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# Differing antibody responses to *Haemophilus influenzae* type b after meningitis or epiglottitis

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## SUMMARY

Two common forms of invasive disease due to *Haemophilus influenzae* type b (Hib) are epiglottitis and meningitis. It is not known why some children develop epiglottitis and others meningitis. To examine the hypothesis that epiglottitis occurs in children who may have been previously exposed to Hib, and who would therefore exhibit a more vigorous antibody response in convalescence, we measured levels of antibody to Hib capsule in 92 children. Geometric mean convalescent-phase IgG, IgA, IgM and total antibody levels were significantly higher in 45 children with epiglottitis than in 47 with meningitis, even after adjustment for age differences (mean total antibody, 95% confidence intervals: meningitis 0·38, 0·34–0·43; epiglottitis: 2·25, 2·0–2·54 $\mu$g/ml). However, contrary to previous reports, a poor antibody response was only observed in a minority of children with meningitis; the antibody response of the majority was indistinguishable from that observed in children with epiglottitis.

## INTRODUCTION

Prior to the introduction of conjugate vaccines, the two most common forms of invasive disease due to *Haemophilus influenzae* type b (Hib) in Western communities were epiglottitis and meningitis [1]. It is not known how Hib is able to cause such different clinical diseases within the same population of susceptible children. Studies of Hib bacterial epidemiology have failed to show that specific Hib subtypes causes specific types of invasive disease [2], suggesting instead that host differences are responsible.

Because children with epiglottitis are generally older than those with meningitis [3–8], we proposed that those with epiglottitis may have acquired 'partial immunity' because of prior exposure to Hib, enabling them to better localise their infection. Alternatively, the different age distributions of the two diseases may indicate that epiglottitis occurs in children capable of a mature response to Hib, while meningitis occurs in those with immature or innately impaired responses. In a previous study designed to test these hypotheses, we compared levels of Hib outer-membrane-protein (OMP) antibody in children with epiglottitis or meningitis but were unable to demonstrate differences in OMP antibody levels in acute sera or in the ability to respond in convalescence (3). In this study, we compared levels of antibody to Hib polysaccharide in a larger group of children with either epiglottitis or meningitis.

## PATIENTS AND METHODS

### Patients

Parents of all children admitted to the Royal Children's Hospital with confirmed epiglottitis or meningitis between February 1988 and August 1990 were approached for permission to enter their children

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into this study. The study was approved by the Ethics in Human Research Committee of the Royal Children's Hospital, and all work was carried out in accordance with the committee's guidelines.

As previously described [3, 4, 9], cases were defined as a compatible clinical illness occurring in a child aged under 16 years plus isolation of Hib from blood or cerebrospinal fluid (CSF), detection of Hib antigen in urine or CSF, or isolation of Hib from the throat of a child in whom epiglottitis had been confirmed by laryngoscopy. Cases of epiglottitis were also included without bacteriological confirmation if epiglottitis was confirmed by laryngoscopy and the clinical features were judged to be typical by two experienced paediatricians.

Acute sera were collected during hospital admission and convalescent sera were collected at follow-up review, approximately 6 weeks after discharge from hospital. Seventy-five of the convalescent sera (including 28 with paired acute sera) were obtained during a prospective study of cases of all invasive Hib disease admitted to the Royal Children's Hospital from February 1988 to February 1990 [10]. An additional 17 sera (including 15 paired sera) were obtained from children admitted to the hospital from February 1990 to August 1990 according to the same criteria. Sera were aliquoted and stored at −70 °C until use.

No children in this study had received Hib vaccine.

### Hib capsular-polysaccharide antibody ELISA method

Antibody levels were measured by ELISA as described by Phipps and colleagues [11]. A standard serum, the specific anti-PRP IgG, IgA and IgM content of which had been measured, was used to generate a standard curve for each assay (serum kindly supplied by Dr Carl Frasch of the Office of Biologics, FDA, Bethesda, MD, USA).

The limit of detectability of the assay, originally published as 0·1 μg/ml [12], has been shown by limiting dilution in our laboratory to be better than 0·02 μg/ml for each of the antibody isotypes (unpublished data). Serial dilution of positive test sera showed parallel dilution curves with the standard serum.

### Statistical methods

Results were transformed to log values before analysis. Zero values were accommodated by arbitrarily adding 0·01 μg/ml to all results (one-half of the limit of detectability of this assay).

