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MAJOR ARTICLE

Haemophilus influenzae Type b Vaccine Failure in Children Is Associated with Inadequate Production of High-Quality Antibody

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Background. Despite the excellent immunogenicity of *Haemophilus influenzae* type b (Hib) conjugate vaccines, breakthrough cases of Hib disease still affect a small proportion of vaccinated children in the United Kingdom. We performed a retrospective study to compare the avidity of antibody directed against the Hib polysaccharide capsule (PRP) in children who experienced Hib vaccine failure in the United Kingdom among 3 historical cohorts and with age-matched healthy control subjects.

Methods. Serum samples from vaccinated children with invasive Hib disease were collected beginning in 1992 as part of enhanced surveillance for Hib disease following vaccine introduction. A total of 251 children who experienced Hib vaccine failure were identified from 3 historical cohorts (1992–1995, 1996–1999, and 2000–2003). The anti-PRP antibody concentration and avidity from healthy age-matched control subjects was obtained for the 3 contemporary time points (1995, 1999, and 2002). Serum anti-PRP antibody concentration was measured in each of the samples using a standard Hib ELISA, and antibody avidity was determined using thiocyanate elution.

Results. Within the first 60 days after disease onset, there was no change in the anti-PRP antibody avidity, and there was no statistically significant difference in the geometric mean Hib antibody avidity over the 3 study periods. However, the children who experienced Hib vaccine failure had significantly lower Hib antibody avidity than did healthy control subjects, despite a marked antibody response following infection.

Conclusions. Children who experience Hib disease despite vaccination appear to have a defect in immunological priming, leading to a qualitative difference in Hib-specific memory B cells. Low anti-PRP antibody avidity decreases the functional activity of anti-PRP antibody in the sera of these children experiencing vaccine failure, leading to disease susceptibility.

The implementation of *Haemophilus influenzae* type b (Hib) conjugate vaccine into routine vaccination schedules has led to a >90% decrease in invasive Hib disease [1, 2]. In the United Kingdom from 1992 through 1998, the overall vaccine efficacy was estimated to be 98%, and the disease incidence remained at a very low level (0.65 cases per 100,000 children), with a 3-dose accelerated primary vaccination schedule and no booster in the second year of life [3]. Subsequently, an increase in the number of cases was noted in 1999, and a rate of 4.6 cases per 100,000 children was reported in 2002 [4]. This increase in the rate of invasive Hib disease coincided with the distribution of a Hib combination vaccine containing acellular pertussis with diphtheria and tetanus (DTPa-Hib). Analysis of risk factors for invasive Hib disease revealed a dose-dependent reduction in Hib antibody concentration in children receiving DTPa-Hib rather than the whole-cell pertussis vaccine that had been used previously [5]. Various other factors may have contributed to the increase in cases, but the resurgence of cases was controlled by offering a booster dose of Hib vaccine in 2003 to all chil-

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dren aged <4 years. A booster in the second year of life was introduced in 2006, to improve the persistence of population immunity against Hib.

Coincident with the introduction of the Hib conjugate vaccine for all infants in the United Kingdom in 1992, with a catch-up campaign for all children aged <4 years, active surveillance was commenced to prospectively identify cases of Hib vaccine failure. Because of the high vaccine efficacy achieved and the excellent immunogenicity in healthy children [1, 6, 7] and in those with chronic conditions (e.g., children with sickle cell disease [8] or congenital asplenia [9] and children who have received bone marrow transplants [10]), the rare occurrence of vaccine failure highlighted the presence of a subset of the population with susceptibility to Hib disease despite vaccination. True vaccine failure (TVF) for Hib was defined as invasive Hib disease occurring >2 weeks after receipt of a single dose of Hib conjugate vaccine in a child aged >1 year or invasive Hib disease occurring >1 week after receipt of at least 2 doses of vaccine in a child aged <1 year.

