Antibodies to Capsular Polysaccharides of Group B *Streptococcus* in Pregnant Canadian Women: Relationship to Colonization Status and Infection in the Neonate

H. Dele Davies,1,2,3,4,5 Carol Adair,5,6 Alison McGeer,6 Doreen Ma,7 Sheila Robertson,4 Melissa Mucenski,6 Laura Kowalsky,8 Gregory Tyrell,5 and Carol J. Baker7,8

In a cohort study of 1207 pregnant women in Alberta, Canada, the serotype distributions of vaginal-rectal group B *Streptococcus* (GBS) isolates were compared with all isolates from neonates with invasive GBS disease identified by population-based surveillance. Serum concentrations of IgG to types Ia, Ib, II, III, and V capsular polysaccharide (CPS)-specific were determined, according to serotype of the vaginal-rectal colonizing GBS strain. GBS colonization was detected in 19.5% (235 of 1207) of women. Serotype III accounted for 20.6% (48 of 233) of colonizing strains available for typing but for 37% (27 of 73) of invasive isolates from neonates (*P*<.01). Maternal colonization with type III was least likely to be associated with moderate concentrations of III CPS–specific IgG. Serotype III GBS is more invasive than other serotypes in this population; this may be due, at least in part, to poor maternal type III CPS–specific antibody response.

Group B *Streptococcus* (GBS) is an important cause of invasive infection in humans, especially in infants ≤3 months old [1]. On the basis of information from an active population-based surveillance in the United States, >15,000 cases and >1300 deaths due to invasive GBS disease were estimated to occur each year before 1990 [2], with just over half (7600) occurring in infants. In Canada, rates of neonatal GBS disease before implementation of guidelines to prevent early-onset (age <7 days) disease were 0.44–1.2/1000 live births [3]. The primary source of infection in the neonate is the GBS-colonized mother, with transmission occurring shortly before or during the birth process. Estimates of GBS colonization rates among pregnant women in North America are 15%–40% [4–6]. GBS is transmitted to ~50% of newborns, and an estimated 1%–2% of neonates develop early-onset disease. Overall rates of infant disease are 0.2–5/1000 live births [7–12].

Antibodies to type-specific capsular polysaccharides (CPSs) of GBS in the serum of experimental animals and human neonates correlate with protection from GBS disease [13, 14]. Up to 90% of pregnant women may be deficient in type III CPS–specific antibody, which suggests a lack of priming by this antigen [13, 15]. In addition, age-related differences in antibody response have been described, with school-aged girls more likely than older women to have lower levels [16]. The precise concentration of CPS-specific antibody needed for protection of neonates may differ by serotype, bacterial inoculum, and possibly other factors [13, 17–19].

We hypothesized that certain serotypes of GBS may cause early-onset neonatal GBS disease at rates higher than expected from their prevalence in colonized mothers. By use of a prospective cohort study and population-based surveillance for early-onset neonatal GBS disease, we compared the serotype distribution of vaginal-rectal GBS isolates from women in Calgary, Alberta, with that of all isolates from neonates with invasive GBS disease in Alberta. We also measured serum concentrations of CPS-specific IgG in women colonized with the same GBS type, to determine whether certain serotypes were...
more or less likely to be associated with measurable CPS-specific IgG than others.

Patients and Methods

Population. The surveillance area for identification of cases of neonatal early-onset GBS disease was the province of Alberta. This is a mixed urban-rural province with defined geographic boundaries easily identified by the first digit of the postal code (T) and a population, as of 1999, of 2,964,689 (Statistics Canada Census data). The city of Calgary in Alberta, population 933,700, has 3 hospitals that recorded 11,931 births in 1999.

Laboratory surveillance procedures. Prospective, population-based active surveillance for invasive neonatal GBS disease has been operating in Alberta since 1 January 1995. Invasive disease was defined as infection associated with isolation of GBS from a sterile site (e.g., blood; cerebrospinal fluid; joint, pleural, or peritoneal fluid; and surgical tissue). Early-onset neonatal GBS disease was defined as isolation of GBS from a sterile site in any infant during the first 6 days of life. Before the study began, an investigator or study nurse visited all microbiology laboratories to explain the study protocol and to orient the staff. Cases were reported to the study office whenever GBS was isolated from a normally sterile site or from a specimen that the laboratory staff believed might be from a sterile site. A toll-free telephone number was provided for this purpose.

