Group B Streptococcal Colonization and Serotype-Specific Immunity in Healthy Elderly Persons

Morven S. Edwards,¹ Marcia A. Rench,¹ Debra L. Palazzi,¹ and Carol J. Baker¹,²

¹Section of Infectious Diseases, Department of Pediatrics, and ²Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas

Background. The burden from group B streptococcal (GBS) disease in elderly persons (age, ≥65 years) has increased. Rates of colonization and prevalence of antibodies against capsular polysaccharides (CPS) that might confer protection against invasive GBS disease in such persons are not defined.

Methods. A cross-sectional survey was conducted in an outpatient setting in Houston. GBS colonization rates in this convenience sample were assessed by self-obtained vaginal and rectal specimens (for women) and rectal and urine specimens (for men). The CPS type distribution among GBS isolates was determined, and CPS-specific antibodies against GBS types Ia, Ib, II, III, and V were quantified by enzyme-linked immunosorbent assays.

Results. The GBS colonization rate among 254 healthy elderly participants (mean age, 73 years) was 21.7%. CPS types Ia (22.8%), III (12.3%), and V (47.3%) predominated, and 12.3% of colonizing isolates were nontypeable. Random selection of 1 member of 33 participating married couples did not alter the overall colonization rate (21.7%) or GBS serotype distribution. The geometric mean concentrations of CPS-specific IgG in serum specimens were low and were significantly lower for GBS type V, compared with other serotypes (P < .001).

Conclusions. Adults ≥65 years of age are colonized with GBS at a rate similar to that of younger persons, but older adults are significantly more likely to carry type V, the leading cause of invasive disease in elderly persons, and to lack type V CPS-specific serum IgG. The CPS of type V GBS should be included in candidate GBS vaccines so that adults ≥65 years of age theoretically could be protected against invasive disease.

The past 2 decades have witnessed a 2–4-fold increase in the incidence of invasive group B streptococcal (GBS) disease among nonpregnant adults. Invasive disease usually occurs in adults with underlying medical conditions or advanced age [1]. The success of maternal intrapartum antimicrobial prophylaxis has shifted the disease burden from young infants to nonpregnant adults. Active surveillance indicates that more than two-thirds of cases of invasive GBS disease occur among nonpregnant adults and that a majority of these patients are ≥65 years of age [2]. In 2002, the case-fatality rate from invasive GBS infection among elderly persons was substantial (13.2%) and was comparable to that associated with invasive pneumococcal infection (20.6%) [3, 4]. The case-fatality rate for GBS disease among elderly persons exceeds that among infants (3%–5%) [2, 5]. Approximately one-half of the deaths from invasive GBS disease in the United States occur among adults ≥65 years of age [2].

The distribution of the GBS capsular polysaccharide (CPS) types causing invasive disease has shifted during the past decade to include CPS type V. Multicenter, prospective surveillance in the United States in the mid-1990s revealed that types Ia and III predominated in invasive perinatal GBS disease [6]. GBS serotype V, first recognized in the early 1990s, accounted for 14% of invasive disease cases in neonates <7 days of age and for 23% of such cases in pregnant women [6]. In nonpregnant adults, types Ia, III, and V account for 66%–83% of isolates causing invasive infection [1, 7]. The most commonly identified type in nonpregnant adults has been serotype V, accounting for 24%–31% of invasive isolates [1, 7, 8].

GBS CPS–specific IgG in maternal serum specimens obtained at the time of delivery has a well-defined role in protecting neonates and young infants from invasive
disease, and the concept that colonization precedes the development of invasive infection is well understood [8–10]. Candidate GBS CPS–protein conjugate vaccines under development offer potential for prevention of perinatal disease and also for disease in nonpregnant adults, including elderly persons [11–13]. This investigation aimed to define the prevalence of GBS colonization, the distribution of CPS type among isolates, and the frequency of naturally acquired CPS-specific antibodies against common GBS types in serum specimens obtained from healthy men and women aged ≥65 years.

