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Alpha C Protein-Specific Immunity in Humans with Group B Streptococcal Colonization and Invasive Disease

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Abstract

Alpha C protein, found in 76% of non-type III strains of group B *Streptococcus* (GBS), elicits antibodies protective against α C-expressing strains in experimental animals, making it an appealing carrier for a GBS conjugate vaccine. We determined whether natural exposure to α C elicits antibodies in women. Geometric mean concentrations of α C-specific IgM and IgG were similar by ELISA in sera from 58 α C GBS strain colonized and 174 age-matched non-colonized women (IgG 245 and 313 ng/ml; IgM 257 and 229 ng/ml, respectively), but acute sera from 13 women with invasive α C-expressing GBS infection had significantly higher concentrations (IgM 383 and IgG 476 ng/ml [$p=0.036$ and 0.038 , respectively]). Convalescent sera from 5 of these women 16–49 days later had high α C-specific IgM and IgG concentrations (1355 and 4173 ng/ml, respectively). In vitro killing of α C-expressing GBS correlated with total α C-specific antibody concentration. Invasive disease but not colonization elicits α C-specific IgM and IgG in adults.

Keywords

Group B *Streptococcus*; Alpha C Protein; Conjugate Vaccine

1. Introduction

Group B *Streptococcus* (GBS) continues to be a leading cause of neonatal sepsis and meningitis and of invasive disease in pregnant women and non-pregnant adults with underlying medical conditions, despite implementation of intrapartum antibiotic prophylaxis and advances in diagnosis and treatment [1,2]. The best prevention strategy lies in the development of an effective GBS vaccine [3,4]. The capsular polysaccharide (CPS) antigens are the major targets of antibody-mediated immunity. Conjugation to a protein carrier enhances immunogenicity of GBS CPS polysaccharides [5]. To date, phase 1 and 2 trials of GBS glycoconjugate vaccines

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have used tetanus toxoid almost exclusively as the protein carrier [4]. However, new focus has been placed on the development of a vaccine that includes a GBS surface protein. In addition to enhancing the immune response of the CPS, a GBS surface protein could serve as a carrier that would elicit antibodies protective against GBS disease caused by strains expressing the specific protein [4]. Furthermore, among the 9 CPS types of GBS that have been identified, cross-protection has not been demonstrated. Antibodies to a GBS surface protein, however, could protect against strains of multiple CPS types expressing the protein.

The α C protein is the most frequently expressed surface protein of GBS; it is found in up to 57% of isolates and 76% of non-type III CPS strains [6,7]. The α C protein is the prototype of a family of streptococcal surface proteins that are characterized by the presence of: (1) conserved amino terminal domains, (2) long tandem repeating elements, and (3) carboxy-terminal domains containing the highly conserved consensus sequence LPXTGX associated with attachment of these proteins to the cell wall [8]. The most frequent form of α C protein found in nature contains 9 identical 246 bp repeating elements and a 33 bp partial repeat and has a predicted molecular weight of 108,705 Da. Alpha C protein binds host cell surface glycosaminoglycan and mediates translocation of GBS across epithelial barriers, facilitating invasive GBS infection [9,10].

Animal studies have demonstrated that α C protein can function as an effective carrier and simultaneously induce protective immunity against strains of multiple CPS types expressing this surface protein [11]. Thus, α C protein is an attractive candidate GBS vaccine component. Although naturally occurring α C protein-specific antibodies have been found in human sera [12,13], no work published to date has addressed the antibody response after exposure to α C-containing GBS strains through colonization or invasive disease or its role in immunity against GBS infection in humans.

We performed a case-control analysis to quantify concentrations of α C-specific IgM and IgG in sera from α C-expressing GBS colonized and non-colonized women at delivery. We also analyzed sera from adult women with GBS bacteremia and from mothers of neonates with early-onset sepsis caused by α C-expressing GBS. The purpose of our investigation was to determine if natural exposure to α C protein of GBS elicits antibodies in humans and if high maternal α C-specific serum antibody at delivery is associated with protection against neonatal disease.

