Prevention of *Haemophilus influenzae* Type b Infections in Apache and Navajo Children

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Prospective surveillance of *Haemophilus influenzae* type b (Hib) disease has been done since 1981 in two high-risk populations, White Mountain Apaches and Navajos. The attack rate in children <5 years of age is 5–10 times higher than in the general US population. Three vaccines were evaluated. Unconjugated Hib capsular polysaccharide produced lower antibody responses in 18- and 24-month-old Apache infants than in white infants. HbOC (Hib oligosaccharide covalently linked to the nontoxic mutant diphtheria toxin CRM_{197}) produced low antibody responses in Navajo infants after one or two doses but induced responses similar to those in whites after three doses. The responses of 18-month-old Navajos to HbOC were lower than those of whites, but most achieved protective levels. PRP-OMP (Hib capsular polysaccharide linked to the outer membrane protein complex of *Neisseria meningitidis*) produced good immune responses in 2-month-old Navajo and Apache infants after a single dose. This vaccine was >90% efficacious in protecting Navajo infants from Hib disease when given at 2 and 4 months of age. Even a single dose achieved a high protective efficacy.

The incidence of *Haemophilus influenzae* type b (Hib) disease has been documented to be 5–10 times higher in Navajo and Alaskan natives than in the general US population [1–6]. We have identified another high-risk group, the White Mountain Apaches in Arizona [7]. In the past 10 years, we confirmed the high rates of Hib disease in Navajo and Apache children, evaluated their antibody responses to a pure polysaccharide vaccine and two conjugate vaccines, and demonstrated the protective efficacy of one conjugate vaccine in Navajo infants.

Epidemiology of Hib Disease in Apaches and Navajos

We retrospectively reviewed Hib meningitis at the White Mountain Apache Reservation from January 1973 to December 1981 [7] and then maintained a prospective surveillance of Hib meningitis from October 1981 to December 1982. The attack rates in children <5 years of age were 134–692/100,000, which was 5–10 times higher than the attack rate in the general US population. About 40% of cases occurred before 6 months of age in Apache infants.

Before routine administration of Hib vaccines on the Navajo Reservation, the incidence of invasive Hib diseases in children <5 years of age was 214/100,000 [3] and the incidence of meningitis was 152–173/100,000 [3, 4]. These rates are ~10-fold higher than rates in the general US population during the same period. Between July 1988 and May 1990, more than half of the cases occurred before 9 months of age (figure 1) and ~30% before 6 months.

Immunogenicity of Hib Vaccines

Capsular polysaccharide. We compared the antibody responses to Hib capsular polysaccharide (PRP) vaccine in healthy Apache and white children. [8] Total concentrations of antibody to PRP 1 month after immunization with PRP were ~10 times lower in 24-month-old Apache children than in white children of similar age (table 1). IgG, IgM, and IgA antibodies were all lower in Apache children.

Hib oligosaccharide–mutant diphtheria toxin conjugate. We randomly assigned 2-month-old Navajo infants to one of three groups. The controls were not immunized against Hib ($n = 25$); one group received Hib oligosaccharide conjugated to nontoxic mutant diphtheria toxin CRM_{197} (HbOC [HibTITER]; Praxis Biologics, Rochester, NY) at 2, 4, and 6 months of age ($n = 26$); and another group received HbOC at the same ages and also received hyperimmune globulin (bacterial polysaccharide immune globulin [BPIG]; Massa-

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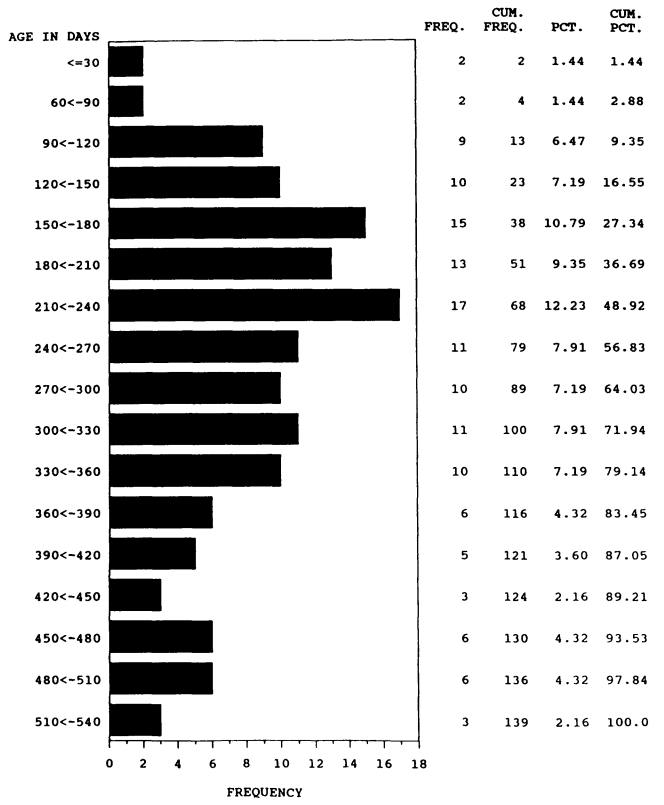

Figure 1. Cases of *Haemophilus influenzae* type b disease on the Navajo Reservation in children up to 18 months of age, 1 July 1988–31 May 1990.

chusetts Public Health Biologic Laboratories, Boston) at 2 months of age (n = 24) [9].

