21. Käyhty H, Karanko V, Peltola H, Sarna S, Mäkelä PH. Serum antibodies to capsular polysaccharide vaccine of group A *Neisseria meningitidis* followed for three years in infants and children. J Infect Dis 1980; 142:861-8.
22. Käyhty H, Peltola H, Eskola J. Immunogenicity and reactogenicity of four *Haemophilus influenzae* type b capsular polysaccharide vaccines in Finnish 24-month-old children. Pediatr Infect Dis J 1988; 7:574-7.
23. Orenstein WA, Bernier RH, Hinman AR. Assessing vaccine efficacy in the field: further observations. Epidemiol Rev 1988; 10:212-41.
24. Armitage P. Statistical methods in medical research. Oxford, England: Blackwell Scientific, 1971:139.
25. Käyhty H, Jousimies-Somer H, Peltola H, Mäkelä PH. Antibody response to capsular polysaccharides of groups A and C *Neisseria meningitidis* and *Haemophilus influenzae* type b during bacteremic disease. J Infect Dis 1981; 143:32-41.
26. Käyhty H, Peltola H, Karanko V, Mäkelä PH. The protective level of serum antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b. J Infect Dis 1983; 147:1100.
27. Anderson P. The protective level of serum antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b. J Infect Dis 1984; 149:1034-5.
28. Ward JI, Lum MK, Hall DB, Silimperi DR, Bender TR. Invasive *Haemophilus influenzae* type b disease in Alaska: background epidemiology for a vaccine efficacy trial. J Infect Dis 1986; 153:17-26.
29. Takala AK, Eskola J, Palmgren J, et al. Risk factors of invasive *Haemophilus influenzae* type b disease among children in Finland. J Pediatr 1989; 115:694-701.
30. Ward JI, Brenneman G, Lepow M, Lum M, Burkhart K, Chiu CY. *Haemophilus influenzae* type b anticapsular antibody responses to PRP-pertussis and PRP-D vaccines in Alaska native infants. J Infect Dis 1988; 158:719-23.
31. Takala AK. Epidemiology and risk factors of invasive *Haemophilus influenzae* type b (Hib) disease among children in Finland. Helsinki, Finland: National Public Health Institute, 1989. (Publication no. 82.)
32. Ward JI, Margolis HS, Lum MK, Fraser DW, Bender TR, Anderson P. *Haemophilus influenzae* disease in Alaskan Eskimos: characteristics of a population with an unusual incidence of invasive disease. Lancet 1981; 1:1281-5.
33. Mäkelä PH, Eskola J, Peltola H, Takala AK, Käyhty H. Clinical experience with *Haemophilus influenzae* type b conjugate vaccines. Pediatrics 1990; 85:Suppl:651-3.

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 50 times higher in the Native American children.[1,2,4,6] 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.[2]

The reasons for these high rates of infection are not well understood. In Alaskan Eskimos, genetic markers such as uridine monophosphate kinase 3[10] and combinations of immunoglobulin allotypes and HLA-DR markers[11] 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 capsular polysaccharide.[10,11]

Since antibody to the capsular polysaccharide is a major determinant of protection from invasive *H. in-*

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fluenzae type b disease,[12,13] 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.[14] 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 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 allotypes were measured; in 79, selected only 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 Cynsie Johnson (Praxis Biologics). Subjects 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 allotypes. 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, Praxis 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.[15,16]

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 radioimmunoassay[17] 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 anticapsular antibody per milliliter, obtained from Dr. Carl Frasch (Center for Biologics 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 F. Skvaril (Bern, Switzerland).

Total immunoglobulin was measured by nephelometry[20] and IgG subclasses by solid-phase competitive radioimmunoassay with use of sheep antiserum to human IgG subclass proteins[21] 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 were below the 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.

