Class Specificity of Naturally Acquired and Vaccine-Induced Antibody to Type III Group B Streptococcal Capsular Polysaccharide: Determination with a Radioimmunoprecipitin Assay

MORVEN S. EDWARDS,1,* PAMELA A. FUSELIER,1 MARCIA A. RENCH,1 DENNIS L. KASPER,2 AND CAROL J. BAKER1

Department of Pediatrics, Myers-Black Section of Infectious Disease, Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030,1 and Division of Infectious Diseases, Beth Israel Hospital, and Channing Laboratory, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts 021152

Received 21 November 1983/Accepted 25 January 1984

A radioimmunoprecipitin test was developed to determine the immunoglobulin class distribution of naturally acquired and vaccine-induced antibody to the native capsular polysaccharide of type III group B streptococci (III-GBS). In sera from adults and pregnant women with naturally acquired antibody, the mean percentage of antigen bound by immunoglobulin G (IgG) was 74.9 and 78.6, respectively, whereas antigen bound by IgM comprised less than 10% of the total. In contrast, early-convalescent-phase sera (mean, 16.3 days) from neonates responding to III-GBS infection with an increase in specific antibody had significantly more IgM (mean, 36%; P < 0.001, unpaired t test). However, in late convalescence, the immunoglobulin class distribution in sera from these neonates was similar to that of naturally immune adults. Four weeks after immunization with III-GBS polysaccharide vaccine, sera from adults with low (<2 µg/ml) preimmunization antibody levels in their sera and from those with moderate (mean, 5.5 µg/ml) preimmunization levels contained specific antibody predominantly of the IgG class. Although the percentage of IgG-specific antibody was greater in sera from naturally immune adults than in that from vaccinees with a presumed primary immune response, the major portion of antigen bound by sera at 4 weeks postimmunization (62.5%) was associated with IgG. These observations support the opinion that immunization of pregnant women with III-GBS capsular polysaccharide could be efficacious for the prevention of invasive neonatal III-GBS disease.

The correlation between low levels of maternally derived antibody to the capsular polysaccharide of type III group B streptococci (III-GBS) in neonatal sera and susceptibility to invasive infection due to III-GBS, which was described initially by Baker and Kasper (4), has been corroborated by others (11, 23). Immunization of women with purified III-GBS polysaccharide during or before pregnancy, therefore, has been proposed as one means by which passive protection of neonates from these infections might be accomplished (1). Although the purified native capsular polysaccharide of III-GBS appears to be safe and immunogenic in healthy adults (2), the potential of this candidate vaccine for passive immunization of neonates via placental transport of protective antibody remains to be demonstrated. Since maternally derived immunoglobulin acquired by neonates is restricted to the immunoglobulin G (IgG) isotype, elucidation of the immunoglobulin class specificity of antibody to III-GBS acquired naturally as compared with that elicited in response to immunization is pertinent to predicting the potential efficacy of type-specific antibody in the prevention of III-GBS disease.

The radioactive antigen-binding assay (RABA) developed by Baker and Kasper (4) for the determination of antibody to the III-GBS capsular polysaccharide provides a means whereby specific antibody can be quantitated precisely. With this assay, concentrations of antibody to III-GBS in serum have been determined in the normal population, in neonates with III-GBS infection, and in vaccine recipients (2–4, 6). The major limitation of this assay has been its failure to delineate by immunoglobulin class the antibody detected, since labeled antigen–antibody complexes of all classes are precipitated by the ammonium sulfate method as described originally by Farr (9). Minden et al. (17) and Schalch and Parker (19) have shown that soluble immune complexes may be precipitated as effectively by the use of heterologous anti-immunoglobulins as by the ammonium sulfate method. This procedure, also referred to as the double-antibody, coprecipitation, or radioimmunoprecipitin (RIP) method (17), has been adapted to determine the class specificity of antibody to capsular polysaccharides of bacteria other than GBS e.g., group A meningococci (15).

