Serotypes VI and VIII Predominate among Group B Streptococci Isolated from Pregnant Japanese Women


Infection by group B streptococcus (GBS) is an important cause of bacterial disease in neonates, pregnant women, and nonpregnant adults. Whereas serotypes Ia, Ib, II, III, and V are most commonly associated with colonization and disease in the United States, strains of other serotypes have been isolated from patients in Japan. By use of an inhibition ELISA, the serotypes of 73 vaginal colonizing GBS strains isolated from healthy pregnant Japanese women were investigated. Twenty-six (35.6%) were type VIII, 18 (24.7%) were type VI, and the remaining 29 were distributed among more traditional serotypes. Strains were also tested by immunoblot for the presence of GBS surface proteins. Fifty-three (72.6%) of the 73 strains expressed one or more laddering GBS proteins. These data show that type VI and VIII GBS strains are common vaginal isolates in pregnant Japanese women and that one or more laddering proteins are present in most GBS strains.

Infants perinatally infected by group B streptococci (GBS), primarily through exposure to organisms colonizing the birth canal, are at risk for invasive disease, most commonly sepsis, pneumonia, and meningitis. A major research effort is the development of polysaccharide-based GBS vaccines to be offered to women before or during pregnancy [1]. Transplacental passage of vaccine-induced antibodies should protect the majority of at-risk infants from invasive GBS disease.

GBS are classified according to the presence of type-specific capsular polysaccharides (CPS), of which nine have been identified. Before the 1990s, most perinatal GBS disease was caused by strains of types Ia, Ib, II, and III [2]. Recently, serotype V has emerged as an important disease-associated serotype [3, 4]. Strains of serotypes IV, VI, VII, and VIII have rarely been isolated from patients in the United States [3, 4]. Ongoing monitoring of GBS serotype distribution will be important for determining the components of GBS vaccines.

Surface protein analysis is a useful adjunct to polysaccharide serotyping in the classification of GBS strains. Most type Ia, Ib, II, III, and V strains express at least one surface protein antigen belonging to a putative family of laddering GBS proteins [5–7]. Whereas antibody to CPS is the principal determinant of immunity to GBS in young infants, limited data suggest a role for the laddering proteins in immunity to GBS [5, 8].

We report the serotypes of vaginal GBS strains isolated from healthy pregnant women in Kawasaki, Japan. Our results show a striking predominance of strains of serotypes VI and VIII and, with previous reports from the Japanese literature [9, 10], suggest an emergence of these serotypes over the last decade in Japan. The strains were also evaluated by Western blot for the presence of GBS surface proteins, including an α-like protein purified from a type VIII strain. A majority of the strains expressed at least one laddering surface protein.

Materials and Methods

Bacterial strains, antigens, and antisera. Strains 090, H36B, 18RS21, M781, A909, D136C, and the Compton 25/60 strain were originally obtained from Rebecca Lancefield (Rockefeller University, New York, NY). Strain CJB-110 was provided by Carol Baker (Baylor College of Medicine, Houston). Strains 3139, SS1214, 7271, and JM9-130013 were provided by J. Jelinkova (Institute of Hygiene and Epidemiology, Prague).

GBS were isolated from vaginal cultures obtained during routine...
medical visits from 441 pregnant women sequentially attending a single urban obstetric practice in Kawasaki, Japan, between May 1992 and June 1994. Rectal cultures were not obtained. Samples for culture were obtained during the first, second, or third trimester and, for some patients, at multiple times during pregnancy. Isolates suspected of being GBS were tested with a latex agglutination test (Denka Seiken, Tokyo) and confirmed by the API system (BioMérieux, Marcy l’etoile, France). In this way, 109 GBS isolates were cultured from 71 (16.1%) of 441 women. Fifty of the 109 isolates were tested for the presence of CAMP factor; all were positive.

Purified CPSs of all known serotypes were prepared from prototype GBS strains by methods described for serotype III [1]. Rabbit antisera raised to types Ia, Ib, II, III, and V CPS coupled to tetanus toxoid have been described [11]. Rabbit antisera raised to GBS strains of types IV, VI, and VII, and adsorbed with strains of heterologous serotypes to reduce the amount of surface protein antibodies were produced at the GBS Molecular Reference Laboratory at the University of Minnesota (Minneapolis), as were rabbit R1 and R4 antisera [12]. Rabbit antiserum raised to protein Rib was provided by Gunnar Lindahl (University of Lund, Lund, Sweden) [6]. Monoclonal antibody 4G8, a murine monoclonal antibody directed to the repeat region of the α C protein [13], and rabbit antisera to an α-like protein purified from type V strain CJB-110 [7] and to the β C protein [14] have been described. A laddering α-like protein from type VIII strain JM9-130013 and corresponding rabbit antiserum were produced by methods described, for the α-like protein from type V strain CJB-110 [7]. The final product showed a laddering pattern on SDS-PAGE, with a predominant band of ~90 kDa and a difference between adjacent bands of ~8 kDa. Protein-specific IgG titer of rabbit serum obtained 2 weeks after the third vaccination was 512,000, a 250-fold increase over preimmunization titers.

