Haemophilus influenzae Type b Conjugate Vaccine (Meningococcal Protein Conjugate) (PedvaxHIB®): Clinical Evaluation

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Although systemic infections caused by *Haemophilus influenzae* type b occur worldwide, detailed epidemiologic data are available in but a few countries.[1-3] The public health impact of morbidity, mortality, and serious sequelae from disease caused by *H influenzae* type b has stimulated the search for control strategies. In the United States now, active immunoprophylaxis is largely favored over treatment or prophylaxis with antibiotics. This preference stems from three major observations: that high mortality and morbidity persist despite the availability of potent antimicrobial agents, that antibiotic-resistant strains of *H influenzae* type b have emerged, and that implementation of antimicrobial prophylaxis on a large scale has been unsatisfactory. Moreover, universal vaccination has been projected as offering a higher economic benefit than other control strategies.[4]

A matter of more proximate importance, however, is the search for *H influenzae* type b vaccines that will confer protection to all age groups, including infants younger than 18 months of age and subpopulations specifically at risk for invasive disease caused by *H influenzae* type b. *Haemophilus* b conjugate vaccine (meningococcal protein conjugate), PedvaxHIB® (PRP-OMPC), is a conjugate *H influenzae* type b vaccine developed at Merck Sharp & Dohme Research Laboratories that now is undergoing extensive clinical evaluation to assess its prospects for disease control when first administered in early infancy. This is an interim report of results obtained in studies conducted in diverse locations throughout the United States.

METHODS

Study protocols were approved by Merck Sharp & Dohme Research Laboratories and the Institutional Review Board of each center. Written informed consent was obtained from the parents of subjects at the time of enrollment into each study. The study designs were open and noncomparative; only healthy subjects from 2 months to five years of age were eligible to participate. Administration of other vaccines was deferred for at least 1 week from the day of entering the study, except in those studies designed to evaluate the effect of concurrent vaccination.

Vaccine and Vaccination Schedule

The vaccine was prepared by Merck Sharp & Dohme Research Laboratories as described previously[5] and supplied as a lyophilized product that was reconstituted with an aluminum hydroxide diluent to yield a sterile suspension for intramuscular injection. Each dose was formulated to contain the following: 15 μg of *H influenzae* type b polyribosylribitol-phosphate (PRP); *Neisseria meningitidis* serogroup B outer membrane protein complex (OMPC), at a PRP to OMPC ratio maintained in the range of 0.05 to 0.10; approximately 225 μg of aluminum; thimerosal at 1:20 000 as a preservative; and 1 to 2.5 mg of lactose. Vaccine lot numbers 1069/C-P241, 1072/C-P298, 1080/C-P749, and 1085/C-R132 were used throughout the study. Subjects 12 months of age and older received a single injection of vaccine and those younger than 12 months received a second injection 2 months later. The regimen of two injections was later extended up to 17 months of age.

Safety Evaluation

Subjects were observed for a period of 15 minutes in the clinic for immediate postvaccination reactions. In addition, daily temperature, injection site, and systemic reactions were recorded for 5 days on

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standardized vaccination cards by parents. Serious adverse events occurring up to 14 days postvaccination were noted, irrespective of their association with vaccination.

Antibody Assays

Serum *H influenzae* type b anti-PRP was measured by a Farr-type radioimmunoassay and anti-PRP IgM, IgG, IgG1, and IgG2 antibodies by enzyme-linked immunosorbent assay.[6] Vaccine-induced antibody functional activities were assessed in assays for bactericidal activity,[6] opsonization plus intracellular bacterial killing,[7] and passive protection of infant rats.[6] All assays were performed at Merck Sharp & Dohme Research Laboratories with the exception of the assay for opsonization and intracellular bacterial killing, which was performed by Dr Barry Gray.[7]

Data Analysis

Statistical summaries and analyses of antibody titers were performed on log-transformed data as these variables appear normally distributed in the log scale. For the calculation of anti-PRP geometric mean titers, undiluted serum samples with undetectable antibody were assigned titer values equal to one half of the lowest detectable titer. Geometric mean titers were compared among lots using an analysis of covariance, with the log-transformed baseline titer as the covariate. Age as a category (2 to 6 months, 7 to 11 months, 12 to 17 months, 18 to 23 months, and ≥24 months) was included as a factor in the model. A paired *t* test was used to compare postvaccination to prevaccination levels or postinjection 2 to postinjection 1 levels of anti-PRP antibodies.

RESULTS

Only subjects with data available as of May 16, 1988, were included in this report. Subjects were recruited from five age groups (Table 1) to ensure a broad base of clinical experience, but with emphasis on the age groups at highest risk for disease caused by *H influenzae* type b. The racial composition of subjects was approximately 53% white, 23% black, 11% Native American (Navajo and Apache), 10% Hispanic, and 3% other. The male to female ratio was approximately 1:1.