The purposes of the present study were to develop a modification of RABA which permitted the measurement of immunoglobulin class-specific antibodies to the III-GBS polysaccharide and to determine the class specificity of antibody acquired naturally by adults and passively or in response to III-GBS infection by neonates. In addition, to more accurately predict the potential of immunoprophylaxis as a means by which III-GBS disease in neonates might be prevented, a comparison was made of the class distribution of antibody to III-GBS in adults with naturally acquired antibody with the class distribution in those immunized with III-GBS polysaccharide.

MATERIALS AND METHODS

Serum specimens. Sera from healthy adults were obtained as preimmunization specimens, and those containing ≥2 µg of antibody to III-GBS per ml were selected to represent naturally acquired immunity. Sera from nonpregnant adult volunteers were collected 4 weeks after immunization subcutaneously with a single 50-µg dose of purified III-GBS...
polysaccharide vaccine. Informed consent for participation in this III-GBS vaccine trial was obtained from all volunteers. Maternal sera were collected at the time of admission to the hospital for delivery, and cord sera were obtained at birth from the neonate of each participating mother. All cord sera tested were obtained from neonates at term gestation based upon a birth weight of at least 2.500 g and an estimated age of at least 38 weeks. The mean birth weight for these infants was 3.378 g (range, 2.610 to 4.080 g). Sera from neonates or adults with III-GBS disease were collected at various intervals after the diagnosis of infection was established. Whole blood from each of the study subjects was allowed to clot at 25°C for 30 min, and sera then were separated by centrifugation and stored at −70°C before antibody testing.

**RIP assay.** The assay developed to determine the distribution by immunoglobulin classes of antibody to the III-GBS polysaccharide is a modification of the previously described RABA, in which purified native III-GBS polysaccharide labeled intrinsically with tritium is the antigen employed (4). The labeled III-GBS antigen consists of a repeating unit of four monosaccharides (glucose, galactose, glucosamine, and sialic acid) (5, 13) and has specificity only for type III antibody. The reaction mixture for the RIP assay contained 100 μl of serum and 100 ng of 3H-labeled antigen in 100 μl of borate-buffered saline. After overnight incubation at 4°C, goat anti-human IgG, IgA, or IgM (Cappel Laboratories, Cochranville, Pa.) was added to precipitate immune complexes, and the mixtures were placed in a shaking water bath at 37°C, vortexed at 1-min intervals for 1 h, and then incubated for 16 h at 4°C. The maximum precipitating capacity of the anti-human globulins was determined in a dose-response manner with adult serum with a moderate concentration of specific antibody (16.9 μg/ml) by RABA. The precipitating capacity of the anti-human globulins was enhanced by the addition of polyethylene glycol (PEG; molecular weight, 20,000) at a final concentration of 4% (14, 22). The precipitated immune complexes were centrifuged, and the pellets were washed twice in borate-buffered saline (pH 8.3), inverted to dry, and dissolved with 2 ml of Protosol (New England Nuclear Corp., Boston, Mass.). The percentage of antibody bound then was determined in the same manner as with RABA (5, 6).

The total concentration of antibody to the III-GBS polysaccharide present in each serum specimen tested was determined by performing RABA as described previously (5, 6) in conjunction with the RIP assay. The efficiency of the binding capacity of the heterologous anti-immunoglobulins was determined by comparing the ratio of the total percentage of antigen bound by anti-human IgG, IgA, and IgM with that of antigen bound by saturated ammonium sulfate.

**Binding specificity studies.** Human IgG, IgA, and IgM (lot no. 18475, 18673, and 19392, respectively; Cappel Laboratories) were employed to confirm the binding specificities of the anti-human immunoglobulins utilized in the RIP assay. These proteins were commercially purified chromatographically from human plasma (IgG and IgA) or myeloma-pooled human serum (IgM), and their purity was confirmed by immunoelectrophoresis. After overnight incubation of serum and labeled antigen, purified human IgG, IgA, or IgM at concentrations equaling those contained in the homologous anti-immunoglobulins was added to reaction mixtures in conjunction with the addition of precipitating antiglobulins. A control tube to which borate-buffered saline was added was included in each assay. The procedure was completed as described above. The results were expressed as the percent inhibition of binding by each purified immunoglobulin for its homologous and heterologous antiglobulin.