One possible explanation for vaccine failure is that such cases simply occur in children who, for technical reasons (e.g., poor vaccine administration technique or poorly maintained vaccine stock), were never adequately primed by their Hib vaccination series. However, previous studies have clearly revealed an anamnestic antibody response following Hib disease in vaccinated children [11, 12], indicating that the children had developed disease despite immunological priming by the vaccine. Because the antibody concentration before disease onset cannot be measured, it is not possible to determine whether the lack of a protective level of antibody against the Hib polysaccharide capsule (anti-PRP antibody) is the cause of Hib vaccine failure. The serum anti-PRP antibody concentration decreases rapidly after primary vaccination [4], and the absolute level does not reflect the functional activity of the antibody [7, 11, 13]. However, anti-PRP antibody avidity correlates with bactericidal activity [14] and immunological priming, even with low antibody concentration [8, 15–17]. Thus, the functional activity of Hib antibody after vaccination and the competence of priming might be better reflected by evaluation of antibody avidity. In this retrospective study, the avidity of anti-PRP antibody in both healthy children and in children experiencing TVF in the United Kingdom was investigated.

METHODS

Samples. Following Hib vaccine implementation in the UK vaccination schedule in 1992, clinical information and serum samples from children experiencing TVF were collected as part of a national active surveillance study by the British Paediatric Surveillance Unit in collaboration with the Health Protection Agency Haemophilus Reference Unit and the Oxford Vaccine Group. From 2000 onward, the study continued to be directed by the latter 2 organizations. Selection of samples for inclusion in this study is described in Results. Ethical approval for this study was obtained from the Central Office for Research Ethics Committee in Oxford, United Kingdom (REC: O4/Q1606/114).

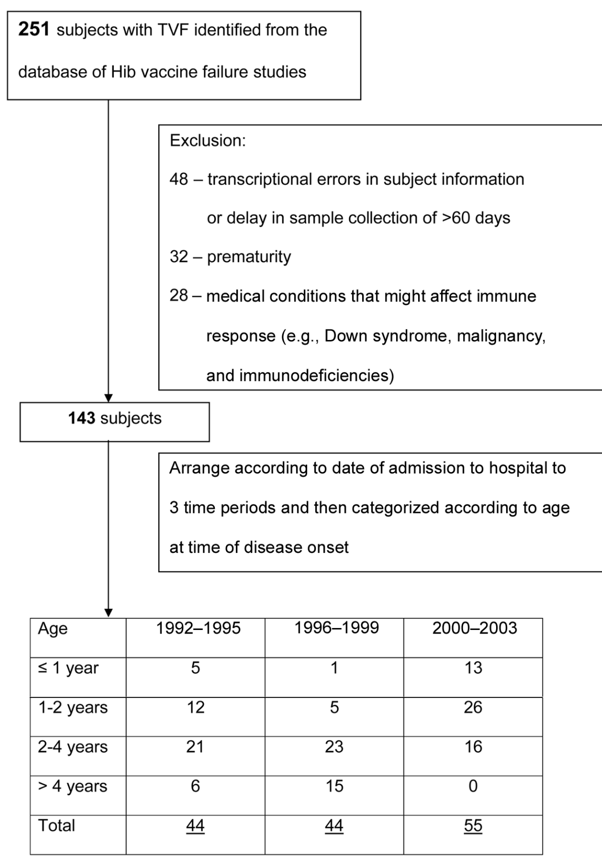
Controls. The anti-PRP antibody concentration and avidity level in serum samples from healthy UK children (aged 3–4 years), collected in 1995, 1999, and 2002 [18–20], were selected as control values for the 3 historical cohorts (1992–1995, 1996–1999, and 2000–2003, respectively). These children had received 3 doses of Hib conjugate vaccine as primary vaccination during infancy and were enrolled in studies performed by the Oxford Vaccine Group. The blood samples were obtained 3–4 years after primary vaccination, and a single serum sample was available for each child. Ethical approval for the use of the samples was obtained from the Oxfordshire's Research Ethics Committee (OXREC number: C02.013).

ELISA assay. Because of the difficulty in interpreting historical data obtained using different methods, the anti-PRP antibody concentration of all samples was remeasured using the standard Hib ELISA protocol [21]. The anti-PRP antibody avidity was then determined using a thiocyanate elution assay, as described elsewhere [22]. The antibody avidity, the relative strength of antibody binding to the antigen, was displayed as the avidity index (AI) corresponding to the molar concentration of thiocyanate required to produce a 50% reduction in optical density. The antibody response to tetanus toxoid and its avidity were also measured using a tetanus ELISA protocol and a thiocyanate elution assay, as describe elsewhere [23, 24].