Study staff initiated a case tracking form and a laboratory information form at the time of the initial telephone report and assigned a study code number, which was affixed to all the data sheets. If there was incomplete information from the laboratory at the time of the first telephone call, a follow-up call was made 5–15 days later. All participating laboratories in Alberta were audited by an investigator every 6 months, to ensure complete case ascertainment. For hospitals with electronic laboratory information systems, the laboratory contact person was asked to generate a printout of sterile site isolates (including date of isolate and organism identified), which was reviewed on site. The rest of the audits were performed by manual examination of laboratory records.

Cohort study procedures. From November 1998 through May 2000, a cohort of 1215 pregnant women was enrolled into the study from 16 representative offices of obstetric care practitioners (family doctors and obstetricians) across Calgary, with a mix of low-risk, medium-risk, and high-risk deliveries. During the last prenatal visit (usually at 32–33 weeks’ gestation), before the routine 36-week assessment, eligible pregnant women were given an introductory letter describing the study and a copy of the consent form and were asked to indicate interest in participating in the study. The study nurse tracked all patients, called those who indicated interest, and explained the study procedures in detail. Consenting mothers were asked to take the form back to their physicians’ offices. During the 36-week prenatal visit, intent to participate was confirmed by the collaborating physician, and consent was obtained. The physicians then collected a combined vaginal-rectal swab specimen for GBS culture in Amies transport medium. The specimen was transported to Calgary Laboratory Services for processing. After the 36-week visit, the participant also had blood collected for GBS serologic testing at a later time.

Cohort study laboratory procedures. All vaginal-rectal swab specimens were inoculated into Todd-Hewitt broth supplemented with colistin and gentamicin and were processed for isolation of GBS by means of standard laboratory procedures [20]. During the cohort study, all the remaining microbiology laboratories in the Province of Alberta were asked to submit 9 consecutive GBS isolates from vaginal-rectal specimens (118 total from 13 operational laboratories; one facility sent 10 specimens). These GBS isolates were serotyped at the National Center for Streptococcus (Edmonton, Alberta), to compare with specimens obtained from the Calgary cohort of pregnant women. Grouping was performed by the capillary precipitin test, with Lancefield hot acid extracts and group-specific antisera (Difco Laboratories) [21]. Serotyping was performed on the hot acid extracts by Ouchterlony immunodiffusion with type-specific antisera prepared in rabbits at the National Center for Streptococcus [22]. The typing set included antisera for polysaccharide antigens Ia, Ib, II, III, IV, V, VI, VII, and VIII.

All serum samples obtained from participating mothers were frozen at −70°C at Calgary Laboratory Services. Serum samples from the first 10 GBS-colonized women and further randomly sampled from 219 women (66 colonized and 153 noncolonized) were tested for concentrations of GBS Ia, Ib, II, III, and V CPS–specific IgG, as described elsewhere [23].

Sample size calculations. On the basis of previous Alberta data (K. Aziz, unpublished data), it was anticipated that 17% of the women would be colonized with GBS. Assuming a colonization rate as low as 10%, a sample size of 1200 women was calculated to ensure a minimum of 100 colonized women (95% predictive interval of 120 from 1200 women, or 100–143 women). The sample size of 117 consecutive GBS screening specimens collected from across Alberta was chosen to ensure that the serotype distribution of the women in the Calgary cohort was representative of other pregnant women in Alberta.

Statistical analysis. All data were entered into an Access database (version 7.0; Microsoft) and analyzed by Statistica programs (version 5.0; StatSoft). χ2 tests or Fisher’s exact tests were used to compare the serotype distribution of colonizing GBS strains with that of early-onset GBS disease isolates. Group means were compared with that of early-onset GBS disease isolates. Group means were compared with the Student’s t test. The medians of GBS CPS–specific IgG levels in serum samples and the ranges were calculated in colonized and noncolonized mothers. The antibody comparisons between these 2 groups of women were calculated by χ2 tests and Fisher’s exact tests for categorical variables and by Kruskal-Wallis (K-W) tests to compare medians. P < .05 was considered to be significant, except when multiple comparisons were being made. For the latter scenario, P values that were still <.05 after multiplying by the number of comparisons being made were considered to be significant (Bonferroni adjustment). For example, if 5 comparisons were being made, P < .01 would be considered significant.