Table 1. *The table shows the squared semi-partial correlation ($sr^2$) and P value for the variables diagnosis, age and serum collection time. The $sr^2$ value indicates the relative influence that each variable had on the measured level of antibody (see text)*

| Variable | $sr^2$ (%) | *P* |
|---|---|---|
| Total acute-phase antibody | | |
| Serum collection | 33·3 | < 0·001 |
| Age | 0·1 | 0·82 |
| Diagnosis | 5·3 | 0·1 |
| Total convalescent-phase antibody | | |
| Serum collection | 0·14 | 0·65 |
| Age | 19·1 | < 0·001 |
| Diagnosis | 5·8 | 0·004 |

Diagnosis (epiglottitis or meningitis), patient age and serum collection time were all possible influences on the level of Hib antibody. The relative importance of these three variables was therefore estimated by calculating the $sr^2$ value for each variable [13]. The $sr^2$ value estimates the contribution of each predictor variable to the overall coefficient of determination ($r^2$) when all variables are analysed together by multiple regression. The $sr^2$ value can be regarded as indicating the unique influence that diagnosis (epiglottitis or meningitis), age, or serum collection time had upon the measured level of antibody in each serum sample (Table 1).

To adjust for differences in age and serum collection time between groups, acute and convalescent-phase antibody levels for each patient were mathematically corrected by analysis of covariance (ANCOVA) with a computer statistical software package (Minitab Version 8·2). In principle, the ANCOVA adjusted each antibody result so that the confounding effect of age and serum collection time were removed. After correction, the adjusted antibody levels from children with epiglottitis or meningitis were directly compared.

For clarity, adjusted geometric mean results have been expressed as the mean and 95% confidence interval after conversion back from log values. Other comparisons were made by two tailed *t* tests or Fisher's exact test as appropriate.

## RESULTS

### Comparison of groups

Convalescent-phase sera from 92 children, 47 with meningitis (26 males, 21 females) and 45 with epiglottitis (25 males, 20 females) were tested. Acute-

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Table 2. *Adjusted geometric mean acute and convalescent-phase antibody levels in children with meningitis or epiglottitis (95% confidence intervals are shown in brackets). Antibody levels were adjusted for age and serum collection time before comparison*

| Acute-phase | Meningitis ($n = 17$) | Epiglottitis ($n = 26$) | $P$ |
|---|---|---|---|
| IgG acute | 0·04 (0·03–0·05) | 0·08 (0·06–0·1) | 0·4 |
| IgA acute | 0·02 (0·01–0·03) | 0·09 (0·07–0·1) | 0·09 |
| IgM acute | 0·03 (0·02–0·05) | 0·05 (0·04–0·06) | 0·6 |
| Total acute* | 0·09 (0·06–0·11) | 0·36 (0·3–0·43) | 0·1 |
| **Convalescent-phase** | **Meningitis ($n = 47$)** | **Epiglottitis ($n = 45$)** | **$P$** |
| IgG conv. | 0·25 (0·22–0·28) | 1·01 (0·89–1·15) | 0·03 |
| IgA conv. | 0·04 (0·03–0·05) | 0·46 (0·42–0·51) | 0·001 |
| IgM conv. | 0·06 (0·05–0·07) | 0·24 (0·22–0·26) | 0·001 |
| Total conv.* | 0·38 (0·34–0·43) | 2·25 (2·0–2·54) | 0·004 |

* Total antibody was not separately measured and is the sum of IgG, IgA and IgM.

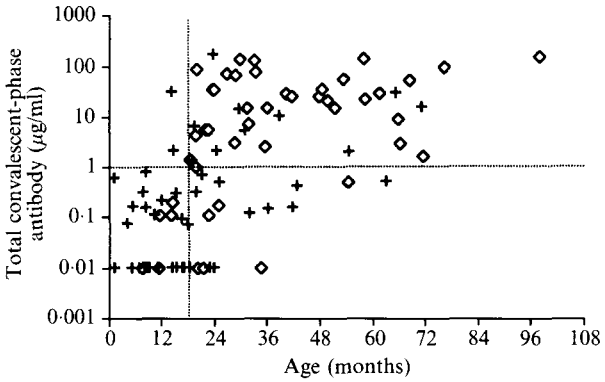


**Fig. 1.** Unadjusted, convalescent-phase total anti-capsular antibody in 47 children with meningitis (+) and 45 with epiglottitis (◇). The vertical axis shows micrograms per ml of total antibody on a logarithmic scale, the horizontal axis shows age in months. The dashed lines divide the data according to age (greater or lesser than 18 months) and antibody level (greater or less than 1·0 μg/ml). Eighty-three percent of children older than 18 months with epiglottitis had levels of convalescent antibody above 1·0 μg/ml, but this only applied to 45% of those with meningitis.

phase sera were also collected from 43 of these 92 children (17 meningitis, 26 epiglottitis).

Of the children with meningitis, all but one had positive cultures for Hib from blood or CSF or both. A single patient with meningitis had negative cultures but had a typical illness, abnormal CSF findings and a positive CSF Hib antigen test. Of those with epiglottitis, 32 (71%) had positive blood cultures. Of the remaining 13, eight had positive throat swabs for Hib, three had positive Hib urine antigen tests only and two had typical clinical features but no bacteriological confirmation of Hib.

