IMPAIRED ANTIBODY RESPONSE TO HAEMOPHILUS INFLUENZAE TYPE b POLYSACCHARIDE AND LOW IgG2 AND IgG4 CONCENTRATIONS IN APACHE CHILDREN

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Abstract Background and Methods. Because Native American children are at much higher risk for invasive Haemophilus influenzae type b infection than white children, we compared the antibody responses to H. influenzae type b polysaccharide vaccine in healthy Apache and white children.

Results. The concentrations of H. influenzae type b antibody after immunization with polysaccharide vaccine were approximately 10-fold lower in 24-month-old Apache children than in whites of a similar age (P<0.01). The decreased response involved H. influenzae type b antibodies of the IgG, IgM, and IgA classes. Concentrations of IgG antibody to tetanus toxoid did not differ significantly, and IgG antibodies to diphtheria toxoid were only twofold lower (P=0.028). Although total IgG, IgM, and IgA levels were higher in two-year-old Apaches than in whites (all P<0.001), IgG2 and IgG4 subclasses were lower (both P<0.001). Among the Apaches, individual immunoglobulin levels and allotypes were not significantly correlated with their antibody responses to H. influenzae type b polysaccharide.

Conclusions. Apache children have significant impairment of their antibody response to H. influenzae type b polysaccharide and little or no impairment of their antibody responses to protein toxoids. This immunodeficiency may explain the high incidence of H. influenzae type b infection in this population. (N Engl J Med 1990; 323: 1387-92.)

NATIVE American children, including Alaskan Eskimos,1,2 Canadian Inuits,3 Navajos,4,5 and Apaches,6 are at greater risk of invasive bacterial infections than white children. Bacteremic infections due to Haemophilus influenzae type b and Streptococcus pneumoniae occur at rates 10 to 30 times higher in the Native American children.1,2,4 In addition, a higher proportion of H. influenzae type b bacteremia in Native Americans occurs early in infancy, when meningitis is a frequent and devastating complication.7 Native American groups also appear to be at greater risk of other infections, including severe and recurrent otitis media,8 enteric infections,9 and pneumonia.10

The reasons for these high rates of infection are not well understood. In Alaskan Eskimos, genetic markers such as uridine monophosphate kinase 310 and combinations of immunoglobulin allotypes and HLA-DR markers11 have been correlated with susceptibility to H. influenzae type b infections. However, these markers were not correlated with levels of antibody to the H. influenzae type b capsule polysaccharide.10,11 Since antibody to the capsule polysaccharide is a major determinant of protection from invasive H. in-
fleuzae type b disease, we compared antibody responses to H. influenzae type b polysaccharide vaccine in Apache children and in whites of a similar age.

**METHODS**

**Patient Population**

The study was approved by the Joint Committee on Clinical Investigation of the Johns Hopkins University School of Medicine, the Indian Health Service, the Health Boards of the U.S. Public Health Service Indian Hospital at Whiteriver, Arizona, and the Tribal Council at Whiteriver. Written informed consent was obtained from parents.

One hundred twelve healthy Apache children residing on the White Mountain Apache Reservation were enrolled in the study. These children were scheduled for well-child clinic visits and routine immunizations. Children with chronic underlying illnesses or previous bacteremic infections were not enrolled. The nutritional status of the White Mountain Apaches has improved during the past two decades, and protein-calorie malnutrition is currently rare among the children at Whiteriver. The weights of the study children reflected the distribution expected in a healthy population. The mean weight of the participants was in the 48th percentile for age, and only 4 percent fell below the 5th percentile. All the children received a routine diphtheria, tetanus, and pertussis vaccine at the age of 18 months, and 25 μg of H. influenzae type b polysaccharide vaccine at the age of 18 (n = 17) or 24 (n = 95) months. Serum samples were obtained before and one month after immunization with H. influenzae type b polysaccharide. Because the amount of serum was limited, complete serologic studies could not be performed for all the 24-month-old children. In all 95, total immunoglobulin levels and isotypes were measured; in 79, selected on the basis of the availability of an adequate amount of serum collected before and after immunization, total H. influenzae type b antibody was measured by radioimmunoassay; in 33, tetanus and diphtheria antibodies were measured by enzyme-linked immunosorbent assay (ELISA); and in 23, class-specific H. influenzae type b antibody was measured by ELISA.