2. Material and Methods

2.1. Maternal and infant sera and GBS strains

Sera previously collected from pregnant women at delivery and cord sera from their neonates in Houston, Texas, were used for this study [14]. The pregnant women had been assessed for GBS colonization by cultures obtained from vaginal and rectal sites at hospital admission for delivery and GBS isolates were serotyped in the investigators' laboratory. In addition, the database of the Streptococcal Immunology Laboratory was reviewed to identify adults with invasive GBS disease for whom acute and convalescent sera were available. GBS isolates and sera from neonates with early-onset sepsis and their mothers were acquired by active laboratory-based surveillance of Texas Children's Hospital, Ben Taub General Hospital, St Luke's Episcopal Hospital, and The Methodist Hospital in Houston, TX. Early-onset sepsis was defined as isolation of GBS from a normally sterile site in a newborn less than seven days of age. With the exception of convalescent sera, samples were collected from mothers and neonates with or without GBS infection at delivery or at the time of initial sepsis evaluation. All but one infected neonate underwent sepsis evaluation within 24 hours of delivery. All sera and GBS isolates were maintained at -80°C until testing. Sera were de-identified for the

purpose of this study. The study was approved by the Institutional Review Board of Baylor College of Medicine and affiliated hospitals.

2.2. Determination of CPS and α C protein expression

GBS CPS type was determined by capillary precipitin method using hyperimmune rabbit antisera specific for types Ia, Ib, and II through VIII [15]. Isolates not typeable were further tested using a 10-fold concentration of extracted CPS or by genotyping [16]. Expression of α C protein was defined for all GBS strains using similar methods with rabbit antisera specific for α C protein antigen (performed at Baylor College of Medicine or through the courtesy of Patricia Ferrieri at the University of Minnesota School of Medicine, Minneapolis) [6]. With the exception of sera from non-colonized controls, only sera from patients with GBS isolates expressing α C protein were included in this study.

2.3. Measurement of α C protein-specific IgM and IgG

Alpha C-specific IgG concentrations in sera were determined by quantitative enzyme-linked immunosorbent assay (ELISA) adapted from previously described methods [17]. Purification of α C protein containing 9 tandem repeats was performed at the Channing Laboratory as described by Gravekamp et al [18]. Optimal concentrations of coating antigen and secondary antibody were determined by checkerboard ELISA. Wells of microtiter plates (Intermountain Scientific Corp. BioExpress) were initially coated with purified α C protein at a 781 ng/ml concentration. Serial two-fold dilutions of serum samples in incubation buffer (10mM PBS with 0.05% Brij [Sigma], 2.5% newborn calf serum [BioWhittaker], and 0.02% azide) were added to duplicate wells starting at 1:100. Alkaline phosphatase-labeled goat anti-human IgM (BioSource) diluted 1:1000 or IgG diluted 1:3000 in incubation buffer was added, and absorbance was read at 405 nm (A_{405}) (Dynex Technologies, Revelation Software version 4.25, 2003).

The standard curve was generated as described [17] except that goat F(ab)₂ anti-human IgM or IgG (Accurate Chemical & Scientific Corp.) diluted to 781 ng/ml in 0.1M carbonate buffer (pH 9.8) was used to coat wells followed by serial two-fold dilutions of unlabeled affinity-purified human IgM or IgG (Accurate Chemical & Scientific Corp.) in incubation buffer starting at 100 ng/ml. Concentrations of α C-specific IgM measured in sera from 9 non-GBS-immunized healthy adult volunteers ranged from 47 to 1409 ng/ml. Serum samples that contained concentrations of 81 and 1409 ng/ml were designated low and high internal controls, respectively. Concentrations of α C-specific IgG measured in sera from 22 non-GBS-immunized healthy adult volunteers ranged from 51 to 3027 ng/ml. Serum samples with the minimal and maximal values were designated low and high internal controls, respectively. Internal controls were included in each ELISA. If the high control deviated by more than 15%, the assay was repeated. A linear relationship between absorbance and α C-specific IgM and IgG existed to a concentration of 20 and 15 ng/ml, respectively. Values below this lower limit of detection were recorded as 10 and 8 ng/ml, respectively, for statistical analysis.

