

ORIGINAL ARTICLE

Immunologic memory in *Haemophilus influenzae* type b conjugate vaccine failure

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Aims: To compare the convalescent antibody response to invasive *Haemophilus influenzae* type b (Hib) disease between conjugate vaccine immunised and unimmunised children, to look for evidence of priming for immunologic memory.

Methods: Unmatched case-control study in the UK and Ireland 1992–2001 and Victoria, Australia 1988–1990. A total of 93 children were identified as having invasive Hib disease following three doses of conjugate vaccine in infancy through post licensure surveillance throughout the UK and Ireland; 92 unvaccinated children admitted to an Australian paediatric hospital with invasive Hib disease were used as historical controls. Convalescent serum was taken for measurement of Hib antibody concentration, and clinical information relating to potential disease risk factors was collected. The geometric mean concentrations of convalescent Hib antibodies were compared between immunised and unimmunised children, using raw and adjusted data.

Results: Hib conjugate vaccine immunised children had higher serum Hib antibody responses to disease (geometric mean concentration (GMC) 10.81 µg/ml (95% CI 6.62 to 17.66) than unimmunised children (1.06 µg/ml (0.61 to 1.84)) ($p < 0.0001$). However, following adjustment for the significant confounding influences of age at presentation and timing of serum collection, a difference persisted only in children presenting with meningitis (vaccinated GMC 3.78 µg/ml (2.78 to 5.15); unvaccinated GMC 1.48 µg/ml (0.90 to 2.21); $p = 0.003$).

Conclusions: Higher antibody responses to invasive Hib disease in vaccinated children with meningitis reflect priming for immunologic memory by the vaccine. Although a majority of children in the UK are protected from Hib disease by immunisation, the relative roles of immunologic memory and other immune mechanisms in conferring protection remain unclear.

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Routine immunisation with *Haemophilus influenzae* type b (Hib) conjugate vaccines was introduced in the UK in October 1992, and comprised a three dose accelerated infant schedule at 2, 3, and 4 months, with no booster dose. In consequence, invasive infections caused by Hib have become extremely rare, but cases of clinical vaccine failure do occur. An understanding of the mechanisms involved in conjugate vaccine failure is relevant to this and other vaccines.

Serum bactericidal activity against Hib correlates with the presence of specific antibodies against the capsular polysaccharide polyribosylribitol phosphate (PRP).¹ Like most bacterial capsular polysaccharides, PRP does not stimulate antigen specific T cells, which are required for the induction of immunologic memory. Hib conjugate vaccines are produced by the chemical conjugation of PRP with proteins such as tetanus and diphtheria toxoids, converting them to antigens capable of eliciting T cell help. As a result, memory B cells are formed in germinal centres, which then persist in lymphoid tissues and can be readily reactivated to produce highly specific antibodies with enhanced functional activity on re-exposure to Hib.² Children primed with Hib conjugate vaccines in infancy show large increases in antibody following a booster dose of vaccine in the second year of life, consistent with the induction of immunologic memory.³

Studies conducted before the introduction of conjugate vaccines into the primary immunisation schedule suggested that a serum anti-PRP antibody concentration of at least 0.15 µg/ml and 1.0 µg/ml might correlate with short and long term protection respectively from invasive Hib disease.⁴ The need for a higher titre to confer longer term protection reflects the fact that antibody levels decline in the months and years following infant immunisation.⁵ An issue not addressed by these figures is the additional role of immunologic memory in pro-

viding protection against disease in children immunised with Hib conjugate vaccines. A recent study of the seroprevalence of "protective" Hib serum antibody levels in UK children found that 32% of individuals fell below the threshold of 0.15 µg/ml by the age of 6 years, without an increase in the incidence of disease in this age group.⁵ A large number of children in the UK can thus be presumed to be critically dependent on recall antibody responses for protection.

