Seroepidemiologic Studies of Serotype VIII Group B Streptococcus in Japan

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Levels of antibody to serotype VIII group B Streptococcus (GBS) were surveyed in serum samples from 583 pregnant women, from 461 neonates born to these women, and from 4 mother-and-neonate pairs with early-onset serotype VIII sepsis. Colonization by serotype VIII GBS was associated with significantly higher serum concentrations of serotype-specific antibodies (geometric mean [GM], 5.53 μg/mL), compared with both noncolonization (1.53 μg/mL) and colonization with other serotypes (2.19 μg/mL). There was excellent correlation between antibody levels in mothers and those in their neonates. The prevalence of positive antibody levels, when arbitrarily defined, according to antibody levels in neonatal sepsis (GM, 0.49 μg/mL) as >1.0 μg/mL, was 58% of all pregnant women and 85% of the women colonized by serotype VIII. This high serotype prevalence may explain, at least in part, why serotype VIII causes early-onset neonatal disease at rates lower than those which would be expected on the basis of its prevalence in mothers in Japan who are colonized by GBS.

Group B Streptococcus (GBS) is an important pathogen that causes neonatal sepsis and meningitis, with high mortality and morbidity [1]. Although many factors influence the rate of vertical transmission, one of the most important is the concentration of specific serum IgG antibodies to the different capsular polysaccharides (CPSs) of GBS [2–7]. A low concentration of serotype III CPS–specific antibody in maternal serum is associated with susceptibility to serotype III infection in neonates [2, 3]. Similar correlations have been reported for serotypes Ia, Ib, II, and V [4–7].

In Japan, serotypes VI and VIII have been increasingly recognized among isolates from pregnant women since the early 1980s. At present, serotype VIII accounts for 36% of all GBS isolates [8]. Compared with the carrier rate in pregnant women, the frequency of isolation of serotype VIII in early-onset neonatal infection is low. In a nationwide surveillance of neonatal GBS infection from 1993 to 1997, the most frequent serotype among 43 strains examined was serotype III (28%), followed by serotypes VI (19%), II (12%), and V (12%) [9]. However, only 2 neonates (5%) had serotype VIII invasive disease [9]. The reason for the discrepancy is not obvious. We hypothesized that the rarity of serotype VIII GBS disease in Japanese neonates may be due to a high prevalence of serotype-specific antibodies in maternal serum that protect the neonates against invasive disease. To address this issue, we investigated the distribution of IgG antibody to serotype VIII GBS in maternal and neonatal serum samples, using an ELISA.

Subjects, Materials, and Methods

Subjects. The subjects included pregnant women (n = 583) attending Nishi-Kobe Medical Center from June 1999 to May 2000 and, subsequently, their neonates (n = 461). We excluded pregnant women with multiplets. There were no neonates with invasive GBS infection during this study period. We also analyzed levels of antibody to serotype VIII in 4 mother-and-neonate pairs from the previously reported (1985–1998) cases of early-onset serotype VIII sepsis [10]. Blood samples were collected from pregnant women at 28 weeks of gestation, when these women were routinely examined for anemia, and culture from the distal vagina was sampled. Cord blood was collected from a doubly clamped section of the umbilical cord. Serum samples from subjects in this study and from the previously reported cases [10] were stored at −80°C until analyzed.