2.4. Opsonophagocytosis assay

Serum samples containing varying concentrations of combined α C-specific IgM and IgG were tested for their ability to promote opsonization, phagocytosis by human polymorphonuclear neutrophils (PMNs), and killing of an α C protein-expressing GBS strain that did not express CPS. The GBS strain had been isolated from a neonate with early-onset sepsis. The strain carried the type Ia CPS-specific gene detected by genotypic methods [19] but did not express CPS as it did not react with the standard CPS antisera. Polymerase chain reaction confirmed presence of *Alp1*. We used an opsonophagocytosis assay described elsewhere [20] but modified it for the α C protein experiments. Reaction mixtures for opsonization consisted of 50 μ l of bacteria (6×10^6 colony-forming units [cfu]), 100 μ l of heat-inactivated serum, 30 μ l of infant

rabbit complement (Serotec), 50 μ l of PMNs (1.5×10^6) from a healthy adult, and 70 μ l of minimum essential medium (Invitrogen) with 5% newborn calf serum (BioWhittaker). Results were expressed as the mean $\log_{10}$ decrease in cfu of GBS before and after incubation at 37°C for 40 minutes and represented the mean of at least 3 experiments.

2.5. Statistical analysis

Power analyses were performed using PASS (version 6.0) at the start of the study. With alpha set to 0.05, our study had a power of 90% to detect a statistically significant difference of 100 ng/ml by matching GBS colonized women to non-colonized controls in a 1:3 ratio. Additionally, comparison of available sera from 42 mothers of neonates with invasive GBS infection to 58 GBS colonized mothers of healthy neonates had a power of 80% to detect a significant difference of 100 ng/ml. Statistical analyses were performed using SPSS (version 10.0) and were 2-tailed with $P < 0.05$ considered significant. Analyses included t-tests on log transformed data for comparing IgM and IgG concentrations between two groups, analyses of variance for comparison of multiple groups, and Spearman rho correlation analyses for comparison of maternal and cord serum concentrations.

3. Results

3.1. Measurement of α C-specific IgG and IgM in α C colonized pregnant women

A case-control design was used in which each delivery serum from an α C-expressing GBS colonized woman at delivery was matched by age within 2 years to 3 non-colonized women. Alpha C-specific IgM and IgG concentrations were compared between the two groups. All mothers in the colonized (case) and non-colonized (control) groups delivered healthy infants. Among 570 mothers of healthy neonates with available sera, 156 were colonized with GBS at vaginal and/or rectal sites and 58 (37.2%) were colonized with GBS strains that expressed α C protein. This accounted for 48.7% of non-type III CPS strains. CPS types for these strains are displayed in Table 1. Mean age of these 58 pregnant women was 24.5 years (range 13–38) and mean gestation was 39 weeks (range 35–42). Eleven (19.0%) were white, 17 (29.3%) were black, 29 (50.0%) were Hispanic and one (1.7%) was Asian. Among the 174 non-colonized women (controls), mean age was 24.6 years (range 14–39) and mean gestation was 39 weeks (range 34–43). Twenty-nine (16.7%) were white, 29 (16.7%) were black, 114 (65.5%) were Hispanic and 2 (1.1%) were Asian. Age, weeks of gestation, and racial/ethnic groups did not differ statistically between the colonized and non-colonized women.

IgM or IgG concentrations did not differ in colonized women when stratified by age, race, gestation at delivery, or CPS type of colonizing strain. Serum concentrations of β C-specific IgG and IgM were similar in the 58 colonized and 174 non-colonized women (Table 2).

3.2. α C-specific antibodies in sera from women with invasive GBS infection

Alpha C-specific IgM and IgG concentrations were determined in acute sera from 13 women with peripartum bacteremia with GBS expressing α C protein. Bacteremia persisted for 1 to 3 days. Ten of the 13 also were diagnosed clinically with chorioamnionitis and/or endometritis. Bacteremia elicited significantly higher concentrations of α C-specific IgM and IgG compared to the serum concentrations found in non-colonized women (Table 2). Alpha C-specific IgM concentrations in acute sera from women with bacteremia also were elevated compared to asymptomatic colonized women (Table 2).

Of the 13 bacteremic women with acute sera at delivery, 10 gave birth to healthy neonates. Two mothers had elective abortions. One mother gave birth to a stillborn neonate who did not undergo an autopsy or have cultures performed. Her serum contained low α C-specific IgM and IgG concentrations of 103 and 283 ng/ml, respectively. Sera were obtained from seven

healthy neonates born to women with invasive infection and compared to sera from ill neonates with invasive GBS (Table 2).