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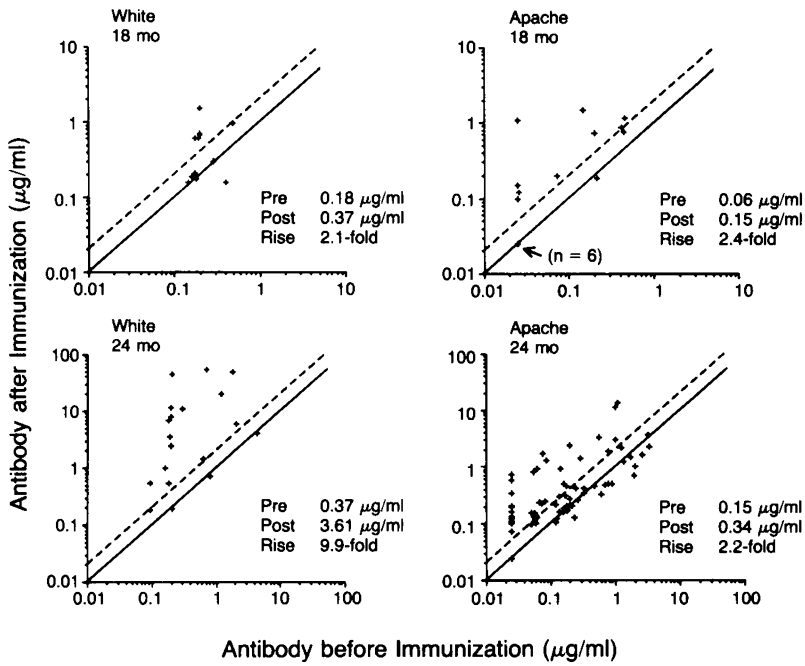

Figure 1. Antibody Responses to *H. influenzae* Type b Capsular Polysaccharide in 18- and 24-Month-Old Apache and White Children.

The solid lines indicate no response, and the dashed lines a twofold response. Values before immunization (Pre) and after immunization (Post), and the magnitude of change (Rise) are geometric means.

3.6 μg per milliliter, P<0.01 by t-test). The geometric mean antibody concentrations before immunization were also lower in the Apaches (0.15 μg per milliliter, vs. 0.37 in the whites; P<0.01 by the Mann–Whitney test). The antibody response expressed as multiples of increase was greater in the whites than in the Apaches (9.9-fold vs. 2.2-fold, P<0.01 by t-test).

Both Apache and white 18-month-olds had much lower antibody responses to *H. influenzae* type b polysaccharide than the corresponding 24-month-old children. Again, Apache children had lower antibody concentrations than whites before and after immunization. However, only the values before immunization differed significantly (0.06 vs. 0.18 μg per milliliter, 0.01<P<0.05 by the Mann–Whitney test).

A remarkable finding was that the geometric mean *H. influenzae* type b antibody concentrations in the Apache children after immunization were lower than those in the whites before immunization in each age group.

The responses were also analyzed to determine the proportion of children who had antibody concentrations >0.15 μg per milliliter, the level estimated to protect most children at a given point in time,[12] and >1.0 μg per milliliter, the post-immunization level predictive of longer-term vaccine efficacy.[13] Among the 18-month-old children, only 8 of 17 Apaches (47 percent) reached antibody concentrations ≥0.15 μg per milliliter, as compared with 13 of 13 whites (100 percent, P<0.01); among the 24-month-olds, only 16 of 79 Apaches (20 percent) reached post-immunization antibody concentrations ≥1 μg 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

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

Children	Antibody Concentration*		
	IgG	IgM	IgA
	ELISA units $\times 10^{-3}$		
Apache (n = 23)	0.154 (4.4)	0.146 (3.9)	0.033 (3.0)
White (n = 19)	0.718 (11.0)	0.339 (4.7)	0.117 (9.6)
P value†	0.023	0.074	0.034

*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.

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

CHILDREN	ANTIBODY CONCENTRATION†	
	TETANUS TOXOID	DIPHTHERIA TOXOID
	ELISA units $\times 10^{-3}$	
Apache (n = 33)	34.9 (2.6)	6.96 (2.9)
White (n = 15)	41.1 (2.2)	12.3 (1.9)
P value	>0.05‡	0.028§

*The antibodies were measured a mean of 222 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.

significantly higher. The concentrations of IgG2 and IgG4, however, were selectively and significantly lower in the Apaches than in the whites ($P<0.001$).

