SEROPREVALENCE OF ANTIBODIES TO GROUP B STREPTOCOCCAL POLYSACCHARIDES IN GAMBIAN MOTHERS AND THEIR NEWBORNS

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In developing countries, little is known about the relationship between group B streptococcal (GBS) colonization in pregnant women and serum antibody levels to capsular polysaccharide antigens of these organisms. This study examined the prevalence of antibodies to two polysaccharides of GBS, Ia and III, in 124 Gambian women with known GBS colonization at delivery and their newborns. Mean antibody levels in maternal-cord serum pairs were 4.06±0.25 μg/mL and 2.64±0.20 μg/mL for type Ia GBS, and 1.1±0.52 μg/mL and 0.78±0.43 μg/mL for type III GBS. Women colonized with type V GBS had significantly higher antibody levels to type III GBS than did noncolonized women, but no difference was found when these groups were compared for antibody levels to type Ia GBS. Women ≥20 years had significantly higher antibody levels to type III GBS compared with younger women and those colonized by other GBS serotypes. Maternal antibodies to types Ia and III GBS were transferred across the placenta to newborns. The rarity of GBS disease in Gambia and other developing countries may be due to the prevalence of maternally derived GBS antibodies, the low prevalence of colonization with serotype III strains, or other undefined factors. (*J Natl Med Assoc.* 1998;90:109-114.)

Key words: group B streptococci ◆ pregnancy ◆ Gambia, Africa

The common occurrence of group B streptococcal (GBS) disease in industrialized countries has been well-documented.[1] Maternal factors predisposing to invasive neonatal infection include high genital inoculum with GBS at delivery, delivery <37 weeks gestation, rupture of membranes >18 hours, chorioamnionitis, GBS bacteriuria, age <20 years, and African-American race.[1] A low level of maternal antibodies to GBS capsular polysaccharide also is an important determinant of infant susceptibility to invasive disease.[2]

In the very premature infant, deficiency of type-specific antibody is uniform due to diminished placental transfer of maternal IgG antibodies prior to 32 weeks gestation. Baker et al[3,4] showed that sera from most pregnant women contain low levels of antibody to type III polysaccharide antigen (<1 μg/mL), but

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that sera from women colonized with type III GBS and delivering healthy babies contained significantly higher antibody levels.[3,4] In a study by Vogel et al,[5] <10% of sera from 200 pregnant women had titers of type III GBS antibodies that were associated with protection against lethal challenge to chicken embryos. In another study of pregnant women of lower socioeconomic status in United States, the GBS colonization rate was 28.6%, with type III strains accounting for about 40% of the isolates.[6] However, antibody levels to type III capsular polysaccharide in sera from noncolonized women were significantly lower than those in sera from women with type III GBS colonization.[6] Others have reported low levels of antibody in sera from pregnant women, regardless of colonization status.[7]

In developing countries, little is known about the relationship between maternal GBS colonization and serum antibodies to GBS capsular polysaccharide antigens. Recently, we reported that Gambian women at delivery often (22.4%) were colonized with GBS, but isolation of type III strains was uncommon (6%) while the newly described serotype V accounted for 40% of GBS isolates.[8] This distribution of GBS serotypes was distinct from that reported in studies of pregnant women living in industrialized countries.[1-12] Further, despite maternal GBS colonization and vertical transmission rates similar to those reported in the United States, invasive GBS disease is rare in Gambia and other African countries.[8,9] The explanation for this is not obvious. It is possible that the lower prevalence of the virulent GBS serotype III accounts, at least in part, for this finding.

We hypothesized that the rarity of GBS disease in Gambian neonates may be due to a high prevalence of type-specific antibodies in maternal sera that protects newborns from invasive disease. This study was undertaken to determine the distribution of type-specific antibodies to GBS capsular types Ia and III in the sera of Gambian mothers and their newborns.

MATERIALS AND METHODS

One hundred thirty-six Gambian women and their newborns comprised the study population. Maternal delivery-cord pair serum samples as well as maternal and infant swabs were obtained at the time of delivery. Twenty-seven mothers were colonized with GBS. Ten had serotype V, and the remainder were colonized with other serotypes (5 mothers with Ia or Ia/c, 1 mother with Ib/c, 7 mothers with II or II/c, 2 mothers with III, and 1 mother with IV; 1 mother was nontypable).

The characteristics of the study population and methods of sample collection have been described previously in detail.[8] Delivery serum samples were collected from 124 mothers and cord sera from 114 of their newborns. There were 107 maternal-cord pairs available, but not all samples had sufficient volumes to assay for antibodies to both GBS serotypes. After clotting at 24°C, blood samples were centrifuged, and separated sera were stored at -20°C until testing.

