Clinical and Immunological Risk Factors Associated with *Haemophilus influenzae* Type b Conjugate Vaccine Failure in Childhood

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***Haemophilus influenzae* type b (Hib) conjugate vaccines have proved extremely efficacious in healthy children. True Hib vaccine failures are rare. Hib conjugate vaccines were introduced for routine immunization in the United Kingdom and the Republic of Ireland in 1992. Coincident with this, active prospective and national surveillance via pediatricians, microbiologists, and public health physicians was commenced to assess the clinical and immunological factors associated with vaccine failure. During the 6 years of the study, 115 children with true vaccine failure were reported. Of the children who were vaccinated before 12 months of age, a clinical risk factor was detected in 20%, an immunological deficiency was detected in 30%, and one or both were detected in 44%. Children who were vaccinated after 12 months of age were more likely to have one or both factors (67%). Thirty percent (33 of 105) of children with true vaccine failure had a low Hib antibody response (concentration, <1.0 μg/mL) after disease, but the majority then responded to a further dose of Hib vaccine. Children who develop Hib disease despite vaccination deserve further clinical and immunological evaluation.**

The first vaccine to be developed against *Haemophilus influenzae* type b (Hib) was composed of polyribosylribitol phosphate (PRP), the organism's capsular polysaccharide. An early trial of this vaccine demonstrated >90% efficacy in children who received vaccine when they were ⩾18 months of age, but it provided no protection in younger children [1]. The conjugation of PRP to protein has lead to the development of Hib vaccines with enhanced immunogenicity in infants and the ability to induce immunological memory. The subsequent implementation of these vaccines has resulted in dramatic reductions in the rate of Hib disease [2].

Despite the impressive efficacy of Hib conjugate vaccines, a very few vaccinated children still develop invasive Hib disease and are therefore considered to have vaccine failure. Given the excellent immunogenicity of conjugate vaccines in healthy children as well as in children with underlying diseases (e.g., sickle cell disease [3] and congenital asplenia [4]) and those with bone marrow transplants [5], rare vaccine failures are worthy of further investigation. Holmes and Granoff [6] detected hypogammaglobulinemia in 40% of 25 children with conjugate vaccine failure; others describe associations with severe burns [7], HIV [8], a CSF shunt [9], coeliac disease, Down's syndrome, and holoprosencephaly [10].

Since 1 October 1992, Hib conjugate vaccines have been used routinely in the United Kingdom (UK) and the Republic of Ireland (ROI). The Hib polysaccharide-tetanus conjugate PRP-T (Pasteur-Merieux, Lyon, France) was used initially in the UK for primary vaccination of children <12 months of age, and the mutant diphtheria toxoid conjugate HbOC (Wyeth-Lederle, Pearl River, NY) was used as a single dose in a catch-up program for children 12–48 months of age. In the ROI, HbOC was used for both primary and catch-up vaccination. In the UK, primary vaccination is recommended at 2, 3, and 4 months of age, and in the ROI, it is recommended at 2, 4, and 6 months of age. A further dose is not given in either schedule.

After the introduction of routine vaccination against Hib, we initiated a prospective national study to detect cases of invasive *H. influenzae* (Hi) disease in vaccinated children. This allowed us to assess the clinical and immunological factors associated with Hib conjugate vaccine failure in childhood.

Patients and Methods

This study was performed under the auspices of the British Paediatric Surveillance Unit (BPSU) of the Royal College of Paediatrics and Child Health. The BPSU has a program of active surveillance for selected rare pediatric conditions in the UK and the

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This study was approved by the Central Oxford Research Ethics Committee (Oxford, United Kingdom).

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Table 1. Clinical presentations of 106 children with true vaccine failure who were vaccinated against *Haemophilus influenzae* type b at ≤1 year of age.

Clinical presentation	Patients, no. (%)[a]
Meningitis	65 (61)
Epiglottitis	20 (19)
Pneumonia	6 (6)
Septic arthritis or osteomyelitis	6 (6)
Bacteremia	5 (5)
Cellulitis	3 (3)
Pericarditis	1 (1)

[a] The sum of the percentages exceeds 100% because of rounding.

