

# Safety and immunogenicity of a *Haemophilus influenzae* type b conjugate vaccine in a high risk American Indian population

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The safety and immunogenicity of a *Haemophilus influenzae* type b polysaccharide conjugate vaccine linked to the outer membrane protein complex of *Neisseria meningitidis* (Hib-OMP) were evaluated among Apache and Navajo infants and children. One dose of the Hib-OMP was given to 42 children who were from 12 and 60 months of age. Ninety-two infants 6 to 8 weeks old were given one dose of Hib-OMP at the time of enrollment. A subsequent dose of the vaccine was given 2 months later and a third dose was offered between 12 and 15 months of age. All the 12- to 60-month-old children achieved a protective antibody concentration ( $>1 \mu\text{g/ml}$ ) 1 month postvaccination. Among the 6- to 8-week-old infants only 11% of the Apaches and 8% of Navajos had a protective anti-PRP antibody concentration prevaccination. One month post vaccination 68% of the Apaches and 69% of the Navajos had protective anti-PRP antibody concentrations. One month after the second immunization 67% of the Apaches and 75% of Navajos had protective anti-PRP concentrations. Among the infants that received the third (booster) immunization ( $N = 28$ ) 74% had protective anti-PRP antibody titers just before the booster immunization. One month after the booster immunization all of the infants had protective concentrations of anti-PRP antibody. We conclude that the Hib-OMP

is safe and highly immunogenic among Apache and Navajo infants and children.

## INTRODUCTION

In the United States *Haemophilus influenzae* type b (Hib) is the leading cause of meningitis in infants younger than 5 years of age.<sup>1-3</sup> In this age group the attack rate of systemic Hib disease among certain Native American populations such as Alaskan Eskimos, Navajos and Apaches is 5 to 10 times higher than that in the general United States population.<sup>4-7</sup> Moreover approximately 35 to 40% of cases occur before 6 months of age.

Recently a number of Hib conjugate vaccines, prepared by covalently linking the Hib capsular polysaccharide, polyribosylribitol phosphate (PRP), to various protein carriers have been evaluated. Most of these vaccines elicit reliable antibody responses in infants older than 6 months of age but induce poor responses below that age.<sup>7-10</sup> A conjugate vaccine composed of PRP linked to the outer membrane protein complex of *Neisseria meningitidis* (OMP) has been prepared by Merck Sharp and Dohme Research Laboratories, West Point, PA.<sup>11</sup> This vaccine (Hib-OMP) has been shown to produce what is considered a protective antibody response after one dose of the vaccine among infants ages 2 to 3 months in the general United States population.<sup>12-14</sup> We report the safety and immunogenicity of Hib-OMP among infants and children in different age groups from two Native American populations in Arizona.

## METHODS

**Study population.** Apache and Navajo infants and children were recruited from the White Mountain Apache Indian and Navajo Indian Reservations in the state of Arizona. The study protocol was approved by the Committee on Human Volunteers at the Johns Hopkins University, School of Hygiene and Public Health, the Indian Health Service, the Tribal Council of the White Mountain Apache Tribe and the Health

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**Vaccine.** The preparation and characterization of Hib-OMP have been described previously.<sup>11</sup> The vaccine consists of PRP (molecular weight approximately 50 000 by gel filtration on Sepharose Cl-4B) covalently coupled to a meningococcal outer membrane protein complex. Each dose of the vaccine was provided lyophilized in individual vials. The lots of the vaccine used were 1069/c-p 241, 1072/c-p 298 and 1080/c-p 149. Lot 1069 was stored at  $-70^{\circ}\text{C}$ . Lots 1072 and 1080 were stored at  $2-8^{\circ}\text{C}$ . The vaccine was rehydrated with a diluent containing aluminum hydroxide before use. The dosage of administration (intramuscular) for Lot 1069 was 0.75 ml/dose. Lots 1072 and 1080 were given in doses of 0.5 ml. Each dose of the reconstituted vaccine contained 15  $\mu\text{g}$  of capsular polysaccharide, 97 to 251  $\mu\text{g}$  of meningococcal protein and 0.3 mg of lactose.

**Recruitment.** Healthy children were recruited into the study by obtaining written informed consent from parents/guardians. Infants were excluded from the study if they met one of the following conditions; suspected immunodeficiency; history of serious adverse reactions to routine childhood immunizations; history of receiving diphtheria, pertussis and tetanus toxoid vaccine in the preceding 10 days; and history of receiving any Hib vaccine.

**Schedule of immunization.** Children ages 12 to 60 months (Group A) were given one dose of the vaccine at the time of recruitment. Infants ages 6 to 8 weeks (Group B) were also given one dose of the vaccine at the time of recruitment, followed by an additional dose 2 months after the initial immunization. A third dose of the vaccine (booster dose) was offered between 12 and 15 months of age to infants in Group B whose parents provided additional written consent for booster immunization.