Adverse Reactions

Vaccine safety was assessed in all subjects by the investigator, the parents, or both to ensure that significant adverse reactions were detected. However, specific safety data presented below were derived from subjects who completed their adverse reaction cards. The vaccine was well tolerated; no serious reactions related to vaccination were reported. At 6 hours, 24 hours, or 48 hours postvaccination, fever (temperature >38.3°C) was reported in ≤2.1% of vaccinees and local reactions (ery-

TABLE 1. Age Distribution and Number of Subjects Immunized With Each Vaccine Lot

Age, mo	Vaccine Lot					Total
	1069/C-P241	1072/C-P298	1080/C-P749	1085/C-R132	Other	
2–6	196	250	178	47	106	777
7–11	71	99	72	22	36	300
12–17	92	132	185	26	41	476
18–23	46	70	112	21	0	249
24–71	91	158	87	25	0	361
Total No. Vaccinated	496	709	634	141	183	2163

TABLE 2. Subjects With Fever or Indicated Local Reactions 6, 24, and 48 Hours After Vaccination

Age, mo	Reaction	Injection 1				Injection 2			
		No. of Subjects	6 h*	24 h*	48 h*	No. of Subjects	6 h*	24 h*	48 h*
2–17	Fever >38.3°C	1108	1.3	1.5	0.7	540	1.3	1.4	1.8
	Erythema >2.5 cm diameter	1191	0.2	0.8	0.3	573	0.8	1.2	0.7
	Swelling/induration >2.5 cm diameter	1191	0.5	1.3	1.2	573	0.7	1.9	2.4
18–71	Fever >38.3°C	420	2.1	1.4	2.1	...	...	...	...
	Erythema >2.5 cm diameter	454	0.0	0.4	0.2	...	...	...	...
	Swelling/Induration >2.5 cm diameter	454	0.4	0.9	0.9	...	...	...	...

* Values given in percentages.

thema, swelling/induration) in ≤2.5% (Table 2). The majority of these reactions subsided during the 5-day observation period, with no evidence of increases after a second injection. Systemic adverse reactions were reported less frequently among one-dose recipients than two-dose recipients. The most frequent systemic reactions within 48 hours postvaccination were irritability (19.9% to 25.9%), drowsiness (14.1% to 18.8%), diarrhea/nausea/vomiting (6.4% to 10.0%), miscellaneous respiratory symptoms (2.8% to 5.5%), and rash (1.5% to 2.9%). As with fever and local reactions, no increases in systemic reactions were observed after a second injection of vaccine.

Anti-PRP Responses

Of the vaccine lots tested, three (1072/C-P298, 1080/C-P749, and 1085/C-R132) manufactured to demonstrate clinical consistency were randomly administered to a subset of subjects in each age group. No statistically significant differences were observed among the three lots with respect to anti-PRP antibody levels induced; accordingly, the data for all lots were combined.

Anti-PRP geometric mean titers increased significantly from prevaccination to postvaccination values in all subjects from 2 months to 71 months of age after a single dose of vaccine (Figure). There was also a significant increase in geometric mean titer from postinjection 1 to postinjection 2. Detailed anti-PRP data are shown in Table 3. Antibody responses were age dependent, consistent with immunologic maturation. The vaccine induced greater than a twofold rise in anti-PRP antibody levels in ≥90% of subjects; ≥98% and 78% to 96% responded with >0.15 μg/mL and >1.0 μg/mL, respectively, after one injection. After completion of the recommended doses, 91% (range 88% to 100%)

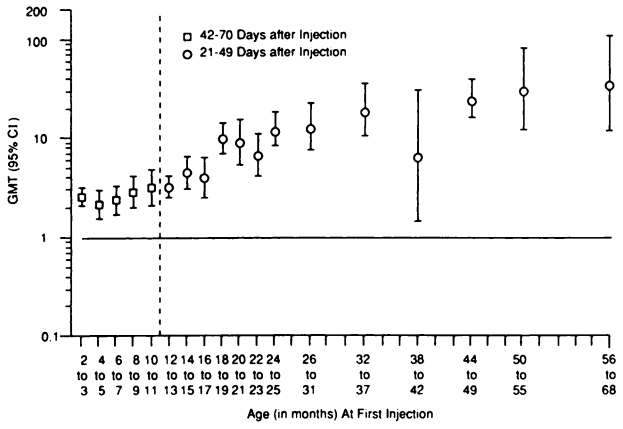

Figure Antipolyribosylribitol-phosphate response (micrograms per milliliter) to one dose of PedvaxHIB by age. GMT, geometric mean titer; CI, confidence interval.