Two groups of white children served as controls. Thirty-two healthy white children scheduled for well-child evaluation and immunization were immunized with 25 μg of H. influenzae type b polysaccharide vaccine at the age of 18 (n = 13) or 24 (n = 19) months. Serum samples drawn before and one month after immunization were kindly provided by Cynnie Johnson (Praxia Biologics). Sera were selected only on the basis of the availability of an adequate amount of serum collected before and after immunization. Sufficient serum for assays of tetanus and diphtheria antibodies was available in 15 of the 24-month-old children.

Two hundred fifteen white children from 6 months to 10 years of age who were admitted to Children’s Hospital, Boston, for elective surgical procedures served as controls for immunoglobulin allootypes. Children with primary immunodeficiency diseases, conditions associated with secondary immunodeficiency diseases, or previous bacteremic infections were not enrolled. A subset of 51 of these children (mean age, 23 months) served as a control group for total immunoglobulin and IgG subclass levels.

**Vaccine**

Licensed H. influenzae type b polysaccharide vaccine from a single manufacturer (bCAPSA, Praxia Biologics) was used for all the children. Comparative studies have shown no significant differences in the immunogenicity of licensed H. influenzae type b polysaccharide vaccines prepared by different manufacturers.5,16

**Laboratory Studies**

Assays of antibodies to H. influenzae type b, tetanus toxoid, and diphtheria toxoid were performed in a single laboratory. Total antibody to H. influenzae type b polysaccharide was measured by radioimmunoassay17 with use of tritiated polysaccharide (lots 8/86, 3/87, and 4/87) provided by Dr. Porter Anderson (University of Rochester, Rochester, N.Y.). The assay was calibrated with a standard plasma pool (lot 1983) assigned a concentration of 70 μg of antiscalar antibody per milliliter, obtained from Dr. Carl Frasch (Center for Biologic Evaluation and Research, Food and Drug Administration). The assay protocol was similar to the final protocol recommended by Frasch (H. influenzae type b radioimmunoassay protocol of April 6, 1987), except that the diluent for serum was 5 percent fetal calf serum (instead of 100 percent) and the reaction volumes were 50 μl of test serum and 25 μl of labeled polysaccharide (instead of 25 and 50 μl, respectively).

IgG, IgM, and IgA antibodies to H. influenzae type b polysaccharide were measured by ELISA with use of polysaccharide tagged with tyramine, as previously described.17,18 Antibody concentrations were expressed in ELISA units.

IgG antibodies to tetanus and diphtheria toxoids were measured by ELISA with reagents similar to those used for the H. influenzae type b polysaccharide assay. ELISA plates were coated with 1 μg of tetanus or diphtheria toxoid per milliliter obtained from the Massachusetts Public Health Biologic Laboratories. The assay was calibrated by the method of Zollinger and Boslego in ELISA units.19 One ELISA unit was the optical density of 1 ng of IgG in a parallel titration curve on wells coated with anti-IgG antibodies.

IgG1 and IgG2 antibodies to tetanus toxoid were measured in the same way, with murine monoclonal antibodies to IgG1 (clone NL-16) and IgG2 (clone HP6014) as detecting antibodies. The isotype and subclass specificity of conjugates was verified with myeloma proteins kindly provided by P. Skvaril (Bern, Switzerland).

Total immunoglobulin was measured by nephelometry20 and IgG subclasses by solid-phase competitive radioimmunoassay with use of sheep antiserum to human IgG subclass proteins21 in the laboratory of one of the authors. The World Health Organization serum pool (67/97) was used as a reference standard.

Immunoglobulin allotypes were determined by hemagglutination-inhibition assays in microtiter plates.22

**Statistical Analysis**

Data organization and analysis were performed on PROPHET, a national computer system sponsored by the Chemical/Biological Information Handling Program of the National Institutes of Health. The concentrations of total immunoglobulin, IgG subclasses, and specific antibodies were often not normally distributed. Conversion to logarithms usually resulted in normal or nearly normal distributions. The logarithms of the concentrations were therefore used for all statistical calculations. Antibody concentrations that fell below the lower limit of sensitivity of an assay were assigned values equal to one half the lower limit. The lower limits of sensitivity were 0.05 μg per milliliter for the H. influenzae type b radioimmunoassay, 16 ELISA units per milliliter for IgG anti–H. influenzae type b, 2 ELISA units per milliliter for IgM anti–H. influenzae type b, and 6 ELISA units per milliliter for IgA anti–H. influenzae type b. All concentrations of tetanus and diphtheria antibodies were above the lower limits of sensitivity of the assays.