Serotyping by inhibition ELISA (iELISA). CPS extracts were prepared from cultures of each clinical isolate grown to an optical density at $A_{600}$ of 0.3–0.5 and used in an iELISA modified from previous methods [15]. A 1:50 dilution of each capsular extract was incubated with type-specific antisera diluted 1:50,000 (types Ia, Ib, and II), 1:25,000 (type III), 1:15,000 (type IV), 1:3000 (type V), 1:300 (type VI), 1:1000 (type VII), or 1:2000 (type VIII) in PBS and added to microtiter plates coated with a 1-μg/mL solution of homologous CPS linked to poly-L-lysine. Percent inhibition for each test extract was calculated as follows: \[ \frac{[A_{\text{asc}} \text{ of uninhibited control} - A_{\text{asc}} \text{ of uninhibited control}]}{A_{\text{asc}} \text{ of each well}} \times 100. \] The $A_{600}$ of the positive control (purified homologous polysaccharide diluted to 10 μg/mL or 1:50 dilution of capsule extract of the homologous reference strain) was subtracted from the $A_{600}$ of each well; thus, 100% inhibition was defined as the inhibition corresponding to the positive control. For the serotype VIII iELISA, a positive result was defined by a percent inhibition of ≥60. For all other iELISAs, a positive result was defined by a percent inhibition of ≥70. Each strain was tested for the presence of CPS types Ia, Ib, II, III, IV, V, VI, and VIII. Nontypeable strains were retested at an extract dilution of 1:10 for all known CPS types, including type VII.

Serotyping by immunodiffusion. A subgroup of 20 strains was serotyped in a blinded fashion by double-diffusion in agarose (Ouchterlony method) [16].

Surface protein analysis. Western blotting was performed on SDS extracts of whole GBS as described [7]. Antisera were diluted to 1:500 (4G8 ascites) or 1:1000 (immune sera).

Results

Serotyping. From 71 (16%) of 441 women, 109 GBS isolates were cultured. In all but 2 cases, isolates collected at different times from a single patient were of the same CPS serotype and were considered to be the same strain. In 1 patient, a type VIII and a type III strain were collected at 20 and 30 weeks of gestation, respectively. In another patient, a type VIII strain was isolated at 20 weeks of gestation, and a nontypeable strain was isolated at 30 weeks of gestation. Thus, 73 vaginal GBS strains were isolated from 71 patients.

Serotyping of the GBS strains was performed by iELISA (figure 1). None of the strains were positive for >1 serotype. The 20 strains whose serotypes were validated by the Ouchterlony method included at least 1 strain of each serotype identified in this study and 5 of the 7 strains that were nontypeable by iELISA. The results using the two methods were concordant in all cases.

Surface protein analysis. SDS extracts of the 109 Japanese isolates were examined for the presence of immunoreactive proteins by Western blot with antisera reactive with various GBS proteins. Fifty-three (72.6%) of the 73 strains expressed one or more laddering GBS proteins (table 1). For 21 of the 27 patients from whom multiple GBS isolates of homologous CPS serotype were collected, Western blot results were identical for all isolates, indicating the presence of the same strain at different times during pregnancy. In 6 other patients from whom multiple isolates were obtained, a laddering protein was seen in some iso-
lates but not others. The nonreactive isolates were not designated as distinct strains.

Discussion

This study shows a high prevalence of serotypes VI and VIII among colonizing GBS strains isolated from pregnant women in Japan. Previous reports in the Japanese literature have indicated the presence of these serotypes in Japan. Murai et al. [9] examined the serotypes of 268 invasive GBS strains collected from 1974 to 1990 and found a trend toward an increasing frequency of serotypes VI and VIII: whereas the first type VIII and VI strains were noted in 1983 and 1985, respectively, 6 of 12 strains collected in 1990 were either type VI or type VIII. In a multicenter survey study of neonatal GBS infection from 1988 to 1992, Hoshina et al. [17] examined the serotypes of 99 of 301 invasive GBS strains isolated from infants with early-onset disease; 11 (11.1%) were type VI, and 9 (9.1%) were type VIII. A recent multicenter study reported the relative frequency of type VI and VIII strains among vaginal GBS isolates to be 28.1% and 24.8%, respectively, data similar to ours [10]. The high frequency of type VI and VIII GBS strains in Japan has not been previously reported in the English language literature.