TABLE 3. Anti-PRP Responses by Age*

Age, mo	Prevaccination			Postinjection 1†			Postinjection 2‡			
	% (Proportion) With Anti-PRP >0.15 μg/mL	% (Proportion) With Anti-PRP >1.0 μg/mL	GMT, μg/mL	% (Proportion) With Anti-PRP > 0.15 μg/mL	% (Proportion) With Anti-PRP > 1.0 μg/mL	GMT, μg/mL	% (Proportion) >2-fold Rise	% (Proportion) With Anti-PRP > 0.15 μg/mL	% (Proportion) With Anti-PRP > 1.0 μg/mL	GMT, μg/mL	% (Proportion) >2-fold Rise
2–6	38 (95/248)	2 (6/248)	<0.13	99 (236/239)	78 (186/239)	2.41	90 (216/239)	99 (186/187)	88 (164/187)	4.63	96 (179/187)
7–11	22 (26/121)	1 (1/121)	<0.13	98 (118/120)	78 (93/120)	2.91	91 (109/120)	100 (94/94)	94 (88/94)	7.41	100 (94/94)
12–17	29 (53/182)	2 (4/182)	<0.13	100 (182/182)	84 (152/182)	3.76	97 (177/182)	100 (32/32)	100 (32/32)	>8.99	100 (32/32)
18–23	43 (46/110)	5 (6/110)	0.14	99 (109/110)	96 (106/110)	8.88	99 (109/110)				
24–71	58 (84/145)	19 (28/145)	0.27	99 (144/145)	96 (139/145)	16.60	98 (142/245)				

* PRP, polyribosylribitol-phosphate; GMT, geometric mean titer.
† Response measured 1 month postvaccination in children 12–71 months of age or 2 months postvaccination in infants 2–11 months of age and 32 infants 12–17 months of age.
‡ Responses measured 1 month after injection.

TABLE 4. Anti-PRP Isotype and Subclass Antibody Responses by Age*

Age, mo	IgM			IgG			IgG1			IgG2		
	No. of Subjects	GMT, μg/mL		No. of Subjects	GMT, μg/mL		No. of Subjects	GMT, μg/mL		No. of Subjects	GMT, μg/mL	
		Pre	Post		Pre	Post		Pre	Post		Pre	Post
2–6	38	<0.10	0.13	36	<0.10	1.03	40	<0.60	5.22	17	<0.25	<0.25
7–11	24	<0.10	0.27	24	<0.10	1.77	21	<0.60	13.03	13	<0.25	<0.25
12–17	79	<0.10	0.65	78	<0.10	0.71	72	<0.60	3.94	43	<0.25	<0.25
18–23	42	<0.12	0.98	42	<0.10	2.57	47	<0.60	10.68	23	<0.25	0.32
≥24	60	<0.20	1.38	59	<0.10	3.57	60	<0.60	14.61	32	<0.25	0.41

* PRP, polyribosylribitol-phosphate; GMT, geometric mean titer; Pre, preimmunization; Post, postimmunization. IgG is not the sum of IgG1 and IgG2 because of the different assays used. Sensitivity of assays: 0.10 μg/mL for IgM and IgG, 0.6 μg/mL for IgG1, 0.25 μg/mL for IgG2.

of subjects younger than 18 months old achieved anti-PRP antibody levels of >1.0 μg/mL.

The vaccine induced anti-PRP antibodies of both IgG and IgM isotypes, with IgG predominating (Table 4). IgG1 response was observed in all age groups. However, only children 18 months of age and older had detectable IgG2 responses.

Vaccine-induced anti-PRP antibodies were biologically active in an in vitro assay for serum bactericidal activity and in an in vivo test for passive protection of infant rats from *H influenzae* type b bacteremia (Table 5). Functional activities appeared to correlate with levels of antibodies and with age of vaccinees but were observed in all groups. Vaccine-induced antibodies were similarly functional in an additional in vitro assay for opsonization and intracellular bacterial killing.[7]

TABLE 5. Biologic Activities of Anti-PRP Induced by PedvaxHIB in the Sera of Vaccinees*

Anti-PRP, μg/mL	Age, mo	Proportion (%) of Vaccinees With Serum Bactericidal Assay Titer >5†	Proportion (%) of Vaccinees With Sera That Passively Protected Infant Rats
0.2–1.0	<12	4/10 (40)	1/5 (20)
	12–17	8/11 (73)	3/3 (100)
	≥18	5/6 (83)	3/4 (75)
1.0–3.9	<12	20/37 (54)	27/31 (88)
	12–17	32/35 (91)	21/21 (100)
	≥18	30/31 (97)	22/24 (92)
4–11	<12	23/30 (76)	1/1 (100)
	12–17	30/30 (100)	3/3 (100)
	≥18	27/27 (100)	1/1 (100)

* PRP, polyribosylribitol-phosphate. Sera were obtained 1 month after the last or only dose.
† Reciprocal serum dilution.