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 $\gamma 3$ allele, G3m (b0b3b5st), which is almost always found in linkage as the Gm haplotype (za;..;b0b3b5st), was found in 42 of 95 Apaches and in none of the whites. This allele is found mainly in Eskimo and other Mongoloid 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 $P \leq 0.05$ to indicate significance) or other allotypes and immunoglobulin levels (considering $P \leq 0.01$ to indicate significance, to compensate for multiple comparisons).

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

CHILDREN	CONCENTRATION*						
	IgG	IgM	IgA	IgG1	IgG2	IgG3	IgG4
	milligrams per milliliter						
Apache (n = 95)	12.2 (1.3)	2.48 (1.5)	0.75 (1.6)	10.6 (1.4)	0.43 (2.5)	0.51 (1.8)	0.037 (2.7)
White (n = 51)	7.05 (1.5)	1.35 (1.6)	0.37 (2.0)	6.12 (1.4)	0.72 (1.7)	0.53 (1.7)	0.10 (3.2)
P value†	<0.001	<0.001	<0.001	<0.001	<0.001	NS	<0.001

*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.

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 μg 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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sponse to the PRP-D–*H. influenzae* type b conjugate vaccine could thus have contributed to the lower efficacy of this vaccine in Alaskan Eskimo[29] than in Finnish[30] 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.[31] Indeed, total immunoglobulin levels decrease in black Africans residing in England to levels that more closely resemble those of whites.[31] 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,[32,33] recurrent sinopulmonary infections,[34,35] and impaired antibody responses to bacterial polysaccharide vaccines.[35-37]

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.[38,39] Total IgG2 levels correlate with the polysaccharide antibody responses of various adult groups.[17,36,40] The correlation, however, is weaker in infants and children[37] 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.[41,42] 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.[24] 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.[17] 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 Apaches. 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.[11]

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[43] and in children with mild degrees of protein-calorie malnutrition.[44] However, deficits in micronutrients that are known to be important to immune function, such as vitamins A and E and zinc,[45] may exist in Native American children[14] 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.[46] 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.