ROI. More than 90% of pediatricians routinely report to the BPSU by completing a report card that is sent to them on a monthly basis.

For the first phase of the study (1 October 1992 to 1 November 1995), pediatricians were requested to report any child <10 years of age who had invasive Hi disease and who had received Hib conjugate vaccine. From 1 November 1995 to 1 October 1998, the case definition was broadened to include all children <16 years of age who had invasive Hi disease, regardless of vaccination status. "Invasive disease" was defined as isolation of Hi from a normally sterile site or as a positive Hib antigen test result combined with a clinical picture compatible with invasive Hi disease. Microbiologists were informed about the study and contributed to surveillance.

Early notification by telephone was requested. The pediatrician was sent a questionnaire requesting clinical, demographic, and laboratory information. In the ROI, this was coupled with active laboratory surveillance in which the investigator telephoned all laboratories serving pediatric populations every 2 weeks. As a result, notification of cases could come from pediatricians, microbiologists, and public health physicians. The dates of all primary immunizations were obtained from the child's general practitioner or, if necessary, from the district child health immunization computer records.

The local microbiologist was contacted and was asked to send the isolate to the Haemophilus Reference Unit, Oxford, where the identity was verified by means of standard slide agglutination and PCR techniques [11]. In the ROI, these were sent via the microbiology department at the Waterford General Hospital, Waterford.

The pediatrician was asked to obtain a serum specimen during both the acute phase (within 2 days of hospital admission) and the convalescent phase of illness and to send them to the Immunology Department, Churchill Hospital, Oxford, where anti-PRP antibody concentrations, total immunoglobulin, and IgG subclass assays were performed.

Definition. "True vaccine failure" was defined as invasive Hib disease occurring either >2 weeks after a single dose of vaccine was given to an infant >1 year of age or >1 week after at least 2 doses were given to a child ≤1 year of age.

Assays. IgG antibody to PRP was quantified by an ELISA, as previously described elsewhere [12]. Immunoglobulin concentrations were measured by nephelometry with the Beckmann array system (Beckmann Coulter, Fullerton, CA). IgG subclasses were measured by radial immunodiffusion with use of monoclonal antibody kits (The Binding Site, Birmingham, UK). "Deficiency of immunoglobulin class" was defined as a concentration >2 SD below the mean for age. "Deficiency of IgG subclasses" was defined as a concentration less than the fifth percentile for the age-adjusted range. Age-stratified normal ranges for the UK are published by the National Supraregional Protein Reference Unit [13].

Statistics. Statistical analysis was performed by use of SPSS (SPSS, Chicago, IL) and Epi Info, version 6 (Centers for Disease Control and Prevention, Atlanta, GA). Ages and time periods are given as medians and ranges and are compared using the Mann-Whitney test. Proportions are compared using the χ^2 test or the Fisher exact test. Logarithmic transformations were performed on anti-PRP antibody concentrations, and these were reported as geometric mean concentrations (GMC) with 95% CIs and were compared with the 2-tailed t test. Antibody concentrations of <0.15 μg/mL were given the value of 0.08 μg/mL. When ≥3 independent groups were compared, analysis-of-variance or Kruskal-Wallis tests were performed. Associations between continuous variables were assessed by use of multiple linear regression, and categorical variables were assessed by use of multiple logistic regression. No corrections were made for multiple comparisons.

Results

A total of 115 children with true vaccine failure were reported to the surveillance study during the 6 years from 1 October 1992 to 1 October 1998. Of these children, 101 were born and were vaccinated in the UK (14 in the ROI). The majority (106) were vaccinated during the first year of life (95 had received 3 doses; 11 had received 2 doses), and the remainder (9) were vaccinated when they were >1 year of age. A total of 112 patients had culture proven cases, and 3 had latex antigen–positive cases with compatible clinical presentations.