Results

During the study period (November 1998–May 2000), 1215 women were enrolled into the study. Complete data collection occurred for 1207 of these women (99.3%). Of these 1207, 235 (19.5%) were colonized with GBS, and 233 of these isolates were available for serotyping. The mean age of enrolled women was 30.2 years, with no significant difference between colonized (30.3 ± 4.8 years) and noncolonized women (30.0 ± 5.1 years;
**Figure 1.** Distribution of group B streptococcal serotypes causing early-onset disease (EOD) in Alberta, compared with maternal colonizing strains from Calgary.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Calgary</th>
<th>Rest of Alberta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>26.7</td>
<td>22.7</td>
</tr>
<tr>
<td>Ib</td>
<td>22.7</td>
<td>5.3</td>
</tr>
<tr>
<td>II</td>
<td>10.7</td>
<td>8</td>
</tr>
<tr>
<td>III</td>
<td>12.9</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>20.6</td>
<td>2.1</td>
</tr>
<tr>
<td>V</td>
<td>37.3</td>
<td>17.3</td>
</tr>
<tr>
<td>Nontypeable</td>
<td>5.3</td>
<td>11.2</td>
</tr>
</tbody>
</table>

GBS CPS-specific IgG levels in serum samples from women selected from the larger cohort are summarized in table 2 as medians. In general, women colonized with a particular serotype were more likely to have higher concentrations of specific IgG to the colonizing serotype than to other serotypes. However, colonization with serotype III GBS was associated with lower concentrations of IgG to this CPS. Although the homologous serum IgG concentration for GBS-colonized women was always significantly higher in women colonized with non–type III strains (trend for serotype II), this was not found for women colonized with serotype III GBS (table 2 and 3).

To confirm this finding, response to serotype III was measured in 32 of the remaining 34 women colonized with this serotype who had serum samples available. The overall median antibody level against type III for all 46 type III–colonized women was 0.39 μg/mL, versus 0.96–4.63 μg/mL for specific antibodies against the remaining colonizing serotypes (\( P = .01 \), overall K-W median test for all types [Ia, Ib, II, III, IV, V, and nontypeable]; \( P = .002 \), K-W median test for type III vs. non–type III). Furthermore, although colonization with the other serotypes was associated with homologous antibody levels >0.5 μg/mL in more than two thirds of women, such levels were seen in <50% of women colonized with serotype III (table 4).

**Table 1.** Comparison of maternal colonizing group B Streptococcus serotypes in Calgary with those in women from the rest of Alberta.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Calgary</th>
<th>Rest of Alberta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>53 (22.7)</td>
<td>24 (20.3)</td>
</tr>
<tr>
<td>Ib</td>
<td>25 (10.7)</td>
<td>10 (8.5)</td>
</tr>
<tr>
<td>II</td>
<td>30 (12.9)</td>
<td>10 (8.5)</td>
</tr>
<tr>
<td>III</td>
<td>48 (20.6)</td>
<td>18 (15.3)</td>
</tr>
<tr>
<td>IV</td>
<td>5 (2.1)</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>45 (19.3)</td>
<td>33 (28.0)</td>
</tr>
<tr>
<td>VIII</td>
<td>1 (0.4)</td>
<td>0</td>
</tr>
<tr>
<td>Nontypeable</td>
<td>26 (11.2)</td>
<td>23 (19.5)</td>
</tr>
</tbody>
</table>

Total 233 118

NOTE. Data are no. (%) of isolates.

\( P = .72 \), Student’s t test). The mean number of previous pregnancies was 1.2 ± 1.4 (median, 1.0 pregnancy; range, 0–7 pregnancies). The serotype distributions of GBS isolates from neonates with early-onset disease (1995–1999) in all of Alberta and of maternal colonizing GBS strains for the cohort of Calgary women (1998–2000) are shown in figure 1. None of the cohort of Calgary women had an affected neonate. There was an overall difference in the distribution of serotypes in maternal colonizing and infant disease isolates \( (P < .01) \). Most of this difference could be attributed to the ∼2-fold number of infant invasive cases caused by serotype III, compared with type III colonization in mothers.

Because the cohort study involved only Calgary women, and because women were enrolled predominately during 1999–2000, it was possible that the colonization status of Calgary women may not be representative of Alberta women as a whole. We compared the GBS-colonizing serotypes for Calgary women with the serotypes of GBS-colonizing strains of women from across Alberta collected during the same time (table 1). The distributions of colonizing serotypes from Calgary were similar to the distributions in the rest of the province. There was also potential for bias in comparing GBS isolates that were collected from mothers during 1999–2000 with strains that caused invasive early-onset disease for a period of 5 years. For this reason, we compared the distribution of GBS serotypes causing invasive disease during 1999 with the maternal colonizing strains during the same period. Eight (57.1%) of 14 neonatal cases during 1999 were caused by serotype III, whereas 48 (20.6%) of 233 maternal colonizing strains in Calgary were serotype III \( (P < .01, \chi^2 \text{ test}) \). The results were similar when maternal colonizing serotype III strains for the rest of Alberta (18 [15.3%] of 118) were compared with those type III strains causing early-onset disease during 1999 (8 [57.1%] of 14; \( P < .001, \chi^2 \text{ test}) \).
Discussion