This study examined the serum antibody response following invasive Hib disease in vaccinated children in the UK in comparison with unvaccinated historical controls. On the basis of the magnitude of the convalescent antibody response following Hib meningitis and epiglottitis, we looked to see whether immunised children had been primed for memory responses to Hib. The contribution of confounding factors to the convalescent antibody level was also considered.

METHODS

Aim

To compare the geometric mean concentration of convalescent PRP specific serum antibodies produced following invasive Hib infection between Hib conjugate vaccine immunised and unimmunised children. In making this comparison, significant confounding factors influencing the immune response to disease were taken into account.

Abbreviations: DTP, diphtheria-tetanus-pertussis; GMC, geometric mean concentration; Hib, *Haemophilus influenzae* type b; HRU, *Haemophilus* reference unit; PHLS, Public Health Laboratory Service; PRP, polyribosylribitol phosphate

Subject populations

UK Hib vaccine failures

Subjects were identified through enhanced surveillance for invasive *Haemophilus influenzae* infections conducted throughout the UK and Eire from October 1992. Sources of data included information on isolates sent to the Public Health Laboratory Service (PHLS) *Haemophilus* Reference Unit (HRU) and laboratory reports collected at the PHLS Communicable Disease Surveillance Centre (CDSC). Clinical reports of Hib cases were directly received through the British Paediatric Surveillance Unit's (BPSU) orange card reporting scheme until October 2000 and continue to be notified through the HRU, CDSC, Scottish Centre for Infection and Environmental Health (SCIEH), and Oxford Vaccine Group (OVG).

The case definition required isolation of the organism from a normally sterile site, with serotype and secondary PCR confirmation of isolates as type b through the PHLS HRU. Vaccination status was confirmed by contacting the child's usual general practitioner, or from computerised records held in local child health departments. All children had received three doses of Hib vaccine in infancy, at 2, 3, and 4 months of age, without a booster dose. The majority of vaccine used in the UK was the polyribosylribitol phosphate-tetanus conjugate (PRP-T), mainly in combination with diphtheria-tetanus-pertussis (DTP) vaccine since 1997. Acellular pertussis vaccine combinations have only been widely available in the UK since 2000.

As part of this study, further clinical information was prospectively collected in relation to cases. This included age at onset of disease, clinical presentation, history of underlying chronic disease, known immunodeficiency, or premature delivery. Serum was collected for measurement of acute and convalescent concentrations of Hib antibody and the timing of collection in relation to disease onset was noted. Measurement of immunoglobulin classes and subclasses was also performed on convalescent specimens and classified as normal or deficient in relation to age appropriate reference ranges. This study was approved by the Central Oxford Research Ethics Committee in 1991 and again by the South East Multi-Centre Research Ethics Committee in 2001.

Unimmunised Australian children with Hib

A cohort of children, previously described,⁶ was selected as an unvaccinated historical control group from among patients admitted to the Royal Children's Hospital, Melbourne, Australia between February 1988 and August 1990 with Hib epiglottitis or meningitis. Of 47 children with meningitis, 46 had positive Hib cultures from blood, cerebrospinal fluid, or both. Forty five cases of epiglottitis were described—32 had a positive blood culture, eight a positive throat swab, and three were diagnosed on latex antigen testing. Two were diagnosed on clinical grounds alone according to the judgement of two experienced clinicians. The geometric mean concentration (GMC) of convalescent Hib antibodies following epiglottitis in the previous study was no different when children without a positive blood culture were included in the case definition, compared with the GMC for the 32 from whom Hib was grown in the blood. No children had received Hib vaccine. Acute and

convalescent serum samples were taken and age at presentation, clinical diagnosis, and timing of serum collection were also noted. The study was approved by the Ethics in Human Research Committee of the Royal Children's Hospital, Melbourne.