Vaccine Failures among Children Receiving Vaccine at ≤1 Year of Age

The clinical presentations are shown in table 1, and the clinical and immunological risk factors found to be associated with cases of true vaccine failure are shown in tables 2 and 3. A clinical risk factor was found in 21 (19.8%) of 106 children, and an immunological deficiency was found in 31 (30.4%) of 102 children for whom all information was available. Overall, deficiencies of immunoglobulins, clinical risk factors, or both were detected in 45 (44%) of 103 children for whom all information was available.

Table 2. Clinical risk factors among 106 children with true vaccine failure who were vaccinated against *Haemophilus influenzae* type b at ≤1 year of age.

Risk factor	Patients, no. (%)
Prematurity	13[a] (12.3)
Malignancy	3 (3)
Dysmorphic or developmental delay	3 (3)
Down's syndrome	2 (2)
Neutropenia	1 (1)

[a] One premature child was also dysmorphic.

Table 3. Immunological deficiencies detected among 106 children with true vaccine failure who were vaccinated against *Haemophilus influenzae* type b at ≤1 year of age.

Deficiency	Patients, no. (%)
Total immunoglobulins	
IgA	9 (9)
IgM	3
IgG	2
≥2 of IgG/IgA/IgM	3
IgG subclasses	
IgG-2	8 (8)
IgG-1	1
Both total immunoglobulins and IgG subclasses	
IgG-2 and ≥1 of IgG/IgA/IgM	5 (5)

A 4-month-old girl who was born at 34 weeks' gestation, who received 3 doses of vaccine, and who developed Hib pneumonia at 4.8 months of age (19 days after administration of the third dose) was the only child to die. She was found to have a low total IgG concentration in a serum specimen drawn during the acute phase of illness.

Vaccine failures after 3 doses. Ninety-five children (85 of whom were from the UK) had Hib disease develop despite receiving 3 doses of vaccine. They were vaccinated at median ages of 2.0 months (range, 1.5–3.9), 3.1 months (range, 2.8–7.4), and 4.4 months (range, 3.5–11.7), and they developed disease at a median age of 23.0 months (range, 4.8–64.0). The median time from receipt of the third dose to onset of disease was 18.6 months (range, 0.6–58.3). Excluding the 10 children from the ROI does not significantly alter these parameters.

Meningitis was the major mode of presentation (58 children; median age, 23.1 months [range, 7.4–60.4]), followed by epiglottitis (19; median age, 24.4 months [range, 12.1–64.0]), bacteremia (5; median age, 29.5 months [range, 23.0–47.5]), cellulitis (5; median age, 22.2 months [range, 12.9–33.8]), pneumonia (4; median age, 12.9 months [4.8–19.7]), septic arthritis or osteomyelitis (3; median age, 22.9 months [range, 21.9–23.2]), and pericarditis (1; age, 38 months). There was no significant difference in age at onset of disease for children with meningitis and those with epiglottitis ($P = .29$). Eleven children were born prematurely, and 6 had other clinical risk factors.

Serum specimens drawn during the acute phase of illness were available for 26 patients: 14 (54%) had an anti-PRP antibody concentration <0.15 μg/mL, and 24 (92%) had a concentration <1.0 μg/mL. The antibody concentration before disease was known incidentally in 1 child. She had a concentration of 0.92 μg/mL measured at 13 months of age (this is greater than the expected GMC of 0.83 μg/mL [14]). This occurred before she commenced chemotherapy and 9 months before she presented with Hib osteomyelitis.

Serum specimens obtained during the convalescent phase of illness were available for 89 (94%) patients and were obtained at a median of 28 days after hospital admission (range, 3–300 days). The anti-PRP antibody concentration during the convalescent phase was correlated with age at onset of disease ($r = .35$, $P = .001$). The following factors were incorporated into a multiple linear regression model: sex, clinical presentation, presence of immunoglobulin deficiency, prematurity, clinical risk factors, and number of days that serum was obtained after hospital admission; only age at onset of disease was significantly correlated ($R = .45$, $P = .002$). Exclusion of the 20 samples collected at 3–14 days after admission did not alter this finding. Convalescent-phase anti-PRP antibody concentrations, according to age at onset of disease, are shown in figure 1. The GMC, according to age group, is detailed in table 4.