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References

1. Ward JI, Margolis HS, Lum MK, Fraser DW, Bender TR, Anderson P. *Haemophilus influenzae* disease in Alaskan Eskimos: characteristics of a population with an unusual incidence of invasive disease. Lancet 1981; 1:1281-5.
2. Wotton KA, Stiver HG, Hildes JA. Meningitis in the central Arctic: a 4-year experience. Can Med Assoc J 1981; 124:887-90.
3. Coulehan JL, Michaels RH, Hallowell C, Schults R, Welty TK, Kuo JS. Epidemiology of *Haemophilus influenzae* type b disease among Navajo Indians. Public Health Rep 1984; 99:404-9.
4. Coulehan JL, Michaels RH, Williams KE, et al. Bacterial meningitis in Navajo Indians. Public Health Rep 1976; 91:464-8.
5. Losonsky GA, Santosham M, Sehgal VM, Zwahlen A, Moxon ER. *Haemophilus influenzae* disease in the White Mountain Apaches: molecular epidemiology of a high risk population. Pediatr Infect Dis 1984; 3:539-47.
6. Yost GC, Kaplan AM, Bustamante R, Ellison C, Hargrave AF III, Randall DL. Bacterial meningitis in Arizona American Indian children. Am J Dis Child 1986; 140:943-6.
7. Wiet RJ, DeBlanc GB, Stewart J, Weider DJ. Natural history of otitis media in the American native. Ann Otol Rhinol Laryngol Suppl 1980; 89:14-9.
8. Santosham M, Yolken RH, Wyatt RG, et al. Epidemiology of rotavirus diarrhea in a prospectively monitored American Indian population. J Infect Dis 1985; 152:778-83.
9. Davidson M, Schraer CD, Parkinson AJ, et al. Invasive pneumococcal disease in an Alaska Native population, 1980 through 1986. JAMA 1989; 261:715-8.
10. Petersen GM, Silimperi DR, Scott EM, Hall DB, Rotter JI, Ward JI. Uridine monophosphate kinase 3: a genetic marker for susceptibility to *Haemophilus influenzae* type b disease. Lancet 1985; 2:417-9.
11. Petersen GM, Silimperi DR, Rotter JI, et al. Genetic factors in *Haemophilus influenzae* type b disease susceptibility and antibody acquisition. J Pediatr 1987; 110:228-33.
12. Robbins JB, Parke JC Jr, Schneerson R, Whisnant JK. Quantitative measurement of "natural" and immunization-induced *Haemophilus influenzae* type b capsular polysaccharide antibodies. Pediatr Res 1973; 7:103-10.
13. Käyhty H, Peltola H, Karanko V, Mäkelä PH. The protective level of serum antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b. J Infect Dis 1983; 147:1100.
14. Owen GM, Garry PJ, Seymoure RD, Harrison GG, Acosta PB. Nutrition studies with White Mountain Apache preschool children in 1976 and 1969. Am J Clin Nutr 1981; 34:266-77.
15. Greenberg DP, Ward JI, Burkart K, Christenson PD, Guravitz L, Marey SM. Factors influencing immunogenicity and safety of two *Haemophilus influenzae* type b polysaccharide vaccines in children 18 and 24 months of age. Pediatr Infect Dis J 1987; 6:660-5.
16. Käyhty H, Peltola H, Eskola J. Immunogenicity and reactogenicity of four *Haemophilus influenzae* type b capsular polysaccharide vaccines in Finnish 24-month-old children. Pediatr Infect Dis J 1988; 7:574-7.
17. Ambrosino DM, Schiffman G, Gotschlich EC, et al. Correlation between G2m(n) immunoglobulin allotype and human antibody response and susceptibility to polysaccharide encapsulated bacteria. J Clin Invest 1985; 75:1935-42.
18. Ambrosino DM, Barrus VA, DeLange GG, Siber GR. Correlation of the Km(1) immunoglobulin allotype with anti-polysaccharide antibodies in Caucasian adults. J Clin Invest 1986; 78:361-5.
19. Zollinger WD, Boslego JW. A general approach to standardization of the solid-phase radioimmunoassay for quantitation of class-specific antibodies. J Immunol Methods 1981; 46:129-40.
20. Alper CA. Plasma protein measurements as a diagnostic aid. N Engl J Med 1974; 291:287-90.
21. Morell A, Van Loghem E, Nef M, Theilkäes L, Skvaril F. Determination of IgG subclasses and Gm allotypes in culture supernatants of pokeweed mitogen-stimulated human blood lymphocytes. J Immunol 1981; 127:1099-102.
22. Schanfield MS, van Loghem E. Human immunoglobulin allotypes. In: Weir DM, ed. Handbook of experimental immunology. Vol. 3. Genetics and molecular immunology. 4th ed. Oxford, England: Blackwell Scientific, 1986:94.1-94.18.
23. Granoff DM, Suarez BK, Pandey JP, Shackelford PG. Genes associated with the G2m(23) immunoglobulin allotype regulate the IgG subclass responses to *Haemophilus influenzae* type b polysaccharide vaccine. J Infect Dis 1988; 157:1142-9.
24. Granoff DM, Pandey JP, Boies E, Squires J, Munson RS Jr, Suarez B. Response to immunization with *Haemophilus influenzae* type b polysaccharide-pertussis vaccine and risk of *Haemophilus* meningitis in children with the Km(1) immunoglobulin allotype. J Clin Invest 1984; 74:1708-14.