**Statistical methods.** The portion of total antigen bound by anti-human globulins was compared among groups of sera with Student’s unpaired t test (21). The Spearman rank correlation was used to assess the relationship between the placental transfer of antibody and the concentration or class specificity of maternal antibody (21).

**RESULTS**

**Standardization of the RIP assay.** The precipitating capacities of the anti-human IgG, IgA, and IgM were determined with adult serum with a moderate concentration of naturally acquired antibody to the III-GBS polysaccharide antigen, and the results represented the mean of 10 determinations. Maximum precipitation occurred at 40, 3, and 7 mg of anti-IgG, anti-IgA, and anti-IgM, respectively, per ml of serum. The percentage of total antigen bound by anti-IgG, anti-IgA, and anti-IgM was 95.6% (range, 88.8 to 106.2%) of that bound by RABA. Of the total antigen bound, 68.6% was associated with IgG (range, 64.1 to 74.7%), 23.5% was associated with IgA (range, 20.1 to 26.5%), and 7.9% was associated with IgM (range, 4.9 to 10.6%).

To assess the specificity of the goat anti-human immunoglobulins for precipitating immune complexes containing homologous versus heterologous classes of antibody, competitive binding experiments were performed. Purified human IgG, IgA, or IgM was added to reaction mixtures containing serum and antigen at equivalence concurrently with the addition of homologous or heterologous anti-human globulins. The concentration of the purified human immunoglobulins added as competitive inhibitors of binding was equal to that of the precipitating anti-immunoglobulins and exceeded that in the human serum by 2.5 - to 5-fold. The percent inhibition of binding observed after the addition of purified IgG, IgA, or IgM to reaction mixtures in which immune complexes were precipitated with homologous or heterologous classes of antiglobulins is shown in Table 1. The percent inhibition of binding observed when heterologous classes of antibody were added to the reaction mixture ranged from 0 to 13%. When the homologous class of antibody was added, competitive binding to the anti-human globulin ranged from 40.4 to 53.8%.

**Immunoglobulin class specificity of naturally acquired antibody to III-GBS capsular antigen in adults and neonates.** The distribution of immunoglobulin classes of naturally acquired antibody to the III-GBS capsular polysaccharide in 18 adult sera with levels ranging from 3.7 to 116 μg/ml (mean, 24.7 μg/ml) by RABA is summarized in Table 2. Immunospecific antibody was primarily IgG (mean, 74.9%), but the percentage of antigen bound by IgG varied considerably in individual sera (39.0 to 97.6%). With the exception of serum from one adult whose immunospecific IgM comprised 25.9% of total antibody, the amount of antigen bound by IgM was negligible (<10%).

The percentage of specific antibody of the IgG class and therefore available for placental transfer did not differ significantly when sera from naturally immune adults were compared with those from parturients (t = 0.73, P > 0.05, unpaired t test). The mean amount of maternally derived antibody in cord sera was 70.8%, and this placentally transferred antibody was, as would be expected, virtually all IgG (mean, 90.8%). However, among individual maternal-cord serum pairs, the percentage of maternally acquired antibody varied greatly (31.7 to 135%). Although a significant correlation existed between the absolute concentration
of III-GBS-specific antibody in maternal and neonatal sera (r = 0.66, P < 0.05, Spearman rank correlation), the percentage of maternal antibody bound by IgG did not correlate significantly with the percentage acquired transplacentally by neonates (r = 0.4, Spearman rank correlation).

Sera from 11 neonates with III-GBS osteomyelitis (5 infants) or meningitis (6 infants) who developed a rise in specific antibody during convalescence were tested (Table 2). During early convalescence (mean, 16.3 days; range, 5 to 24 days after diagnosis), a significant portion of immunospecific antibody was of the IgM class (mean, 36%; range, 29.2 to 48.1%). The IgM portion was significantly greater during early convalescence in neonates than in naturally immune adults (t = 7.41, P < 0.001, unpaired t test). Sera collected during late convalescence (mean, 4.6 months after diagnosis) had a distribution of immunoglobulins similar to that observed in naturally immune adult and maternal sera. The highest percentage of IgA (27.3%) was observed in acute-phase sera from adults convalescing after a definite or presumed III-GBS infection. However, the number of sera available for testing was insufficient to permit determination of the possible significance of this observation.