Type-specific antibodies to GBS capsular polysaccharides were measured by radioactive antigen-binding assays.[2] Purified type Ia and III GBS polysaccharide antigens extrinsically labeled with $[H]^3$ were obtained through the courtesy of Dennis L. Kasper, MD (Channing Laboratory, Harvard Medical School, Boston, Massachusetts). The assays for types Ia and III were quantitated as reported previously,[2] and the results were reported as μg/mL of antibody.

Data were analyzed using SPSS software. Concentrations of antibodies were normalized by logarithmic transformation. Geometric mean antibody concentration was calculated as the antilogarithm of the mean of antibody concentrations after logarithmic transformation. Sera with concentrations of antibody to GBS polysaccharides below the lower limit of detection were assigned half that value for the purpose of statistical analysis. These sera were excluded in the analysis of mean transmission of type III antibody. The mean transmission of antibody to the newborn from the mother was defined as mean ratio of antibody concentration in cord sera to that of maternal sera using maternal-cord pairs only.

Comparison of proportions and means was performed using Mantel-Haenzel chi-square or Fisher's exact test; Student's *t*-test was used where appropriate. Spearman's rank correlation was used to determine the correlation between antibody levels in paired maternal-cord sera. Statistical significance was defined as $P<.05$ using a two-tailed test.

RESULTS

Sera from 124 women, 114 newborns, and 105 maternal-cord pairs were assayed for antibody to type III GBS polysaccharide antigen. The geometric mean antibody level in maternal delivery sera was 1.1 ± 0.52 μg/mL (range: 0.41 to 40.7 μg/mL). The Table summarizes maternal serum antibody concentrations to type III GBS capsular polysaccharide at delivery by colonization status.

Table. Maternal Serum Antibody Concentrations to Type III GBS Polysaccharide at Delivery by Colonization Pattern

	No. Women	Geometric Mean Level (μg/mL)	No. (%) Sera ≥2 μg/mL
Colonized with any serotype	27	1.1±0.50	12 (44)*
Not colonized	97	1.0±0.50	21 (22)
Colonized with type V GBS	10	3.28±0.67†	7 (70)‡
Colonized by other GBS serotypes	17	1.04±0.42	5 (29)
All patients	124	1.1±0.52	33 (27)

Abbreviations: GBS=group B streptococcal.
*Difference between women colonized with any GBS type and noncolonized women ($P<.02$).
†Difference between serum levels in type V GBS colonized women versus women colonized by other GBS types ($P<.03$).
‡Difference between women colonized with type V GBS and those colonized by other serotypes or not colonized ($P<.05$).

Women colonized with any serotype of GBS had antibody levels (1.1±0.50 μg/mL) that were similar to noncolonized women (1.0±0.50 μg/mL). Of interest, women colonized with type V GBS had significantly higher geometric mean antibody levels to type III GBS polysaccharide in their sera than did women colonized by other GBS serotypes (3.28±0.67 versus 1.04±0.42 μg/mL; $P<.03$). The geometric mean antibody level to type III GBS polysaccharide was significantly higher in women >20 years than those in younger women (1.66±0.58 versus 0.73±0.45 μg/mL; $P<.02$).

Previous studies have noted a correlation between ≥2 μg/mL of antibody to type III GBS in maternal delivery sera and the absence of neonatal invasive infection.[2] Thus, the proportion of women with this amount of antibody in their sera were examined by colonization status and age. A significantly higher proportion of women colonized with type V GBS had ≥2 μg/mL antibody to type III GBS in their sera when compared with those colonized by other serotypes ($P<.05$). However, 58 (45%) women had concentrations below the lower limit of assay detection (<0.82 μg/mL). Sera from women <20 years were significantly more likely to have these low levels of antibody to type III GBS polysaccharide when compared with sera from their older counterparts.

The geometric mean level of antibody to type III GBS polysaccharide in cord sera was 0.78±0.43 μg/mL (range: 0.41 to 20.4 μg/mL), and there was a significant correlation between antibody levels in maternal-cord serum pairs ($r=0.86$; $P<.0001$) (Figure). Infants born to women colonized with any GBS serotype had a similar geometric mean antibody level to type III GBS capsular polysaccharide (0.89±0.52 μg/mL) as those born to noncolonized women (0.75±0.39 μg/mL).

Type III antibody concentrations in cord sera from infants delivered to women who had three or more previous children were significantly higher than those found in cord sera obtained from those of women who had fewer previous children (1.1±0.50 versus 0.70±0.39 μg/mL; $P=.03$). Cord sera from the latter group also were significantly more likely to have antibody level below the lower limit of detection of the assay (57% versus 33%; $P=.02$).