The presence of any immunoglobulin deficiency was associated with a lower age at onset of disease. For the 29 children with immunoglobulin deficiency, the median age at onset of disease was 20.5 months (range, 4.8–48), compared with 25.6 months (range 7.4–64) for the 62 children without immunoglobulin deficiency ($P = .02$). The major contributor to this difference was IgG subclass deficiency. For the 13 children with IgG subclass deficiency, the median age at onset of disease was 16.9 months (range, 9.8–39.3), compared with 23.9 months (range, 4.8–64.0) for the 82 children without such deficiency ($P = .02$).

The 11 infants born prematurely (median gestational age, 34 weeks [range, 29–36]) were slightly younger at onset of disease than were the term infants (median age, 20.2 months [range, 4.8–34.6] vs. 23.6 months [range, 7.4–64.0]) ($P = .14$). The 9 premature infants for whom antibody was available had a significantly lower antibody response than the term infants (GMC, 1.22 μg/mL [95% CI, 0.33–4.54 μg/mL] vs. 5.63 μg/mL (95% CI, 3.40–9.32 μg/mL) ($P = .04$). However, when age at onset of disease was entered in the multiple regression model, prematurity was not significantly associated with a lower antibody response ($P = .12$).

The 18 children presenting with epiglottitis had a higher con-

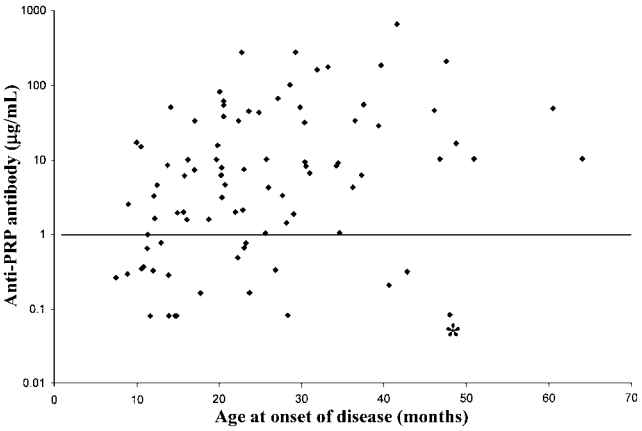

Figure 1. Convalescent-phase anti–polyribosylribitol phosphate (PRP) antibody concentration, according to age at onset of *Haemophilus influenzae* type b disease (true vaccine failures occurring after 3 doses). Horizontal line indicates a concentration of 1.0 μg/mL; asterisk indicates an outlier.

Table 4. Convalescent-phase anti-PRP antibody concentration: Age groups according to age at onset of *Hemophilus influenzae* type b disease (with true vaccine failure occurring after 3 doses).

Age group *(n)*	GMC of anti-PRP antibody, μg/mL (95% CI)
<12 months (12)	0.91 (0.31–2.64)
12–23 months (38)	3.69 (1.85–7.38)
24–35 months (22)	9.04 (3.50–22.91)
36–47 months (12)	17.52 (3.64–84.28)
>48 months (5)	5.72 (0.27–123.4); 16.65 (5.15–53.77)[a]

NOTE. GMC, geometric mean concentration; PRP, polyribosylribitol phosphate. Analysis of variance: $F = 3.56$, $P = .01$ (linear trend, $F = 4.84$, $P = .03$).
[a] Excluding outlier (see figure 1). Analysis of variance: $F = 4.08$, $P = .005$ (linear trend, $F = 8.68$, $P = .004$).

valescent-phase anti-PRP antibody response than did the 58 children with meningitis (GMC, 11.93 μg/mL [95% CI, 6.26–22.74 μg/mL] vs. 4.13 μg/mL [95% CI, 2.15–7.93 μg/mL]) ($P = .09$). In addition, a significantly greater proportion of the children with epiglottitis had an anti-PRP antibody concentration >1.0 μg/mL (18 of 18 vs. 41 of 58; $P = .009$).