Statistical analysis. Both antibody and avidity data were log-transformed before analysis. Comparisons of the 3 historical cohorts were determined using regression analysis, with and without adjustment for possible confounding factors, such as age and the interval between disease onset and day of sample collection. Comparison between the case data and control data was also performed using regression analysis, with adjustment for age and historical cohorts. The relative difference between the 2 cohorts was calculated by the exponent of the coefficient obtained from the regression model. All analyses were performed using Stata, version 9.2 (Stata).

RESULTS

Sample selection. Serum samples from a total of 251 children who experienced TVF were identified (figure 1). Of these subjects, 48 were excluded, either because of transcriptional errors in subject information or because of a delay in sample collection of >60 days after admission to the hospital for Hib disease. Sixty subjects were excluded because of medical conditions that might affect their immune response, such as prematurity, Down syndrome, malignancy, or immunodeficiency. The remaining 143 subjects were categorized into 3 historical cohorts as follows: 1992–1995 (the period after vaccine introduction, when



Age	1992–1995	1996–1999	2000–2003
≤ 1 year	5	1	13
1-2 years	12	5	26
2-4 years	21	23	16
> 4 years	6	15	0
Total	44	44	55

Figure 1. The process of sample selection for this study. Hib, *Haemophilus influenzae* type b; TVF, true vaccine failure.

the prevalence of invasive Hib disease was decreasing), 1996–1999 (a period of low Hib disease prevalence), and 2001–2003 (a period with a marked increase in Hib disease prevalence).

Children who experienced TVF and had multiple serum samples obtained. There were 37 individuals with 2 samples of sufficient volume for analysis that had been collected during the first 60 days after the onset of Hib disease. The first serum samples were collected at 4 days after disease onset (range, 0–15 days), and the second samples were collected at 31 days after disease onset (range, 8–59 days). The serum AI in the same individuals revealed no systematic difference between the 2 separate samples over the first 60 days after disease onset (figure 2).

Antibody concentration and avidity in serum samples from children who experienced TVF. The anti-PRP antibody concentration was measured for all available serum samples ($n = 143$; figure 3). Antibody avidity could be measured in serum samples from 108 individuals. For the remaining samples, there was either insufficient serum volume available or the antibody concentration was too low for avidity measurement (<0.3 μg/mL). Antibody concentration correlated closely with the levels previously recorded using routine assays for each individual at the time of disease (data not shown). The children in the 3 cohorts had comparable age distribution (although there were more younger children in the later cohort) (figure 1). The median durations from disease onset to the day of sample collection for the 3 cohorts (1992–1995, 1996–1999, and 2001–2003) were 8 days (range, 0–57 days), 6 days (range, 0–50 days), and 12 days (range, 0–51 days), respectively. There was marginal evidence of statistical significance of anti-PRP antibody among the 3 cohorts ($P = .04$ and $P = .07$, before and after adjustment for confounding factors, respectively) (figure 3 and table 1). In particular, the mean antibody concentration in the 1996–1999 cohort was 4-fold higher than the mean antibody concentration in the 1992–1995 cohort (table 1). There was no evidence of difference observed in antibody avidity among the 3 groups (figure 4).

Comparison of serum samples from children who experienced TVF with serum samples from healthy control subjects. Because of the discrepancy in age distribution between the patients with Hib disease and healthy control subjects, only samples from patients who experienced TVF who were >3 years of age at the time of sampling (42 children) were selected for initial comparison (avidity analysis was possible for 33 of these samples). As shown in figure 5, the antibody level in the samples from children who experienced TVF was significantly higher than that in the samples from control subjects ($P < .001$), with geometric mean concentrations of 4.46 μg/mL and 0.29 μg/mL, respectively. However, the AI of the samples from children who experienced TVF was significantly lower than that of samples from control subjects ($P < .001$, figure 5), with a geometric