Antibody responses to PRP are shown in table 2. Infants who received BPIG simultaneously with their first dose of HbOC at 2 months had significantly higher antibody concentrations than did the other two groups at 4 months ($P <$ .001). The antibody responses to the second and third injections of HbOC were similar in the groups that received HbOC alone and HbOC plus BPIG.

One infant who received HbOC alone developed Hib meningitis at age 3 months, 6 weeks after his first immunization. The antibody concentration before immunization was 0.18 μg/ml and 1 month after onset of disease was undetectable. He did not generate an antibody response to the first dose or to natural infection but developed antibody concentrations of 0.33 and 1.56 μg/ml after the second and third doses, respectively. The patient's antibody response was the fourth lowest of those receiving HbOC alone.

Because a large proportion of Hib disease occurs before age 6 months in Navajo infants, we concluded that HbOC alone would not be optimal prophylaxis in this population or others with similar epidemiology of Hib disease.

Next, we compared the antibody responses to HbOC of Navajo and white children.

Anti-PRP antibody responses to a single dose of HbOC at 18 months were significantly lower in Navajo than in white children (4.1 vs. 29 μg/ml; $P <$ .001) (figure 2). IgG, IgM, and IgA class antibodies were all lower ($P <$ .05 for all) (table 3).

Table 1. Total and immunoglobulin class antibodies to *Haemophilus influenzae* type b capsular polysaccharide 1 month after immunization of 24-month-old Apache and white children with polysaccharide vaccine.

Antibody	Apache (n = 55)	White (n = 19)	P
Total	0.29 (0.23–0.37)*	3.61 (1.52–8.56)	.0001
IgG	0.315 (0.19–0.52)	1.320 (0.41–4.28)	.01
IgM	0.130 (0.08–0.21)	0.244 (0.12–0.51)	.165
IgA	0.091 (0.06–0.14)	0.279 (0.09–0.83)	.026

NOTE. Antibody concentrations are geometric means (total [assessed by radioantigen binding assay], μg/ml; class, ELISA units × 10^{-3}); 95% confidence intervals are in parentheses. P values were determined by t test. Data are from [8] (used with permission).

* n = 94.

Table 2. Antibody responses to *Haemophilus influenzae* type b capsular polysaccharide of 2-, 4-, and 6-month-old Navajo infants immunized with HbOC alone or in combination with hyperimmune globulin.

Group, age (months)	Geometric mean concentration (μg/ml)	% with concentration ≥0.15 μg/ml	% with concentration ≥1 μg/ml
Control (n = 25)			
2	0.23	56	24
4	0.11	36	4
6	0.11	44	4
7	0.11	42	4
12	0.075	21	0
HbOC alone (n = 26)			
2	0.19	58	12
4	0.12	42	4
6	1.28*	88*	58*
7	10.5*	100*	92*
12	1.43*	96*	70*
HbOC + BPIG (n = 24)			
2	0.16	46	8
4	0.76†	100†	33‡
6	1.53*	83§	58*
7	8.82*	100*	83*
12	2.06*	95*	73*

NOTE. HbOC, *H. influenzae* type b oligosaccharide covalently linked to nontoxic mutant diphtheria toxin CRM_{197}; BPIG, bacterial polysaccharide immune globulin. Data are from [9] (used with permission).

* $P <$ .01 compared with controls.

† $P <$ .001 compared with group receiving HbOC alone.

‡ $P <$ .05 compared with group receiving HbOC alone.

§ $P <$ .05 compared with controls.

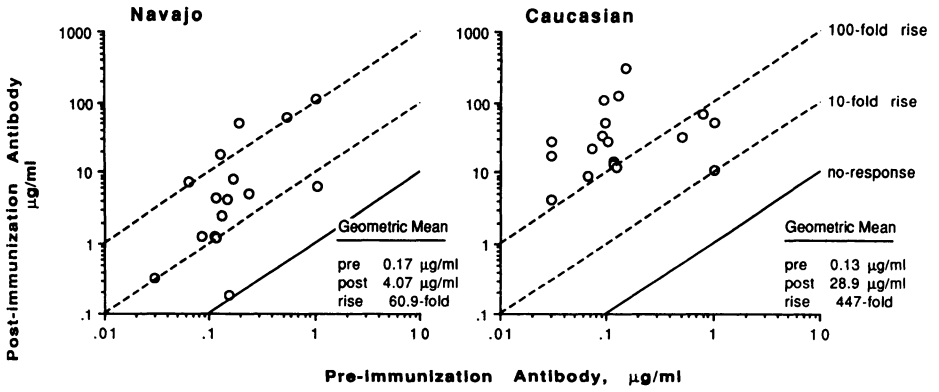

Figure 2. Concentrations of antibodies to *Haemophilus influenzae* type b (Hib) capsular polysaccharide in 18-month-old Navajo and white children before and after immunization with Hib oligosaccharide–mutant diphtheria toxin conjugate.