Antibody concentrations to type III GBS polysaccharide were somewhat higher in cord blood obtained from infants of women who were ≥20 years (0.91±0.44 μg/mL) than in cord blood obtained from infants whose mothers were younger (0.58±0.34 μg/mL) but not significantly so. The mean transmission of antibody to type III GBS polysaccharide across the placenta was 80% (range: 7% to 220%).

The concentration of antibodies to type Ia GBS polysaccharide was measured in sera from 117 women and 108 newborns; there were 97 maternal-cord pairs. The geometric mean antibody level in maternal delivery sera was 4.06±0.25 μg/mL (range: 1.83 to 31.77 μg/mL). As for antibodies to type III GBS, concentrations to type Ia GBS polysaccharide were higher in sera from women colonized with type V GBS compared with those from women colonized by other serotypes, but the difference was not significant (5.1±0.38 versus 3.26±0.08; $P=.08$). Also, levels of antibodies to type Ia GBS in maternal sera were not influenced by maternal age or parity. The geometric mean antibody level in cord blood was 2.64±0.20 μg/mL (range: 1.82 to 24.55 μg/mL) and was similar in cord blood obtained from GBS-colo-

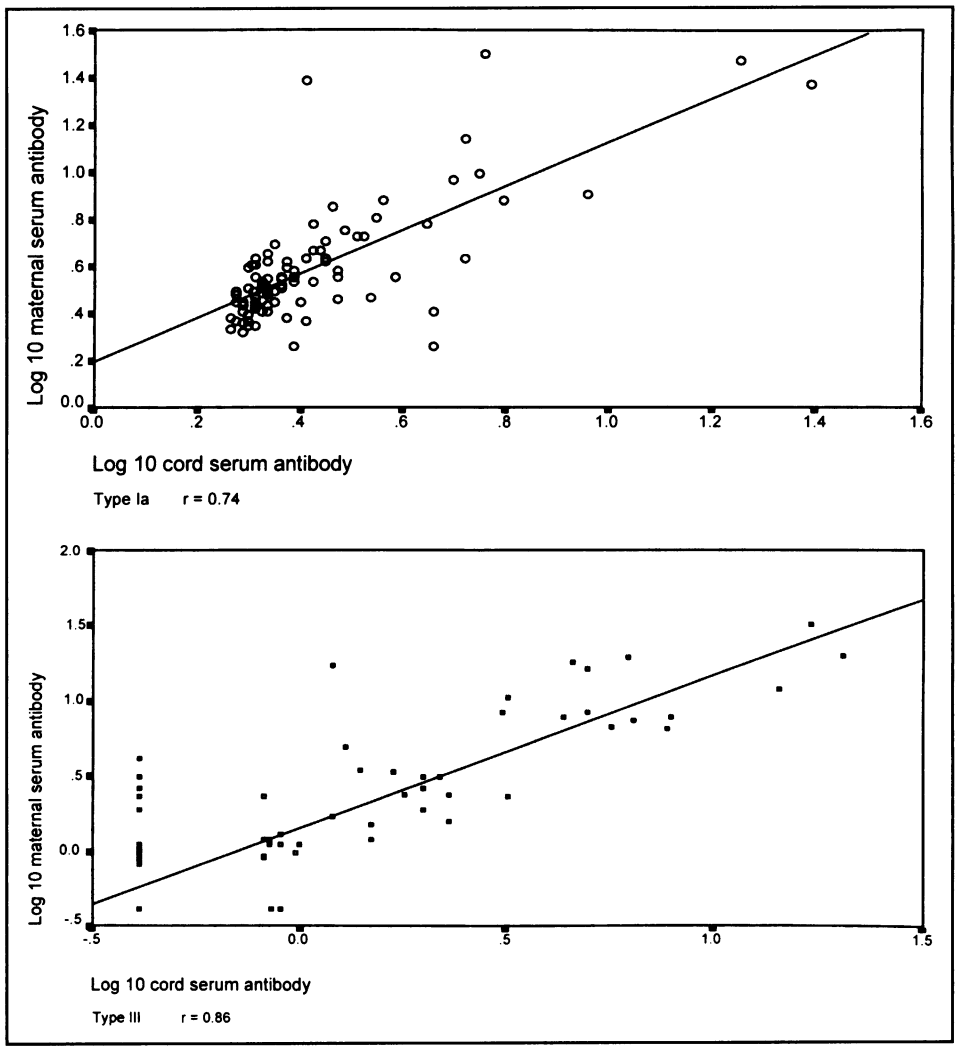

Figure.
The correlation between $\log_{10}$ of antibody concentration to types Ia (○) and III (■) GBS polysaccharides in maternal-cord serum pairs. Levels are expressed as μg/mL. There was a significant correlation ($P<.0001$) between antibody levels in maternal-cord serum pairs ("r" denotes Spearman's correlation coefficient).

nized and noncolonized mothers (2.93±0.27 and 2.56±0.17 μg/mL, respectively). Cord antibody levels also were not affected by maternal age or parity. There was a significant correlation between antibody level to type Ia GBS in maternal-cord serum pairs (r=0.74; $P<.0001$) (Figure). The mean transmission of antibody to type Ia GBS polysaccharide across the placenta was 70% (range: 11% to 249%).

