oxidized CPS and monomeric TT were covalently coupled by reductive amination and purified by gel filtration chromatography using methods described previously for the manufacture of type III conjugate vaccine [9]. Bottled GBS Ia-TT and Ib-TT conjugates were composed of 66% (wt/wt) carbohydrate and 34% (wt/wt) protein as determined by the methods of Dubois et al. [19] using purified Ia CPS as the standard and of Larson et al. [20] using TT as the standard.

GBS Ia-TT conjugate vaccine was bottled in single-dose vials at Ia CPS concentrations of 60 $\mu\text{g}/0.5$ mL (lot 93-1a) and 15 $\mu\text{g}/0.5$ mL (lot 93-1b) in PBS, pH 7.2, containing 0.01% thimerosal. Uncoupled Ia CPS (lot 93-C) also was bottled in single-dose vials with the same diluent at a Ia CPS concentration of 55 $\mu\text{g}/0.5$ mL. GBS Ib-TT conjugate vaccine was bottled in single-dose vials at Ib CPS concentrations of 63 $\mu\text{g}/0.5$ mL (lot 93-2a) and 15.75 $\mu\text{g}/0.5$ mL (lot 93-2b) in PBS, pH 7.2, containing 0.01% thimerosal. Uncoupled Ib CPS (lot 93-3S) also was bottled in single-dose vials with the same diluent at a Ib CPS concentration of 53 $\mu\text{g}/0.5$ mL. Placebo (lot 0293), 0.9% saline containing 0.01% thimerosal, was provided by NIAID (National Institute of Allergy and Infectious Diseases, Bethesda, MD). All vaccines were stored at 4–8°C prior to use.

GBS vaccines were bottled and tested in adherence to good manufacturing practices, regulation 21 Code of Federal Regulations (CFR), part 58, at the Experimental Vaccine Preparation Laboratory (Program Resources, Rockville, MD). Final container tests on these vaccines followed the guidelines established by the US Food and Drug Administration for general biologic products standards as found in 21 CFR, parts 610.11 (general safety), 610.12 (microbial sterility), and 610.13 (pyrogenicity). All GBS type Ia and type Ib vaccines satisfactorily passed these tests.

Clinical trials with GBS Ia and Ib vaccines. We performed four separate phase 1 or 2 trials. Study participants were women who met each of the following criteria: age, 18–40 years; good health without acute or chronic illness; use of an acceptable birth control method throughout the study; negative serum pregnancy test at study enrollment; not breast-feeding; no TT immunization within the prior 12 months; no prior immunization with a GBS Ia or Ib vaccine; and no allergy to the preservative, thimerosal. For some studies, limited physical examinations were done for vital signs.

Type Ia vaccines were evaluated in two trials. Study 1 evaluated the safety and immunogenicity of Ia-TT conjugate and uncoupled Ia CPS vaccine. Subjects were randomized to receive one of the two vaccines ($n = 15/\text{group}$) or placebo ($n = 5$). Study 2 further assessed the safety and immunogenicity of Ia-TT conjugate and uncoupled Ia CPS vaccine while determining the optimal dose of Ia-TT conjugate vaccine. Subjects were randomized to receive one of three 4-fold decreasing doses of Ia-TT conjugate ($n = 15/\text{dose}$), uncoupled Ia-CPS ($n = 15$), or placebo ($n = 10$). All Ia CPS-containing vaccines (and the saline placebo) were delivered as a single 0.5-mL intramuscular injection in the deltoid region. The 105 women enrolled in these two studies included 70 (66.6%) Cau-

casians, 13 (12.4%) Hispanics, 13 (12.4%) Asians, and 9 (8.6%) African Americans.

Type Ib vaccines also were evaluated in two trials that were almost identical in design to those for type Ia vaccines. In study 3, in which we evaluated the safety and immunogenicity of Ib vaccines, volunteers were randomized to receive either Ib-TT ($n = 15$) or uncoupled Ib CPS ($n = 15$) vaccines or placebo ($n = 5$). Study 4 determined the optimal dose of Ib-TT conjugate vaccine. Subjects were randomized to receive one of three 4-fold decreasing doses of Ib-TT conjugate vaccine ($n = 15/\text{dose}$) or placebo ($n = 5$). All Ib CPS-containing vaccines (and saline) were delivered as a single 0.5-mL intramuscular injection in the deltoid region. The 85 women enrolled in these two trials included 55 (64.7%) Caucasians, 13 (15.3%) Hispanics, 11 (12.9%) African Americans, and 6 (7.1%) Asians.

For assessment of reactogenicity, subjects and study personnel (except for the nurse administering the injections) were blinded to vaccine group assignment. Subjects were observed by study personnel in the clinic for 15–30 min after vaccination. They were interviewed by telephone the following day and examined in the clinic 2 days after vaccination. Systemic symptoms, including temperature and injection site signs and symptoms, when present, were recorded by subjects daily for 8 days. Complete blood counts (CBC) and differentials and a panel of 20 serum chemistry tests before and 48 h after vaccination were done in women participating in studies 1 and 3.