After two doses of HbOC given with DTP (diphtheria and tetanus toxoids and pertussis vaccine) at 2 and 4 months, Navajo infants had significantly lower total anti-PRP antibody concentrations than did whites (0.90 vs. 7.6 µg/ml; $P < .001$) (table 4). In contrast, anti–tetanus toxoid antibody concentrations after two doses of tetanus toxoid were similar in Navajo and white 6-month-olds (4.21 vs. 4.95 × 10^{-3} ELISA units/ml). At 7 months of age, after a third dose of HbOC, Navajo infants had concentrations of antibody to PRP similar to those of whites (9.2 vs. 17 µg/ml; not significant).

Although it appears that Navajo children were less responsive than white children to HbOC, two factors may have contributed to the differences observed. First, the experimental lots of HbOC may have differed in immunogenicity. A variety of investigational lots of HbOC (lots 5, 8, 9, 10, 11, and 12) were used. We detected no significant lot-to-lot variation in immunogenicity among them [9], but the power to detect differences was limited.

Second, Navajo children typically received their first immunization at 6 weeks of age, whereas whites received theirs at 2–3 months of age. To examine the effect of this age difference, we evaluated a second cohort of 20 white children who had all received their first immunization before 60 days of age (table 4). The responses of the Navajo infants to two doses of HbOC remained lower than those of the age-matched whites (0.90 vs. 3.16 µg/ml; $P < .05$).

Our studies suggest that Navajo children may be significantly less responsive than white children to PRP, even when this antigen is presented as a covalent conjugate with a protein carrier. However, definitive comparison awaits a study using the same vaccine lot. Despite their delayed response, Navajo infants had antibody responses after three doses of HbOC that were similar to responses of whites and at levels associated with protection from Hib disease.

PRP–outer membrane protein conjugate. The safety and immunogenicity of a conjugate vaccine composed of PRP linked to the outer membrane protein complex of *Neisseria meningitidis* (PRP-OMP [PedvaxHIB]; Merck Sharp & Dohme, West Point, PA) was evaluated in Apache and Navajo children [10]. When 42 children were first vaccinated at 12–60 months of age with PRP-OMP, 100% attained levels of antibody to PRP ≥1.0 µg/ml, with a geometric mean of 15.57 µg/ml (95% confidence interval [CI], 10.8–22.4) 4 weeks postvaccination [10].

Table 3. Antibody responses to *Haemophilus influenzae* type b capsular polysaccharide after immunization with HbOC in 18-month-old children.

Antibody	Navajo (n = 18)	White (n = 18)	P
IgG	3.11 (1.24–7.78)	15.8 (6.87–36.3)	<.01
IgM	1.43 (0.75–2.71)	3.20 (1.88–5.44)	<.05
IgA	0.71 (0.27–1.85)	2.26 (1.13–4.52)	<.05

NOTE. HbOC, *H. influenzae* type b oligosaccharide covalently linked to nontoxic mutant diphtheria toxin CRM_{197}; antibody concentrations are geometric means in ELISA units × 10^{-3}; 95% confidence intervals are in parentheses.

Table 4. Antibody responses to *Haemophilus influenzae* type b capsular polysaccharide after immunization with HbOC in Navajo and white infants.

Group, mean age (days) at first dose	n	Geometric mean concentration after 2-month dose	4-month dose	6-month dose	7-month dose
Navajo, 45	17	0.27 (0.10–0.73)	0.14 (0.07–0.27)	0.90 (0.39–2.1)	9.24 (4.4–19.0)
White, 81	18	0.17 (0.06–0.49)	0.24 (0.13–0.46)	7.55 (4.0–14.0)*	17.3 (11.0–28.0)†
White, 52	20	0.16 (0.06–0.36)	0.16 (0.08–0.32)	3.16 (1.3–7.9)‡	6.81 (3.0–16.0)

NOTE. HbOC, *H. influenzae* type b oligosaccharide covalently linked to nontoxic mutant diphtheria toxin CRM_{197}; antibody concentrations (in µg/ml) were determined by radioactive antigen binding; 95% confidence intervals are in parentheses. Compared with Navajo infants, P = *.0005; †.134; ‡.044.

Table 5. Antibody responses to *Haemophilus influenzae* type b capsular polysaccharide after immunization of infants 6–8 weeks old with PRP-OMP.