**Monitoring adverse reactions.** All infants were observed in the clinic for 30 minutes after each immunization. A member of the study staff visited the homes of the participants 4 to 6 hours after immunization and every 24 hours thereafter for 1 week. At these home visits a standard questionnaire was administered to the parents/guardians in order to detect any adverse reactions experienced by the infant. In addition the infant's temperature was recorded and the injection site was inspected.

**Serum samples.** For infants ages 12 to 60 months of age 5 ml of venous blood was drawn before immunization and 4 weeks after immunization. For infants 6 to 8 weeks old 5 ml of venous blood were drawn before each immunization, at 5 months of age and at 12 months of age. Infants that received a booster dose of the vaccine at 12 months of age had another sample of blood drawn 4 weeks later.

## LABORATORY METHODS

**Serology.** Serum antibody to the capsular polysaccharide of Hib (anti-PRP) was detected by a standardized radioimmunoassay procedure as described previously.<sup>15</sup>

**Data analysis.** Data were entered into a computer on site in Whiteriver using the FOXPLUS database package and then converted in Baltimore for analysis using the SAS software package. The analyses were conducted using contingency tables to compare grouped levels of antibodies and *t* tests, correlation and analysis of variance to compare the logarithm of the antibody values. The minimum concentration of antibody reported by the laboratories was 0.125  $\mu\text{g}/\text{ml}$  which was converted to 0.10  $\mu\text{g}/\text{ml}$  for these analyses.

## RESULTS

Nine (3%) of 303 infants in whom a temperature was recorded had a temperature greater than  $101^{\circ}\text{F}$  during the 1 week follow-up period and 1 (0.3%) had temperature  $>102^{\circ}\text{F}$ . Reactions, believed to be related to the vaccine, were reported in 46 (13.2%) of 348 vaccinations. None was serious. Forty-five of these were localized swelling or similar reactions at the vaccination site. One child was noted to be fussy. Four children experienced a second reaction in addition to localized swelling. These reactions were irritability, crying, fever and diarrhea, respectively. All of these symptoms resolved within 24 hours of onset.

Forty-two Apache children between 12 and 60 months were enrolled in the study. No Navajos were enrolled in this age group. The proportion of infants with anti-PRP antibodies of  $\geq 1.0$   $\mu\text{g}/\text{ml}$  and the geometric mean antibody titers before and after immunization in these infants are shown in Table 1. All participants had an anti-PRP antibody titer of  $\geq 1.0$   $\mu\text{g}/\text{ml}$  4 weeks after immunization. The age for vaccination did have a statistically significant ( $P < 0.001$ ) impact on antibody response. Even after controlling for prevaccination antibody concentrations the effect remained significant ( $P < 0.001$ ) and an increase of 1 month of age resulted in an average increase of 6.0% antibody response.

Ninety-two infants (64 Navajo and 28 Apache) ages

**TABLE 1.** Anti-PRP antibody response of 12- to 60-month-old Apache children after one dose of Hib-OMP vaccine ( $N = 42$ )

Anti-PRP Antibody ( $\mu\text{g}/\text{ml}$ )	Anti-PRP Antibody Concentrations	
	Preimmunization no.	Postimmunization no.
<0.15	14 (33) <sup>a</sup>	0
0.15 $\leq$ 1	22 (52) <sup>a</sup>	0
$\geq 1.0$	6 (14) <sup>a</sup>	42 (100) <sup>a</sup>
Geometric mean	0.34 (0.24, 0.49) <sup>b</sup>	15.57 (10.8, 22.4) <sup>b</sup>

<sup>a</sup> Numbers in parentheses, percent.

<sup>b</sup> Numbers in parentheses, 95% confidence interval.

6 to 8 weeks were enrolled in the study. The distribution of anti-PRP antibodies before first and second immunizations and 1 months and 8 months after second immunization is shown in Table 2 and Figure 1. There were no statistically significant differences in the antibody titers between the Apache and Navajo infants at any of the time points. There was some variation in the interval between vaccinations. However, this never accounted for more than 5.0% of the variation in antibody response and was never statis-

tically significant, even after controlling for prior antibody concentrations and age at first vaccination. Age at first vaccination did not significantly affect the serum response until 12 months of age ( $P = 0.02$ ) where it accounted for 9.7% of the variation. This effect remained after controlling for other factors such as response at 5 months of age.