METHODS

Participants, setting, and procedures. The study was conducted at Baylor College of Medicine in Houston from October 2001 through June 2002. Participants were healthy, ambulatory adults ≥65 years of age who were recruited from the community by letter, advertisements in local newspapers, or word of mouth. The letter was sent to persons who had previously participated in a study of a vaccine against shingles and had indicated willingness to be notified of future studies of potential interest to them. Each potential volunteer was interviewed by telephone or in person to ascertain eligibility. Potential subjects were excluded if they had a chronic medical condition known to enhance risk for invasive GBS disease. Exclusion criteria were diagnoses of diabetes mellitus, chronic liver or kidney disease, active malignancy, immunodeficiency, or immunosuppression. Receipt of a blood product or experimental medication or vaccine ≤30 days before study entry, a febrile illness ≤3 days before entry, or antibiotic use at the time of enrollment also precluded participation. Those who met inclusion criteria and were willing to volunteer came to Baylor College of Medicine for an outpatient visit. After eligibility and current medications were documented, written informed consent was obtained. Subjects were compensated for participation with $25. A small cohort of 40 of these subjects has been described in a previously published report [14] of the ability of neutrophils and endogenous, CPS-specific IgG to ingest and kill type V GBS. The Institutional Review Board for Human Subject Research at Baylor College of Medicine approved the present study.

Specimen collection and laboratory methods. After instruction from study personnel, male volunteers self-obtained a rectal swab specimen and initial void urine specimen. Female subjects self-obtained rectal and lower vaginal swab specimens [15]. Swabs were placed into a selective broth medium (Baltimore Biologics Laboratories), incubated overnight, inoculated onto sheep blood agar (Remel BAP), and streaked for isolation of GBS. A sterile, disposable, 10-μL loop was dipped into the urine sample and streaked onto sheep blood agar for isolation of GBS [16]. After incubation, β-hemolytic colonies were confirmed as GBS by latex agglutination (Streptex; Murex).

GBS isolates were serotyped by the capillary precipitin method [17]. Nontypeable isolates were further tested by the capillary precipitin method using a 10-fold concentration of extracted CPS [18] and by PFGE of SmaI chromosomal DNA digests [19] (as modified by Green et al. [20]), with use of control strains of known CPS types. Representative type Ia, II, and III GBS isolates from previous studies [20, 21] and type V GBS isolates with the 4 most common PFGE profiles [19] (kindly provided by John Elliott, Centers for Disease Control and Prevention, Atlanta) were used as controls. The similarity of GBS isolates that were not serologically typeable was determined by visual comparison of PFGE bands for these isolates with those for control strains. Strains were considered to be identical on the basis of criteria adapted from Tenover et al. [22] if they differed by ≤1 band from a control serotype pattern. Serum was stored at −80°C until testing by ELISA with use of purified CPS covalently linked to human serum albumin (kindly provided by Lawrence C. Paoletti and Dennis L. Kasper, Channing Laboratory, Boston) as the coating antigen. Quantitative determination of CPS-specific IgG (types Ia, Ib, II, III, and V), IgM (types II and V), and IgA (types II and V) were assessed as described elsewhere [23, 24]. Values that were below the lower limit of detection for the ELISA were assigned a concentration that was one-half that of the lower limit.

Statistical analysis. Data were analyzed with the statistical package SPSS, version 11.5 (SPSS). SEs were calculated for all mean data results. The χ² test with 2-tailed P values was used to compare proportions of subjects who were associated with a selected range of CPS-specific antibody concentrations. The Wilcoxon signed rank test was used to compare different CPS-specific antibody and isotype concentrations in serum specimens for all subjects, and the Mann-Whitney U test was used to compare these factors according to sex, age, or colonization status [25]. P<.05 was considered to be statistically significant. Random selection of 1 member of each married couple was accomplished by evaluation of sequential dates of study enrollment and alternate selection of the husband or wife for inclusion.

RESULTS

Participant characteristics. Of the 284 adults interviewed, 30 did not participate (16 did not meet inclusion criteria, and 14 either declined to participate or did not keep a scheduled appointment) and 254 were enrolled (125 men [49.2%] and 129 women [51.8%]). Ninety-eight subjects (38.6%) were recruited by letter, 95 (37.4%) by word of mouth, and 42 (16.5%) by advertisement, and the recruitment method was not specified for 19 (7.5%). The mean age was 72.6 years for men (range, 65–87 years) and 72.7 years for women (range, 65–89 years). Ninety-five percent were white, 3% were black, and 2% were Asian. The subjects had been prescribed a mean of 2.6 medications (range, 0 to 10), most commonly for treatment of hy-
pertension (46% of subjects), hyperlipidemia (26%), and gastroesophageal reflux disease (15%).