Group, sample	*n*	Antibody concentration	% with concentration >0.15	% with concentration >1
Apache				
Prevaccination	27	0.26 (0.17–0.41)	59.3	11.1
2 months after dose 1	28	1.69 (1.14–2.5)	100	67.9
1 month after dose 2	27	2.59 (1.52–4.40)	100	66.7
8 months after dose 2	26	0.49 (0.29–0.83)	69.2	23.1
Navajo				
Prevaccination	63	0.22 (0.18–0.28)	54.0	7.9
2 months after dose 1	64	2.53 (1.80–3.54)	96.9	68.7
1 month after dose 2	61	2.75 (1.95–3.88)	98.4	75.4
8 months after dose 2	28	0.53 (0.31–0.93)	71.4	28.6
1 month after dose 3	27	8.38 (4.86–14.46)	100	88.9

NOTE. PRP-OMP, *H. influenzae* type b capsular polysaccharide linked to outer membrane protein complex of *Neisseria meningitidis*. Antibody concentrations (μg/ml, measured by radioactive antigen binding) are geometric means; 95% confidence intervals are in parentheses. Dose 1 was given at 2 months of age, dose 2 at 4 months, and dose 3 (booster) at 12–15 months). Data are from [10] (used with permission).

Antibody responses were evaluated in 92 infants (64 Navajo and 28 Apache) who received PRP-OMP at 6–8 weeks and 4 months concurrently with DTP and oral poliovirus vaccine (OPV) and a booster at 12–15 months. After the first dose, almost 100% developed antibodies to PRP >0.15 μg/ml and 68% developed antibodies >1.0 μg/ml. The geometric mean concentrations and the proportions reaching 0.15 and 1.0 μg/ml increased only minimally with the second dose at 4 months. Eight months after the second dose, the mean concentrations decreased to 0.49 and 0.53 μg/ml, respectively in Apaches and Navajos. However, 1 month after a booster dose, they increased to >8 μg/ml, and almost 90% of children had >1 μg/ml (table 5).

We also evaluated the immune responses in Navajo and Apache infants to lower doses of PRP-OMP at 2, 4, and 6 months of age. The standard dose contains 15 μg of PRP covalently coupled to 250 μg of OMP complex absorbed at the time of use to 0.5 ml of aluminum hydroxide diluent containing 225 μg of aluminum. The dose-response studies were done by diluting lyophilized PRP-OMP with larger volumes of diluent (1.4 ml, 2.1 ml, and 3.3 ml) to give PRP-OMP doses containing 7.5, 5.0, and 3.2 μg of PRP, respectively. Thus, the amount of PRP-OMP was reduced but the amount of aluminum hydroxide was kept constant.

Navajo infants were administered 7.5-μg doses only, but Apache infants were randomized to doses of 7.5, 5.0, and 3.2 μg. The proportion of infants responding with antibodies >0.15 μg and >1.0 μg/ml and the geometric mean concentrations were not significantly different between the groups receiving 7.5-μg doses (table 6) and standard doses (15 μg) (table 5). We tested Apache children receiving nonstandard doses for a dose-response effect by evaluating the proportion achieving 1.0 μg of antibody after the first dose of vaccine. As determined by logistic regression, the effect was significant ($P = .03$) after the first but not after the second and third doses.

Efficacy of PRP-OMP in Navajo Infants

Since PRP-OMP elicited levels of antibody to PRP predictive of sustained protection (≥1 μg/ml) in ~70% of Navajo infants aged 6–8 weeks, we evaluated the efficacy of PRP-OMP in preventing Hib disease in this population [11].

Navajo infants were randomized to receive PRP-OMP or lactose placebo (2 mg) between 42 and 90 days of age. The second injection of vaccine or placebo was given between 70 and 146 days, at least 28 days after the first injection. Infants who received the second dose after 146 days were excluded from the "strict protocol" efficacy analysis. However, they were part of the "intent-to-treat" analysis.

DTP and OPV were administered simultaneously with PRP-OMP or placebo. Before April 1990, the Indian Health

Table 6. Antibody responses to *Haemophilus influenzae* type b capsular polysaccharide after vaccination with various doses of PRP-OMP in Apache and Navajo infants.

Group (dose), sample	*n*	Antibody concentration	% with concentration >0.15	% with concentration >1
Navajo (7.5 μg)				
Prevaccination	59	0.14	37	5
2 months after dose 1	94	3.19	100	81
2 months after dose 2	10	5.89	100	100
Apache (7.5 μg)				
Prevaccination	26	0.09	54	4
2 months after dose 1	26	1.81	100	73
2 months after dose 2	23	3.11	91	82
1 month after dose 3	21	2.83	86	76
Apache (5.0 μg)				
Prevaccination	23	0.18	48	9
2 months after dose 1	22	1.55	96	64
2 months after dose 2	23	3.09	96	78
1 month after dose 3	21	4.99	100	86
Apache (3.2 μg)				
Prevaccination	28	0.12	36	4
2 months after dose 1	27	1.08	100	44
2 months after dose 2	26	3.45	100	65
1 month after dose 3	20	3.96	95	85

NOTE. PRP-OMP, *H. influenzae* type b capsular polysaccharide linked to outer membrane protein complex of *Neisseria meningitidis*. Antibody concentrations (μg/ml) are geometric means. Dose 1 was given at 2 months of age, dose 2 at 4 months, and dose 3 at 6 months of age.