Immunoglobulin class specificity of vaccine-induced antibody to III-GBS capsular polysaccharide antigen. The distribution of specific antibody isotype elicited in response to immunization with purified III-GBS polysaccharide is shown in Table 3. Sera were evaluated from individuals who responded to immunization with at least a 1.0-µg/ml rise in antibody concentration at 4 weeks after immunization. Ten adults with low preimmunization antibody levels (<2 µg/ml) and 13 with preimmunization levels of 2 µg/ml or greater were tested. Although the mean III-GBS antibody concentration was higher for sera with moderate levels of antibody before immunization than for those with low levels, there was no significant difference in the immunoglobulin class distribution between the two groups.

Since adults with low concentrations of antibody to III-GBS would be the target population for immunophrophylaxis, a comparison of vaccine-induced with naturally acquired antibody to III-GBS was made for the sera in which immunization elicited a presumed primary immune response. The percentage of antigen bound by IgG for these 10 sera at 4 weeks postimmunization (Table 3) did not differ significantly from the percentage of that bound by the group of 18 naturally immune adults (Table 2) (t = 1.94, P = not significant). However, when these 10 sera were compared with the naturally immune adult sera and the maternal sera, the percentage of antigen bound by IgG was greater in naturally immune sera than in the others at 4 weeks postimmunization (t = 2.56, P < 0.05, unpaired t test).

**DISCUSSION**

The importance of naturally acquired antibody to the III-GBS capsular polysaccharide in modulating neonatal susceptibility to III-GBS disease has been documented in previous studies in which the RABA developed by Baker and Kasper (4) was employed to quantitate specific antibody. Immunogenicity of the purified native capsular polysaccharide in adults also was demonstrated by RABA (2). However, since RABA detects immune complexes without regard to immunoglobulin class, more cumbersome techniques such as 2-mercaptoethanol treatment were required to confirm the predominance of IgG in naturally acquired and vaccine-induced antibody to III-GBS in adults (2, 6).

The potential usefulness of vaccination to induce antibodies to III-GBS which will cross the placenta prompted the development of the RIP assay described in the present study. By means of the technique described, immunoglobulin class-specific antibodies to III-GBS were determined in unfractionated sera, thereby overcoming the major limitation of RABA. In addition, modification of RABA to a RIP assay ensured adequate quality control for the new assay by permitting comparison of data with the standard assay.

Initial experiments performed to determine the precipitating capacity of the anti-human immunoglobulins indicated that, even in excess, the efficiency of the antiglobulins was inadequate when their total precipitation of soluble immune complexes was compared with that achieved by the ammonium sulfate method. The addition of PEG at a final concentration of 4% enhanced the precipitating capacity of the anti-human globulins to a level comparable with the precipitating capacity of RABA and obviated the need for routine dilution of sera before assay. PEG-associated precipitation of immune complexes occurs by an indirect mechanism in which PEG causes cross-linking and aggregation of immune complexes without binding directly to them (7, 14, 22). With the

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**TABLE 1.** Specificity of anti-human immunoglobulins for precipitating immune complexes

<table>
<thead>
<tr>
<th>Anti-human immunoglobulin</th>
<th>% Inhibition of binding after the addition of purified:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>IgA</td>
</tr>
<tr>
<td>IgG</td>
<td>40.4</td>
<td>0</td>
</tr>
<tr>
<td>IgA</td>
<td>0</td>
<td>42.7</td>
</tr>
<tr>
<td>IgM</td>
<td>0</td>
<td>2.4</td>
</tr>
</tbody>
</table>

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**TABLE 2.** Immunoglobulin classes of naturally acquired antibody to the capsular polysaccharide of III-GBS in neonates and adults