The apparent emergence of types VI and VIII has implications for GBS vaccine development. Current efforts are underway to construct a multivalent GBS vaccine composed of the major serotypes (Ia, Ib, II, III, and V) conjugated to a protein carrier. Ongoing surveillance of serotype distribution among GBS strains in diverse geographic areas will be an important aspect of a GBS vaccine program. If additional epidemiologic studies demonstrate a high contribution of serotypes VI and VIII to neonatal GBS disease, inclusion of these serotypes in future vaccine constructs should be considered, at least for areas in which these serotypes are prevalent.

GBS strains isolated from Japanese women were also evaluated for the expression of members of an apparent family of laddering GBS proteins. Among type VI and VIII strains, the major serotypes found in this study, reactivity profiles were well conserved. For example, type VIII strains reacted either with antiserum to R1, R4, Rib, and the Α-like proteins from types V and VIII GBS, or with no antiserum. This immunoreactivity profile was not seen with strains of other serotypes, suggesting that the Α-like protein from type VIII GBS may represent a novel protein. Evidence supporting a family of GBS surface proteins includes phenotypic similarities of these proteins [7] and genetic similarities between the Α-C protein and protein Rib [18]. The data presented here suggest a close relationship among several GBS proteins, presumably based on conserved epitopes serving as common links. It is also possible that several of the different antiseras identify identical proteins, rather than merely shared epitopes. The relationship among these proteins will likely be elucidated through genetic analysis.

Among 6 patients from whom multiple GBS isolates of homologous CPS type were collected at different points in pregnancy, a laddering protein was detected in some isolates but not others. Although these findings could be explained by the presence of two different strains or by technical limitations of the Western blot technique, we suspect the differences in protein expression result from a gene mutation (as has been demonstrated for the Α-C protein [5]) or a difference in gene regulation.

Consistent with studies from other areas, these data show that a majority of strains express at least one laddering surface protein. Consideration has been given to including a GBS protein antigen in a GBS conjugate vaccine [14]. Conjugation of a GBS surface protein to one or more GBS polysaccharides should provide an enhancement of the immune response to the CPS similar to that provided by the currently used tetanus toxoid and might also improve vaccine efficacy by eliciting antibodies to multiple epitopes and by extending coverage to strains that contain the protein antigen but none of the known CPSs. A protective epitope conserved among the laddering GBS proteins would be a candidate for this role.

The iELISA is a useful method for serotyping GBS strains, which is traditionally performed by use of the Lancefield capsular precipitin method or related immunoprecipitation assays. Of importance, our iELISAs used highly characterized reagents. A significant advantage of the iELISA is that less antiserum is required than for the immunoprecipitin method. Moreover, comparison to a standard curve of known CPS concentrations would allow for quantifiable results [15]. The 100% concordance between the results of the iELISA and the immunoprecipitin methods supports the iELISA as an alternative serotyping method.

Acknowledgments

We thank Carol Baker for type V GBS strain CJB-110 and Gunnar Lindahl for antiserum to the Rib protein.

Table 1. Laddering motifs of 73 GBS isolates from pregnant Japanese women.

<table>
<thead>
<tr>
<th>Antiserum reactivity</th>
<th>Ia</th>
<th>Ib</th>
<th>III</th>
<th>V</th>
<th>VI</th>
<th>VIII</th>
<th>NT</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a/AL5</td>
<td>1</td>
<td>7</td>
<td>7</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL5/AL8</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a/AL5/AL8</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a/AL5/AL8/R1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a/AL5/AL8/R1/R4/Rib</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL5/AL8/R1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL5/AL8/R1/R4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL5/AL8/R4/Rib</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL5/AL8/R1/R4/Rib/Rib</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>21</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>AL8/R4/Rib</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>10</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Total no. of strains 5 6 8 3 18 26 7

NOTE: Α = 4G8, monoclonal antibody raised to repeat region of Α protein; AL5 = antiserum raised to Α-like protein from GBS type V strain CJB-110; AL8 = antiserum raised to Α-like protein from GBS type VIII strain JM9-130013; R1 = R1-specific antiserum; R4 = R4-specific antiserum; Rib = antiserum raised to protein Rib; NR = not reactive.

Three Ib strains also reacted with antiserum to β-C protein.
References