Table 7. Antibody responses to *Haemophilus influenzae* type b capsular polysaccharide in infants vaccinated with PRP-OMP.

Group, sample	*n*	Mean age (months)	Antibody concentration	% with concentration <0.15	% with concentration <1
Vaccine					
Prevaccination	982	1.8	0.16	45	10
After dose 1	879	4.2	0.97	90	51
After dose 2	735	6.3	1.35	91	59
Follow-up 1	331	11.7	0.40	76	24
Follow-up 2	108	17.7	0.40	69	24
Placebo					
Prevaccination	991	1.8	0.16	44	8
After dose 1	905	4.2	0.09	19	1
After dose 2	735	6.3	0.08	13	1
Follow-up 1	336	11.8	0.10	24	4
Follow-up 2	113	17.7	0.11	29	6

NOTE. PRP-OMP, *H. influenzae* type b capsular polysaccharide linked to outer membrane protein complex of *Neisseria meningitidis*. Antibody concentrations (μg/ml, measured by radioactive antigen binding) are geometric means. Dose 1 was given at 2 months of age and dose 2 at 4 months; follow-up 1 was at 12 months and follow-up 2 at 15–18 months, before booster dose. After vaccination, antibody concentrations were always significantly higher in vaccine group than in placebo ($P < .001$).

Service routinely administered one of the licensed conjugate vaccines, PRP-D (a diphtheria toxoid conjugate [ProHIBiT]; Connaught Laboratories, Swiftwater, PA), at 18 months of age. After April 1990, when the recommendations were revised by the American Academy of Pediatrics and the Immunization Practices Advisory Committee, either PRP-D or HbOC was given at 15 months of age.

Methods for serology, microbiology, monitoring of safety and adverse reactions, and data analysis have been described previously [11].

Enrollment. From 1 July 1988 to 2 August 1990, ~90% of the 9038 births at our study sites were available for recruitment. We could not obtain permission to follow the other 10%. Therefore, few data were collected on those who refused to participate. Among them, the male-to-female ratio did not differ significantly from that among participants. Of the 5190 who were enrolled and received vaccine ($n = 2588$) or placebo ($n = 2602$), 4161 (80%) received the second dose of vaccine ($n = 2056$) or placebo ($n = 2105$). There were no statistically significant differences between groups in the male-to-female ratio, mean age, ethnic background, number of doses of vaccine or placebo received, interval between doses, or length of follow-up.

Adverse reactions. There were eight deaths in each group. Seizures occurred in nine infants in the vaccine group and seven in the placebo group. Neither seizures nor deaths were clustered by time after immunization. No serious adverse reactions were attributed to the vaccine [11].

Antibody response to PRP. Of the infants, 51% and 59% had antibody levels >1 μg after the first and second doses of vaccine, respectively (table 7). The geometric mean concentration 2 months after the second dose was 1.35 μg/ml; 5 months later it dropped to 0.40 μg/ml.

We evaluated lot-to-lot variability in antibody responses to the vaccine (table 8). Geometric mean concentrations differed significantly between lots after dose 1 and dose 2. Differences were no longer evident on follow-up at 12 or 15–18 months of age. The magnitude of the differences was small and probably not clinically significant.

Incidence of invasive Hib disease. Incidence rates of Hib disease were analyzed up to 15 and 18 months of age to reflect the change in Indian Health Service vaccination policy. At 15 or 18 months of age, Navajo babies received the regularly scheduled Hib vaccination and were no longer considered to be at risk for purposes of the analysis.

As shown in table 9, when all enrollees were included, the intent-to-treat analysis of cases before 18 months yielded 1 case in the vaccine and 22 in the placebo group ($P < .001$; point estimate of efficacy [PEE], 95%; CI, 72%–99%). Includ-

Table 8. Antibody responses to *Haemophilus influenzae* type b capsular polysaccharide in infants vaccinated with different lots of PRP-OMP.

Lot, sample	*n*	Antibody concentration	% with concentration >0.15	% with concentration >1
CP298				
Prevaccination	210	0.16 (0.13–0.18)	44	9
After dose 1	174	1.27 (1.02–1.57)	92	57
After dose 2	144	1.69 (1.31–2.17)	92	66
Follow-up 1	79	0.42 (0.33–0.55)	84	22
Follow-up 2	24	0.25 (0.15–0.43)	63	13
CP749				
Prevaccination	668	0.16 (0.15–0.18)	45	10
After dose 1	614	0.87 (0.77–0.97)	89	49
After dose 2	516	1.23 (1.08–1.40)	91	56
Follow-up 1	221	0.39 (0.32–0.48)	74	23
Follow-up 2	71	0.47 (0.33–0.68)	73	27
CR132				
Prevaccination	103	0.19 (0.15–0.23)	53	10
After dose 1	92	1.18 (0.87–1.61)	91	57
After dose 2	72	1.64 (1.12–2.40)	92	65
Follow-up 1	30	0.39 (0.24–0.63)	77	33
Follow-up 2	13	0.33 (0.14–0.76)	62	31