Paired acute-phase and convalescent-phase anti-PRP antibody concentrations were available for 24 patients. The median increase in antibody between pairs was ~10-fold (range, 0.25–2275-fold increase). However, this group may not be representative of all cases, because the convalescent-phase GMC anti-PRP level was 1.59 μg/mL (95% CI, 0.65–3.85 μg/mL), compared with 7.27 μg/mL (95% CI, 4.23–12.49 μg/mL) for patients without acute sera ($P = .005$).

Twenty-three children (26%) had convalescent-phase anti-PRP antibody responses that were <1 μg/mL (figure 1, table 5). Three of 23 had clinical risk factors, and 3 were born prematurely, in comparison with 1 and 6, respectively, of the 66 patients with a satisfactory convalescent response ($P = .05$ for comparison of clinical risk factors and $P = .09$ when prematurity is included). Low convalescent-phase response was not correlated with sex or the presence of immunoglobulin deficiency. By use of a conventional age cutoff (for responses to polysaccharide antigens) of 18 months, 51 of 60 children >18 months of age had an anti-PRP response >1.0 μg/mL versus 15 of 29 children <18 months of age ($P = .001$).

Sixteen patients who were low responders received a further dose of Hib conjugate vaccine. Fourteen patients (88%) had a good response (concentration, >1 μg/mL), whereas 2 required 2 doses to achieve this concentration.

Vaccine failures after 2 doses. Eleven children (9 in the UK) developed Hib disease after receiving 2 doses of vaccine in the first year of life. Included in this group of children is 1 child who received the third dose 1 day before presentation.

Vaccines were given at a median age of 2.0 months (range, 1.7–3.4) and 3.8 months (range, 2.8–9.2), and onset of disease occurred at a median of 5.9 months of age (range, 3.8–18.7), at a median of 1.7 months (range, 0.5–11.3) after administration of the second dose of vaccine. Six children had associated clinical risk factors (prematurity in 2) or immunological risk factors. Acute-phase serum levels were available for 3 patients (all levels were <0.15 μg/mL), and convalescent-phase serum levels were available for 10 patients (specimens were obtained on median day 27) and were <1 μg/mL for 8 of these 10 patients. An antibody concentration of <0.15 μg/mL was measured 4 months before onset of disease in 1 child.

In comparison with children who had true vaccine failure after 3 doses, the children with vaccine failure after 2 doses had a lower age at onset of disease (median age, 5.9 months [range, 3.8–18.7] vs. 23.0 months [range, 4.8–64.0]) ($P < .001$) and a lower convalescent anti-PRP antibody response (0.21 μg/mL [0.09–0.48] vs. 4.82 μg/mL [3.00–7.75]) ($P < .001$). They were also more likely to have a clinical risk factor, including prematurity (5 of 11 children vs. 17 of 95; $P = .05$). In a multiple linear regression model in which convalescent-phase anti-PRP antibody response was used as the outcome, both age at onset of disease and receipt of 2 doses of vaccine were significantly correlated, but presence of a clinical risk factor was not ($R = 0.52$; β [2 doses] $= 0.26$, $P = .007$; β [age] $= 0.35$, $P < .001$).

Vaccine Failures among Children Receiving Vaccine at >1 Year of Age

Nine children (7 in the UK and 2 in the ROI) developed invasive Hib disease despite receiving vaccine when they were >1 year of age. Median age at vaccination was 16.8 months (range, 12.8–43.8), and age at onset of disease was 26.3 months (range, 16.0–76.9). The median time between vaccination and onset of disease was 9.9 months (range, 1.2–37.4). One child died. She had been born after 32 weeks' gestation, had been vaccinated at age 18 months, and had presented with meningitis at age 23 months. Four children had clinical risk factors, 2 others were born prematurely, and 3 of 7 for whom blood test results were available had an immunoglobulin deficiency. Six had a convalescent-phase blood specimen drawn at a median of 20 days (range, 5–30) after admission. Two had low responses: 0.36 μg/mL (on day 30) and 0.3 μg/mL (on day 16). Overall, 7 (78%) of 9 children had clinical or immunological risk factors or a poor convalescent-phase antibody response.