Antigen purification. Five prototype strains of GBS, designated “090” (Ia), “H36B” (Ib), “18RS21” (II), “6331” (III), and “130669” (VIII), were obtained from the Czech National Type Culture Collection and were cultured for 36 h at 37°C in Todd-Hewitt broth medium. After 2 washes with PBS (pH 7.0), the packed cells were resuspended in PBS and were heated in a boiling-water bath for 30 min. The solution of the serologically active fraction was eluted over a DEAE-Sepharose column (CL-6B; Pharmacia Biotech) equilibrated with a linear gradient of 0.02–0.5 M (NH4)2CO3, then over a gel-filtration column (TOYOPEARL HW-65; Tosoh) equilibrated with PBS, and finally over a gel-filtration column (TOYOPEARL HW-55) equilibrated with PBS after treatment with pronase (Wako Chemicals). The CPS antigens purified in this fashion...
Values were read at 405 nm (OD 405). All assays were performed in N9.5 (Sigma) in 0.01 M Tris buffer including 0.5 M diethanolamine (pH 9.5) and were interrupted with 1.0 M NaOH, and optical-density values were read at 405 nm (OD405). ELISA was performed by incubation of 100 μL of each human serum sample, diluted 1:40 with PBS containing 0.25% bovine serum albumin (Sigma Chemicals), for 1 h at room temperature. After 3 washes with PBS containing 0.05% Tween 20, alkaline phosphatase–conjugated goat antibody to human IgG (Cappel Laboratories) was added to the wells, at a dilution of 1:200. The phosphatase–conjugated goat antibody to human IgG was added to the125I-labeled IgG–coated microplates. The concentration of serum antibody levels of both the full-term neonates (37–42 weeks of gestation, , , ; ) ( ) or colonized by GBS and those in serum from her neonate. The ages of the women in the 3 groups were not significantly different (mean age of all enrolled women, 29.7 ± 4.3 years). There were no significant differences, in serotype VIII–specific antibody levels, across the 6 maternal-age groups (5-year intervals; data not shown).

**Figure 1.** Levels of antibody to serotype VIII, in association with group B Streptococcus–colonization status of pregnant women. Solid lines and dotted lines represent geometric means and 95% confidence limits (±1.96 SE) about the means, respectively.

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<thead>
<tr>
<th>Colonization status</th>
<th>Type VIII</th>
<th>Other types</th>
<th>Noncolonized</th>
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<tr>
<td>Type VIII (13)</td>
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<td>Noncolonized (535)</td>
<td>y = 1.07x</td>
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**Results**

**Colonization status.** Of the 583 women, 48 (8.2%) were colonized by GBS at 28 weeks of gestation. The serotype distribution, in order of decreasing frequency, was VIII (n = 13); VI (9); Ia (6); III (5), Ia, II, and nontypeable (4 each); and V (3).

**Effect of colonization of serotype VIII GBS on serotype-specific antibody levels.** Figure 1 shows the serotype VIII–specific antibody levels in serum samples from 583 women. The serotype VIII–specific IgG levels in the pregnant women colonized by serotype VIII (geometric mean [GM] ± SE, 5.53 ± 2.79 μg/mL [range, 0.47–33.85 μg/mL]; n = 13) were significantly higher than those in pregnant women either not colonized by any serotype of GBS (GM ± SE, 1.53 ± 0.32 μg/mL [range, 0.07–104.97 μg/mL]; n = 538) (P < .01) or colonized by GBS serotypes other than serotype VIII (GM ± SE, 2.19 ± 1.57 μg/mL [range, 0.21–50.21 μg/mL]; n = 35) (P < .05). The ages of the women in the 3 groups were not significantly different (mean age of all enrolled women, 29.7 ± 4.3 years). There were no significant differences, in serotype VIII–specific antibody levels, across the 6 maternal-age groups (5-year intervals; data not shown).
0.08) and their preterm counterparts (32–36 weeks of gestation, \( n = 13, r = 0.95, P < .001; y = 0.54x – 0.69 \) were correlated significantly with those of their mothers (\( x = \) antibody level in mother; \( y = \) antibody level in neonate).

**Antibody levels in neonates with early-onset serotype VIII sepsis and in their mothers.** The serotype VIII antibody level in serum samples from the 4 historical cases of serotype VIII sepsis was \( GM \pm SE \) 0.49 ± 0.12 \( \mu g/mL \) (range, 0.35–0.84 \( \mu g/mL \), in contrast to \( GM \pm SE \) 0.41 ± 0.07 \( \mu g/mL \) (range, 0.26–0.57 \( \mu g/mL \)) in maternal serum at delivery. To analyze the antibody response in neonates with invasive serotype VIII infection, we serially evaluated serotype VIII antibody in 1 infant at birth and at days 7, 14, and 60 and found levels of 0.58, 0.45, 0.31, and 0.15 \( \mu g/mL \), respectively. This poor immune response was concordant with those observed for serotypes II and III infections in neonates [4, 5].

