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Immunological Characterization of Conjugated *Haemophilus influenzae* Type b Vaccine Failure in Infants

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Infant vaccination with conjugated *Haemophilus influenzae* type b (Hib) vaccine is highly effective in protecting against invasive Hib infections, but vaccine failures do occur. Twenty-one vaccine failures are reported since the introduction of the Hib conjugate vaccine in The Netherlands. Of the 14 evaluable patients, 6 children showed no antibody response to Hib polysaccharide in convalescent-phase serum (immunoglobulin [Ig] G anti-Hib level <1.0 μg/mL), including 1 child with hypogammaglobulinemia and 1 child with IgG2 deficiency. After revaccination, almost all children developed anti-Hib antibodies. In case of Hib vaccine failure, case investigation should be performed, including measurement of serum Ig concentrations as well as specific anti-Hib antibodies. Invasive Hib disease after infant conjugate Hib vaccination may be the presentation of an underlying immunodeficiency, but more often, only a decreased antibody response to Hib is found; revaccination with conjugated Hib vaccine is advised.

Infant vaccination with conjugated *Haemophilus influenzae* type b (Hib) vaccine is highly effective in protecting against invasive Hib infections [1, 2]. In The Netherlands, vaccination with conjugate Hib vaccine has been incorporated into the National Vaccination Program for children born after 1 April 1993. At the time of introduction, the Hib vaccine was given at the ages of 3, 4, 5, and 11 months, concomitantly with the diphtheria, tetanus, and pertussis vaccine and the poliomyelitis vaccine. Unlike the policy of several other countries, no catch-up vaccinations were given for children aged <5 years. Since January 1999, infants have been vaccinated at 2, 3, 4, and 11 months of age.

In the years after the introduction of Hib vaccination, the number of cases of invasive Hib disease dropped impressively [3, 4]. But invasive Hib disease did not disappear completely. This might be expected, because cases of invasive Hib disease still occur in infants who are not yet fully vaccinated, and vaccine coverage in The Netherlands, although high (95.5% of all infants received at least 3 doses of Hib vaccine; January 1997), is not complete. But it is also true that vaccine failures occurred in children who were completely (3 or 4 times) vaccinated with the conjugate vaccine. We here report the clinical and immunological follow-up for the 19 children who developed invasive Hib disease despite having received at least 3 vaccinations as an infant.

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METHODS

Surveillance of invasive Hib infections. Since 1975, invasive Hib isolates and surveillance data on Hib meningitis in The Netherlands are collected by The Netherlands Reference Laboratory for Bacterial Meningitis

Table 1. Clinical characteristics and serum Ig concentrations for 14 patients for whom conjugated *Haemophilius influenzae* type b vaccine failed.

Patient	Sex	Age, mo	Hib disease	Ig concentrations, g/L						
				IgA	IgG	IgM	IgG1	IgG2	IgG3	IgG4
A	F	24	Meningitis	0.04[a]	3.3[a]	0.73	2.8[a]	0.15[a]	0.25	<0.1
B	M	13	Meningitis	0.65	9.9	0.9	8.2	0.9	0.55	0.15
C[b]	M	10	Meningitis	0.17	5.5	1.37	4.0	0.3	0.2	<0.1
D	M	27	Meningitis	0.83	9.5	1.16	5.9	1.65	0.6	0.55
E	F	36	Meningitis	1.23	7.7	1.5	7.3	1.05	0.1	0.1
F	M	11	Meningitis	0.36	6.4	0.94	5.1	<0.1[a]	0.45	<0.1
G	M	33	Epiglottitis	0.75	6.0	1.5	5.7	0.9	0.4	<0.1
H	F	28	Meningitis	0.45	11.7	0.95	9.4	1.3	0.6	0.3
I	M	62	Meningitis	1.11	11.7	1.84	9.8	0.9	0.5	<0.1
J	F	18	Meningitis	0.78	9.9	2.83	8.2	1.41	0.74	<0.05
K	M	56	Epiglottitis	0.91	9.0	0.96	5.3	1.65	0.25	0.65
L	M	38	Meningitis	1.00	9.8	1.65	7.3	1.5	0.2	0.1
M	M	25	Epiglottitis	0.60	9.3	1.3	9.1	1.5	0.2	0.4
N	M	42	Meningitis	3.62	12.8	1.5	10.4	2.61	0.27	0.25
O[c]	F	28	Meningitis	—	—	—	—	—	—	—
P	F	33	Meningitis	—	—	—	—	—	—	—
Q	F	9	Meningitis	—	—	—	—	—	—	—
R	M	25	Meningitis	—	—	—	—	—	—	—
S	F	13	Cellulitis	—	—	—	—	—	—	—
T[d]	M	22	Meningitis	—	—	—	—	—	—	—
U	M	8	Meningitis	—	—	—	—	—	—	—