Serologic methods. Blood samples were obtained immediately before and at 2, 4, 8, and 52 weeks after vaccination. Twenty-one and 28 subjects in studies 1 and 3, respectively, also had blood specimens collected 104 weeks after vaccination. Type Ia and Ib CPS-specific IgG in serum samples were measured by ELISA. Ia or Ib CPS covalently linked to human serum albumin (HSA) was the coating antigen. Methods for these ELISAs were essentially as described previously for the quantitative determination of type III CPS-specific IgG [18]. The same concentration of uncoupled Ia CPS and Ia-HSA conjugate inhibited 50% of the binding (IC_{50}) of Ia CPS-specific antibodies to the Ia-HSA-coated plate. Similarly, the IC_{50} for uncoupled Ib CPS and Ib-HSA conjugate as inhibitors of Ib CPS-specific IgG to Ib-HSA coated plates was identical. These competitive inhibition experiments indicate that the conjugation of CPS to HSA does not alter epitope specificity. The lower limit of detection for these Ia and Ib CPS-specific IgG ELISAs were 0.05 and 0.1 $\mu\text{g/mL}$, respectively. The results were expressed as geometric mean concentrations (GMC) of Ia and Ib CPS-specific IgG, 95% confidence intervals (CIs), and ranges. Previously described radioactive antigen-binding assays employing purified Ia or Ib CPS extrinsically labeled with tritium were used to quantify CPS-specific antibodies in sera [18].

Opsonophagocytic assays. Pre- and 4-week postimmunization sera from selected vaccine recipients of Ia-TT conjugate vaccines (all doses, $n = 27$) and of uncoupled Ia CPS vaccine ($n = 15$) were tested for their ability to promote opsonization of GBS type Ia strain 515 for phagocytosis and killing by adult polymorphonuclear leukocytes in vitro [21]. The same opsonophagocytic assay was used to assess selected sera from recipients of Ib-TT conjugate (all doses, $n = 32$) and of uncoupled Ib CPS ($n = 8$) vaccines with type Ib GBS strain H36B. All sera were processed within 1 h of collection to preserve endogenous complement and were tested at a final

concentration of 10% in the reaction mixture. Results were expressed as the differences between the number of colony-forming units (cfu) of GBS before and after incubation for 40 min at 37°C and as the mean log reduction. Correlation coefficients (r) between the $\log_{10}$ decrease in cfu and the CPS-specific IgG concentrations in these sera were determined by Spearman's rank correlation.

Statistical analysis. Because the clinical trials for serotypes Ia and Ib used the same vaccine lots, performance statistics for each vaccine preparation were derived from measurements in all recipients of that preparation. Natural logarithms of antibody concentrations were assumed to be normally distributed. Antibody concentrations below the lower limits of detection by the IgG ELISAs were designated as censored, that is their exact value is unknown but they are in the interval 0, 0.05 or 0, 0.1 $\mu\text{g/mL}$. Maximum likelihood estimation of antibody GMCs and associated CIs was based on the results of experiments in which some observations were censored (S-Plus version 3.4; Mathsoft, Cambridge, MA). Vaccine response distributions were illustrated with the reverse cumulative distribution plot [22]. Geometric mean distributions of antibody concentrations by vaccine group were compared non-parametrically by a modification of the Wilcoxon rank sum statistic [23]. Differences between proportions of vaccinees exhibiting certain response characteristics (reactogenicity or 4-fold increase in antibody concentrations at intervals after vaccination) were evaluated by Fisher's exact test.

Results

Characterization of experimental GBS type Ia and Ib conjugate vaccines. Previous experiments with GBS type III CPS coupled to TT revealed that highly cross-linked preparations were more potent immunogens in animals [15]. With this observation in mind, we synthesized a series of type Ia- and Ib-TT conjugates that differed in degree of CPS-to-protein cross-linking so that the design of these vaccines for clinical trials might be optimal. The extent of cross-linking is controlled by varying the fraction of sialic acid residues on the CPS that are oxidized prior to coupling, since each oxidized sialic acid res-

Table 1. Composition of experimental GBS polysaccharide-tetanus toxoid conjugate vaccines.

Vaccine	Sialic acid residues oxidized (%) ^a	Amount of vaccine (% dry wt) as	
		Carbohydrate	Protein
Type Ia			
Ia _{6%} -TT	6	63	37
Ia _{12%} -TT	12	46	54
Ia _{27%} -TT	27	46	54
Ia _{50%} -TT	50	ND	ND
Ia _{81%} -TT	81	ND	ND
Type Ib			
Ib _{1%} -TT	<2	69	31
Ib _{2%} -TT	2	57	43
Ib _{15%} -TT	15	26	74
Ib _{41%} -TT	41	21	79
Ib _{70%} -TT	70	ND	ND

NOTE. ND, not determined.