Comparison of geometric means was performed with the two-sided t-test for normally distributed values and the Mann-Whitney test for values that were not normally distributed. Variation about geometric means is expressed as the antilog of the standard deviation of the logarithms of the values. Multiplication and division by this number give the bounds of 1 SD above and below the geometric mean, respectively. Comparisons of proportions were performed with a two-sided Fisher’s exact test.

**RESULTS**

**Antibody Response to H. influenzae Type b Polysaccharide Vaccine**

The H. influenzae type b antibody responses measured by radioimmunoassay are summarized in Figure 1. Twenty-four-month-old Apache children had 10-fold lower geometric mean antibody concentrations after immunization than white children (0.34 vs.
of 79 Apaches (20 percent) reached post-immunization antibody concentrations \( \geq 1 \mu g \) per milliliter, as compared with 14 of 19 whites (74 percent, \( P<0.01 \)). Four of 19 white 24-month-olds (21 percent) did not have a twofold response, as compared with 42 of 79 Apaches (53 percent, \( P = 0.02 \)).

Measurements of the immunoglobulin class of the antibody after immunization in 24-month-old children by ELISA revealed that concentrations of IgG, IgM, and IgA classes of antibody were all lower in the Apache group (Table 1).

**Concentrations of Antibodies to Tetanus and Diphtheria Toxoids**

As compared with the concentrations of polysaccharide antibody, the concentrations of IgG antibodies to two protein antigens, tetanus and diphtheria toxoid, were more similar in 24-month-old Apache and white children (Table 2). The geometric mean concentrations of IgG antibody to tetanus toxoid did not differ significantly, but the geometric mean concentration of IgG antibody to diphtheria toxoid was approximately 1.8-fold lower in the Apaches than in the whites (\( P = 0.028 \)).

**Total Immunoglobulin and IgG Subclass Concentrations**

In order to determine whether the low *H. influenzae* type b polysaccharide antibody response of the Apache children was related to a general B-cell immunodeficiency, we compared the total immunoglobulin and IgG subclass concentrations of the 24-month-old children.

Total IgG, IgM, and IgA concentrations were significantly higher in Apache than in white children (\( P<0.001 \)) (Table 3). IgG1 concentrations were also

![Graph showing antibody responses to *H. influenzae* type b polysaccharide in 18- and 24-month-old Apache and White Children.](image)

**Table 1. Immunoglobulin Composition of *H. influenzae* Type b Polysaccharide Antibody One Month after Immunization of 24-Month-Old Apache and White Children.**

<table>
<thead>
<tr>
<th>Category</th>
<th>IgG (ELISA units × 10^4)</th>
<th>IgM (ELISA units × 10^4)</th>
<th>IgA (ELISA units × 10^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apache</td>
<td>0.154 (4.4)</td>
<td>0.146 (3.9)</td>
<td>0.033 (3.0)</td>
</tr>
<tr>
<td>White</td>
<td>0.718 (11.0)</td>
<td>0.339 (4.7)</td>
<td>0.117 (9.6)</td>
</tr>
</tbody>
</table>

*Geometric mean concentration. Values in parentheses are the antilogs of the standard deviations, multiplying and dividing by these values give the upper and lower bounds, respectively, of 1 SD.*

†By t-test.
Table 2. IgG Antibodies to Tetanus and Diphtheria Toxoids in 24-Month-Old Apache and White Children.*

<table>
<thead>
<tr>
<th>CHILDREN</th>
<th>ANTIBODY CONCENTRATION†</th>
<th>TETANUS TOXOID</th>
<th>DIPHTHERIA TOXOID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apache (n = 33)</td>
<td>34.9 (2.6)</td>
<td>6.96 (2.9)</td>
<td></td>
</tr>
<tr>
<td>White (n = 15)</td>
<td>41.1 (2.2)</td>
<td>12.3 (1.9)</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>&gt;0.05</td>
<td>0.028</td>
<td></td>
</tr>
</tbody>
</table>

*The antibodies were measured a mean of 22 days (Apaches) and 208 days (whites) after diphtheria–tetanus–pertussis immunization.
†Geometric mean concentration. Values in parentheses are the antilogs of the standard deviations; multiplying and dividing by these values give the upper and lower bounds, respectively, of 1 SD.
§By Mann-Whitney rank-sum test.
©By t-test.

