Identification of a Highly Encapsulated, Genetically Related Group of Invasive Type III Group B Streptococci

Shinji Takahashi, Elisabeth E. Adderson, Yukiko Nagano, Noriyuki Nagano, Mark R. Briesacher, and John F. Bohnsack

Type III group B streptococci (GBS) isolated from Tokyo and Salt Lake City were classified according to the similarity of HindIII and Sse83871 restriction digest patterns (RDPs) of bacterial DNA. The bacteria were clustered into three RDP types, with excellent correlation between subtyping based on the two enzymes. The majority (91%) of invasive isolates obtained from neonates were RDP type III-3. The mean sialic acid content of the III-3 strains was higher than that of other type III strains. Closely related isolates were concordant for expression of the bacterial enzyme C5a-ase, but invasive strains were no more likely to be C5a-ase positive than were strains isolated from the genitourinary tract of pregnant women. These data indicate that a group of genetically related organisms with increased capsule production causes the majority of invasive type III GBS disease.

Group B streptococci (GBS) are an important cause of serious bacterial disease in neonates, pregnant women, and patients with underlying illnesses [1]. GBS are subclassified into serotypes according to the immunologic reactivity of the polysaccharide capsule. Of the nine serotypes, types I, II, III, and, more recently, type V and VIII GBS cause the majority of neonatal human GBS disease [1–3]. Serotype III GBS are particularly important because type III cause a significant percentage of early-onset disease (within the first week of life) and the majority of late-onset disease (after the first week of life) in human neonates and also cause the vast majority of neonatal GBS meningitis [1].

GBS can be further subclassified into restriction digest pattern (RDP) types by electrophoretic analysis of fragments produced by restriction enzyme digestion of bacterial DNA. RDP typing of clinical isolates in Japan by use of the restriction enzyme HindIII showed that a single RDP type (RDP type III-3) causes most serotype III neonatal sepsis, suggesting the existence of a genetically related subgroup of serotype III GBS that are intrinsically more virulent than other serotype III strains [4]. The purpose of these studies was to determine whether invasive serotype III isolates from Salt Lake City, as well as more recently isolated clinical isolates from Japan, are also RDP III-3. We also validated the HindIII typing by typing the GBS with a second restriction endonuclease, Sse83871, and determined whether other putative virulence factors, including capsular sialic acid content, R protein, and C5a-ase expression, correlated with RDP type.

Materials and Methods

Bacteria. GBS were isolated from routine vaginal swabs from women seen at the University of Utah Hospital during 1993–1995. GBS isolated from blood or cerebrospinal fluid or from a sterile body site at autopsy were obtained from the Microbiology Laboratories at the University Medical Center and at Primary Children’s Medical Center, a regional children’s referral hospital, from 1993 to 1995. Most Japanese isolates were collected from patients cared for at the Funabashi Medical Center, as well as other hospitals in the Tokyo area, between 1988 and 1995. GBS were serotyped with commercially available antisera (Denka Seiken, Tokyo). Colonies of GBS were isolated from blood-agar plates, grown in Todd-Hewitt broth, and stored at −70°C until analysis.

RDP typing. DNA was extracted from GBS suspended in agarose gel plugs (InCert; FMC BioProducts, Rockland, ME) according to the manufacturer’s instructions except that mutanolysin and SDS containing protease K were used for digestion of the bacteria. DNA in the agarose gel plugs was digested with HindIII, extracted from the agarose with phenol, and redigested with HindIII. The DNA sample was then subjected to electrophoresis in a conventional ethidium bromide–agarose gel. The similarity between densitometric RDPs from individual strains was expressed as a Pearson product moment correlation coefficient (PPMCC) and clustered by the unweighted pair group method average as previously described [4]. RDPs from a single isolate always resemble each other with a PPMCC ≥0.99, while RDPs of GBS isolates of the same serotype always resemble each other with a PPMCC >0.93 (data not shown). Therefore, strains with RDPs that resemble each other with PPMCCs ≥0.99 are considered identical, and isolates that resemble each other with PPMCCs >0.93 are clustered into subtypes.

Sse83871 RDP typing was done similarly to HindIII typing except that Sse83871-digested DNA fragments were separated by
pulsed-field gel electrophoresis. Similarity coefficients between all possible pairs of RDPs were calculated as follows: similarity = (number of shared bands × 2)/total number of bands. The RDPs were then clustered by unweighted pair group method average. RDPs from strains of the same serotype resemble each other with a similarity coefficient >0.45 (data not shown). Thus, isolates were clustered into subtypes in which the isolates resembled each other with a similarity coefficient >0.45.