25. Lepow ML, Peter G, Glode MP, et al. Response of infants to *Haemophilus influenzae* type b polysaccharide and diphtheria-tetanus-pertussis vaccines in combination. J Infect Dis 1984; 149:950-5.
26. Coulehan JL, Hallowell C, Michaels RH, Welty TK, Lui N, Kuo JS. Immunogenicity of a *Haemophilus influenzae* type b vaccine in combination with diphtheria-pertussis-tetanus vaccine in infants. J Infect Dis 1983; 148:530-4.
27. Ward JI, Brenneman G, Lepow M, Lum M, Burkhart K, Chiu CY. *Haemophilus influenzae* type b anticapsular antibody responses to PRP-pertussis and PRP-D vaccines in Alaska native infants. J Infect Dis 1988; 158:719-23.
28. Siber GR, Santosham M, Priehs CM, et al. Impaired antibody (Ab) response to *H. influenzae* b (Hib) capsular polysaccharide (CP) in Native American children. In: Program and abstracts of the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy, New York, October 4–7, 1987. Washington, D.C.: American Society for Microbiology, 1987. abstract.
29. Ward JI, Brenneman G, Letson G, Heyward W, Alaska Vaccine Efficacy Trial Study Group. Limited protective efficacy of an *H. influenzae* type b conjugate vaccine (PRP-D) in native Alaskan infants immunized at 2, 4 and 6 months of age. Presented at the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, October 23–26, 1988. abstract.
30. Eskola J, Peltola H, Takala AK, et al. Efficacy of *Haemophilus influenzae* type b polysaccharide–diphtheria toxoid conjugate vaccine in infancy. N Engl J Med 1987; 317:717-22.
31. Hobbs JR. Immune globulins in some diseases. Br J Hosp Med 1970; 3:669-80.
32. Schur PH, Borel H, Gelfand EW, Alper CA, Rosen FS. Selective gamma-G globulin deficiencies in patients with recurrent pyogenic infections. N Engl J Med 1970; 283:631-4.
33. Oxelius V-A, Laurell A-B, Lindquist B, et al. IgG subclasses in selective IgA deficiency: importance of IgG2-IgA deficiency. N Engl J Med 1981; 304:1476-7.
34. Hammarström L, Smith CI. IgG subclasses in bacterial infections. Monogr Allergy 1986; 19:122-33.
35. Umetsu DT, Ambrosino DM, Quinti I, Siber GR, Geha RS. Recurrent sinopulmonary infection and impaired antibody response to bacterial capsular polysaccharide antigen in children with selective IgG-subclass deficiency. N Engl J Med 1985; 313:1247-51.
36. Siber GR, Schur PH, Aisenberg AC, Weitzman SA, Schiffman G. Correlation between serum IgG-2 concentrations and the antibody response to bacterial polysaccharide antigens. N Engl J Med 1980; 303:178-82.
37. Shackelford PG, Granoff DM, Nahm MH, Scott MG, Suarez B, Nelson SJ. Correlation of serum immunoglobulin subclass concentrations with antibody responses of children to immunization with *Haemophilus influenzae* type b polysaccharide-pertussis vaccine. J Clin Immunol 1985; 5:390-5.
38. Yount WJ, Dorner MM, Kunkel HG, Kabat EA. Studies on human antibodies. VI. Selective variations in subgroup composition and genetic markers. J Exp Med 1968; 127:633-46.
39. Riesen WF, Skvaril F, Braun DG. Natural infection of man with group A streptococci: levels; restriction in class, subclass and type; and clonal appearance of polysaccharide-group-specific antibodies. Scand J Immunol 1976; 5:383-90.
40. Morell A, Vassalli G, deLange GG, Skvaril F, Ambrosino DM, Siber GR. Class and subclass composition of natural antibodies to group A streptococcal carbohydrate: correlations with serum immunoglobulin concentrations and allotypes. J Immunol 1989; 142:2495-500.
41. Insel RA, Anderson PW. IgG subclass distribution of antibody induced by immunization with the isolated and protein-conjugated polysaccharide of *H. influenzae* b and G2m(n) distribution of serum IgG2 in man. Monogr Allergy 1988; 23:128-37.
42. Granoff DM, Weinberg GA, Shackelford PG. IgG subclass response to immunization with *Haemophilus influenzae* type b polysaccharide-outer membrane protein conjugate vaccine. Pediatr Res 1988; 24:180-5.
43. Price P. Responses to polyvinyl pyrrolidone and pneumococcal polysaccharide in protein-deficient mice. Immunology 1978; 34:87-96.
44. Greenwood BM, Bradley AK, Blakebrough IS, Whittle HC, Marshall TF, Gilles HM. The immune response to a meningococcal polysaccharide vaccine in an African village. Trans R Soc Trop Med Hyg 1980; 74:340-6.
45. Keusch GT. Immune function in the malnourished host. Pediatr Ann 1982; 11:1004-14.
46. Käyhty H, Jousimies-Somer H, Peltola H, Mäkelä PH. Antibody response to capsular polysaccharides of groups A and C *Neisseria meningitidis* and *Haemophilus influenzae* type b during bacteremic disease. J Infect Dis 1981; 143:32-41.

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