NOTE. Serum samples used for determination of Ig concentrations were obtained at onset of disease for all patients except patients D, G, I, and N, for whom convalescent serum samples were used. Serological data were not available for patients O–U. Normal values for serum Ig concentrations for children in different age groups are as follows: children aged 15 months–4 years: IgA 0.30–0.90 g/L; IgG 4.6–7.8 g/L; 12–24 months: IgG1 2.0–6.5 g/L; IgG2 0.35–2.2 g/L; and 6–12 months: IgG2 0.10–0.50 g/L.

[a] IgG and IgG2 values below the normal range for age.

[b] Patient C experienced a first meningitis (unknown cause) at the age of 3 months; he received antibiotic prophylaxis from 10 to 33 months of age.

[c] Patient O died from complications of *Haemophilus influenzae* type b meningitis.

[d] Patient T had trisomy 16p.

(RBM) in Amsterdam. Since 1994, *H. influenzae* isolates from all normally sterile sites are sent to the RBM. These isolates are identified and typed for capsular polysaccharide serotype by use of coagglutination [5]. Capsular polysaccharide serotyping was controlled by PHLS Haemophilus Reference Unit, Oxford, United Kingdom.

Collection and testing of serological data. The laboratory of the department of Immunology, Wilhelmina Children's Hospital, University Medical Center Utrecht, is a central facility for anti-Hib antibody determinations. Serum samples from patients with invasive Hib infections are sent in by clinicians. Since 1999, active immunological follow-up of patients with invasive Hib infections despite vaccination has been done by combining the serological data with data from the RBM system.

We determined anti-Hib antibody titers for IgG, IgG1, and IgG2 (and IgM; data not shown) by use of ELISA, as described elsewhere [6], then calibrated with a standard anti-Hib hyperimmune serum containing 27.2 μg/mL IgG–anti-Hib, 4.05 μg/mL IgG1–anti-Hib and 9.48 μg/mL IgG2–anti-Hib. Total concentrations of IgG, IgA, IgM, and IgG subclasses were determined by use of radial immunodiffusion in serum samples obtained at onset of illness, except for patients D, G, I, and N, for whom the serum samples obtained at convalescence were used.

Vaccine failures. Cases of invasive Hib infection are defined as attributable to vaccine failure when they occur despite at least 3 vaccinations with the conjugated Hib vaccine (or after 1 vaccination in a child aged >1 year). In The Netherlands, the Hib vaccine used is the PRP-T vaccine (polyribosylribitol-phosphate conjugated to tetanus toxoid; Pasteur Mérieux Sérums et Vaccins)

Subjects. We obtained additional clinical information and evaluated the immune status of patients who developed invasive Hib disease despite 3–4 vaccinations. Invasive Hib infection

was considered proven if Hib was isolated from culture of blood or CSF samples, or both. Vaccination records were checked. Serum samples were taken at the time of disease and after reconvalescence (3–4 weeks after the onset of the disease). For patients with IgG anti-Hib antibody concentrations <1.0 μg/mL in serum samples obtained 3–4 weeks after the onset of the disease, revaccination with the Hib vaccine was recommended. Booster vaccination was also given when anti-Hib antibodies were slightly above this level. In both cases, informed consent was obtained. Three to four weeks after revaccination, IgG, IgG1, IgG2, and IgM anti-Hib antibody concentrations were determined again. Vaccination was repeated in case of persistently low antibody concentrations.

RESULTS

Hib vaccine failures. In the 7 years after the introduction of the Hib vaccination program for infants in The Netherlands, a total of 1.13 million children received at least 3 doses of Hib vaccine. Twenty-one true failures of the vaccine were reported: 17 cases (81%) of Hib meningitis, 1 case (5%) of Hib cellulitis, and 3 cases (19%) of Hib epiglottitis. Bacterial isolates taken from 19 of these patients (90%) were directly sent to the RBM. From 2 additional (Hib epiglottitis) patients (10%), bacterial isolates from blood were typed at the local clinical microbiology laboratories, and serum samples of these patients were sent to the laboratory of the Department of Immunology, University Medical Center Utrecht. Checking of vaccination data confirmed that these 2 patients failed to respond to vaccine too. The mean age of the patients with meningitis was 26 months (range, 9–62 months; median, 25 months); the mean age of the patients with epiglottitis was 38 months (range, 25–56 months; median, 33 months).