^a Determined by gas chromatography-mass spectrometry analysis of trimethylsilyl derivatives of oxidized polysaccharide.

Table 2. Efficacy of experimental GBS capsular polysaccharide-tetanus toxoid conjugate vaccines in a maternal vaccination–neonatal mouse model.

Vaccine	No. of dams	No. of pups survived/ no. of pups challenged	Survival (%)
Type Ia			
Ia _{6%} -TT	3	30/36	83
Ia _{12%} -TT	4	46/49	94
Ia _{27%} -TT	4	44/49	90
Saline	2	0/21	0
Type Ib			
Ib _{1%} -TT	5	32/70	46
Ib _{2%} -TT	5	47/60	78
Ib _{15%} -TT	4	38/46	83
Ib CPS	1	0/10	0
Saline	5	0/59	0

idue contains an aldehyde group that potentially can form a covalent bond with the carrier protein. By varying the concentration of sodium periodate used to oxidize the CPS, a series of Ia and Ib CPS samples were prepared with different degrees of sialic acid oxidation. All five oxidized derivatives of type Ia CPS ($M_r > 1 \times 10^6$) were successfully coupled to TT. However, only the Ia CPS with 6%, 12%, or 27% oxidation remained soluble after the conjugation reaction was complete. The conjugates prepared with 50% and 81% oxidation formed insoluble gels (table 1). Analysis of the three soluble vaccines showed that the Ia_{6%}-TT contained more carbohydrate than protein and the Ia_{12%}-TT and Ia_{27%}-TT vaccines were composed of equal amounts of each component. Similarly, the Ib-TT vaccines prepared with 1%, 2%, or 15% of the sialic acid oxidized residues (M_r of Ib CPS = 800,000) resulted in soluble conjugates, but those prepared with the two most highly oxidized residues formed a thick slurry (Ib_{41%}-TT) or an insoluble gel (Ib_{70%}-TT). The carbohydrate-to-protein ratio was high in Ib-TT conjugate vaccines prepared with lower percentages of sialic acid oxidation and was lower in those prepared with a high degree of oxidation.

As observed with type III conjugate vaccines [15], the degree of cross-linking correlated with immunogenicity of the Ia and Ib conjugate vaccines. The GMC of Ia CPS-specific IgG in sera from rabbits given Ia_{6%}-TT, Ia_{12%}-TT, or Ia_{27%}-TT vaccines rose from $<0.1 \mu\text{g/mL}$ to 18, 155, and 327 $\mu\text{g/mL}$, respectively, following 3 doses of vaccine. The Ib_{1%}-TT and the Ib_{2%}-TT vaccines did not evoke a Ib CPS-specific antibody response. However, rabbits given Ib_{15%}-TT had a 10-fold increase in Ib CPS-specific IgG after vaccination (GMC change of 2 to 22 $\mu\text{g/mL}$). Each of the 6 soluble conjugate vaccines were evaluated for protective efficacy in a maternal vaccination–neonatal challenge model in mice [13]. More than 80% of pups born to dams vaccinated with any of the 3 soluble Ia-TT vaccines survived a type Ia GBS challenge (table 2). The most efficacious Ib-TT vaccines were those generated with 2% and 15% oxidized CPS; relatively poor efficacy was observed for the Ib_{1%}-TT vaccine. There were no survivors after GBS challenge in litters born to control dams vaccinated with uncoupled Ib CPS or

with saline. Based on these results, a type Ia-TT conjugate vaccine for clinical trial was synthesized using Ia CPS in which 25% of sialic acid residues were oxidized. A type Ib-TT conjugate was prepared from Ib CPS that had 9% of its sialic acid residues oxidized. Both of these conjugate vaccines contained 66% carbohydrate and 34% protein by weight.

Clinical testing of type Ia and type Ib conjugate vaccines. In general, type Ia-TT or Ib-TT conjugates and the uncoupled Ia or Ib CPS vaccines were well-tolerated when given to 165 women as single intramuscular doses. No serious adverse effects or vaccine-associated systemic symptoms were reported. Most of the women receiving a conjugate vaccine at any dose had only mild tenderness at the injection site or no discernable local symptoms or signs (table 3). Moderate pain or mild redness or swelling were observed somewhat more frequently among recipients of the 2 higher doses of the conjugate vaccines than among recipients of uncoupled CPS or saline. These small differences, however, were not statistically significant. One woman, who received a 63- μg dose of Ib-TT vaccine, described severe arm pain that radiated into her shoulder that persisted ≤ 24 h after vaccination. Another who received the same vaccine had moderate erythema and mild swelling at the injection site that resolved within 48 h of vaccination. In the 70 women enrolled in study 1 (Ia vaccines) and study 3 (Ib vaccines), no significant changes in CBC or blood chemistry values were noted 2 days after vaccination.