Detection of plasmid DNA. DNA was extracted from agarose plugs, alkaline-denatured, and subjected to conventional agarose gel electrophoresis to detect plasmids.

Sialic acid content. Bacteria were grown in Todd-Hewitt broth, harvested at mid-log phase to maximize capsular and sialic acid content [5], and washed by centrifugation. The capsule was extracted by hydrolysis with 0.1 N HCl at 84°C for 20 min, and the sialic acid content of the capsular extract was determined by the thiobarbituric acid method [6].

C5a-ase activity. Functional C5a-ase activity was determined as previously described by use of a quantitative neutrophil adhesion assay [7].

Results

The 62 isolates were divided into three HindIII RDP types, as in the previous study [4]. The majority of the isolates (41) were found to be RDP type III-3, while 18 and 3 of the remaining isolates were RDP type III-2 and III-1, respectively (figure 1). Isolates clustered into five Sse83871 RDP types, with the exception of isolate 59 (figure 1). Bacteria within these 5 groups of bacteria, designated III-1, III-2a, III-2b, III-3a, and III-3b, were found in the corresponding HindIII RDP types (figure 1). Furthermore, bacteria that clustered into Sse83871 RDP type III-3a (strains 1–27) and III-3b (strains 28–41) also clustered into these subtypes within the HindIII dendrogram (figure 1). Strains 42–54 in the HindIII-2 dendrogram were III-2a except strain 45, which was III-2b, while strains 55–58 were all III-2b, except strain 58, which was III-2a. Thus, the two methods of RDP typing clustered the type III isolates into virtually identical groups, strongly supporting the validity of these two methods for subtyping type III GBS. Isolates that resembled each other with similarity coefficients >0.97 by HindIII typing, or that had identical RDPs by Sse83871 typing, were always isolated from the same city (not shown). No plasmids were detected in any of the isolates.

Overall, 35 of the GBS were isolated from a normally sterile body fluid (blood or cerebrospinal fluid). The overwhelming majority of these strains (91%; 32 strains) were RDP type III-3, whereas only 9, or 33%, of the vaginal isolates were III-3. In contrast, 59% (16) of the vaginal isolates were RDP type III-2 strains, while only 6% (2) of the invasive strains were III-2. RDP type III-3 strains were significantly more likely to be invasive isolates than to be vaginal isolates, while type III-2 strains were significantly more likely to be vaginal isolates than to be invasive (P < .01, χ², Yates’s modification).

For 27 isolates, the patient’s age at the onset of disease was available. Two of the 27 isolates were from older children (2 and 18 years of age). The remaining 25 were from neonates with either early- (n = 12) or late-onset (n = 12) disease. Both of the III-2 isolates were from infants with late-onset disease. The III-3 strains were still significantly more likely to be invasive than to be vaginal isolates (P < .01, χ², Yates’s modification), even when the isolates from older children and from those patients for which clinical information was lacking were excluded.

The mean sialic acid content of the type III-3 strains was significantly greater than the sialic acid content of either the III-2 or III-1 strains (P < .05, Student’s t test; table 1), although 2 of the III-2 strains had sialic acid contents greater than the mean sialic acid content of the III-3 strains. The sialic acid content of the invasive III-3 isolates was not significantly greater than the sialic acid content of colonizing III-3 strains (6.19 ± 1.14 vs. 5.83 ± 1.65 mg of sialic acid/mg of cell dry weight), nor was the sialic acid content of the invasive III-2 or III-1 isolates elevated compared with that of the isolates of the same RDP type.

While 100% of the III-2 and III-1 isolates were C5a-ase–positive, only 63% of the III-3 strains were C5a-ase–negative (table 1). All of the III-3a strains were C5a-ase–positive except 1 (isolate 9), and all of the III-3b strains were C5a-ase–negative. Overall, 76% of the type III GBS expressed functional C5a-ase activity, but there was no correlation between C5a-ase activity and whether the organism was invasive or colonizing: 69% (24/35) of the invasive strains expressed C5a-ase, while 85% (23/27) of the vaginal isolates expressed C5a-ase. Consistent with a previous report [8], all of the strains expressed R protein, except for the III-1 strains and 1 of the III-3 isolates.