Antibody measurement

In the UK, PRP antibody measurements were performed initially using the Farr type radioimmunoassay⁷ with a change to the CDC standard protocol HbO-HA ELISA⁸ in 1999. Equivalency studies performed at the time were favourable, with some discordance noted in the lowest ranges only (H Griffiths, personal communication). All assays in Australia were conducted using the HbO-HA ELISA. Both assays were standardised using reference sera supplied by the Food and Drug Administration, USA.^{6,7}

Statistical analysis

For descriptive statistics, the Wilcoxon rank sum test was used to compare group medians. In the comparison of Hib antibodies, a minimum concentration of 0.08 µg/ml was assigned for values <0.15 µg/ml. Convalescent antibody measurements were log transformed in order to achieve a normal distribution and described as GMCs. For the pooled data on vaccinated and unvaccinated children, simple regression analysis was performed to assess the relations between convalescent antibody and both age at presentation and timing of serum collection. These were then incorporated into a multiple linear regression model, which was used to predict adjusted convalescent antibody responses. Student's *t* test was used to assess the significance of any difference in raw and adjusted GMCs between vaccinated and unvaccinated children. The same comparisons were made with children presenting with either meningitis or epiglottitis considered separately.

Further exploration of potential confounders was performed using the UK vaccinated dataset. The effects of the various clinical and immunologic variables measured on logged convalescent antibody concentrations were first assessed using simple linear or logistic regression analysis depending on the nature of variables assessed. Their relative contributions were then further measured in a multivariate regression model in which all potential confounders were included. All statistical analyses were performed using STATA 7.0.⁹

RESULTS

From October 1992 to 1 January 2001, 185 reports of Hib vaccine failure following three doses in infancy were notified through surveillance in the UK and Eire (171 in the UK). Forty nine of these presented with illnesses other than epiglottitis or meningitis, such as pneumonia, bacteraemia, cellulitis, or bone and joint infections, and were excluded. Children born prematurely or on whom gestational information was unavailable were considered separately (3 of 38 with epiglottitis, and 13 of 98 with meningitis). Children with incomplete antibody data, for either concentration or day of collection (a further 27), were also excluded. This left 25 vaccinated children presenting with epiglottitis and 68 with meningitis. A

Table 1 Demographic characteristics of vaccinated and unvaccinated children

Vaccination status	Clinical presentation	Number of cases	% female	Median day of convalescent serum collection (range)	Median age in months (range)	% less than 18 months
Vaccinated	Epiglottitis	25	56	41 (9–300)	36.89 (11.93–63.12)	12
	Meningitis	68	46	28 (8–135)	25.23 (7.27–91.30)	31
Unvaccinated	Epiglottitis	45	44	43 (27–78)	31.79 (7.50–98.20)	11
	Meningitis	47	46	52 (16–112)	16.87 (1.41–71.64)	53

Table 2 Convalescent Hib antibody geometric mean concentrations, with 95% CI

Vaccination status	All cases		Meningitis cases only		Epiglottitis cases only	
	Raw	Adjusted	Raw	Adjusted	Raw	Adjusted
Vaccinated (no. cases)	10.81 (6.62 to 17.66)	3.86 (2.96 to 5.03)	8.32 (4.47 to 15.46)	3.78 (2.78 to 5.15)	22.08 (11.18 to 43.61)	4.06 (2.32 to 7.12)
Unvaccinated (no. cases)	1.06 (0.61 to 1.84)	3.01 (2.14 to 4.23)	0.33 (0.18 to 0.60)	1.48 (0.90 to 2.21)	3.57 (1.60 to 7.99)	6.32 (3.92 to 10.20)
Student's <i>t</i> test <i>p</i> value*	<0.0001	0.25	<0.0001	0.0003	0.003	0.25

Raw data and adjusted data from regression model controlling for age and timing of serum collection shown.