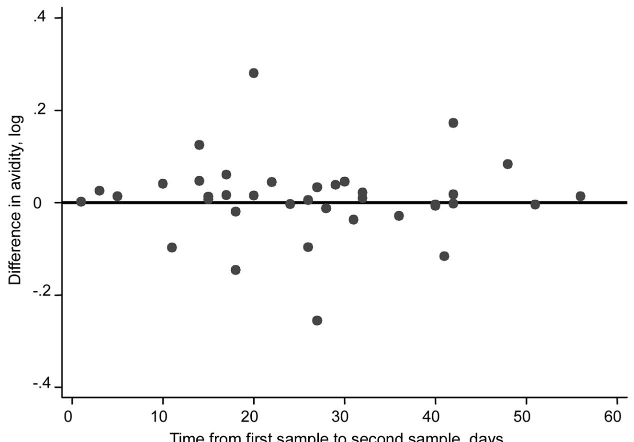


Figure 2. The variation of avidity levels of antibody against *Haemophilus influenzae* type b polysaccharide capsule (anti-PRP antibody) in relation to the time between the first and second sample collection ($n = 37$) after hospital admission for invasive *Haemophilus influenzae* type b disease.

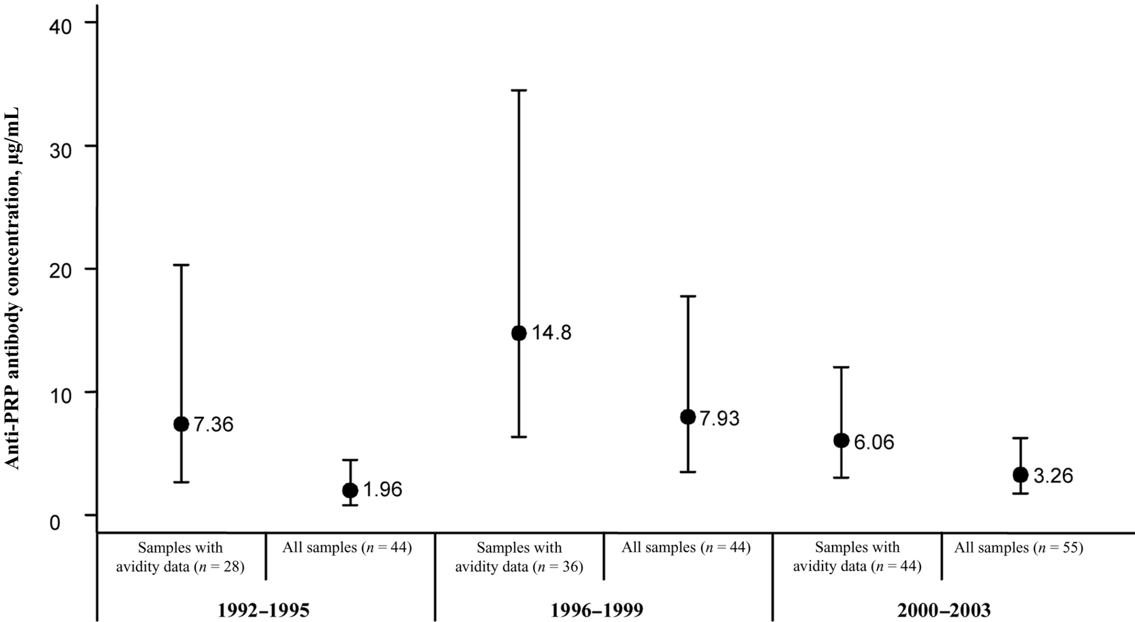


Figure 3. Concentration of antibody against *Haemophilus influenzae* type b polysaccharide capsule (anti-PRP antibody) in serum samples from children who experienced true vaccine failure across 3 historical cohorts. The dots represent the geometric mean value, and the lines represent 95% CIs.

mean AI of 0.12 and 0.24, respectively. This outcome remained statistically significant, even when all case patients ($n = 143$; geometric mean antibody concentration, 3.66 μg/mL; geometric mean avidity index, 0.10) were considered for analysis, compared with all control subjects ($P < .001$). There was no statistically significant difference in either the antitetanus antibody concentration or avidity between the children who experienced TVF and healthy control subjects (data not shown).