Discussion

In the studies reported here, we demonstrate that type III GBS from both Salt Lake City and Tokyo can be clustered into three subtypes on the basis of HindIII RDPs. The validity of the HindIII subtyping is strongly supported by the Sse83871 typing and is further corroborated by correlation with several different phenotypic determinations. First, the III-3 isolates had a significantly higher sialic acid content than did the III-2 or III-1 isolates. Second, the III-3a, III-2, and III-1 isolates all expressed C5a-ase activity (with 1 exception), while the III-3b isolates did not. Third, the 3 III-1 strains in this study were uniformly R protein negative, as were the 4 III-1 strains studied in our previous study [4]. In contrast, the overwhelming majority of III-2 and III-3 strains were found to express R protein both in this study and in our previous report [4]. This suggests that lack of R protein expression is a unique phenotypic characteristic of III-1 strains, although the small number of strains identified as III-1 limits the power of this observation. Additional support for the validity of the RDP typing is derived from the fact that isolates identified as closely related or identical by RDP typing always originated from the same city.
Studies from several laboratories have suggested that most invasive type III GBS disease is caused by related strains of bacteria [9, 4, 10], although a distinct group of type III GBS with increased virulence could not be identified in one study [11]. Our data indicating that the vast majority of invasive isolates in this sample were RDP type III-3 suggest that RDP type III-3 strains are the most common type III GBS to cause invasive disease. These studies therefore support the existence of a subtype of type III GBS with increased pathogenic potential.

We previously proposed that the bacterial enzyme C5a-ase contributes to the pathogenic potential of GBS by its ability to rapidly inactivate the potent complement-derived neutrophil agonist C5a [12, 13]. Data presented here, however, suggest that expression of C5a-ase is not the basis for increased virulence of the III-3 strains, since there was no correlation between C5a-ase activity and invasiveness. These studies do not address the possibility that expression of the gene encoding C5a-ase is down-regulated under the in vitro conditions used in these experiments, leaving open the possibility that C5a-ase contri-
Table 1. Sialic acid content, C5a-ase activity, and R protein expression of type III GBS.

<table>
<thead>
<tr>
<th>RDP type</th>
<th>No. of isolates</th>
<th>Sialic acid content*</th>
<th>C5a-ase positive (%)</th>
<th>R protein positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>III-1</td>
<td>3</td>
<td>4.03 ± 0.23</td>
<td>3 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>III-2†</td>
<td>18</td>
<td>3.45 ± 1.66</td>
<td>18 (100%)</td>
<td>18 (100%)</td>
</tr>
<tr>
<td>III-2a</td>
<td>13</td>
<td>3.71 ± 1.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III-2b</td>
<td>4</td>
<td>2.83 ± 0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III-3</td>
<td>41</td>
<td>6.11 ± 1.25</td>
<td>26 (63%)</td>
<td>40 (98%)</td>
</tr>
<tr>
<td>III-3a</td>
<td>27</td>
<td>6.18 ± 1.05</td>
<td>26 (96%)</td>
<td>26 (96%)</td>
</tr>
<tr>
<td>III-3b</td>
<td>14</td>
<td>5.97 ± 1.62</td>
<td>0</td>
<td>14 (100%)</td>
</tr>
</tbody>
</table>

* Mean sialic acid content ± SD, expressed as µg/mg of cell dry weight, of all isolates in indicated restriction digest pattern type.
† Significantly less than sialic acid content of III-3 strains (P < .05, Student’s t test).
‡ III-2 isolate 59 is neither III-2a nor III-2b.

butes to the pathogenesis of all type III GBS in vivo. Further investigations are underway to determine the molecular basis for the lack of C5a-ase expression in the III-3b strains.

Our observation that the average sialic acid content of the III-3 strains is higher than that of the III-2 and III-1 strains indicates that III-3 strains are more encapsulated than are III-2 and III-1 strains and suggests that there is a common genetic basis for the greater encapsulation of the III-3 strains. Resistance to opsonization by complement probably contributes to the increased virulence of the III-3 strains, because resistance of type III GBS to opsonization is largely due to the sialic acid content of the capsule and is the major virulence factor known in type III GBS [14, 15].

While the sialylated polysaccharide capsule is critical for type III GBS to evade host defenses, it seems likely that additional bacterial factors contribute to the virulence of type III GBS in human neonates. Our ability to identify more virulent, genetically related strains of GBS by RDP typing should facilitate identification of such virulence factors.

Acknowledgments

We thank Andrew Pavia for helpful comments and assistance with statistical analysis, Karen Carroll and Judy Daly for supplying bacterial samples, and Harry Hill for discussion.

References

5. Paoletti LC, Ross RA, Johnson KD. Cell growth rate regulates expression of all isolates in indicated restriction digest pattern type.