Table 4 summarizes the immunogenicity of the type Ia-TT conjugate vaccine at 3 4-fold decreasing doses compared with uncoupled Ia CPS and placebo (saline). Before vaccination, members of each of the 5 study groups had similarly low serum concentrations of Ia CPS-specific IgG ($\leq 0.6 \mu\text{g/mL}$). Among the 30 recipients of the highest Ia-TT dose, the Ia CPS-specific GMC increased to 21.6 $\mu\text{g/mL}$ 2 weeks after vaccination, peaking at 26.2 $\mu\text{g/mL}$ 6 weeks later. One year after vaccination, Ia CPS-specific antibody concentrations fell by $\sim 50\%$ to 12.4 $\mu\text{g/mL}$. Ten subjects in study 1 who received the highest Ia-TT dose had Ia CPS-specific IgG GMCs of 0.3, 13.3, 6.4, and 6.1

Table 3. Reactogenicity of type Ia and type Ib CPS-TT conjugate vaccines in healthy women.

Vaccine ^a	Dose (μg)	No. of women	Pain ^b (%)				Redness or swelling ^c (%)			
			0	1	2	3	0	1	2	3
Ia-TT	60	30	33.3	40	26.7	0	70	10	20	0
	15	15	40	46.7	13.3	0	73.3	20	6.7	0
	3.75	15	80	13.3	6.7	0	93.3	6.7	0	0
Ia CPS	55	30	70	13.3	16.7	0	100	0	0	0
Saline		15	80	6.7	13.3	0	93.3	0	6.7	0
Ib-TT	63	30	23.3	33.3	40.1	3.3	86.7	10	0	3.3
	15.75	15	40	20	40	0	93.3	6.7	0	0
	3.94	15	46.7	33.3	20	0	93.3	6.7	0	0
Ib CPS	53	15	33.3	40	26.7	0	100	0	0	0
Saline		10	80	20	0	0	100	0	0	0

^a All vaccines and saline were delivered in 0.5-mL volumes.

^b 0, no pain; 1, tender to touch; 2, sore with movement; 3, unable to move arm.

^c 0, none; 1, 1–3 cm; 2, 3–5 cm; 3, >5 cm.

Table 4. Immunogenicity of GBS type Ia vaccines in women.

Vaccine	Dose (μg)	No. of recipients	Geometric mean concentration (μg/mL) of type Ia CPS-specific IgG, 95% CI, and range at indicated week after vaccination				
			0	2	4	8	52
Ia-TT	60	30	0.5 ^a	21.6 ^{b,c}	25.5 ^{b,c}	26.2 ^{b,c}	12.4 ^{b,d}
			0.3–1.1	10.7–43.7	12.6–51.4	13.0–52.9	5.9–25.9
			≤0.05–140.0	0.1–548.2	0.3–517.1	0.5–497.9	0.3–310.0
	15	15	0.5 ^a	9.7 ^c	13.1 ^{b,c}	18.3 ^{b,c}	9.1 ^e
			0.1–1.4	3.2–29.6	4.3–39.8	6.0–55.4	2.9–28.6
			≤0.05–19.8	0.1–476.4	0.5–503.9	0.7–469.4	0.37–234.8
Ia CPS	3.75	15	0.2 ^f	1.3 ^f	1.5 ^f	1.9 ^f	1.5 ^f
			0.1–0.7	0.5–3.5	0.6–4.3	0.7–5.4	0.6–4.3
			≤0.05–18.6	0.1–184.7	0.1–143.7	0.1–152.8	0.1–75.4
	55	30	0.2 ^a	2.1	2.4	2.4 ^g	2.4 ^h
			0.1–0.7	0.8–5.9	0.9–6.6	0.8–6.8	0.8–6.9
			≤0.05–17.5	≤0.05–306.9	≤0.05–303.0	≤0.05–244.3	≤0.05–182.5
Placebo		15	0.6 ^a	0.5	0.6	0.6	1.4 ⁱ
			0.2–2.4	0.1–2.0	0.1–2.3	0.2–2.4	0.2–6.9
			≤0.05–69.1	≤0.05–78.9	≤0.05–76.3	≤0.05–22.4	≤0.05–38.7

NOTE. CI, confidence interval.

^a Not significant at $P \geq .05$ compared with one another.^b $P \leq .05$ vs. Ia CPS.^c $P \leq .05$ vs. saline.^d $n = 27$.^e $n = 14$.^f $P \leq .05$ vs. 60- or 15-μg dose of Ia-TT.^g $n = 29$.^h $n = 29$.ⁱ $n = 9$ of 10 from study 2.