<table>
<thead>
<tr>
<th>Type of serum</th>
<th>No. of patients</th>
<th>Mean (range) of III-GBS antibody (µg/ml)</th>
<th>% of total antigen (range) bound by:</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>18</td>
<td>24.7 (3.7-116)</td>
<td>74.9 (39.0-97.6)</td>
<td>18.6 (2.4-58.8)</td>
<td>6.5 (0-25.9)</td>
<td></td>
</tr>
<tr>
<td>Maternal</td>
<td>11</td>
<td>12.5 (7.1-29.0)</td>
<td>78.6 (56.7-89.6)</td>
<td>12.9 (4.4-27.4)</td>
<td>8.5 (4.9-15.9)</td>
<td></td>
</tr>
<tr>
<td>Neonatal cord</td>
<td>11</td>
<td>9.2 (2.6-24.6)</td>
<td>90.8 (80.6-98.7)</td>
<td>6.4 (1.3-13.8)</td>
<td>2.8 (0-6.1)</td>
<td></td>
</tr>
<tr>
<td>Early neonatal</td>
<td>11</td>
<td>12.3 (2.8-23.5)</td>
<td>41.1 (24.6-52.3)</td>
<td>22.9 (13.7-29.5)</td>
<td>36.0 (29.2-48.1)</td>
<td></td>
</tr>
<tr>
<td>Convalescent</td>
<td>9</td>
<td>23.9 (3.7-51.4)</td>
<td>65.7 (41.7-88.2)</td>
<td>20.5 (11.8-32.2)</td>
<td>13.8 (0-28.9)</td>
<td></td>
</tr>
<tr>
<td>Late neonatal</td>
<td>3</td>
<td>17.1 (4.6-38.8)</td>
<td>57.1 (51.8-67.5)</td>
<td>27.3 (21.2-37.2)</td>
<td>15.6 (11.0-24.6)</td>
<td></td>
</tr>
</tbody>
</table>

a Sera obtained at a mean of 16.3 days (range, 5 to 24 days) after diagnosis of III-GBS disease.
b Sera obtained at a mean of 4.6 months (range, 2 to 8 months) after diagnosis of III-GBS disease.
c Sera obtained at 4 days, 12 days, and 3 weeks after definite or presumptive diagnosis of III-GBS infection.
TABLE 3. Immunoglobulin classes of antibody to the III-GBS capsular antigen in response to immunization with purified III-GBS polysaccharide

<table>
<thead>
<tr>
<th>Adult preimmunization antibody in serum (µg/ml)</th>
<th>No. of sera</th>
<th>mean (range) of III-GBS antibody (µg/ml)</th>
<th>% of total antigen (range) bound by:</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>10</td>
<td>1.3 (1.1–1.9)</td>
<td>IgG&lt;sup&gt;a&lt;/sup&gt; 62.5 (35.3–89.7)</td>
</tr>
<tr>
<td>≥2</td>
<td>13</td>
<td>5.5 (2.4–10.2)</td>
<td>IgA 23.9 (6.6–45.7)</td>
</tr>
</tbody>
</table>

<sup>a</sup> The difference between groups is not significant (t = 0.6, P > 0.05).

PEG modification, the maximum precipitating capacity of the anti-human globulins was shown to equal that of ammonium sulfate. A mean of 96.5% of the antigen bound by RABA (mean of 10 experiments) was detected by the RIP assay. Further, minimal inhibition of binding was evident when heterologous purified human immunoglobulins were added to the reaction mixtures (Table 1).

Upon examination of the immunoglobulin class specificity of naturally acquired antibody to III-GBS in adult and maternal sera, IgG was the predominant isotype present (Table 2). There was a considerable variation among individual sera in the percentage of antigen bound by IgG. However, similarly wide ranges among individual sera have been described in studies assessing the classes of antibodies to other encapsulated bacteria. For example, among 10 group A meningococcal carriers, a mean of 48.9% of antigen was bound by IgG, with a range from 21.4 to 73.3% (15). No difference was anticipated in the percentage of specific antibody of the IgG class between adults and parturients, and none was found. For both groups, a negligible portion of antigen was bound by anti-human IgM (mean, 6.5 and 8.9% for adults and parturients, respectively). Although the latter sera were evaluated without regard to colonization status, this finding is at variance with the data of Anthony et al. (B. F. Anthony, S. A. McGearry, and N. F. Concepcion, Pediatr. Res. 16:234A, 1982). Employing an enzyme-linked immunosorbent assay, these investigators described a striking prevalence of IgM antibody to III-GBS which was more pronounced in carriers than in noncarriers of the organism during pregnancy. The percentage of bound antigen which was associated with IgA was higher in sera obtained during convalescence after a III-GBS infection and in sera obtained after immunization with the III-GBS capsular antigen than in sera from naturally immune adults. This suggests, but does not prove, that specific antibody of the IgA class may be elicited in response to a recent stimulus with III-GBS antigen.