DISCUSSION

This large study provides compelling evidence that the occurrence of Hib disease in vaccinated children may be related to the failure of normal immunological priming, leading to impairment of avidity maturation of Hib-specific B cells or lack or loss of B cells producing high-avidity antibody, despite evidence that priming has permitted significant antibody responses to be achieved following invasive Hib disease. The antibody concentration was higher in the serum samples from children who experienced TVF than in samples from the healthy age-matched control subjects, even at the earliest time points after disease onset, indicating an anamnestic response after the exposure to Hib [11]. However, the low AI in the children who experienced TVF suggests an intrinsic defect in anti-PRP antibody avidity maturation, which increased their susceptibility to disease. In support of this, in a series involving 12 children who experienced TVF in The Netherlands, Breukels et al. [13] also found lower serum antibody avidity. The low avidity does not appear to reflect a general defect in production of high-avidity antibody, because we found no difference in avidity to tetanus toxoid between the children who experienced TVF and control subjects.

In accordance with our findings, Breukels et al. [13] documented no significant changes in the avidity index of samples from children who experienced TVF at disease onset and during the convalescent phase (3–4 weeks after disease onset). Similarly, the AI does not change during the month after a booster dose of Hib vaccine in healthy children [17], indicating that the serum AI of children who experience TVF is likely to reflect their immune status prior to Hib disease. Thus, low levels of

Table 1. Comparison of the relative difference in antibody concentration among 3 cohorts of patients who experienced true vaccine failure, with and without adjustment, using the 1992–1995 cohort as reference ($n = 143$).

	Unadjusted		Adjusted[a]	
Variable	Estimated relative difference (95% CI)	*P*	Estimated relative difference (95% CI)	*P*
Group		.04		.07
1992–1995	Reference		Reference	
1996–1999	4.05 (1.36–12.08)	.01	3.69 (1.19–11.4)	.02
2000–2003	1.67 (0.59–4.69)	.6	2.12 (0.71–6.34)	.2

[a] Adjusted for age at hospital admission and interval between disease onset and day of sample collection.

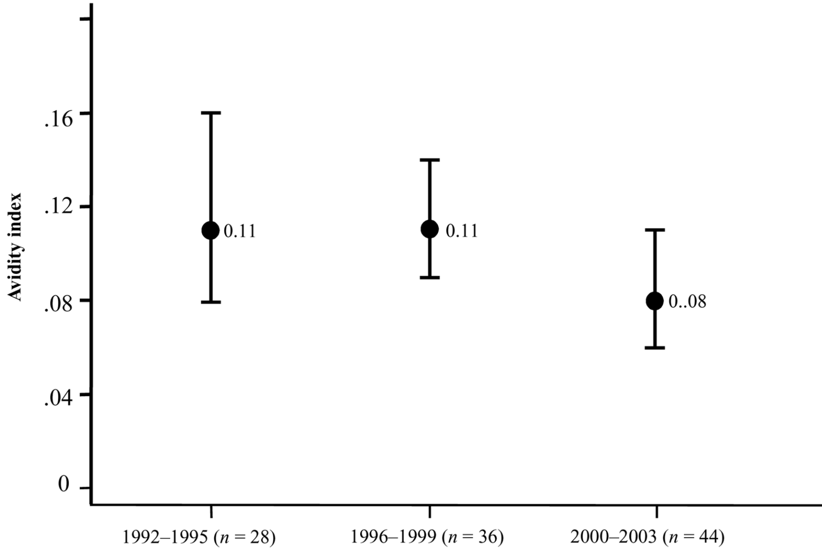


Figure 4. Avidity of antibody against *Haemophilus influenzae* type b polysaccharide capsule (anti-PRP antibody) in serum samples from children who experienced true vaccine failure across 3 historical cohorts. The dots represent the geometric mean value, and the lines represent 95% CIs. For both comparisons, $P < .001$, after adjustment for age and historical cohorts.

functional antibody may provide the explanation for their disease susceptibility.