Rate of colonization and CPS type distribution of GBS isolates. Overall, 55 (21.7%) of 254 study participants were colonized with GBS (table 1). The rate of colonization was nearly identical for men and women. Two-thirds of women were colonized at both vaginal and rectal sites. Seventy-eight percent of men and 14% of women carried GBS at the rectal site only.

Among the 76 GBS isolates obtained from the 55 colonized subjects, the most frequent CPS types were V (47.3%) and Ia (22.8%). Strains that were nontypeable constituted 12.3% of the total. CPS types Ib (3.5%), III (12.3%), and IV (1.8%) were identified among the remainder. No subject had colonization with type II GBS. Overall, 85.9% of these colonizing GBS isolates were type Ia, Ib, III, or V. Rates of colonization did not differ significantly between subjects <75 years of age and subjects ≥75 years of age. In cases in which both body sites were colonized, the GBS types were concordant in 19 (90.5%) of 21 subjects. One woman had type V rectal and type III vaginal colonization. One man had type Ia/c rectal colonization, with a type V strain isolated from a voided urine specimen.

GBS was isolated from one or both members of 10 (30.3%) of 33 married couples who participated in the study. In 6 couples, only the husband (2 couples) or wife (4 couples) was colonized. There was a concordance of GBS types for the 4 couples in which both subjects were colonized (1 couple each had types III and V, and 2 couples had nontypeable strains). The rate of colonization among these married subjects (21.2%) did not differ significantly from that for the entire group (21.7%). To assess for sampling bias potentially introduced by data for married couples, the prevalence of colonization was analyzed after randomly selecting 1 member of each married couple for inclusion in the analysis. Overall, 21.7% of subjects (48 of 221) were colonized with GBS at ≥1 body site. This prevalence estimate did not differ from that for the total subject group. Similarly, the distribution of GBS isolates was not affected after correction for the potential bias associated with inclusion of married couples. The most frequent CPS types remained V (46.9%) and Ia (24.5%). Strains that were nontypeable comprised 10.2% of the isolates.

Forty-seven (61.8%) of 76 isolates were serotyped by the Lancefield capillary precipitin method; 5 additional isolates (6.6%) were typed by use of a 10-fold concentration of the CPS extract. Analysis of extracted DNA by PFGE allowed provisional assignment of a CPS type to 15 (19.7%) of the remaining 24 isolates; 10 were type V, 4 were type Ia, and 1 was type III. GBS isolates that are genetically identical may have different serotypes, and PFGE alone cannot be used to definitively confirm a serotype. Because of this, the serotype distribution also was analyzed with the isolates assigned a serotype by PFGE alone considered as nontypeable. The most frequent CPS types for isolates from the 55 colonized subjects still were V (35.1%) and Ia (19.3%). Strains that were nontypeable constituted 29.8% of the total number isolated.

Distribution of naturally acquired CPS-specific serum antibodies. The concentration of CPS-specific antibodies against the 5 major CPS types of GBS in serum specimens obtained from the 254 study participants is summarized in table 2. The geometric mean concentration of CPS-specific IgG was <1 μg/mL for all GBS types. CPS-specific IgG levels were significantly lower for type V, compared with the other types (P < .001). Although GBS type Ia, Ib, and III CPS–specific IgM and IgA are typically nondetectable in human serum [11, 12], type II and V often elicit these immunoglobulin isotypes [24]. The geometric mean concentration of type II CPS–specific IgM (0.7 μg/mL) was actually higher than that of type II CPS–specific IgG (0.6 μg/mL). The geometric mean concentrations for type V CPS–specific IgM (0.2 μg/mL) and IgA (0.1 μg/mL) were significantly lower than those for type II CPS–specific IgM (0.7 μg/mL) and IgA (0.3 μg/mL) (P < .001 for both). There was a trend toward higher type V CPS–specific IgM but not IgG or IgA concentrations when the 27 type V–colonized subjects were compared with all other subjects (P = .052). The proportion of older adults with CPS-specific IgG at a serum concentration ≥1 μg/mL was lower for type V (11.8%) than for each of the other GBS types (24.8%–39.4%) (P < .001). Similarly, the percentage of subjects who had a CPS-specific IgM concentration ≥1 μg/mL was significantly lower for type V GBS (7.9%), compared with type II GBS (34.3%).

Men had higher concentrations of GBS type Ib CPS–specific IgG (0.5 vs. 0.3 μg/mL; P = .02) and higher GBS type II CPS–specific IgA (0.3 vs. 0.2 μg/mL; P = .02) than did women, but these differences are not likely to have biological significance.