Convalescent sera were obtained from five women with invasive GBS disease. Their diagnoses and serum antibody concentrations are summarized in Table 3. Sera 16 to 49 days after invasive GBS disease contained α C-specific IgM and IgG geometric mean concentrations of 1355 (95% CI, 323–5679) and 4173 (95% CI, 70–249,287) ng/ml, respectively. Alpha C-specific IgM and IgG concentrations in sera from the 5 women varied in relation to their diagnosis and the duration between the likely onset of infection and presentation for medical evaluation and treatment ranging from 1 to 136 days. Alpha C-specific IgG concentrations in convalescent sera were significantly higher than in acute sera ($p = 0.047$).

3.3. α C-specific IgM or IgG in sera from neonates with early-onset GBS disease and their mothers

Alpha C-specific antibody concentrations in sera were compared in mothers of neonates who developed early-onset GBS infection caused by an α C protein expressing strain and healthy neonates exposed to α C-expressing GBS by maternal colonization. Antibody concentrations also were determined in cord blood of affected neonates. Evaluation of 135 GBS isolates from neonates with early-onset sepsis between 1994 and 2005 identified 42 (31.1% of isolates) that expressed α C protein. This represented 41.2% of non-type III CPS strains. Their CPS types shown in table 1 did not change significantly over time. Mean gestational age at delivery was 38 weeks (range 29–43). Mean time of ruptured membranes prior to delivery was 11.3 hours (range 0–32). Twenty-four mothers had a temperature $\geq 100.4^\circ\text{F}$ within two hours before or after delivery, but none had documented bacteremia. Eighteen mothers had a clinical diagnosis of chorioamnionitis. All infants were bacteremic for one day and four also grew GBS from cerebrospinal fluid. Sera were available from 39 of the 42 infected infants. The 42 mothers of the sick neonates had serum concentrations of α C-specific IgM and IgG similar to 58 mothers of healthy neonates but significantly lower IgM than women with documented invasive GBS infection (Table 2).

3.4. Maternal-infant placental transport

Correlation between maternal and neonatal serum α C-specific IgG was high, suggesting efficient placental transport ($r_s = 0.91$, $p < 0.001$). As expected, maternal IgM was not transferred to the neonate ($r_s = 0.18$, $p = 0.25$).

3.5. Functional activity of human α C-specific antibodies

Sera from selected controls with varying concentrations of α C-specific IgM and IgG were tested for functional activity against a nontypeable (non CPS-expressing) GBS strain expressing α C protein by use of an opsonophagocytosis assay. Log kill of GBS correlated with the amount of α C-specific antibodies in the reaction mixture ($r_s = 0.88$, $p = 0.002$) (Figure). Significant killing ($>1 \log_{10}$ reduction in cfu after 40 minutes incubation) was achieved in sera containing naturally-acquired antibody at concentrations greater than 540 ng/ml. This represented at least 90% killing of the initial inoculum.

4. Discussion

We demonstrate here for the first time that invasive disease caused by an α C-expressing strain of GBS elicits α C-specific IgM and IgG in adults. Acute sera from young adult women systemically infected with α C-expressing GBS have significantly higher concentrations of serum α C-specific IgM and IgG than non-infected, non-colonized women. Acute sera from these women also reveal a trend toward higher α C-specific IgM concentrations compared to colonized controls as would be expected since IgM rises faster than IgG during the acute phase

of infection. In sera obtained during convalescence, we found a further significant elevation in both α C-specific IgM and IgG. The total IgM and IgG concentrations in both the acute and convalescent phase of illness were higher than concentrations necessary to achieve substantial opsonophagocytic killing in vitro.

In 1975, Rebecca Lancefield first demonstrated that immunization of rabbits with C protein containing GBS strains elicited antibodies that provided passive protection against lethal challenge in mice with strains containing this protein [21]. Investigators later determined that the C protein was comprised of α and β components and found that rabbit antibodies to either component of C protein conferred passive protection in mice [22]. In the 1990's, a monoclonal antibody to α C protein was described that induced opsonic killing of GBS and protected mice from lethal challenge with GBS [23]. Further investigations showed that gene recombination led to variation in the number of repeats and protein size; these cloned or purified α C proteins elicited protective antibody in mice challenged with α C-expressing GBS strains [18,24,25]. An α C protein conjugated to type III CPS used as a vaccine in mice demonstrated that α C protein functioned as a carrier for type III CPS and as an antigen that stimulated a brisk immune response protective against non-type III α C-expressing strains [11].