μg/mL, respectively, before and 8, 52, and 104 weeks after vaccination, demonstrating the durability of the antibody response to this conjugate vaccine. Ninety-three percent of the 30 women who received the 60-μg dose and 80% of those given the 15-μg dose had ≥ 4 -fold increases in serum Ia CPS-specific IgG 8 weeks after vaccination. Type Ia CPS-specific antibodies in sera before and 8 weeks after vaccination quantified by IgG ELISA and by radioactive antigen-binding assay correlated signifi-

cantly ($r = .793$ and $r = .955$, respectively; $P < .001$), indicating that the coupling of Ia CPS with human serum albumin for the IgG ELISA does not affect epitope specificity.

The response to the Ia-TT conjugate vaccine was dose-dependent. The 60- and 15-μg doses evoked significantly higher concentrations of Ia CPS-specific IgG than did the 3.75-μg dose ($P < .05$), but the difference between the 15- and 60-μg doses was not significant ($P = .58$). Among the 30 women who

Table 5. Immunogenicity of GBS type Ib vaccines in women.

Vaccine	Dose (μg)	No. of recipients	Geometric mean concentration (μg/mL) of type Ib CPS-specific IgG, 95% CI, and range at indicated week after vaccination				
			0	2	4	8	52
Ib-TT	63	30	0.4 ^a	13.3 ^b	14.2 ^b	12.9 ^b	5.8 ^{b,d}
			0.2–1.1	5.4–32.9	5.8–35.0	5.2–32.0	1.6–20.7
			≤0.1–39.3	≤0.1–445.3	≤0.1–495.4	≤0.1–443.0	0.3–211.8
	15.75	15	0.6 ^a	8.5 ^b	10.7 ^b	11.1 ^b	7.0 ^{b,d}
			0.2–2.2	2.5–28.3	3.2–35.7	3.3–37.0	2.1–23.4
			≤0.1–14.7	≤0.1–206.8	0.2–317.2	0.2–188.5	0.1–169.2
Ib CPS	3.94	15	0.6 ^a	2.5 ^{b,c}	2.9 ^{b,c}	2.9 ^{b,c}	2.2 ^{b,d}
			0.2–1.4	1.0–6.3	1.1–7.1	1.2–7.1	0.9–5.5
			≤0.1–62.0	≤0.1–65.1	≤0.1–71.3	≤0.1–71.0	0.2–56.6
	53	15	0.4 ^a	3.7 ^b	4.4 ^b	4.7 ^b	ND
			≤0.1–2.2	0.8–17.6	0.9–20.9	1.0–22.3	ND
			≤0.1–32.5	≤0.1–96.7	≤0.1–122.9	≤0.1–152.3	ND
Placebo		10	0.3 ^a	0.3	0.3	0.3	0.3
			≤0.1–1.2	≤0.1–1.2	≤0.1–1.2	≤0.1–1.1	≤0.1–2.0
			≤0.1–10.0	≤0.1–11.2	≤0.1–10.4	≤0.1–10.0	≤0.1–7.6

NOTE. CI, confidence interval; ND, not determined.

^a Not significant at $P \geq .05$ compared with one another.^b $P \leq .05$ vs. saline.^c $P \leq .05$ vs. 63-μg dose of Ib-TT.^d $n = 15$ from study 4 (see Results for complete data).

received uncoupled Ia CPS vaccine, the serum concentrations of Ia CPS-specific IgG 4 and 8 weeks after vaccination were only 2.4 $\mu\text{g/mL}$. As expected, Ia CPS-specific IgG concentrations in sera from the 15 women in the placebo group (studies 1 and 2) remained at preimmunization values through 8 weeks. The 10 women in study 2 who received saline had a GMC of 0.5 (range, <0.05–22.4 $\mu\text{g/mL}$) 8 weeks later, but 3 had significant spontaneous rises in Ia CPS-specific IgG by 52 weeks (table 4).

Table 5 summarizes the immunogenicity of the Ib-TT conjugate at 1 of 3 different doses and of the uncoupled Ib CPS vaccine. Similar to the findings for the type Ia vaccine studies, each of the study groups had very low serum concentrations of type Ib CPS-specific IgG before vaccination. Recipients of the highest dose of Ib-TT conjugate vaccine had an increase in Ib CPS-specific IgG from a GMC of 0.4 before vaccination to 13.3 $\mu\text{g/mL}$ 2 weeks later. The antibody response peaked at 14.2 $\mu\text{g/mL}$ 4 weeks after vaccination and changed little in the ensuing 22 weeks. Recipients of uncoupled Ib CPS had more modest increases in Ib CPS-specific antibodies after vaccination (4.7 $\mu\text{g/mL}$ peak 8 weeks after immunization).