Clinical information was obtained for 18 (86%) of the 21 children (11 boys and 7 girls). One of them (patient C) experienced another episode of meningitis of unknown cause at the age of 3 months. After a second bout of (Hib) meningitis at the age of 10 months, he received antibiotic prophylaxis until the age of 33 months. Patient A did not experience other invasive infections before experiencing Hib meningitis. She received antibiotic prophylaxis after the diagnosis of hypogammaglobulinemia. A third patient (patient T) was known to have trisomy 16p. Apart from a girl aged 2.5 years, (patient O), who died as a result of Hib meningitis, all children recovered without major sequelae. Importantly, they experienced no further serious infections during the follow-up period. All but 1 patient (patient F) were white. Heights and weights of all children were within the normal range (±2 SD) for age according the Dutch standard curves.

Serological investigations. Serum samples were available for 14 patients (67%). Results of the analysis of humoral immunity are shown in table 1. Serum Ig concentrations were within the normal range (±2 SD) for age in 12 (86%) of the 14 patients. In patient F, the serum concentration of IgG2 was >2 SDs below the mean concentration for his age. Patient A was shown to have hypogammaglobulinemia, with low concentrations of IgA and IgG, as well as low concentrations of IgG1 and IgG2.

Table 2 shows specific anti-Hib antibody concentrations in acute-phase serum samples, convalescent-phase serum samples, and in samples obtained after revaccination. Most of the patients had low (or unknown) anti-Hib antibody titers at the time of disease, with the exception of patient M, who had antibody concentrations well above the level considered protective. On the base of analysis of convalescent serum samples, 2 patient groups could be distinguished: group 1, comprised of patients who had persistently low anti-Hib antibody titers after invasive infection (patients A–F); and group 2, comprised of patients who had an IgG anti-Hib antibody response (anti-Hib antibody concentration >1 μg/mL) after the invasive Hib infection (patients G–N). The mean age of patients of group 1 was significantly lower than that of group 2 (20 ± 10 vs. 38 ± 15 months; $P < .05$, t test).

Two (33%) of the 6 patients in group 1 were subsequently lost to serological follow-up. The 4 other patients (67%) were revaccinated. The 3 younger of these 4 (patients B, C, and F) received their fourth Hib vaccination at the regularly scheduled time; the other patient (patient E), who had completed her series of 4 Hib vaccinations 2 years before the onset of disease, received a fifth vaccination with PRP-T. Three patients in group 2 were revaccinated, 2 of them (patients G and H) because their anti-Hib antibody titers had increased only marginally above the minimal protective level. All but 1 (patient F) of the 12 evaluable patients (92%) ultimately achieved IgG anti-Hib antibody concentrations >1.0 μg/mL. The patient that did not respond was the youngest in this series.

Most of the patients who responded who had high antibody titers after their Hib infection (group 2) developed predominantly IgG1 anti-Hib antibodies; only patient J produced IgG1 and IgG2 in equal amounts. In patients B and C, revaccination with PRP-T after invasive infection with Hib resulted in an IgG1 anti-Hib response. Both patients were young at the time of disease and revaccination. In the older patients, relatively more IgG2 was produced after booster vaccination, although only 1 patient (patient E) showed an IgG2-dominated antibody response.

DISCUSSION

Invasive Hib infections such as meningitis and epiglottitis are primarily diseases of early childhood, occurring predominantly in children <2 years of age, although considerable morbidity

Table 2. Concentrations of antibody to *Haemophilus influenzae* type b during the acute phase (0), after convalescence (I), and after revaccination (II and III) for 14 patients for whom *Haemophilius influenzae* type b vaccine failed.

	IgG anti-Hib, μg/mL				IgG1 anti-Hib, μg/mL				IgG2 anti-Hib, μg/mL			
Patient	0	I	II	III	0	I	II	III	0	I	II	III
A	1.5	<	—	—	2.25	<	—	—	<	<	—	—
B	1.2	<	9.1	96	0.90	<	10.4	215.8	<	<	<	4.44
C	<	<	65	—	<	<	75.5	—	<	<	2.73	—
D	—	<	—	—	—	1.35	—	—	—	<	—	—
E	<	<	7.3	—	1.35	<	4.5	—	<	<	16.7	—
F	<	<	<	—	1.80	0.9	0.9	—	<	<	<	—
G	—	1.35	2.7	18	—	1.8	1.8	59.3	—	<	<	11.3
H	<	1.5	27	—	<	0.9	—	—	<	<	—	—
I	—	3.3	—	—	—	2.25	—	—	—	<	—	—
J	<	9	—	—	<	4.05	—	—	<	5.12	—	—
K	<	22	6.4	—	0.90	29.7	12.1	—	<	4.78	3.41	—
L	<	23	—	—	<	23.8	—	—	<	1.02	—	—
M	23	27	—	—	12.6	13.9	—	—	1.37	0.68	—	—
N	—	127	—	—	—	28.8	—	—	—	4.44	—	—