Two previous reports have addressed α C-specific antibody in humans. Bevanger [12] found high antibody activity against α C protein in one patient with septicaemia caused by an α C protein producing strain. Larsson et al. [13] compared antibody concentrations in sera from 13 mothers of neonates infected with α C-expressing GBS concentration in sera from mothers of non-infected neonates and found no association between α C-specific antibody concentration and risk for invasive infection. Colonization status was not assessed in the mothers of non-infected neonates.

We found that colonization with an α C-expressing GBS strain does not elicit a systemic immune response compared to non-colonized controls. In contrast, CPS IgG specific to the colonizing serotype is elicited by GBS colonization [14]. By comparing IgG concentrations in mothers of neonates with early-onset sepsis to colonized mothers of healthy neonates as the control group, multiple investigators have shown that low concentrations of maternal CPS-type specific IgG is highly correlated with susceptibility to neonatal GBS infection [26–28]. Because α C-specific antibody apparently is not produced in response to colonization, colonized mothers of healthy neonates cannot serve as a control group with high antibody concentrations for comparison with mothers of infected neonates to evaluate protection against neonatal early-onset disease. However, we did find that asymptomatic neonates of bacteremic mothers appear to have higher α C-specific IgG concentration than neonates with invasive GBS disease, but the difference was not statistically significant, possibly due to the limited number of available sera.

Our study also shows for the first time that naturally-produced α C-specific IgM and IgG in humans have functional activity in vitro against GBS expressing α C protein. We demonstrated a significant correlation between killing of GBS and combined α C-specific IgM and IgG concentrations by an opsonophagocytosis assay, strongly suggesting that killing is mediated by α C-specific antibodies. Because a GBS strain that did not express CPS was used in the assay, CPS-specific antibody did not contribute to the observed opsonophagocytic killing. The asymptomatic neonates of bacteremic mothers had serum α C-specific IgG concentrations greater than the 540 ng/ml shown to correlate with effective killing of GBS in vitro. In contrast, neonates with invasive infection had less than half of α C-specific IgG concentration necessary to achieve significant opsonophagocytic killing. Although one-half of the mothers of neonates with invasive GBS were symptomatic near the time of delivery without documentation of GBS infection, their serum α C IgM and IgG concentrations were similar to those of non GBS-colonized women and low compared to bacteremic women. One could speculate that quantity

and duration of α C protein stimulation was so brief that IgM and IgG had not yet risen at the time of delivery. Therefore, only low concentrations of IgG were transferred placentally, allowing early-onset sepsis to occur in their neonates.

One factor that potentially contributes to a limitation of this study is the variation in number of repeats in α C protein structure in nature [18,29,30]. Alpha C protein containing 1, 2, 9, and 16 repeats are highly immunogenic in rabbits [18]. Theoretically, our decision to use a 9-repeat α C protein as the coating antigen for the ELISA could have affected binding affinity for serum antibody elicited from α C protein containing differing number of repeats. However, 9-repeat antigens are those found most frequently in nature [8]. In addition, Gravekamp et al. [18] showed via inhibition ELISAs that antibodies elicited by 9- or 16- repeat α C protein recognized conformational epitope(s) on proteins with more repeats (9 or 16) but lost substantial binding affinity for epitopes on proteins with fewer repeats. In contrast, antibodies elicited by 1- or 2-repeat proteins recognized epitope(s) regardless of repeat number, but the concentration of antibody required for binding was inversely proportional to the number of repeats in the α C antigen that elicited the antibody. This observation could account for the variation in the antibody concentrations detected in sera from women with invasive disease. Duration and quantity of antigenic stimulus likely also played a role in the quantity of antibody produced. Nevertheless, the 9-repeat α C protein as the coating antigen in our ELISA is the most likely to detect naturally-occurring antibody elicited by α C proteins containing varying number of repeats.

Another limitation of our study is its retrospective design. The number of cases was restricted to pregnant women with sera available from previous studies or from our ongoing surveillance for invasive GBS disease in adults and neonates.

Alpha C protein typically is not present in type III CPS strains that account for 38% of early-onset and 62% of late onset infection [6,31]. The proportion of α C expressing non-type III CPS isolates in our study population were 48.7% of colonized women and 41.2% of neonates with early-onset sepsis. A previous epidemiologic study of maternal-infant colonization strains showed α C protein expression in 76% of isolates [6]. Conjugation of α C protein to type III CPS in a vaccine could result in protection against up to 85% of invasive early-onset sepsis and 91% of late-onset sepsis strains [6,31]. Even with the frequency of α C protein expression in our study, conjugation of α C protein to only type III CPS in a vaccine still would protect against 64% of invasive early-onset sepsis strains. Therefore, these results affirm that α C protein is an attractive candidate for use in a GBS conjugate vaccine.