DISCUSSION

The presence of sufficient amounts of type-specific antibodies to GBS capsular polysaccharides in maternal sera is an important determinant in protection against GBS invasive disease in newborns.[1,2,4,5] These antibodies are transported across the placenta, presumably providing passive immunity to the newborn during the first weeks of life.[1] The present study examined the prevalence of serologic immunity to GBS in Gambian women at delivery.

The concentration of antibody necessary to protect against GBS disease is unknown. However, Baker et al[2,4] have demonstrated a uniform correlation between an antibody concentration ≥2 μg/mL to type III GBS polysaccharide in maternal delivery serum and the absence of infant disease despite exposure to a mother colonized with type III GBS. This level of antibody also correlates with uniform neutrophil-mediated in-vitro killing.[1]

Other investigators have reported that 0.4 to 2 μg/mL of IgG antibody to type III GBS as determined by ELISA was protective in animal models.[13,14] For the purpose of assessing potential differences between the groups, we chose a level of ≥2 μg/mL for type III, and for GBS type Ia, we arbi-

trarily chose a level of antibody ≥2.97 μg/mL as possible correlates of protection. One third of Gambian women and one fifth of their newborns had antibody concentrations to type III GBS >2 μg/mL in their sera. A greater proportion of Gambian women colonized by type V GBS had these levels when compared with their noncolonized counterparts.

Using a similar assay method, Baker et al[2] observed 12.5 to 22% of sera from pregnant women in Houston, Texas, had this protective level. It also has been observed that a greater proportion of women colonized with other GBS serotypes had these levels when compared with noncolonized women.[2,3,5,15] However, we were unable to evaluate the possible influence of type III GBS colonization on antibody levels since only two Gambian women were colonized with this serotype.

Surprisingly, women colonized with type V GBS had higher levels of antibody to types Ia and III GBS in their sera when compared with sera from women colonized by other types. Possibly, these women had been colonized previously with either type Ia or III and then developed specific antibody that eliminated colonization with these serotypes, and colonization by serotype V then ensued. It also raises the question of whether there is cross-reactivity between the polysaccharides of type V and other GBS serotypes. The GBS polysaccharide antigens used in these assays were antigenically distinct; thus, these findings are not readily explained by polysaccharide antigenic cross-reactivity.[16-19] Unfortunately, an assay for measuring antibody to type V GBS polysaccharide is not yet available.

The significant correlation between antibody levels in maternal-cord serum pairs for the two serotypes of GBS observed in this study indicates that the major proportion of these antibodies were IgG and were available for placental transfer. Taken together, these observations support the speculation that Gambian women often are colonized with GBS during pregnancy, but that sufficient levels of type-specific antibody passively protect their newborns against GBS disease.

Antibody concentrations to type III GBS polysaccharide in maternal and newborn sera were influenced both by maternal age and parity. This is consistent with an earlier observation that adults with symptomatic infection or after immunization with GBS polysaccharides develop significant increases in antibodies to the specific capsular polysaccharide.[20] It also is consistent with recent observations that younger women tend to have lower IgG levels of type III GBS polysaccharide.[21,22] These findings may be relevant to the observation of an enhanced risk for GBS disease in neonates born to teenage mothers.[1] Gambian women tend to marry early, and by age 20 to 25, they tend to have had three or more children.

The newly recognized type V may be becoming increasingly important as a cause of neonatal GBS infections.[10-12] It has been suggested that type V GBS may be similar to serotype Ia in that complement alone may promote killing in vitro.[23] By contrast, type III, the most virulent GBS strain, requires both antibody and complement for optimal opsonization, phagocytosis, and killing in vitro. The observations that the most frequent GBS serotype colonizing Gambian women was type V and that they often had antibodies to Ia and III GBS polysaccharide antigens in their sera may together account for the rarity of GBS disease in Gambian infants. It also may imply that there are other determinants of the relationship between colonization and invasion with GBS that are poorly understood.

SUMMARY

This study has shown that Gambian women often have antibodies to types Ia and III GBS capsular polysaccharides in their sera that are transferred across the placenta to their newborns. The rarity of neonatal GBS infections in Gambia may be due in part to the prevalence of "protective" levels of these GBS type-specific antibodies in maternal sera at delivery. The majority of GBS-colonized women and their newborns were colonized with serotype V; most of these had higher levels of antibodies to types Ia and III GBS polysaccharides than did noncolonized women. Further studies are needed to explore the reasons behind these observations.

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