Immunoglobulin Allotypes

Total immunoglobulin and IgG subclass concentrations and antibody responses to *H. influenzae* type b polysaccharides have been correlated with immunoglobulin allotypes in other populations.\(^{17,18,23,24}\) We therefore examined the immunoglobulin allotypes of 95 Apache children and compared them with those of 215 white children in Boston. Marked differences between the two populations were observed. In particular, the G2m(n) allotype was found in only 5 of 95 Apaches (5 percent), as compared with 148 of 215 whites (69 percent). The γ3 allele, G3m(b0b3b5s5), which is almost always found in linkage to the Gm haplotype (za;...b0b3b5s5), was found in 42 of 95 Apaches and in none of the whites. This allele is found mainly in Eskimos and other Mongolid populations. The Km(1) allotype occurred in 65 percent of the Apaches, but only 16 percent of the whites.

In order to determine whether variation in antibody responses to *H. influenzae* type b polysaccharide in the 24-month-old Apache children was related to their immunoglobulin allotypes or to their total immunoglobulin class or subclass concentrations, we performed correlation analyses, multiple regression analyses, and direct comparisons of concentrations in groups with various allotype phenotypes. No significant correlations were found between *H. influenzae* type b antibody responses and Km(1) and G2m(n) allotypes (considering \(P\leq0.05\) to indicate significance) or other allotypes and immunoglobulin levels (considering \(P\leq0.01\) to indicate significance, to compensate for multiple comparisons).

**DISCUSSION**

The chief finding of this study is that Native American children of the Apache tribe have significantly lower antibody responses to *H. influenzae* type b vaccine than white children of a similar age. Antibody responses to *H. influenzae* type b polysaccharide vaccine at the ages of 18 and 24 months often failed to reach levels considered necessary to provide protection. The decreased response involved IgG, IgM, and IgA antibodies. In contrast, concentrations of IgG antibodies to diphtheria and tetanus toxoids were only slightly lower in the Apache than in the white children (the difference for diphtheria toxoid was significant, however).

Antibody responses of Apache children to polysaccharide antigens have not previously been reported. In Navajo infants immunized with *H. influenzae* type b polysaccharide vaccine mixed with pertussis vaccine, there was no difference from whites in response, but both groups had low antibody responses.\(^{25,26}\) Recently, Ward and colleagues compared antibody responses to a *H. influenzae* type b–diphtheria toxoid conjugate vaccine (PRP-D) in Alaska Native and white New York infants.\(^{27}\) The geometric mean antibody response to three doses of PRP-D was lower in Eskimos than in whites (0.104 vs. 0.232 \(\mu\)g per milliliter), but this difference was not statistically significant. Other measures of antibody response, however, such as the proportion who achieved a fourfold increase in antibody level, were significantly lower in the Eskimo group. The low antibody responses of both groups to PRP-D may have limited the ability to discriminate between them. We recently noted significantly lower antibody responses after immunization with another conjugate vaccine, *H. influenzae* type b oligosaccharide–diphtheria CRM (cross-reactive material) conjugate vaccine, in Navajo infants and 18-month-old children, as compared with whites.\(^{28}\)

Differences in antibody responses to vaccines may contribute to differences in vaccine efficacy in different populations. An impaired re-

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Table 3. Total Immunoglobulin and IgG Subclass Concentrations in Healthy 24-Month-Old Apache and White Children.

<table>
<thead>
<tr>
<th>CHILDREN</th>
<th>CONCENTRATION*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG</td>
</tr>
<tr>
<td>Apache (n = 95)</td>
<td>12.2 (1.3)</td>
</tr>
<tr>
<td>White (n = 51)</td>
<td>7.05 (1.5)</td>
</tr>
<tr>
<td>P value†</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Geometric mean concentration. Values in parentheses are the antilogs of the standard deviations; multiplying and dividing by these values give the upper and lower bounds, respectively, of 1 SD.
†By t-test. NS denotes not significant.
spontaneous to the PRP-D–H. influenzae type b conjugate vaccine could thus have contributed to the lower efficacy of this vaccine in Alaskan Eskimo than in Finnish infants.