Table 1. Rates of group B streptococcal (GBS) colonization among 254 healthy adults ≥65 years of age.

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of subjects enrolled (%)</th>
<th>Source of GBS isolate, no. (%) of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>colonized/total no.</td>
<td>Vagina only</td>
</tr>
<tr>
<td>Female</td>
<td>28/129 (21.7)</td>
<td>6 (22)</td>
</tr>
<tr>
<td>Male</td>
<td>27/125 (21.6)</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Overall</td>
<td>55/254 (21.7)</td>
<td>6 (11)</td>
</tr>
</tbody>
</table>
Table 2. Group B streptococcal (GBS) capsular polysaccharide (CPS)–specific antibody concentrations in serum samples obtained from 254 healthy adults \( \geq 65 \) years of age.

<table>
<thead>
<tr>
<th>GBS type</th>
<th>Antibody isotype</th>
<th>Serum CPS–specific geometric mean concentration, ( \mu g/mL ) (95% CI)</th>
<th>GBS CPS antibody concentration, % of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>IgG</td>
<td>0.6 (0.4–0.7)</td>
<td>(&lt;0.5)  ( \mu g/mL )</td>
</tr>
<tr>
<td>Ib</td>
<td>IgG</td>
<td>0.4 (0.3–0.5)</td>
<td>0.5–1.0  ( \mu g/mL )</td>
</tr>
<tr>
<td>II</td>
<td>IgG</td>
<td>0.6 (0.5–0.7)</td>
<td>&gt;1.0    ( \mu g/mL )</td>
</tr>
<tr>
<td>II</td>
<td>IgM</td>
<td>0.7 (0.7–0.8)</td>
<td>0.5–1.0  ( \mu g/mL )</td>
</tr>
<tr>
<td>II</td>
<td>IgA</td>
<td>0.3 (0.2–0.3)</td>
<td>0.5–1.0  ( \mu g/mL )</td>
</tr>
<tr>
<td>III</td>
<td>IgG</td>
<td>0.7 (0.5–0.8)</td>
<td>0.5–1.0  ( \mu g/mL )</td>
</tr>
<tr>
<td>V</td>
<td>IgG</td>
<td>0.1 (0.1–0.2)</td>
<td>0.5–1.0  ( \mu g/mL )</td>
</tr>
<tr>
<td>V</td>
<td>IgM</td>
<td>0.2 (0.2–0.3)</td>
<td>0.5–1.0  ( \mu g/mL )</td>
</tr>
<tr>
<td>V</td>
<td>IgA</td>
<td>0.1 (0.1–0.2)</td>
<td>0.5–1.0  ( \mu g/mL )</td>
</tr>
</tbody>
</table>

\( * \) \( P < 0.001 \), compared with each of the other serotypes.

\( \dagger \) \( P < 0.001 \), compared with type II GBS.

Subjects <75 years of age (\( n = 168 \)) had significantly higher serum concentrations of type V CPS–specific IgM than did subjects \( \geq 75 \) years of age (\( n = 86 \)) (0.23 vs. 0.18 \( \mu g/mL \); \( P = .04 \)).

DISCUSSION

Several observations emerge from analysis of this convenience sample. First, healthy elderly persons were more likely to be colonized with GBS CPS type V than with other types. Almost one-half carried type V. Even when GBS isolates provisionally assigned by PFGE to the type V group were excluded, more than one-third had CPS type V. This finding contrasts with contemporary data describing the distribution of CPS types among GBS isolates colonizing other populations, particularly pregnant and nonpregnant women and infants, among whom the proportion ranges from \( <15\% \) to 20% [26–28]. Second, the rate of GBS colonization among our elderly study participants (21.7%) did not differ substantially from that observed among pregnant women (15%–35%) [29]. In contemporary studies of colonization in pregnant women and neonates, 3 GBS serotypes are dominant. Type Ia is the most frequent, representing 23%–32% of isolates, type III accounts for 21%–22%, and type V represents 12%–21% [16, 26, 27]. By contrast, type V strains were 2–4-fold more likely to colonize elderly persons than were strains of either type Ia or III.