Table 5. Proportions of patients with convalescent-phase serum anti–polyribosylribitol phosphate concentrations <0.15 μg/mL or <1.0 μg/mL, according to age group, after receiving vaccination against *Haemophilus influenzae* type b (with true vaccine failures occurring after 3 doses).

	No. (%) of patients with a concentration	
Age group *(n)*	<0.15 μg/mL	<1.0 μg/mL
<12 months (12)	1 (8)	8 (67)
12–23 months (38)	3 (8)	10 (26)
24–35 months (22)	1 (5)	2 (9)
36–47 months (12)	0	2 (17)
>48 months (5)	1 (20); 0[a]	1 (20); 0[a]

[a] Excluding outlier (see figure 1).

One of the remaining two patients did not have a serum sample available for testing.

Discussion

We have found that a large proportion of children with Hib conjugate vaccine failure—44% of those who received vaccination at ≤1 year of age and 67% of those who received vaccination at >1 year of age—have associated clinical risk factors (including prematurity) and immunological deficiencies. Because cases were collected as part of active, prospective, population-based surveillance, it is unlikely that case ascertainment was biased toward detection of those with an increased frequency of underlying disorders.

Vaccine failures were analyzed according to the number of doses of vaccine received. The primary vaccine schedule for the UK and the ROI consists of 3 doses; thus, as would be expected, the majority of vaccine failures occurred among those patients who had received 3 doses. A small number of vaccine failures occurred after 2 doses, and these failures occurred among children who were younger, who had a lower antibody response to disease, and who were more likely to have an associated clinical risk factor when compared with patients who had vaccine failure after 3 doses. The lower antibody response is explicable on the basis of the patients' young age and the fewer vaccine doses received. One implication of the apparent association with clinical risk factors is the need for timely completion of the 3-vaccine course in such children.

An association with clinical and immunological factors was even more striking among children with vaccine failure who had previously received 1 dose of vaccine. One dose of Hib conjugate vaccine given to a child >12 months of age is expected to provide protection. That clinical or immunological factors were detected in 78% of such children implies that vaccine failure occurs in this age group in the presence of underlying disorders.

Significance of associated conditions. Because this was an observational study, any associations must be viewed with caution and can do no more than raise hypotheses. In principle, however, it would not be unexpected to find an underlying abnormality in children who have failed to be protected by vaccines that are known to be extremely efficacious when given in infancy.

The most common clinical factor, prematurity, was present in 15 infants. Lower anti-PRP antibody concentrations have been documented in vaccinated preterm infants than in vaccinated term infants [15–18]). A predisposition to vaccine failure therefore seems plausible. This susceptibility may have been further compounded in 2 infants who had received only 2 doses of vaccine and in 5 who had an immunoglobulin deficiency. The latter may also have been a consequence of their prematurity.

It is possible to compare the proportion of total cases that occurred among infants born prematurely with the proportion that might be expected on the basis of rates of prematurity in the general population. This is possible for vaccine failures after 3 doses, because vaccine coverage in the UK has only been estimated for this group. Analysis of these data indicates that although the risk for vaccine failure is higher (10 [11.8%] of 85 children), in comparison with the expected proportion of 6 children (7% of UK births occur at <37 weeks' gestation), the risk does not reach statistical significance (RR, 1.8; $P = .13$). This may reflect the small numbers of patients in the study, and it remains plausible that prematurity might be associated with an increased risk of vaccine failure.

Down's syndrome was detected in 3 of the children in the study group. Unvaccinated infants with Down's syndrome have been shown to have an increased incidence of Hib disease [19], and although there are no data on the immunogenicity of Hib conjugate vaccines in this group, they have a number of immunological abnormalities [20, 21] that might affect their responses to vaccination. Of the 3 children identified in this study, immunoglobulin results were available for 2 and were abnormal (low level of IgM) for 1.

Children with cancer have an increased risk of invasive Hib disease and a lesser response to Hib conjugate vaccines [22]; this was a factor in 5 children in this study. Of the remaining children with clinical risk factors, 3 had dysmorphic syndromes, 1 a duplication of chromosome 12 p, and 1 cyclical neutropenia. None of these are known to be associated with a specific susceptibility to Hib infection or to have abnormal responses to Hib vaccines; however, cyclical neutropenia is associated with an increased risk of bacterial infections [23].