The Apache children were found to have a lower concentration of IgG2 and IgG4 subclasses than the whites, despite higher concentrations of all three major immunoglobulin classes. Hyperglobulinemia has been described in other populations, such as black Africans from areas with high rates of endemic infection. Indeed, total immunoglobulin levels decrease in black Africans residing in England to levels that more closely resemble those of whites. The selectively lower total IgG2 and IgG4 subclass concentrations suggest that Apache children may have a more global defect in their humoral immune system. IgG2 and IgG4 are relatively late maturing subclasses in white children. Many investigators have correlated selective IgG2 or IgG4 deficiencies (or both) with recurrent encapsulated bacterial infections, recurrent sinopulmonary infections, and impaired antibody responses to bacterial polysaccharide vaccines.

Disappointingly, the correlations between low IgG2 levels and hyporesponsiveness to polysaccharide antigens have not shed much light on the mechanisms of this humoral immune defect. The relation was originally examined because of early observations that a relatively high proportion of human adult IgG antibody to several polysaccharide antigens is of the IgG2 subclass. Total IgG2 levels correlate with the polysaccharide antibody responses of various adult groups. The correlation, however, is weaker in infants and children and depends on age. This may be due to the fact that children produce primarily IgG1 antibody to H. influenzae type b polysaccharide and especially to H. influenzae type b conjugate vaccines. In the Apache children, there was no correlation between total IgG2 or IgG4 concentrations and H. influenzae type b antibody responses. It thus appears that selective IgG2 or IgG4 subclass deficiencies are merely surrogate markers for other, yet to be defined immune functions that are important for polysaccharide antibody responses. Like IgG2 and IgG4 concentrations, the immune functions necessary for polysaccharide responses are slow to mature to adult levels.

In other populations, immunoglobulin allotypes have been correlated with antibody responses to H. influenzae type b polysaccharide and with susceptibility to H. influenzae type b infections. For example, the Km(1) allotype was correlated with high H. influenzae type b polysaccharide antibody responses and low susceptibility to H. influenzae type b disease in black children. The G2m(n) allotype is correlated with high responses to pneumococcal and H. influenzae type b polysaccharides in white adults and with low risk of H. influenzae type b infection in white children.

Our current study shows no correlations of the Km(1) and G2m(n) allotypes and H. influenzae type b antibody responses in Apache children. However, the G2m(n) marker was found in only 5 percent of the Apache children. Consequently, too few children with this marker were evaluated to draw firm conclusions. Petersen et al. also found no correlation between Gm and Km allotypes and natural H. influenzae type b polysaccharide antibody concentrations in Alaskan Eskimo children.

Our study thus does not address the mechanism of low antibody response to H. influenzae type b polysaccharide among Native Americans. Although it seems likely that genetic factors play a role in low antibody responses, it is also possible that epidemiologic factors such as frequent infections or crowding and nutritional factors are important. The limited information available suggests that antibody responses to T-cell–independent antigens are well preserved in experimental models and in children with mild degrees of protein-calorie malnutrition. However, deficits in micronutrients that are known to be important to immune function, such as vitamins A and E and zinc, may exist in Native American children and may have a role in their impaired immune function.

We suggest that Apache children may have an immunodeficiency associated with severely impaired antibody responses to T-cell–independent antigens, such as bacterial polysaccharides, and less impaired responses to T-cell–dependent antigens, such as protein toxoids. Evaluation of antibody responses to other polysaccharides and to primary immunization with proteins is needed to delineate this immune defect.

Our studies do not directly indicate whether the low H. influenzae type b polysaccharide responses are causally related to the high rates of H. influenzae type b infection in Apache and Navajo children. Exposure to H. influenzae type b organisms by colonization or infection resembles immunization with the purified H. influenzae type b polysaccharide vaccine, in that infants typically produce only very low concentrations of antibody. It is likely that the outcome of an encounter with H. influenzae type b depends critically on the magnitude of the early antibody response. The lower responses in Native American children may thus be an important factor contributing to their high risk of invasive H. influenzae type b infection.

We are indebted to the staff of the U.S. Public Health Service Indian Hospital at Whiteriver for their assistance and cooperation in carrying out these studies, to Mr. A.M. van Leeuwen for excellent technical assistance with immunoglobulin allotyping, to Dr. Donna Ambrosino for providing serum from healthy white children for allotyping, to Cynnie Johnson for providing serum from healthy white children immunized with H. influenzae type b polysaccharide vaccine, and to Drs. Lou Popejoy and Ed Rothstein, who conducted the immunizations as part of post-licensure clinical studies sponsored by Praxis Biologics.