**Comparison of concentration and prevalence of serum GBS CPS-specific IgG, in all women studied.** We compared the levels of antibodies to serotype VIII and to serotypes Ia, Ib, II, and III, in all women studied. There were significant differences between the mean level of antibody to serotype VIII and those of antibodies to serotypes Ia, Ib, II, and III (table 1). On the basis of the antibody levels in the serum samples of neonates infected by serotype VIII, we arbitrarily estimated that the protective level was >1.0 \( \mu g/mL \). Consequently, 338 (58.0%) of the 583 women had antibody to serotype VIII (table 1). In terms of colonization status, the prevalence was 11 (84.6%) of 13 in women colonized by serotype VIII, 23 (65.7%) of 35 in women colonized by other GBS serotypes, and 304 (56.8%) of 535 women not colonized. When the proportions of women with antibodies (>1.0 \( \mu g/mL \)) to each serotype were compared, the proportion of women with antibody to serotype VIII was significantly higher than the proportion of women with antibody to serotypes Ia, Ib, II, or III (table 1). Even when the threshold for positive status was changed to 0.5 or 2.0 \( \mu g/mL \), the proportion of women with antibody to serotype VIII was different than the proportion of women with antibodies to the other serotypes (table 1).

**Discussion**

To our knowledge, our study is the first to provide epidemiologic data on the prevalence of serum IgG antibody to serotype VIII GBS in pregnant women and, subsequently, in their neonates. Women colonized by serotype VIII had significantly more serum antibodies to serotype VIII CPS than did women who were not colonized. This observation indicates that vaginal colonization by serotype VIII induces a systemic immune response in women of reproductive age. This result is in line with findings in other recent studies, in which colonization with serotypes Ia, Ib, II, III, or V was associated with significantly higher serum concentrations of IgG specific for the CPS of the colonizing serotype compared with noncolonization [3, 5–7].

We also found that the proportion of the women who had antibody to serotype VIII was significantly higher than the proportion who had antibody to serotypes Ia, Ib, II, or III and that the mean concentration of antibody to serotype VIII was much higher than those specific to serotypes Ia, Ib, II, and III. The exact reason is unclear. Many women might have been previously or repeatedly exposed to this prevalent serotype. Similar findings were documented by Suara et al. [14], who showed that antibodies to prevalent strains, serotypes III and Ia, were elevated in substantial proportions of Gambian women regardless of colonization status. In Japan, compared with the high carriage rate among their mothers [8], the frequency of isolation of serotype VIII in early-onset neonatal infection is low [9]. Our findings of high prevalence of maternal serotype VIII antibody and excellent placental transfer may explain better protection against neonatal invasive infection in this serotype.

We did not attempt to propose a precise amount of antibody to serotype VIII CPS needed to protect neonates from infection, because the number of infected infants was limited and because the development of the disease is complex [1]; however, in light of the antibody levels in cord blood from mothers and, subsequently, in serum samples from their neonates with serotype VIII sepsis, it is tempting to speculate that IgG antibody to GBS serotype VIII is protective at >1.0 \( \mu g/mL \). This figure is comparable to the protective concentrations (0.5–2.0 \( \mu g/mL \)) previously determined for serotypes Ia, Ib, II, III, and V [3–7, 14].

Previous investigations compared the concentration of CPS-specific antibody in cord blood with that in maternal serum at delivery [3–7, 14]; however, between early pregnancy and delivery, there was no appreciable change in the concentration of serotype III–specific antibody in maternal serum [4]. In our study, the concentration of serotype VIII–specific antibody in cord blood correlated well with that in maternal serum at 28 weeks of gestation, suggesting that maternal antibody levels at this period of gestation could be used to identify neonates who are highly susceptible to serotype VIII.

A recent research effort focused on the development of CPS-
protein–conjugate vaccines [1, 15]. Our serotype-prevalence data should provide useful information for the formulation of optimal vaccines in Japan.

Acknowledgment

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References