Previously, we have suggested that infants who develop significant levels of antibody to the III-GBS capsular polysaccharide during convalescence after a natural infection elicit IgM antibody (6). This observation was based on serial determinations of antibody levels which indicated that some responding infants had a decline to the base line within 6 to 12 weeks after response (3, 6) and on a loss of antibody which was observed in sera from six infants after treatment with 2-mercaptoethanol (3). The RIP assay permitted further delineation of the antibody response during convalescence after an infection. Early-convalescent-phase sera from nine infants contained a high percentage (mean, 36%) of IgM (Table 2). For infants in whom antibody did not decline to the base line, the class distribution by 2 to 8 months after the diagnosis of III-GBS disease had shifted to a pattern of IgG predominance. Although only three adult convalescent-phase sera were available for study, the magnitude of the IgM response was less pronounced than that observed in neonates.

At 4 weeks after immunization with the III-GBS capsular polysaccharide, IgG was the predominant immunoglobulin class detected in sera from adults with moderate levels of antibody at the preimmunization assessment and from those with very low levels (<2 µg/ml) (Table 3). Although the percentage of IgM was somewhat high in such the latter group, for whom the immune response was presumed to be primary rather than anamnestic, the difference in IgM percentages between the groups was not significant. Similarly, although the percentage of antigen bound by anti-human IgG was somewhat less in sera from adults at 4 weeks postimmunization than in sera from naturally immune adults, the major portion of antibody (62.5%) was associated with IgG. This finding is consistent with the data of Eisenstein et al. (8), in which an enzyme-linked immunosorbent assay technique was employed to determine the isotype specificity of the immune response to the III-GBS capsular polysaccharide in adults. These investigators demonstrated that the majority of the antibody detectable at 2 and 6 weeks after immunization with the polysaccharide antigen was IgG, and they estimated that the contribution of IgM was not more than 10% of the total.

Although the percentage of antigen bound by IgG was significantly greater in sera with naturally acquired antibody than in sera at 4 weeks after a presumed primary immune response (t = 2.56, P < 0.05), the major portion of antibody elicited after immunization was found to be associated with IgG. However, as indicated by the variability in the amount of placental transfer in maternal-cord sera (31.7 to 135%) from neonates at term gestation, factors other than the recognized variable of gestational age (12) which modulate this transfer remain to be elucidated. As noted initially by Baker et al. (6), a significant correlation exists between concentrations of the antibody in the mother and child of maternal-cord sera pairs which permits the estimation of neonatal antibody levels from the maternal level. The present data indicate that the amount of IgG present is not the sole factor determining the efficiency of placental transfer. The subclass specificity of IgG is one such additional factor which may be important in determining placental transfer (10, 18, 20) and is a factor which theoretically could differ between sera with naturally acquired antibody and those with vaccine-induced antibody. In addition, the avidity binding of antibody acquired naturally may differ from that elicited in response to immunization. This factor has been shown to influence the functional efficiency of antibodies to meningococcal polysaccharides (16). Thus, the somewhat lower percentage of IgG available for transport after immunization with III-GBS polysaccharide may be offset by the subclass specificity and functional efficiency of the antibody elicited in response to the purified antigen.

In summary, the human immune response to the III-GBS
capsular polysaccharide antigen has been further characterized by a RIPA assay. With the exception of neonates responding to III-GBS infection with a rise in specific antibody, the immunoglobulin class predominating in sera from naturally immune adults and from recipients of III-GBS polysaccharide vaccine is IgG. These data should provide a further stimulus for the investigation of immunization with III-GBS polysaccharide before or during pregnancy to evaluate the immunoglobulin class and subclass distribution of antibody transferred to infants.

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LITERATURE CITED