Twenty-eight of the Navajo infants consented to have a booster immunization at 12 months of age. The geometric mean antibody concentrations at 12 months

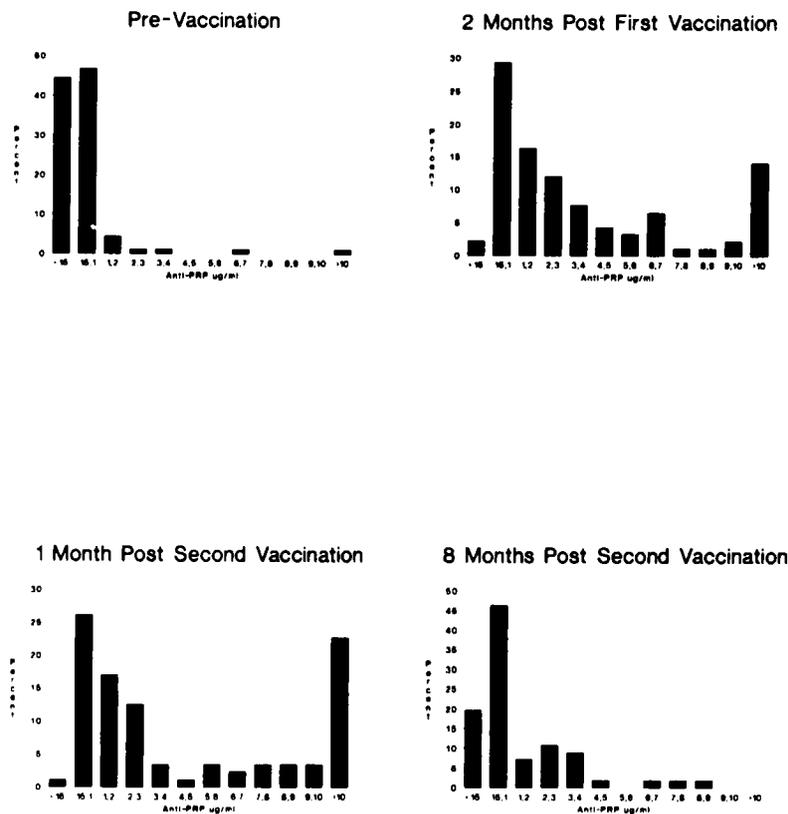
**TABLE 2.** Hib antibody response to Hib-OMP vaccine in 6- to 8-week-old infants

Age (Months)	Serum Sample	Vaccine Dose	No. of Infants	Geometric Mean Anti-PRP	% with Anti-PRP >1 µg/ml
Apache					
2	Prevaccination	1	27	0.26 (0.17, 0.41) <sup>a</sup>	11.1
4	2 months post-Vaccine 1	2	28	1.69 (1.14, 2.5)	67.9
5	1 month post-Vaccine 2		27	2.59 (1.52, 4.40)	66.7
12	8 months post-Vaccine 2		26	0.49 (0.29, 0.83)	23.1
Navajo					
2	Prevaccination	1	63	0.22 (0.18, 0.28)	7.9
			{28} <sup>b</sup>	{0.23 (0.16, 0.35)}	{10.7}
4	2 months post-Vaccine 1	2	64	2.53 (1.80, 3.54)	68.7
			{28}	{1.63 (0.96, 2.78)}	{50.0}
5	1 month post-Vaccine 2		61	2.75 (1.95, 3.88)	75.4
			{27}	{2.51 (1.41, 4.45)}	{77.8}
12	8 months post-Vaccine 2		30		43.3
			{28}	{0.53 (0.31, 0.93)}	{28.6}
{13}	1 month post-Vaccine 3	{3}	{27}	{8.38 (4.86, 14.46)}	{88.9} <sup>c</sup>

<sup>a</sup> Numbers in parentheses, 95% confidence interval.

<sup>b</sup> Statistics in braces are for a subset of 28 Navajo children who received a booster dose at 12 months of age.

<sup>c</sup> 100% of children were above 0.15 µg/ml 1 month after the booster.



**FIG. 1.** Distribution of Anti-PRP antibody concentrations in 2-month-old infants after one and two doses of Hib-OMP (Apache and Navajo combined).

of age (before booster immunization) and 4 weeks post-booster immunization, in the infants that received booster immunization were 0.53  $\mu\text{g}/\text{ml}$  ( $N = 28$ ) and 8.38  $\mu\text{g}/\text{ml}$  ( $N = 27$ ), respectively. Eight of the 28 infants had anti-PRP antibody concentrations  $\geq 1.0 \mu\text{g}/\text{ml}$  at 12 months of age. The other 20 infants had concentrations between 0.15 and 1.0  $\mu\text{g}/\text{ml}$ . Twenty-four of the 27 (88.9%) infants reached a concentration above 1  $\mu\text{g}/\text{ml}$  1 month after receiving the booster dose. The 3 remaining children had prebooster concentrations at or below the undetectable level and 1 month later had concentrations of 0.64, 0.98 and 0.99  $\mu\text{g}/\text{ml}$  respectively. Except for 2 children, all children had 3.8-fold rises or greater after the booster. Importantly the two exceptions had very high concentrations at the time of vaccination, 21.6 and 11.1  $\mu\text{g}/\text{ml}$ , which fell to 13.5 and 7.3, respectively. The mean fold rise was 27.7 with 95% confidence limits of 16.9 to 38.6.