To our knowledge, this is the first study that has attempted to correlate maternal GBS colonizing serotypes, serum antibody levels, and their relationship to invasive disease in neonates. Until recently, when there have been increasing reports of a changing spectrum of invasive GBS disease with the emergence of serotype V [8, 24–26] and other serotypes [27, 28], there had been almost equal distributions reported of all GBS isolates in healthy adults, children, and neonates into 3 major serotypes: I, II, and III [29–33]. Infants with early-onset GBS infection without central nervous system involvement also experienced a similar distribution of serotypes. In contrast, in most reports, up to 90% of isolates from infants with meningitis (irrespective of age at onset) or from infants with late-onset disease (irrespective of site of infection) belonged to serotype III [17, 29, 30, 34–37]. In this study involving a large cohort of women from Alberta, we were able to demonstrate that GBS serotype III colonizes mothers at about half the rate it causes invasive early-onset disease in neonates. Furthermore, we were able to demonstrate that, compared with all other serotypes, vaginal-rectal colonization with serotype III was least likely to be associated with higher serum levels of type III CPS–specific IgG than would be expected in non–GBS colonized women. This implies that serotype III isolates in our population may be more virulent than the other GBS serotypes, which may, at least in part, be explained by lack of maternal IgG that could passively protect their neonates. This has implications for vaccine development, because, although a multicomponent vaccine is likely to be of maximum benefit, a vaccine that contains serotype III alone may protect a disproportionate number of infants in our population from neonatal GBS disease.

The reason why colonization with serotype III was not associated with a CPS-specific IgG response similar to the other serotypes in our population is not immediately obvious. Some studies involving other populations have noted similar findings; other investigators have not recorded a differential response in women to serotype III. Vogel et al. [38] used indirect immuno-fluorescence to measure IgG antibody against the 4 major serotypes of GBS in the serum samples of 200 consecutive pregnant women. Antibody was detectable in 26% of undiluted serum samples against serotype Ia, 52% against serotype Ib, 82% against serotype II, and 45% against serotype III. However, <10% of women had titers of serotype III GBS associated with protection against a lethal challenge to chick embryos. Baker et al. [13, 39] showed that serum samples from most pregnant women contain low levels of antibodies to serotype III CPS (<1 μg/mL). Beachler et al. [40], in a study of pregnant women of lower socioeconomic status, found significantly higher concentrations of type III–specific antibodies in serum samples from women colonized with serotype III GBS than in serum samples from noncolonized or non–type III colonized pregnant women. In a study of 124 Gambian women known to be GBS colonized and their newborns [41], serotype Ia and III CPS–specific IgG serum concentrations were determined. One third of the women and one fifth of their newborns had antibody concentrations to serotype III GBS ≥2 μg/mL in serum samples, compared with 12.5%–22% of Texas women [17] and 5.3% among all GBS colonized women in our study. In a recent study by Campbell et al. [6], colonization with serotype Ia, II, III, or V was associated with significantly higher serum concentrations of IgG specific for the CPS of the colonizing serotype, compared with noncolonization. However, 48% of colonized women had low

<table>
<thead>
<tr>
<th>Colonizing GBS serotype</th>
<th>IA</th>
<th>IB</th>
<th>II</th>
<th>III</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA vs. negative</td>
<td>&lt;0.01*</td>
<td>0.1</td>
<td>0.87</td>
<td>0.46</td>
<td>0.05</td>
</tr>
<tr>
<td>IB vs. negative</td>
<td>0.94</td>
<td>0.07</td>
<td>0.62</td>
<td>0.17</td>
<td>0.97</td>
</tr>
<tr>
<td>II vs. negative</td>
<td>0.05</td>
<td>0.09</td>
<td>&lt;0.01*</td>
<td>0.51</td>
<td>0.04</td>
</tr>
<tr>
<td>III vs. negative</td>
<td>0.20</td>
<td>0.09</td>
<td>&lt;0.01*</td>
<td>0.75</td>
<td>0.12</td>
</tr>
<tr>
<td>V vs. negative</td>
<td>0.10</td>
<td>0.23</td>
<td>0.11</td>
<td>0.78</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>All colonized vs. negative</td>
<td>&lt;0.01</td>
<td>0.01</td>
<td>&lt;0.01</td>
<td>0.62</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

NOTE. Data are P values (Kruskall-Wallis test) for antibody comparisons against colonizing GBS serotypes.