If further investigation establishes that α C specific-IgG in sufficient concentrations confers protection, maternal immunization with a GBS CPS- α C protein glycoconjugate vaccine could result in the protection of the mother and her neonate against disease caused by α C-expressing strains. We have demonstrated that with sufficient antigenic stimulation in adults, α C protein does elicit high serum concentrations. Alpha C-specific antibodies also mediate in vitro killing of GBS expressing α C protein. A high correlation between maternal delivery and neonatal cord α C specific-IgG suggests placental transport would be efficient. These data should stimulate further study of α C protein as a potential component of a GBS glycoconjugate vaccine.

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Figure 1. Opsonophagocytosis of an α C protein-expressing GBS strain in vitro by human PMN in the presence of serum and baby rabbit complement correlated significantly with the amount of α C-specific antibodies in the reaction mixture ($r_s = 0.88$, $p=0.002$).

Table 1
Capsular Polysaccharide (CPS) Types of Colonizing and Invasive α C-Expressing GBS Strains.

Patients [N]	Ia	Ib	II	CPS types [N (%)]	III	IV	V	NT ^a
Colonized Mothers [58]	33 (56.9)	13 (22.4)	10 (17.2)	0	0	0	1 (1.7)	1 (1.7)
Neonates with invasive GBS infection [42]	23 (54.8)	9 (21.4)	8 (19.0)	0	0	0	1 (2.4)	1 (2.4)
Mothers with invasive GBS infection [16]	11 (68.8)	2 (12.5)	2 (12.5)	0	0	1 (6.3)	0	0

^aNT, nontypeable

Table 2
Comparisons of Alpha C Protein-Specific IgM and IgG Concentrations (ng/ml) in Sera at Delivery.

Patients	N	IgM		IgG	
		GMC (95% CI)	P-value	GMC (95% CI)	P-value
Non-colonized mothers	174	257 (234–283)		229 (191–275)	
Colonized mothers of healthy neonates	58	245 (201–299)	0.642 ^a	313 (231–424)	0.090 ^d
Mothers with invasive GBS infection (acute)	13	383 (240–610)	0.036 ^d	476 (241–938)	0.038 ^d
Healthy neonates of mothers with invasive GBS infection	7	17(11–28)	0.059 ^b	553 (212–1440)	0.238 ^b
Mothers of neonates with invasive GBS infection	42	245 (206–292)	0.995 ^b	371 (261–525)	0.462 ^b
Neonates with invasive GBS infection	39	16 (13–20)	0.026 ^c 0.783 ^d	276 (181–388)	0.485 ^c 0.149 ^d

^a Compared to non-colonized mothers
^b Compared to colonized mothers of healthy neonates
^c Compared to mothers with invasive GBS infection
^d Compared to healthy neonates of mothers with invasive GBS infection

α C Protein-Specific IgM and IgG Concentrations in Acute and Convalescent Sera from Women with Invasive Disease Caused By α C-Expressing Strains of GBS

Patient	Age (yr)/ethnicity	Diagnoses	Days of documented bacteremia	CPS ^a type	Days after diagnosis	α C-specific IgM (ng/ml)	α C-specific IgG (ng/ml)
1	26/Black	Bacteremia, chorioamnionitis at delivery of healthy term infant	1	Ia	0	896	79
2	30/Asian	Bacteremia, chorioamnionitis at delivery of healthy term infant	2	Ib	2 23 2	712 742 683	48 97 1245
3	20/Black	Endocarditis with mitral ring abscess; saline abortion 12 days prior to evaluation	1 ^b	IV	23 4	1264 788	6433 620
4	18/White	Septic pelvic vein thrombophlebitis; retained placenta (27 days) following delivery of healthy 32 week neonate; insulin-dependent diabetes	3	Ib	15 30 51 16	616 1364 209 8808	673 1459 1061 771,748
5	36/White	Osteomyelitis of cervical vertebrae diagnosed 87 days after delivery of healthy term infant	0	Ia	49	405	1802

^aCPS, capsular polysaccharide

^bBlood culture was not reassessed until 9 days after initial positive cultures (5 of 5) for GBS on the day of admission