Table 3 Effects of age at disease presentation and timing of convalescent serum collection on convalescent Hib antibody response (vaccinated and unvaccinated children)

Variable	Intercept	Coefficient	Coefficient SE	<i>p</i> value	Adjusted <i>r</i> ²
<i>Simple regression</i>					
Age (months)	-0.97	0.08	0.01	<0.0001	22.6%
Convalescent day of serum collection	2.15	-0.02	0.01	0.002	4.4%
<i>Multiple regression: adjusted <i>r</i>² = 27.8%, <i>p</i><0.0001</i>					
Age (months)		0.08	0.01	<0.0001	
Convalescent day of serum collection		-0.02	0.01	<0.0001	

total of 112 cases were described in the study of unvaccinated children. Clinical presentation was unknown for one, and no convalescent serum was available for 19, leaving 45 children with epiglottitis and 47 with meningitis.

Table 1 shows demographic characteristics of the two groups. Vaccinated children presenting with meningitis were 8.5 months older than their unimmunised counterparts (*p* = 0.005) and had earlier blood sampling at 28 days, compared with 52 days (*p* < 0.001). It should be noted, however, that a wide range of ages and sampling times was observed in both groups. Figure 1 shows raw antibody data for each child, plotted against age at presentation with disease. There was a statistically significantly higher concentration of antibodies in the vaccinated group (*p* < 0.001) (table 2). This was most notable in infants under 18 months of age. Only 13%

of unvaccinated children in this age group made any antibody response to infection, compared with 92% of those who had been immunised. However, age at presentation and timing of serum collection were both significantly related to antibody concentrations in the multiple regression model (*p* < 0.0001 for both) (table 3). Increasing age was associated with an exponential increase in antibody levels, and antibodies were seen to decay exponentially with time from illness. When antibody levels were adjusted for these confounding factors, a significant difference between the convalescent antibody responses of immunised and unimmunised children was only observed in those presenting with meningitis (*p* = 0.0003) (table 2).

Relations between the immune response to Hib meningitis or epiglottitis and the additional clinical risk factors of chronic illness, known immunodeficiency, premature delivery, and deficient immunoglobulins or subclasses described in UK vaccine failures were explored (data not shown). The 93 vaccinated children studied above, as well as 11 prematurely born children with complete data were included. The only additional factor found to influence the magnitude of the convalescent response was a history of premature delivery (coefficient -2.57, coefficient standard error 0.74, *p* = 0.0008), which remained significantly associated with lower antibody levels in the multiple regression model (*p* = 0.047). Comparison of raw Hib GMCs confirmed this finding. Children born prematurely had a GMC of 0.82 µg/ml (95% CI 0.27 to 2.54), while in those born at term it was 10.81 µg/ml (6.62 to 17.66) (*p* = 0.008). This difference persisted after adjusting for age and timing of serum collection: preterm GMC 4.17 µg/ml (2.39 to 7.28); term GMC 8.93 µg/ml (7.00 to 11.38) (*p* = 0.04), although overlapping confidence intervals are noted. For this reason, children delivered prematurely were considered a population of outliers and excluded from the earlier comparisons with the unvaccinated cohort. Their inclusion in the analysis resulted in lower GMC Hib antibody responses in the vaccinated group than those described in table 2. It did not, however, change any of the significant differences observed between groups or conclusions drawn.

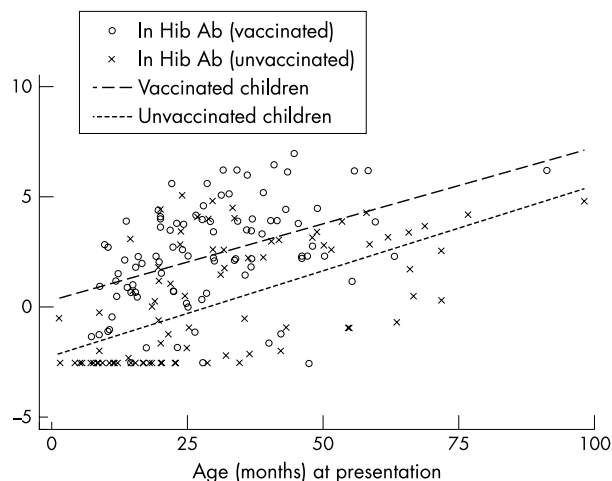


Figure 1 Log of raw convalescent Hib antibody concentrations (µg/ml) for vaccinated and unvaccinated children. Individual data points for vaccinated children are represented by x, with the dashed line indicating line of best fit for this group. Individual data points for unvaccinated children are represented by o, with the dotted line indicating line of best fit for this group.