NOTE. The lowest levels of detection were as follows: for of IgG anti-Hib antibody, 0.9 μg/mL; for IgG1 anti-Hib antibody, 0.45 μg/mL: and for IgG2 anti-Hib antibody, 0.34 μg/mL. Antibody concentrations below these values are shown as <. Concentrations of IgG anti-Hib antibody in control subjects were as follows: after 3 vaccinations in a control group aged 9–10 months (mean age, 9.6 months; median, 10 months; $n = 83$), 2.8 μg/mL (range, 0.05–6.8 μg/mL); after 4 vaccinations in a control group aged 24–43 months (mean age, 31 months; median 31 months; $n = 221$), 3.2 μg/mL (range, 0.05–64.1 μg/mL).

is seen until 5 years of age [7]. This age-related susceptibility to Hib disease correlates with the absence of serum bactericidal antibodies to Hib polysaccharide. Natural antibodies specific for Hib polysaccharide begin to appear at ~2 years of age and reach adult levels by ~5 years. Hib polysaccharide by itself does not function as an effective vaccine in the primary target population [8]. Covalent conjugation of protein carriers to Hib polysaccharide converts it into an effective immunogen, capable of inducing both IgG antibody production and memory in infants [9, 10]. Vaccination with conjugated Hib vaccine has resulted in protection against Hib disease and a rapid decline of the number of cases of invasive Hib disease [11].

In The Netherlands, the Hib conjugate vaccine also proved to be successful, with an efficacy of 99.4% [4]. Before the introduction of the conjugate Hib vaccine in the National Vaccination Program of The Netherlands, it was estimated that there were ~700 invasive Hib infections per year in The Netherlands, of which 90% occurred in children <5 years of age. About 50% of these invasive Hib infections developed into meningitis, and 15%–30% developed into epiglottitis [12]. The highest incidence of Hib meningitis was found in children aged 6–12 months, whereas Hib epiglottitis concerned mainly children 2–3 years of age. After the introduction of Hib vaccination in 1993, the number of invasive Hib infections decreased steadily and sharply: in 1994, 106 cases were reported; in 1995, 37; in 1996, 18; and in 1997, 7. The vaccine truly failed in only a minority of these patients; most of the invasive Hib infections occurred in children who had not been vaccinated because their religion did not allow it, because they were not covered by the health care system, or because they were not yet fully vaccinated because of their age.

We report the 21 real failures of vaccine that have occurred in The Netherlands since the introduction of the Hib conjugate vaccine. The average age of the affected children was higher than that of affected children in the previous unvaccinated population: 26 months for the patients with meningitis and 38 months for patients with epiglottitis. The surveillance data obtained during the first 3 years after the introduction of the Hib vaccine showed an average age of the patients with invasive Hib infections of 7.4 months [4]. The surveillance data obtained during the period from July 1997 to July 2000, however, showed an increase in average age of the Hib patients to 15.5 months (unpublished data). The age shift is most likely due to herd immunity because of reduced circulation of Hib after the introduction of vaccination [13, 14].

In the literature [15], 35%–40% of children who developed invasive Hib disease after a single dose of conjugated Hib vaccine at the age of >15 months were described as having subnormal concentrations of serum Igs. Specifically, a high prevalence of subnormal concentrations of IgG2 was observed [15, 16]. In contrast, reports of Hib polysaccharide vaccine failures in children aged >18 months showed a low incidence of im-

munodeficiency: almost all of these children had normal serum concentrations of Ig, including IgG2 [17]. These data suggested immunological differences between populations of children with polysaccharide vaccine failure and those with conjugate vaccine failure [16]. However, these results were obtained in children vaccinated at >15 months of age. In our series of Hib conjugate vaccine failures after infant immunization, 1 of the 14 evaluable children had hypogammaglobulinemia, and 1 had low serum IgG2 concentrations. Although there are only a few patients, this suggests that the frequency of humoral immunodeficiency among infants with Hib conjugate vaccine failure (14% in our series) is not different than it is among children vaccinated at >15 months of age who have conjugate vaccine failure or among children who have polysaccharide vaccine failure (10%) after 18 months of age [16].