NOTE. PRP-OMP, *H. influenzae* type b capsular polysaccharide linked to outer membrane protein complex of *Neisseria meningitidis*. Antibody concentrations (μg/ml, measured by radioactive antigen binding) are geometric means; 95% confidence intervals are in parentheses. Dose 1 was given at 2 months of age and dose 2 at 4 months; follow-up 1 was at 12 months and follow-up 2 at 15–18 months, before booster dose. *P* values, determined by analysis of variance by lot, were as follows: prevaccination, .439; after dose 1, .003; after dose 2, .042; follow-up 1, .918; follow-up 2, .196.

Table 9. Protective efficacy of *Haemophilus influenzae* type b (Hib) capsular polysaccharide conjugate vaccine (linked to outer membrane protein complex of *Neisseria meningitidis*) in Navajo infants.

Analysis, parameter	No. cases of Hib disease/no. in group		Point estimate of efficacy (%)	*P*	95% confidence interval	
	Vaccine	Placebo			One-sided*	Two-sided
Intent-to-treat						
After ≥1 dose, cases before						
18 months	1/2588	22/2602	95	<.001	77	72–99
15 months	0/2588	21/2602	100	<.001	85	81–100
Cases before second dose	0/2588	8/2602	100	.005	55	41–100
After 2 doses, cases before						
18 months	1/2056	14/2105	93	<.001	61	53–98
15 months	0/2056	13/2105	100	<.001	74	67–100
Strict protocol						
After 2 doses, cases before						
18 months	1/1913	14/1929	93	<.001	61	53–98
15 months	0/1913	13/1929	100	<.001	74	67–100
Cases before second dose	0/2451	6/2430	100	.014	35	15–100

NOTE. Infants who received second dose of vaccine after age specified in protocol were included only in intent-to-treat analysis. Data are from [11] (used with permission).

* Lower limit.

ing only children receiving two doses, there was 1 case in the vaccine ($n = 2056$) and 14 in the placebo group ($n = 2105$) ($P < .001$; PEE, 93%; CI, 53%–98%).

When the intent-to-treat analysis was modified to include only cases occurring before 15 months there were no cases in the vaccine and 21 in the placebo group ($P < .001$; PEE, 100%; CI, 81%–100%). When analysis was restricted to children receiving two doses there were no cases in the vaccine and 13 in the placebo group ($P < .001$; PEE, 100%; CI, 67%–100%).

After the first dose but before the second, no cases occurred in the vaccine group but 8 occurred in the placebo group ($P = .005$; PEE, 100%; CI, 41%–100%). Two were in children who had passed the protocol date for their second dose.

The one case of Hib disease (osteomyelitis) in a vaccine recipient was at 15½ months of age, 368 days after the second immunization. Levels of antibody to PRP were <0.125 μg/ml before first vaccination, 2.86 μg/ml just before second vaccination, 1.49 μg/ml 2 months after second vaccination, 0.14 μg/ml at 1 year of age, and 1.35 μg/ml 42 days after infection. Among the 22 cases of invasive Hib disease in the placebo group, 13 (59.0%) were meningitis.

At the end of the trial, all placebo recipients that could be located (52%, 1347) were given at least one dose of PRP-OMP.

We have continued our surveillance of the entire population on the Navajo Reservation. One additional case of Hib disease occurred in a child who received PRP-OMP in the study at 6 weeks and did not receive a second dose. Hib cellulitis was diagnosed at 7 months of age.

Discussion

Over the past 10 years, we have confirmed the high rates of Hib disease documented 18 years ago in Navajo infants [4] and have documented high rates of Hib disease in the Apache population. In the early 1980s, when Hib vaccines immunogenic in young infants were not available, we evaluated the safety and efficacy of passive immunization with BPIG to prevent Hib disease among Apaches [12]. BPIG was >80% efficacious when administered every 4 months during infancy. Studies of BPIG are summarized elsewhere in this issue [13].

Although hyperimmune globulin was efficacious in this population, its routine use was not feasible over a long time because of its relatively high cost and the need for repeated injections. Therefore, we evaluated antibody responses to several Hib vaccines in these populations.

Initial studies with unconjugated PRP vaccine demonstrated that the immune responses of Apache children were impaired compared to those of whites [8]. Subsequently, we demonstrated that Navajo children may also have lower immune responses to one of the conjugate vaccines (HbOC) compared with those of white children.

We noted that 6-week-old Navajo infants did not respond to one dose of HbOC. After the second and third doses, 58% and 92% of infants had antibody concentrations >1 μg/ml, respectively. In contrast, 68% of Navajo infants 2 months old had concentrations >1 μg/ml after a single dose of PRP-OMP. Since 30%–40% of cases of Hib disease in this population occur before 6 months of age, we conducted an efficacy trial using PRP-OMP.