DISCUSSION

This study confirms others in showing higher raw antibody levels in Hib conjugate vaccine immunised children recovering from invasive Hib disease^{10–12} than in unimmunised children. Much of this difference, however, was due to older age at disease presentation in vaccine failures. After correction for the confounding effects of age and timing of serum collection, a significant difference in the magnitude of the immune response between vaccinated and unvaccinated children was only seen in those presenting with Hib meningitis. We attributed this difference to priming for immunologic memory by infant immunisation, without clinical protection from disease.

For the first time, we have shown no effect of vaccination on convalescent immunity in children recovering from epiglottitis. Prior to the use of conjugate vaccines, it was noted that children with epiglottitis tended to be older and have higher convalescent anticapsular antibody titres than those with meningitis.¹³ It has been suggested that the presence of pre-existing immunity in these cases was enough to contain invasive disease at the epiglottis, perhaps as a result of immunologic priming through prior oropharyngeal Hib carriage.⁶ The absence of a difference in convalescent GMCs between vaccinated and unvaccinated children with epiglottitis may support the notion that both groups were primed. In the Australians, this priming was most likely the result of carriage of Hib or cross reactive organisms, and in the UK cohort, may have been attributable to vaccination with or without carriage. Alternatively, the greater immune response to infection in this group may simply reflect immunologic maturation of the response to capsular polysaccharides with age. It is interesting to note that 20% of the UK Hib vaccine failures identified between October 1992 and March 2001 presented with epiglottitis,¹⁴ compared with only 12% of unvaccinated children in Oxfordshire between 1985 and 1991.¹⁵

Memory immune responses have also been shown in unvaccinated children in the setting of invasive Hib infection. Rising Hib anticapsular antibodies have been detected within as little as 2–3 days of the diagnosis of Hib meningitis, without prevention of disease.¹⁶ Studies of antibodies induced by primary immunisation and subsequent boosting reveal a range of quantitative and qualitative differences between individuals. While the amount of measurable serum antibody produced one month following immunisation is the single correlate of protection on which vaccines are licensed, there is increasing recognition that the avidity and hence function of this antibody differs,¹⁷ as does the rate of avidity maturation over time.¹⁸ If the product of antibody concentration and avidity correlate best with bactericidal activity, some individuals will therefore be potentially susceptible to disease at an earlier stage in the natural decline of serum antibody concentration observed. At the point of recall antibody production, much interest has been directed recently at the time course of the serum antibody response. In one study of the kinetics of serum antibody production following Men C conjugate vaccination in adults, only 21% of subjects had a detectable increase from baseline titres by day 4 of sampling, with most others responding within 10 days of immunisation.¹⁹ In the race against bacterial invasion, hours rather than days may be clinically significant, particularly if antibody is poorly functional because of impaired avidity maturation. Further, it remains questionable whether serum is the most relevant source of protective antibodies, as mucosal immune responses may play a more important role in initial defence. Following pneumococcal conjugate vaccination, mucosal antibodies are produced and achieve peak levels more rapidly than serum responses.²⁰