With respect to immunological deficiencies, Holmes and Granoff [6] measured immunoglobulin concentrations in 25 children with Hib conjugate vaccine failure and found them to be subnormal in 40%, principally showing deficiencies of IgG2, IgM, or both. Those with subnormal immunoglobulin concentrations had lower convalescent Hib antibody responses than those with normal immunoglobulin concentrations. These children were vaccinated at >15 months of age and are comparable to the older vaccinees in our study.

The most frequent immunoglobulin deficiency detected among our cohort was deficiency of IgG2, either alone or in combination (13 [13%] of 102 children). A concentration of 0.3 g/L represents the fifth percentile for IgG2 in children in the UK who are 6 months to 5 years of age [13]. Those with IgG2 deficiency presented at a younger age and had lower convalescent-phase anti-PRP antibody responses than those with normal IgG2 concentrations. Although these differences suggest that IgG2 deficiency may be a real risk factor, this is complicated by the known age dependence of IgG2 and PRP antibody responses. Children who have vaccine failure at a younger age are more likely to have such findings simply because of their younger age, independent of any other predisposition. Even if this is a real phenomenon, the presence of IgG2 deficiency must

be a marker of more widespread immune dysfunction, because IgG1 and IgM predominate in the infant's primary antibody response to Hib conjugate vaccines [24]. Studies that have attempted to differentiate infection-prone IgG2-deficient children from healthy IgG2-deficient children have documented differences in the production of IgG subclasses in response to mitogen stimulation of peripheral blood mononuclear cells [25].

The second most common immunoglobulin deficiency detected was that of IgA, although the age at onset of disease and the convalescent-phase antibody response were no different than those of unaffected children. Selective IgA deficiency may be transient in children and, as with IgG2, might represent a maturational delay in immune development [26].

Finally, we found 3 patients with selective IgM deficiency. Individuals with IgM deficiency may experience infections with encapsulated organisms and appear to have an increased incidence of gram-negative septicemia [26].

Mechanism of susceptibility to Hib disease. Because Hib conjugate vaccines confer protection by eliciting serum antibodies against the capsular polysaccharide, the most likely reason for the development of disease is an inadequate serum concentration of anti-PRP antibody. In this study, the majority of children had a low anti-PRP antibody concentration at the onset of disease. This was also described in children with unconjugated Hib vaccine failure [6] and in children with natural Hib disease [27]. It is possible that this may reflect an artificial lowering of antibody concentration through antigen-antibody binding. The only clear way of defining this is to obtain sera before onset of disease. In this study, this situation arose in 2 children for whom the vaccine had failed. The first child had a satisfactory initial response to vaccination (0.92 μg/mL at 13 months of age) and then underwent chemotherapy before developing Hib disease. This could be classified as secondary vaccine failure. The second child had a low antibody response (<0.15 μg/mL) after receiving 2 doses of vaccine; this would constitute a primary vaccine failure. It is likely that all of the vaccine failures occurring after 2 doses represent primary failures, because the median time between vaccination and disease was short.

Failure to mount an initial antibody response to Hib immunization (primary failure) may be due to a significant immune deficiency, but it may also reflect a maturational delay in responses to polysaccharide antigens. Infants born prematurely may be a model for this, and IgG2 deficiency may be a marker of this maturational delay. A problem with the vaccine or with vaccination is also possible. Indeed, batches of PRP-OMP (PRP conjugated to outer membrane protein of *Neisseria meningitis)* were withdrawn in the United States because of questionable immunogenicity [28]. Analysis of the batch numbers of vaccines given to the children who had vaccine failure in this study did not indicate that a common batch was associated (data not shown).