We evaluated the specific anti-Hib antibody production in our patients. An important question is whether vaccine failure resulted from insufficient levels of circulating antibody after vaccination or from a qualitatively different and suboptimally protective antibody response. In 9 of 10 patients for whom data were available, we found low levels of specific IgG anti-Hib antibodies at the time of disease. These data suggest that these children, similar to children with polysaccharide vaccine failure [16], are low-antibody responders to vaccination. However, low levels of anti-Hib antibodies are more frequently observed in this age group [18] (G.T. Rijkers, unpublished data). In considering the correlation of disease susceptibility with antibody concentrations after Hib conjugate vaccination, account must also be made of the capacity of conjugate vaccines to induce immunological memory [19]. Even infants who fail to show a serum antibody response to immunization with conjugate vaccine may be protected from disease as a result of immunologic priming and the ability to develop a rapid increase in anti-Hib antibody. However, immunologic priming by conjugate Hib vaccine might not always be sufficient to confer protection against invasive Hib disease: some children show high antibody responses to the disease (patient N) [1, 15] and thus were primed to fight Hib, but nevertheless had acquired the invasive Hib infection.

Evidence of priming by the conjugate vaccination was absent in the 6 patients of group 1: the 2 patients with the Ig deficiency (patients A and F), and 4 of 12 children with normal serum Ig concentrations. They had low anti-Hib antibody concentrations in convalescent serum samples after invasive Hib disease. The failure to see an antibody response in younger children after infection (convalescence) is consistent with impaired capacity of young children to generate antibodies to unconjugated polysaccharides. A defective antibody response to Hib infection is also found in children with Hib polysaccharide vaccine failure. In contrast, children with conjugate vaccine failure after vaccination at age >15 months appear to have normal or even increased antibody responses [16], more like the children of group 2.

The serum antibody concentration sufficient to confer protection is not known with certainty, but on the basis of epidemiologic and animal studies, it is estimated to be 0.1–1.0 μg/mL [20–23]. Apart from the antibody concentration, isotype distribution, avidity, and complement mediated bactericidal capacity, all may contribute to the protective antibody concentration [24–26].

The ability to mount an antibody response after Hib vaccination or disease is, in part, genetically determined. This could play a role in the susceptibility to invasive Hib infections [27–29]. However, ultimately, almost all our patients developed anti-Hib antibodies. Populations of several ethnic backgrounds do respond to vaccination with Hib conjugate vaccines but need several extra booster vaccinations [27]. Navajo Indians carry a variant A2 V_κ light chain gene (a gene that is used in 60% of anti-Hib antibodies), which may explain their aberrant pattern of anti-Hib antibody development [30]. Molecular analysis did not reveal this variant light chain gene in patient B or his father, who failed to mount an IgG anti-Hib antibody titer >1.0 μg/mL after a single Hib conjugate vaccination, nor did it indicate the presence of another abnormality in the A2 V_κ light chain gene (data not shown).

The causes of Hib conjugate vaccine failure are probably multiple and complex. Inadequate delivery of the vaccine might be a major reason. In most children, the vaccine's failure does not appear to be the result of the presence of hypogammaglobulinemia or absence of certain V-region genes. Certain Ig allotypes are associated with susceptibility to polysaccharide encapsulated bacteria [31] and risks of vaccine failure [32]. Risk of infection with encapsulated bacteria is also increased in patients with the unfavorable Fc γ receptor IIa polymorphism [33, 34]. The number of vaccine failures in our series for whom we had DNA available was to small to make firm conclusions with respect to a possible relationship of Hib vaccine failure with Ig allotypes or Fc receptor polymorphisms.

In our series of 21 children for whom Hib vaccine failed (of whom 14 had immunological evaluation performed), we found only 1 patient who had a recognized immunodeficiency, hypogammaglobulinemia, that accounted for the failure of the vaccine. For the other patients, the Hib disease apparently was an isolated event. Many of the patients' bodies failed to mount antibody responses to Hib, therefore placing the patient at an increased risk of developing second episodes of the disease. Revaccination was able to overcome this impaired antibody response. We conclude that it is worthwhile to perform case investigation, including measurement of serum Ig levels as well as specific anti-Hib antibodies, in case of Hib vaccine failure. Booster vaccination may be necessary.

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