In a preliminary dose-response study, we reconstituted lyophilized PRP-OMP with larger volumes of aluminum hydroxide adjuvant than the standard formulation. Infants thus received the standard dose (containing 15 μg of PRP) or 50%, 33%, or 25% of this dose absorbed to a constant amount of adjuvant. No significant differences in the geometric mean antibody responses were noted, although the proportion of children achieving 1 μg of antibody/ml declined with increasing doses ($P = .03$). It may be possible to reduce the dose of PRP-OMP without reducing serologic responses. However, before this can be recommended routinely, larger studies must be done to show that the proportion of nonre-

sponders is not increased with lower doses and to confirm that other populations have similar dose responses.

In the efficacy trial, a standard dose of PRP-OMP was given at 2 and 4 months of age. The efficacy of PRP-OMP was estimated to be 93% after two doses and 100% between the first and second dose.

Two concerns have been raised about the PRP-OMP efficacy study. First, can data generated in a population such as the Navajos be extrapolated to the general US population? Indeed, another Hib conjugate vaccine, PRP-D, was estimated to have >90% efficacy in Finnish infants [14, 15], but its efficacy in an Alaskan native population was only 35% [16]. Since Apache and Navajo infants have lower responses than whites to the unconjugated PRP vaccine [8] and may have lower responses to conjugate vaccines, the demonstration of efficacy of PRP-OMP in the Apache and Navajo populations is a rigorous test. One would expect high levels of efficacy in the general US population, in which vaccinees are likely to achieve higher serum anticapsular antibody responses to Hib vaccines than occur in Navajo and Apache populations.

Another concern about PRP-OMP is the three- to fivefold drop in antibody levels between 5 months (1 month after the second dose) and 15–18 months of age. Will cases occur in 12- to 18-month-old infants who do not receive a booster dose at 12 months of age?

The concentration of antibody to PRP that correlates with protection from Hib disease is still debated. The values thought to be predictive of protection are based on limited data. A concentration ≥0.15 μg/ml was estimated to be protective in agammaglobulinemia patients receiving immune globulin prophylaxis [17]. The concentration postulated to be protective against Hib disease, ≥1.0 μg/ml, was based on a large field trial in Finland with unconjugated PRP vaccine [18], a T cell–independent antigen. In that trial, 75% of 18- to 23-month-olds and 90% of 24-month-old children achieved antibody levels ≥1.0 μg/ml. The protective efficacy was calculated to be 90% in these groups. In the Navajo PRP-OMP vaccine efficacy trial, 9% of infants did not achieve levels >0.15 μg/ml and 41% did not achieve levels >1.0 μg/ml at 6 months of age. Nevertheless, the vaccine was >90% efficacious in preventing disease up to 18 months of age. It may be that the concentrations of antibody required for protection after immunization with T cell–independent antigens are higher than the concentrations required for protection after a T cell–dependent antigen, such as the Hib conjugate vaccines.

The one case of Hib disease in the vaccine group was in an infant who had an antibody level of 2.86 μg/ml 2 months after the first dose of vaccine and 1.49 μg/ml 2 months after the second dose but only 0.14 μg/ml at 12 months of age (3½ months before disease).

Recently, HbOC has been shown to provide protection after three doses in an open trial in California [19]. HbOC had high levels of efficacy after three doses and possibly after two doses. However, breakthrough cases of Hib disease occurred after a single dose. HbOC does not induce an antibody response after the first dose in most infants [20].

The reasons for the differences between these two conjugate vaccines are not well understood. HbOC appears to present PRP only as a T cell–dependent antigen. Consequently, T cell priming to the mutant diphtheria toxin protein carrier must occur before an antibody response is produced, usually with the second dose. A strong booster response is induced with the third dose [20].

In contrast, PRP-OMP appears to have properties of both a T cell–dependent and a T cell–independent antigen. The T cell–dependent nature of the vaccine is suggested by booster responses observed in many infants after the second dose [10] and in 18-month-old children who received a primary series of PRP-OMP during infancy [21]. Stein [22] suggested that PRP-OMP also produces direct proliferation of murine lymphocytes and may directly stimulate B cells as described for type 1 T cell–independent antigens. Alternatively, the outer membrane proteins or lipopolysaccharide contained in the OMP carrier may exert adjuvant effects by acting on T cells or macrophages [23].

Regardless of the mechanism of their action, both Hib conjugate vaccines licensed for use in infants have demonstrated a high level of clinical efficacy and promise to produce a dramatic reduction in the incidence of invasive Hib disease. We suggest that the more rapid serologic response to PRP-OMP and its protective efficacy after a single dose at 2 months offer an important advantage in populations with substantial rates of invasive Hib disease in young infants.