DISCUSSION

PedvaxHIB was well tolerated in 2163 subjects who received more than 3200 doses of the vaccine in this multicenter study. Side effects were minor, transient, and self-limited. Subjects who were given a second dose of vaccine did not experience an increase in the spectrum or severity of side effects. In addition, when the vaccine was given concurrently with measles-mumps-rubella (MMRII) or diphtheria-tetanus-pertussis and oral poliovirus vaccines at separate sites, the rates of adverse reactions did not differ from those seen when the individual vaccines were given alone.[8]

The vaccine was highly immunogenic in all age groups tested, including infants as young as 2 months of age. Of infants immunized beginning at 2 to 6 months of age, 99% had anti-PRP antibody levels of >0.15 μg/mL 2 months later, with 78% and 88% of vaccinees having antibody levels of >1.0 μg/mL after one or two injections of vaccine, respectively. Although the precise protective level of vaccine-induced anti-PRP antibodies is unknown, a level of >1.0 μg/mL has been suggested from a large-scale field trial in Finland that confirmed the protective efficacy of *H influenzae* type b polysaccharide vaccine.[9] Based on that estimate, approximately 80% of infants immunized with PedvaxHIB might be expected to be protected from invasive disease caused by *H influenzae* type b from approximately 3 to 4 months of age on the basis of anti-PRP alone. The prospects for such early protection could be of significant import to populations in which the incidence of disease caused by *H influenzae* type b is high in early infancy.

PedvaxHIB is compatible with other pediatric vaccines. In a separate study of subjects ≥15 months old, no impairment of immune responses to individual vaccine antigens tested were noted when PedvaxHIB was given concurrently with measles-mumps-rubella (MMRII) vaccine.

PedvaxHIB has been investigated previously in subpopulations with increased susceptibility to invasive disease caused by *H influenzae* type b or hyporesponsiveness to *H influenzae* type b polysaccharide vaccines. Granoff et al[10] studied 30 children with anti-PRP antibody levels of <2.0 μg/mL in sera obtained after invasive *H influenzae* type b infections despite prior immunization with polysaccharide vaccines. All (10) children immunized with PedvaxHIB, compared with 5 of 16 (31%) reimmunized with a polysaccharide vaccine, achieved

anti-PRP antibody levels of >1.0 μg/mL. PedvaxHIB was also immunogenic in black children who do not express Km(1) immunoglobulin allotype,[11] a condition associated with impaired antibody response to polysaccharide vaccines.[12]

There is considerable evidence that the mechanisms by which anti-PRP antibodies protect against invasive disease caused by *H influenzae* type b include complement-mediated bacteriolysis and opsonization. Serum antibodies induced by PedvaxHIB are functional in in vitro assays for bacteridical activity and opsonization with intracellular bacterial killing. In addition, the antibodies confer protection passively to infant rats experimentally infected with *H influenzae* type b organisms. These findings strongly suggest that PedvaxHIB evokes antibodies relevant for protection against invasive disease caused by *H influenzae* type b.

Other *H influenzae* type b conjugate vaccines are now commercially available (ProHIBit/PRP-D, HibTITER/HbOC) or are under clinical investigation (PRP-tetanus toxoid conjugates). Comparisons of levels of antibodies induced by different conjugate vaccines recently have been reported by investigators who used a single radioimmunoassay procedure for each comparison. In one study, PedvaxHIB induced higher antibody levels after a single injection in infants 2 to 3 months of age than did HibTITER in a comparable age group (anti-PRP geometric mean titer of 1.04 μg/mL vs 0.35 μg/mL).[13] After two injections, there was no significant difference in antibody levels induced by the two vaccines. Ward et al[14] found that PedvaxHIB was more immunogenic after one injection given at 2 months of age (anti-PRP geometric mean titer of 1.77 μg/mL) than ProHIBit after three injections (anti-PRP geometric mean titer of 0.3 μg/mL) given at 2, 4, and 6 months of age in a group of California infants. Thus, *H influenzae* type b conjugate vaccines differ in their immunologic properties and may, therefore, also differ in their ability to protect children and infants against invasive disease caused by *H influenzae* type b. Further studies are in progress to define the potential clinical usefulness of PedvaxHIB in the control of invasive disease caused by *H influenzae* type b in infants who are at highest risk.

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