* To adjust for multiple comparisons of 5 different pairwise comparisons of antibodies vs. specific colonizing serotypes vs. negative, each of these remains significant with P = .01 as significant (P = .05 divided by 5, using the Bonferroni approach).
CPS-specific IgG levels (<0.5 μg/mL) in their delivery serum samples. These data suggest that maternal antibody responses to serotype III vary from region to region, perhaps because of biologic differences in circulating strains or in host response.

Other studies that have examined only maternal GBS colonizing serotype distributions have found very different results. In 441 Japanese women, 16% were GBS positive; serotypes VI (24.7%) and VIII (35.6%) were the most common colonizers, whereas 11.3% were serotype III [42]. In the Netherlands, Jacomina et al. [43] found a 13.9% carrier rate with the following serotype distribution: III (29%), Ib (27%), II (12%), and 1c (10%) [it was not specified whether the serotype was 1a/c or 1b/c]. These authors did not comment on serotypes causing neonatal disease or on antibody levels in maternal serum samples. In a Gambian population with a 22.4% GBS colonization rate but with a negligible rate of neonatal invasive disease, only 6% of colonizing strains were serotype III, and 40% were serotype V [44]. The authors speculated that their low rates of invasive disease might be related, in part, to the infrequency of maternal colonization with serotype III. No antibody measurements were performed in any of these studies, and no comparisons were made of serotype-specific colonizing rates to rates of invasive neonatal GBS disease caused by homologous strains. These studies underscore the importance of local data pertaining to serotype distributions in determining the potential success of any multivalent candidate vaccines.

The potential sources of bias in our study were that the sample of women involved in the cohort study might not represent the general population of women who gave birth in Calgary. The risk of this bias was minimized by enrolling women from all 3 hospitals that perform deliveries in the city and from practices of a wide range of family physicians and obstetricians. Furthermore, by obtaining the subset of vaginal-rectal specimens from laboratories across all of Alberta, we were able to ensure that the serotype distribution of GBS obtained from the Calgary cohort was similar to the serotypes of specimens from women from the rest of Alberta. Finally, by comparing maternal colonizing serotypes obtained during the cohort study period with strains that caused invasive disease during the same time period, we were able to get consistent results that a disproportionate number of serotype III strains cause neonatal disease.

We conclude that serotype III GBS strains invade infants at higher rates than the rates at which they colonize women in this region. This increased pathogenicity may be due, at least in part, to the failure of mounting an adequate antibody response against serotype III colonization.

Acknowledgments

We thank all the community family doctors, obstetricians, and nurses, without whose enthusiastic efforts at enrollment this study could not have been accomplished. We also thank all the microbiology laboratories in Alberta for their cooperation in the ongoing population-based surveillance for group B Streptococcus; Deirdre Church, Heather Semeniuk, and Glennis Doiron (Calgary Laboratory Services) and Marguerite Lovgren (National Center for Streptococcus) for their diligence in various aspects of the study; and Melissa E. Hickman (Streptococcal Immunology Laboratory) for performing the antibody assays.

References


2. Zangwill KM, Schuchat A, Wenger JD. Group B streptococcal disease in the

<table>
<thead>
<tr>
<th>Maternal colonizing serotype</th>
<th>IA</th>
<th>IB</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;0.5</td>
<td>&gt;1.0</td>
<td>&gt;2.0</td>
<td>&gt;0.5</td>
<td>&gt;1.0</td>
</tr>
<tr>
<td>IA</td>
<td>70.6</td>
<td>70.6</td>
<td>52.9</td>
<td>23.5</td>
<td>17.6</td>
</tr>
<tr>
<td>IB</td>
<td>50</td>
<td>37.5</td>
<td>12.5</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>II</td>
<td>69.2</td>
<td>46.2</td>
<td>30.8</td>
<td>46.2</td>
<td>30.8</td>
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<td>III</td>
<td>50</td>
<td>28.6</td>
<td>14.3</td>
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</tr>
<tr>
<td>V</td>
<td>40</td>
<td>33.3</td>
<td>26.7</td>
<td>26.7</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Table 4. Percentage of women with serum group B Streptococcus (GBS) capsular polysaccharide-specific IgG levels, by colonizing GBS serotypes.


46. Kalliola S, Vuopio-Varkila J, Takala AK, Eskola J. Neonatal group B strep-