The response to the Ib-TT vaccine also was dose-dependent. Ib CPS-specific IgG responses were significantly higher in recipients of the 63- μg dose compared with the 3.94- μg dose. There was no statistically significant difference between the response to the 63- and the 15.75- μg doses. The 3.94- μg dose was no more immunogenic than uncoupled Ib CPS. Women who received the 63- μg dose in study 4 ($n = 15$) had type Ib CPS-specific GMCs of 0.7, 9.2, 10.2, 10.8, and 5.8 $\mu\text{g/mL}$ in sera collected before and 2, 4, 8, and 52 weeks, respectively, after vaccination, demonstrating the durability of immune response to this vaccine. Also, in study 3, the Ib CPS-specific IgG concentrations in sera from recipients of a 63- μg dose of Ib-TT conjugate ($n = 13$) and of uncoupled Ib CPS ($n = 15$) 2 years after vaccination were 10.7 $\mu\text{g/mL}$ (range, 0.26–147.7) and 5.0 $\mu\text{g/mL}$ (range, <0.1–100.3), respectively. When immune response was analyzed for ≥ 4 -fold increases in Ib CPS-specific IgG, 78% of the 63- μg and 80% of the 15.75- μg dose groups, respectively, achieved these increases 8 weeks after vaccination. As expected, no increase in Ib CPS-specific antibodies was detected in sera from placebo recipients, and no spontaneous increases were detected. There was a significant correlation between Ib CPS-specific antibodies in sera before ($r = .456$; $P < .001$) and 8 weeks ($r = .925$; $P < .001$) after vaccination quantified by IgG ELISA and by radioactive antigen-binding assay. This suggests that the coupling of the antigen used in the ELISA does not affect epitope specificity.

Figure 1 shows the percentage of women immunized with conjugate vaccines who achieved varying concentrations of GBS Ia or Ib CPS-specific antibodies. Before vaccination, >70% of women receiving the 60- μg dose of Ia-TT conjugate had Ia CPS-specific IgG concentrations of <1 $\mu\text{g/mL}$; this antibody concentration was exceeded in 95% of recipients 8 weeks

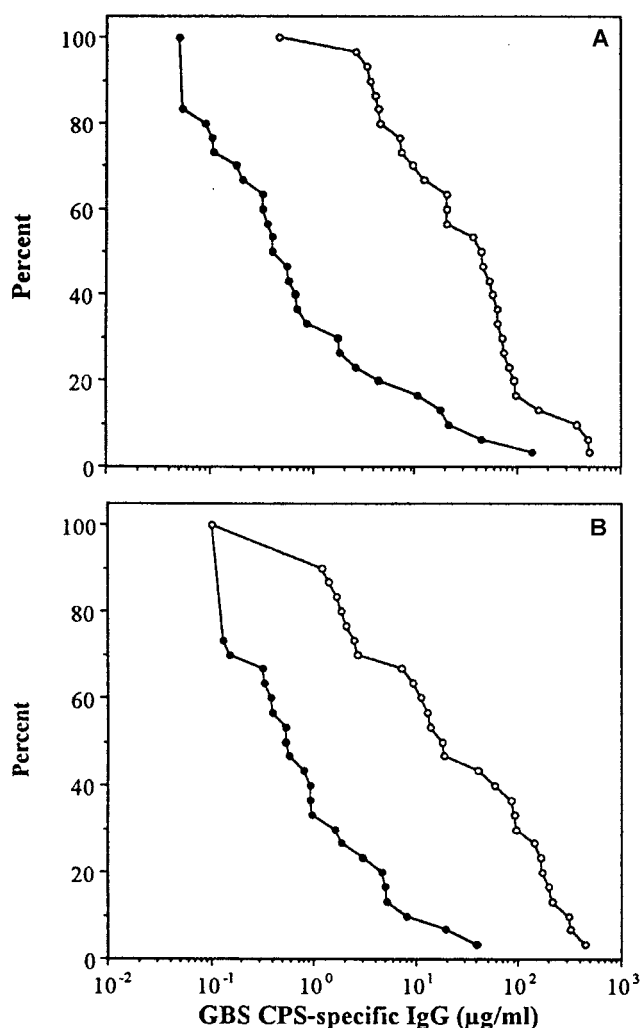


Figure 1. Reverse cumulative distribution plots of capsular polysaccharide (CPS)-specific IgG concentrations in sera from women before (●) and 8 weeks after (○) vaccination with single 60- μg dose of Ia-TT (A) or 63- μg dose of Ib-TT vaccine (B). Lower limits of detection were 0.05 and 0.1 $\mu\text{g/mL}$, respectively, for Ia and Ib CPS-specific IgG ELISAs.

after immunization. Analysis of Ib CPS-specific IgG concentrations in sera from women before and 8 weeks after vaccination with 63 μg of Ib-TT conjugate also showed a shift to the right. Before vaccination, most of the women had Ib CPS-specific IgG concentrations of <1 $\mu\text{g/mL}$, whereas 8 weeks after Ib-TT conjugate vaccination, >90% of vaccinees exceeded this level. One-third of subjects receiving uncoupled Ia or Ib CPS vaccines failed to achieve a rise in CPS-specific IgG of ≥ 4 -fold and a serum concentration of >1 $\mu\text{g/mL}$ 8 weeks later (nonresponders). Conversely, only 12% of subjects who received Ia-TT or Ib-TT conjugates at the high or middle doses were vaccine nonresponsive using this arbitrary definition.