Failure to sustain a measurable antibody response after vaccination (secondary failure) is seen in apparently healthy children. For example, in one UK study, 6% of 95 children had undetectable anti-PRP antibody concentrations at 12 months of age, despite having had an adequate response at 5 months [14]. These children had no obvious immune defects. Whether such "normal" individuals are then susceptible to Hib disease is uncertain, because the presence of immunological memory may compensate for the absence of circulating antibody. Secondary failure may also occur after immunosuppressive treatment and would explain the 5 patients with malignancy recorded in this study.

Antibody response to invasive disease. The majority (74%) of children with vaccine failure after prior receipt of 3 doses of vaccine had a satisfactory convalescent-phase anti-PRP antibody response (>1.0 μg/mL). As would be expected, the age at onset of disease was a significant influence on antibody response [29, 30]. Holmes and Granoff [6] compared convalescent responses of children with conjugate vaccine failure with those of children with natural Hib disease and showed that children with conjugate failure had a similar or increased response. In our cohort, 50% of those <18 months of age had an antibody response >1.0 μg/mL, compared with 0% in a study of unvaccinated children with Hib disease [27]. In another study of natural Hib disease, the GMC of IgG was <0.3 μg/mL for children 0–11 months of age (vs. 0.9 μg/mL in our study), 2.5 μg/mL for those 12–23 months of age (vs. 3.7 μg/mL), and 32.4–37.4 μg/mL for those 2–5 years of age (vs. 10.0 μg/mL) [30]. This would suggest that the antibody response to disease in younger children in whom the vaccine fails is better than that in unvaccinated children of similar age, a finding that is compatible with the presence of immunological memory (although it is apparently insufficient to prevent invasive disease in these cases). It is of interest that this may not be so for the older children in whom the vaccine fails (those >2 years of age). However, the validity of this comparison assumes similar assays and is based on small numbers.

Although there was no striking clinical or immunological difference between children with vaccine failure who responded to disease with an adequate antibody concentration and those with vaccine failure who did not have such a response, it is reasonable to conclude that those with a subnormal response should be viewed as abnormal, because, in addition to having invasive Hib disease, they have failed to respond to 3 doses of Hib conjugate vaccine. It is of interest that those with epiglottitis had a better antibody response than those with meningitis, despite a similar age at onset of disease. This phenomenon is also observed in natural Hib disease [27] and is unexplained.

Clinical presentation of vaccine failures. Children with disease that is the result of vaccine failure present at an older age than do children with natural disease [31], with a peak age at presentation occurring in the second year of life. The distribution of clinical presentations is similar to that seen in un-

vaccinated children; there is a predominance of meningitis, but, compatible with the shift in age at onset of disease, there is a greater proportion of presentations due to epiglottitis. The mortality in this cohort of children with vaccine failures was 1.7% (2 of 115 children), which is less than that recorded in the Oxford region before the introduction of vaccine (4.3%) [31]. We do not have satisfactory data on sequelae such as deafness; however, in general, it would appear that vaccine failure is not associated with more-severe disease, a fear that was raised with unconjugated Hib vaccine failure [32].

Conclusions

We present what is, to our knowledge, the largest published series of children with Hib conjugate vaccine failure and one in which the majority of children were vaccinated in early infancy. A large proportion of patients was shown to have associated clinical risk factors and immunological deficiencies, and although attribution of cause and effect is difficult, certain associations, such as prematurity, Down's syndrome, malignancy, and cyclical neutropenia, seem biologically plausible. Among the immunological deficiencies documented, IgG2 deficiency was associated with children who had vaccine failure at a younger age, and it may therefore be positively linked with vaccine failure. Children who are vaccinated at >1 year of age and who then develop vaccine failure are more likely to have an identifiable underlying defect.

These factors can only explain less than half of all cases, and a number of other predisposing conditions are possible. These include defects of the vaccine or of its storage or administration; maturational delay in immune responses; other deficiencies in the immune system that were not sought in this study, including mannose-binding lectin deficiency; and specific, subtle defects in responses to PRP.

Children with conjugate vaccine failure present at an older age than do those with natural Hib disease, but they present with a similar distribution of clinical presentations. The mortality from vaccine failure is not increased. Approximately 30% do not have a satisfactory antibody response to disease, but the majority then respond to a further dose of conjugate vaccine.

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