It has previously been reported that 30% of children in the UK vaccine failure cohort show minor deficiencies of immunoglobulins or subclasses.²¹ This abnormal immunophenotype has been postulated to be a marker of variant immune

regulation, and may be associated with delayed maturation of B cell responsiveness to polysaccharides and recurrent sino-pulmonary infection.^{21–22} 10 of the 25 children in Holmes and Granoff's study of vaccine failures had low levels of IgG2 subclass and IgM,¹¹ and also showed a reduced convalescent antibody response to disease. In the present study, no such difference in Hib antibody concentrations was noted in children with abnormalities of immunoglobulins or subclasses, using multiple regression analysis. However, the possibility that other defects in immune regulation may be overrepresented among vaccine failures deserves further exploration. A range of polymorphisms in genes responsible for regulating antibody responses, initiating or modulating pathogen recognition or clearance have been implicated in conferring such susceptibility to a range of diseases.²³

A history of premature delivery had a notable effect on antibody response in this study. Some of this may be explained by the lower age of this subset with a median age of 20 months (range 10–51) compared with those delivered at term (median 28 months, range 7–91). However, persistently lower antibody levels were observed, even following adjustment for age and timing of serum collection. These results would strongly suggest defective induction of memory in our population of premature infants. Lower primary antibody responses²⁴ and reduced persistence²⁵ have been observed previously following vaccination of premature infants with conjugate vaccines. A Danish study in which PRP-T was administered at 2, 4, and 12 months confirmed these findings following the first two immunisations.²⁶ Following the booster dose of conjugate vaccine at 12 months, however, preterm and term infants achieved the same antibody levels. The data presented here add further weight to the suggestion that prematurity is a risk factor for vaccine failure. A non-significant trend to increased risk was noted from the UK surveillance data recently, although absolute numbers of cases were small.²⁵ Again, the question is raised of the need for a booster dose of vaccine for this group.

The differences between the vaccinated and unvaccinated cohorts in this study, given their separation in location and time, are acknowledged. It is unfortunate that information on all of the covariates studied in the British cohort was not available for children in the Australian dataset. Further, it is possible that as yet unknown and therefore unmeasured confounders may have produced differences between the antibody responses in the two populations, persisting after the correction performed. The incidence rates of invasive Hib disease among children living in Australia and Britain prior to introduction of the conjugate vaccine were similar up to 12 months of age. Thereafter, the rate of disease in children in Victoria, Australia was approximately twice that seen in Britain, for reasons that are not entirely clear.²⁷ Both countries have predominantly Anglo-Celtic populations, making notable host differences in the immune response to the organism unlikely. Australian Aboriginal infants experience Hib disease much earlier than other Australian children,²⁸ but this ethnic group make up only 0.4% of the population in the state of Victoria (Australian Bureau of Statistics). Given the impact of the Hib vaccine programme in decreasing the prevalence of Hib carriage in the UK,²⁹ the major difference that might be anticipated would be a greater population exposure to the organism in Australia in the late 1980s, which might have resulted in increased background Hib titres in the unvaccinated cohort, rather than the lower levels which we have observed. Further, antibody measurements in the UK and Australia were performed at separate locations and at different times. While this is not ideal, assays in both countries were performed according to recognised protocols, and standardised using reference sera supplied by the United States Food and Drug Administration.

On what basis, then, should we licence vaccines? How often does immunologic memory fail? The denominator from which

our study population is derived must be considered. The UK Hib experience is now based on 22 million child years of follow up over a nine year period. Based on disease incidence rates predating vaccine implementation, 6509 cases would have been expected to occur in the UK over this time. In contrast, only 171 vaccine failures have been reported throughout the entire population following a full course of infant immunisation, with an estimated effectiveness of 97.4% (95% CI 96.9 to 97.8). Although a majority of children in the UK are protected from Hib disease by immunisation, the relative roles of immunologic memory and other immune mechanisms in conferring protection remain unclear. It is important now to determine whether this group of vaccine failures would have been protected by a further vaccine boost to maintain higher antibody titres, or whether they have some other subtle defect in their defence against Hib which is independent of the conjugate vaccine's immunogenicity. The ability of the UK Hib dataset to address issues of this kind highlights the value of ongoing post licensure surveillance.

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