Sera collected before and 4 weeks after vaccination was tested

for functional activity in vitro with an opsonophagocytosis assay. There was a correlation between the concentration of CPS-specific antibody and opsonophagocytic activity of sera both for type Ia ($r = .65$; figure 2A) and type Ib ($r = .80$; figure 2B) vaccinees. At a given antibody concentration, sera from women receiving either GBS vaccine had similar functional capacity, suggesting that the CPS epitope important for opsonophagocytic activity is preserved in the CPS conjugate vaccines. Sera collected from women given saline maintained low levels (0 to $<0.3 \log_{10}$ reduction in cfu/mL) of opsonophagocytic activity before and 4 weeks after "vaccination."

Discussion

After decades of cumulative disease, disability, and death attributable to GBS, prevention of perinatal GBS infection has become a public health priority [24]. While intrapartum antibiotic prophylaxis reduces febrile morbidity in parturients and prevents early onset GBS disease in neonates [25], it is invasive (intravenous administration), temporary (needed for each pregnancy), expensive, may promote emergence of GBS strains resistant to penicillin G, and fails to prevent late-onset infant infection. Prevention of GBS disease through maternal immunization has the potential to overcome most, if not all, of these limitations. Previous studies suggest that GBS-colonized women with sufficient concentrations of CPS-specific IgG in their sera at delivery passively protect their newborns from the GBS exposure that may result in invasive infection [5, 6, 21, 26].

Contemporary surveys of GBS blood culture isolates from neonates, infants, and pregnant women with invasive infections demonstrate some shift in the past decade in the distribution of serotypes. Serotypes Ia and III remain predominant, while type II has diminished in frequency and type V has emerged [16, 17]. Together, serotypes Ia and Ib account for ~35%–40% of the perinatal infections caused by GBS, and type III strains cause an almost equal proportion of disease. These findings are important when considering serotypes to be included in the design of GBS vaccines. Further, they emphasize the need for a pentavalent vaccine formulation that contains CPS from types Ia, Ib, II, III, and V, if prevention of $\geq 95\%$ of GBS disease is to be realized.

In the present report, studies of type Ia and Ib GBS vaccines were undertaken in an effort to design preparations that would provide optimal immunogenicity in humans and to ensure that antibodies evoked by immunization would be functional in vitro and protective in vivo. Despite their similar structures, the Ia and Ib polysaccharides are readily distinguishable by type-specific antisera, although cross-reactions to shared epitopes have been described [27, 28]. In a previous study, antibodies elicited in rabbits to a Ia-TT conjugate vaccine had opsonic and protective activity against Ib GBS; however, the antibodies bound with lower affinity to Ib than to Ia polysaccharides [11].