Ultimately, the degree and duration of protection provided by any of the licensed Hib conjugate vaccines in the general US population can be determined only by conducting postmarketing surveys after widespread use. Nevertheless, both PRP-OMP and HbOC have been licensed to immunize 2-month-old infants in the United States. Both have been shown to be >90% efficacious and have the potential to substantially reduce the morbidity and mortality of Hib disease.

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References

1. Broome CV. Epidemiology of *Haemophilus influenzae* type b infections in the United States. Pediatr Infect Dis **1987**;6:779–82.

2. Schlech WF III, Ward JI, Band JD, et al. Bacterial meningitis in the United States, 1978 through 1981. The National Bacterial Meningitis Surveillance Study. JAMA **1985**;253:1749–54.
3. Coulehan JL, Michaels RH, Hallowell C, et al. Epidemiology of *Haemophilus influenzae* type b 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. Ward JI, Margolis HS, Lum MKW, et al. *Haemophilus influenzae* disease in Alaskan Eskimos: characteristics of a population with an unusual incidence of invasive disease. Lancet **1981**;1:1281–5.
6. Ward JI, Lum MKW, Hall DB, et al. Invasive *Haemophilus influenzae* type b disease in Alaska: background epidemiology for a vaccine efficacy trial. J Infect Dis **1986**;153:17–26.
7. Losonsky GA, Santosham M, Sehgal VM, et al. *Haemophilus influenzae* disease in the White Mountain Apaches: molecular epidemiology of a high risk population. Pediatr Infect Dis **1984**;3:539–47.
8. Siber GR, Santosham M, Reid R, et al. Impaired antibody response to *Haemophilus influenzae* type b polysaccharide and low IgG2 and IgG4 concentrations in Apache children. N Engl J Med **1990**;323:1387–92.
9. Letson GW, Santosham M, Reid R, et al. Comparison of active and combined passive/active immunization of Navajo children against *Haemophilus influenzae* type b. Pediatr Infect Dis J **1988**;7:747–52.
10. Santosham M, Almeido-Hill J, Wolff M, et al. Safety and immunogenicity of a *Haemophilus influenzae* type b conjugate vaccine in a high risk American Indian population. Pediatr Infect Dis J **1991**;10:113–7.
11. Santosham M, Wolff M, Reid R, et al. Efficacy of a *Haemophilus influenzae* type b–*Neisseria meningitidis* outer membrane protein conjugate vaccine in Navajo infants. N Engl J Med **1991**;324:1767–72.
12. Santosham M, Reid R, Ambrosino DM, et al. Prevention of *Haemophilus influenzae* type b infections in high risk infants treated with bacterial polysaccharide immune globulin. N Engl J Med **1987**;317:923–9.
13. Siber GR, Thompson C, Reid GR, et al. Evaluation of bacterial polysaccharide immune globulin for the treatment or prevention of *Haemophilus influenzae* type b and pneumococcal disease. J Infect Dis **1992**:165(suppl 1):S129–33.
14. Eskola J, Kayhty H, Takala AK, et al. A randomized, prospective field trial of a conjugate vaccine in the protection of infants and young children against invasive *Haemophilus influenzae* type b disease. N Engl J Med **1990**;323:1381–7.
15. 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.
16. Ward J, Brenneman G, Letson GW, Heyward WL, Alaska *H. influenzae* vaccine study group. Limited efficacy of a *Haemophilus influenzae* type b conjugate vaccine in Alaska native infants. N Engl J Med **1990**;323:1393–401.
17. 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.
18. Peltola H, Kayhty H, Virtanen M, Makela PH. Prevention of *Haemophilus influenzae* type b bacteremic infections with the capsular polysaccharide vaccine. N Engl J Med **1984**;310:1561–6.
19. Black SB, Shinefield HR, Fireman B, Hiatt R, Polen M, Vittinghoff E. Efficacy in infancy of oligosaccharide conjugate *Haemophilus influenzae* type b (HbOC) vaccine in a United States population of 61,080 children. Pediatr Infect Dis J **1991**;10:97–104.
20. Madore DV, Johnson CL, Phipps DC, et al. Safety and immunogenicity of *Haemophilus influenzae* type b oligosaccharide–CRM_{197} conjugate vaccine in infants aged 15 to 23 months. Pediatrics **1990**;86:527–34.
21. Sood SK, Ballanco GA, Daum RS. Duration of serum anticapsular antibody after a two-dose regimen of an *Haemophilus influenzae* type b polysaccharide-*Neisseria meningitidis* outer membrane protein conjugate vaccine and anamnestic response following a third dose. J Pediatr **1991**;119:652–4.
22. Stein K. Thymus-independent and thymus-dependent responses to polysaccharide antigens. J Infect Dis **1992**;165(suppl 1):S49–52.
23. Donnelly JJ, Deck RR, Liu MA. Immunogenicity of a *Haemophilus influenzae* polysaccharide–*Neisseria meningitidis* outer membrane protein complex conjugate vaccine. J Immunol **1990**;145:3071–9.