Third, a large number of isolates could not be assigned a CPS type by use of a standard serological method. Typically, these nontypeable GBS strains represent \(<5\%\) of invasive isolates [6, 7]. A recent report from metropolitan Atlanta found a somewhat higher proportion of nontypeable GBS isolates (8% of the total number of isolates recovered) among 196 nonpregnant adults with invasive GBS disease [1]. Of interest, 12.3% of the adults in our study were colonized with GBS isolates that were nontypeable by standard methods, by methods maximizing identification of GBS that are low producers of CPS [18], and even by assigning a provisional serotype by means of PFGE. Because the methods were the same as those used to identify invasive GBS isolates, this finding is possibly related to the colonization microenvironment in elderly persons, and in this environment, GBS isolates may produce less capsular material. Davies et al. [27], by use of conventional serotyping methods, found a similarly prominent role for nontypeable isolates colonizing pregnant women in Alberta, Canada. These findings suggest a potential shift toward a higher prevalence of carriage of poor capsule-producing and, on the assumption that the capsule is the major virulence determinant, possibly less virulent GBS strains.

The proportion of older adults with CPS-specific IgG at a serum concentration \( >1 \mu g/mL \) was significantly lower for those with type V isolates (11.8%), compared with those who carried one of the other GBS types (24.8%–39.4%). Maternal Ia, III, and V CPS-specific IgG at concentrations of 0.5–1 \( \mu g/mL \) at delivery have been associated with a significant trend toward protection against neonatal GBS disease (unpublished data). It is not yet known whether CPS-specific IgG has a similarly pivotal role in protecting elderly persons from invasive GBS disease. In a report of 11 adults with GBS bacteremia, 7 had moderate levels of CPS-specific IgG (range, 3.5–49.5 \( \mu g/mL \)) in acute-phase serum specimens [30], reflecting either a rapid increase in CPS-specific IgG or a failure of immune effectors other than CPS-specific IgG. For neonates, a sufficient concentration of CPS-specific IgG and functioning neutrophils is adequate for protection from invasive type III GBS disease [31, 32].

Although this was a convenience sample of healthy elderly persons, we believe that the prevalence estimates are unlikely to be biased. First, with the exception of married couples, there were no related groupings among the study participants. Prevalence estimates and GBS serotype distributions were not affected when analyzed to include only a randomly selected member of a married couple. Second, the subjects were recruited from numerous zip codes throughout the greater Houston area, and recruitment methods included advertisement in Houston’s citywide newspaper. Third, none of the subjects participated because a family member had had GBS disease, which could have placed them at greater risk.

However, there are limitations to our findings. The relatively small sample size could have influenced the CPS type distribution among colonizing GBS isolates. The colonization rate was stable throughout the enrollment period, but larger numbers would be required to affirm the dominance of type V GBS as a colonizer in elderly subjects. The study was conducted at a single site, so the possibility that there are geographic differ-
ences in the distribution of GBS type cannot be excluded. However, locale has not influenced the rates of invasive GBS disease or the CPS type distribution among nonpregnant adults [2], and type V GBS strains have been isolated from diverse surveillance areas throughout the United States [19]. Finally, most of our study participants were white, so the possibility that ethnicity can influence colonization cannot be excluded. Multicenter, population-based surveillance studies have found that black ethnicity enhances the risk of invasive GBS disease in neonates [2, 33] and that black women are more likely to be colonized with GBS during pregnancy than are women from other ethnic groups [16]. Thus, more work is needed to expand the generalizability of our findings.

CPS-specific IgG is crucial to host defense against Streptococcus pneumoniae, another encapsulated gram-positive pathogen [34]. Routine administration of pneumococcal polysaccharide vaccine is recommended for adults ≥65 years of age [35]. In theory, implementation of a prevention strategy against GBS through immunization of elderly persons would be less complex than that for prevention against S. pneumoniae. A multivalent vaccine containing CPS of only 5 GBS types (Ia, Ib, II, III, and V) would encompass >90% of isolates that colonize or cause invasive disease in this population [1, 7, 8]. Currently, >90% of adults ≥65 years of age who develop invasive GBS disease have an underlying medical condition that enhances the risk of GBS disease [36–39]. The age-adjusted annual incidence of GBS disease among nursing home residents is >72 per 100,000 population and is 4-fold higher than that among community-dwelling elderly subjects [5]. The concept that immunization of adults is an important health benefit has gained societal acceptance through the introduction of 23-valent pneumococcal polysaccharide vaccine and through yearly influenza vaccination programs. Addition of another immunization at the same visit to prevent GBS infection could have a major public health impact. Certainly, a focus on GBS and particularly on type V GBS in conjugate vaccine development is warranted and should be a priority for health care research.

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