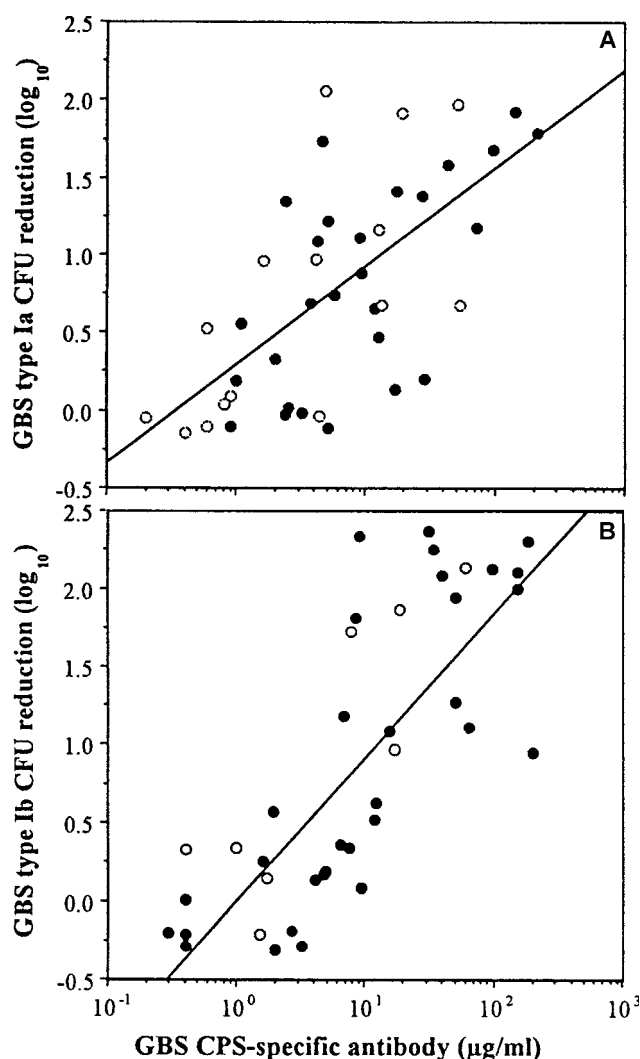


Figure 2. A, Functional activity by opsonophagocytic assay of sera from healthy women 4 weeks after vaccination with Ia-TT vaccine (●) or uncoupled Ia CPS vaccine (○). Correlation coefficients (r) between $\log_{10}$ decrease in colony-forming units (cfu) and capsular polysaccharide (CPS)-specific antibodies elicited by Ia-TT vaccine or by Ia CPS were 0.64 and 0.70, respectively. Shown is regression line of all 42 samples ($r = .65$). B, Functional activity by opsonophagocytic assay of sera from healthy women obtained 4 weeks after vaccination with type Ib-TT vaccine (●) or uncoupled type Ib CPS vaccine (○). Correlation coefficients (r) between $\log_{10}$ decrease in cfu and CPS-specific antibody elicited by Ib-TT or by Ib CPS vaccines were 0.78 and 0.86, respectively. Shown is regression line for all 40 samples ($r = .80$).

In a subsequent study, maternal immunization with Ia-TT protected neonatal mice against Ia GBS challenge but not against Ib challenge [13]. Together, these results indicate that while antibodies evoked by Ia-TT cross-reacted with the Ib polysaccharide, they were not as functionally active against the heterologous serotype. Therefore, on the basis of available evidence, a multivalent GBS vaccine for clinical use should include

both Ia and Ib polysaccharides to provide adequate protection against these serotypes of GBS.

In a study of experimental type III TT conjugates, Wessels et al. [15] reported that immunogenicity in mice was greater when vaccines were constructed using high M_r polysaccharides. The type Ia and Ib CPS in our studies were recovered as very high M_r polysaccharides ($>800,000$) and were used without further size fractionation or depolymerization. Another physicochemical parameter affecting immunogenicity is the extent of polysaccharide-to-protein cross-linking. For type III conjugates, more highly cross-linked vaccines were more immunogenic; however, the most highly cross-linked vaccine (III_{89%}-TT) but not those prepared with lower degrees of cross-linking (e.g., $\leq 66\%$) elicited some antibodies in mice directed against neopeptides [15]. The present studies also demonstrated improved immunogenicity in rabbits given Ia-TT or Ib-TT conjugates with greater degrees of CPS-to-protein cross-linking. However, extensively cross-linked conjugates formed insoluble gels, presumably due to lattice formation. These results led to the preparation of GBS vaccine lots for clinical trials in which the Ia-TT and Ib-TT conjugates were prepared using polysaccharides with 25% (Ia) and 9% (Ib) of their sialic acid residues oxidized.

As reported for III-TT conjugate vaccine [14], the Ia-TT and Ib-TT conjugate vaccines, like the uncoupled CPS, were well-tolerated by healthy women. No systemic symptoms or fever attributable to vaccination were reported, and either no reactions or mild injection site reactions were observed in most recipients. Conjugates were better immunogens than uncoupled polysaccharides. Reverse cumulative distribution plots (figure 1) of 8-week postvaccination CPS-specific IgG concentrations demonstrated that $>90\%$ of women in the conjugate vaccine groups had potentially protective antibody concentrations ($>1 \mu\text{g/mL}$). Dose-response studies with the conjugates indicated the superiority of the 2 higher doses over the 3.75- and 3.94- μg doses of Ia and Ib polysaccharides, respectively, with no statistically significant differences between the responses to the higher doses.

CPS-specific IgG responses to conjugate vaccines were rapid (noted 2 weeks after vaccination) with modest increases that peaked 2–6 weeks later. This prompt increase in CPS-specific IgG, the antibody class that is assured of placental transport, is important if a multivalent GBS vaccine is to be administered in the third trimester of pregnancy. Further, we demonstrated a significant correlation between vaccine-induced CPS-specific antibody and opsonization, phagocytosis, and killing of homologous GBS strains in vitro. This correlation between CPS-specific antibodies and opsonic activity of sera from vaccinees was consistent whether antibodies were induced by conjugate or by uncoupled CPS vaccine. Recipients of GBS III-TT conjugate vaccine had similar antibody response characteristics [14].

In summary, GBS types Ia and Ib conjugate vaccines are well tolerated and suitably immunogenic. They evoke IgG-spe-

cific antibodies to the capsular polysaccharides within 2 weeks of vaccination, and these antibodies persist at $\sim 50\%$ of peak concentrations through 1 year. Vaccine-induced antibodies are functional in vitro and reach concentrations $>1 \mu\text{g/mL}$ in $>90\%$ of subjects. These results suggest that Ia-TT and Ib-TT conjugates of a design similar to those tested in this study will be effective in GBS disease prevention and should be included in the formulation of a pentavalent GBS vaccine.

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