The geometric mean anti-PRP antibody titers before immunization with different lots of the vaccine were similar. However, in older infants, recipients of Lot 1069 tended to have higher titers 1 month after immunization compared with the other 2 lots (Lot 1069 vs. Lot 1072,  $P = 0.025$ ; Lot 1069 vs. Lot 1080,  $P = 0.066$ ). After controlling for age (using analysis of variance), Lot 1069 continued to have better responses ( $P = 0.0142$ ).

The anti-PRP antibody responses of Apaches and Navajos were similar. There were also no differences in responses between males and females or between individual study sites.

## DISCUSSION

These data indicate that the Hib-OMP vaccine was highly immunogenic after a single dose in a majority of Apache and Navajo infants. Moreover the vaccine produced very few adverse reactions.

In our population all infants older than 1 year of age produced an antibody response of  $\geq 1 \mu\text{g}/\text{ml}$ . This titer has been postulated to correlate with providing long term protection against Hib.<sup>16</sup> Sixty to 69% of the 6- to 8-week-old infants had antibody titers of  $\geq 1 \mu\text{g}/\text{ml}$  after a single dose. After a second dose 66 to 75% of infants achieved this titer. In previous studies the Hib-OMP has been shown to produce anti-PRP antibody concentrations above 1  $\mu\text{g}/\text{ml}$  in 64 to 80% of infants less than 6 months of age after a single dose.<sup>12-14</sup>

In a previous study<sup>7</sup> we evaluated the anti-PRP antibody responses of 6- to 8-week-old Navajo infants to a conjugate vaccine termed HbOC (Praxis Biologics), composed of PRP covalently linked to non-toxic diphtheria toxin-cross-reactive material. In that study 4% of the infants achieved anti-PRP antibody titers of  $\geq 1 \mu\text{g}$  after one dose. After the second and

third doses 58 and 92% of infants had antibody concentrations of  $\geq 1 \mu\text{g}$ , respectively. In the general United States population the HbOC and another Hib conjugate vaccine conjugated to tetanus toxoid (PRP-T) produced by Institute Merrieux have been shown to produce a poor immune response after a single dose, but both these vaccines elicit excellent immune responses after the second and third doses.<sup>10, 17</sup> Because approximately 25 to 40% of Hib infections in the Apache and Navajo populations occur before 6 months of age, a vaccine that produces a good immunogenic response after a single dose may provide protection for the maximum number of infants in these populations. Since Hib-OMP appears to meet this criterion, we are evaluating its efficacy in preventing Hib disease among Navajo infants in a prospective randomized double blind placebo-controlled study.

At 12 months of age (8 months after the second immunization) there was a 5-fold drop in anti-PRP antibody titer in Apache infants and a 3-fold drop in titer among Navajo infants. However, all infants who received the third dose of Hib-OMP 8 to 12 months after the first dose had an excellent booster response to the vaccine. This finding indicates that this vaccine produces immunologic priming and induces a T-dependent or memory antibody response as shown for other Hib conjugate vaccines. The responses seen in our population to the booster dose of the vaccine are similar to the responses seen in a study from Missouri.<sup>13</sup>

We conclude that the Hib-OMP is a highly immunogenic vaccine that produces excellent primary and booster responses. Therefore this vaccine may be efficacious in preventing Hib disease among young infants.

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## Interleukin 6 activity in infants and children with bacterial meningitis

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**Concentrations of interleukin 6 (IL-6) in cerebrospinal fluid (CSF) and serum of infants and children with bacterial meningitis were deter-**

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mined and correlations were sought with other indices of inflammation and with outcome. Forty-two patients ages 1 month to 15 years (mean, 2.5 years) were studied. IL-6 activity was detectable (>50 units/ml) in 30 of 36 CSF samples collected at admission from patients with meningitis and in 1 of 23 controls with fever and normal CSF findings. Mean values were 36 000 units/ml (range, 151-156 000). IL-6 activity in CSF persisted during the first 5 days of illness. IL-6 concentrations at admission were not associated with clinical findings, CSF leukocyte, protein and glucose concentrations, serum C-reactive protein concentration and neurologic complications or se-