The lower Hib antibody concentration in the 1992–1995 cohort in our study may reflect the high levels of herd immunity at the time of the Hib vaccine catch-up campaign in 1992 and low rates of natural boosting by nasopharyngeal carriage of the organism. AIs were low in all 3 cohorts of patients who experienced TVF from the early 1990s through 2003, despite differences in population immunity and disease epidemiology during these periods. Previous studies have suggested that many of the cases of TVF in the 2001–2003 cohort may have been associated with deleterious effects on Hib immunogenicity caused by interaction with acellular pertussis in a combined DTPa-Hib vaccine [5, 15, 25–27]. We have previously found that use of this combined DTPa-Hib vaccine was associated with lower Hib antibody avidity in healthy children. Because the avidity of Hib antibody in the general population appears to have been lower during 2001–2003 as a result of this interaction, the observed increase in cases during this period may

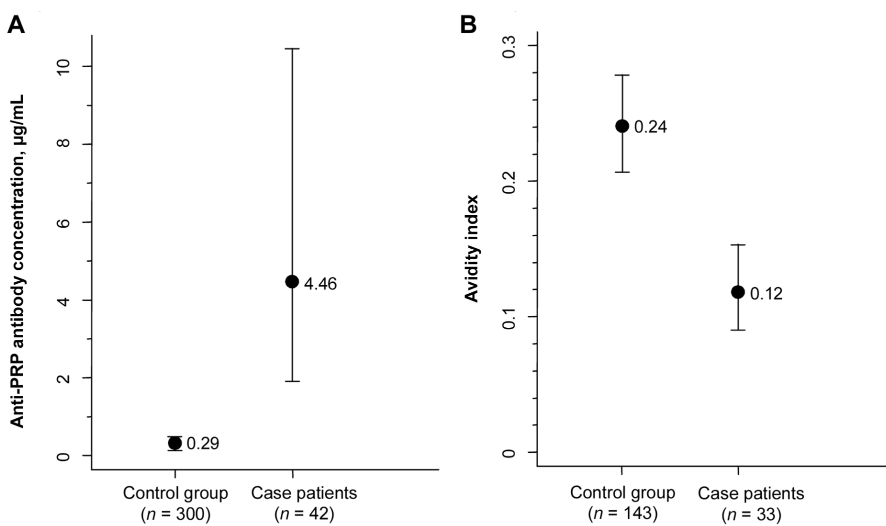


Figure 5. Comparison of the concentration *(A)* and avidity *(B)* of antibody against *Haemophilus influenzae* type b polysaccharide capsule (anti-PRP antibody) in serum samples from children who experienced true vaccine failure (case patients) and in samples from healthy control subjects (control group) during the period 1992–2003.

reflect an avidity-related increase in population susceptibility, further supporting our observations.

We were concerned that handling of the serum samples may have varied among the samples obtained since 1992. However, the lack of change in avidity among the 3 cohorts and the similar tetanus avidity between case samples and control samples do not indicate any significant effects of sample storage on the Hib analysis.

This study provides strong evidence suggesting that defective priming leads to a reduction in either the number or the quality of memory B cells induced by immunization or a loss of avidity in matured B cells prior to disease onset. The lack of high-avidity anti-PRP antibody results in susceptibility to Hib disease. Pichichero et al. [17] studied a group of children who initially had low anti-PRP antibody concentrations and AIs, despite 3-dose priming with Hib vaccine, who showed a larger increase in antibody avidity following a booster, compared with other children. Earlier studies also confirmed that children who experience TVF respond to an additional dose of conjugate vaccine [12, 13]. These observations indicate that the problem may have been related to the vaccine (loss of vaccine epitopes through inadequate handling or storage or uncoupling of conjugation affecting immunogenicity), rather than to a specific defect in the infant's ability to have a normal immune response, or that these children had a maturational defect in their response to this conjugate vaccine that later recovered. In either instance, the defective maturation of antibody avidity might be overcome with an additional dose of Hib conjugate vaccine, providing support for introduction of a booster dose of Hib vaccine for all children in the second year of life, as was finally commenced in the United Kingdom in Autumn 2006.

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