The children with epiglottitis were significantly older than those with meningitis, although there was overlap in age between the groups (mean, median and range: epiglottitis 37·4, 32, 7·5–98 months; meningitis: 21·3, 17, 1·4–72 months, $P < 0·001$).

The majority of acute-phase sera from children with epiglottitis were collected within 1 day of admission to hospital (mean, 95% confidence interval: 1·04, 0·7–1·4 days after admission). However, for children with meningitis (who typically spend considerably longer in hospital than children with epiglottitis), samples were, in retrospect, collected significantly later (mean, 95% confidence interval: 3·2, 2·3–4·1 days after admission). Convalescent-phase sera were collected a mean of 49·1 days (95% c.i. 46·0–52·1 days) after admission for the whole group but, again in retrospect, samples from children with meningitis were collected a mean of 10 days later than from those with epiglottitis (95% c.i. 4·6–16·2 days later).

### Acute-phase antibody levels

The majority of children in both groups had very low level or undetectable antibody in acute-phase sera. In those with detectable acute-phase antibody, the level was strongly correlated with serum collection time, but not with age (Table 1). Adjusted acute-phase antibody levels did not differ significantly between the groups (Table 2).

### Convalescent-phase antibody levels

Convalescent-phase antibody levels were strongly correlated with age but not with serum collection time (Table 1, Fig. 1).

All but two children younger than 18 months either had no detectable antibody during convalescence or a level of total antibody less than 1·0 μg/ml. In contrast, 69% of all children aged over 18 months had levels of total antibody greater than 1·0 μg/ml (43 of 62). However, while this applied to 83% of those with epiglottitis (33 of 40), only 45% of those with meningitis (10 of 22) had levels greater than 1·0 μg/ml in convalescence ($P = 0·001$ Fisher's exact test). The only child over 2 years with no detectable convalescent antibody was a girl of 35 months with clinical epiglottitis from whom no positive cultures were obtained but who had Hib antigen detected in her urine. To ensure that our case definition of epiglottitis had not biased our results by the inclusion of non-Hib

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cases, the data set was reanalysed using results obtained only from children with positive blood cultures. Mean antibody levels in the 32 children with positive blood cultures were almost identical to those obtained for the whole epiglottitis group.

Adjusted geometric mean convalescent-phase anti-capsular antibody levels for children with epiglottitis or meningitis are shown in Table 2. Geometric mean IgG, IgA, IgM and total antibody levels in convalescent-phase sera were significantly higher in children with epiglottitis than in those with meningitis. Despite this difference, several children with meningitis also had quite high convalescent-phase levels, including one child aged 24 months who had the highest result of all (167 $\mu$g/ml) (Fig. 1).

### Clinical associations with antibody response in older children with meningitis

To determine whether a poor antibody response in meningitis was related in any way to the severity of the clinical illness, children older than 18 months with meningitis were arbitrarily divided into two groups. Group 1 comprised children older than 18 months with meningitis with convalescent-phase total antibody levels less than 1·0 $\mu$g/ml, and group 2 comprised those with antibody levels greater than 1·0 $\mu$g/ml. The two groups were then compared for levels of CSF glucose and total CSF white cell count on initial lumbar puncture, duration of fever, length of hospital stay and clinical outcome 6 weeks after discharge from hospital.

The 12 children in group 1 had significantly lower mean CSF glucose levels at the time of diagnosis than those in group 2 (group 1, $n = 12$: 1·02, 0·47–1·6, group 2, $n = 10$: 2·47, 1·3–3·5 mmol/l; $P = 0·05$). Furthermore, seven of 12 children in group 1 compared with 0 of 10 in group 2, had more than 7 days of fever during hospitalization ($P = 0·005$). No significant differences were observed between the groups in numbers of white cells in the initial CSF sample, duration of hospital admission or the presence of complications at outpatient review.

### Correlation between immunoglobulin isotypes

There was a strong positive correlation between all three immunoglobulin isotypes and between each isotype and total antibody in convalescent-phase sera, indicating that the depressed response shown by some children was not due to a failure to produce a particular immunoglobulin isotype.

## DISCUSSION

There are several epidemiological differences between epiglottitis and meningitis which suggest that patient age and disease incidence affect the clinical expression of Hib disease. The mean age of children with epiglottitis is higher than for those with meningitis [3–8], epiglottitis does not occur in populations with high rates of early onset disease [1, 14–20] and before the introduction of Hib conjugate vaccines, epiglottitis was as common as meningitis in some populations with low overall incidences of Hib disease [1].

Bacterial virulence factors may also influence the manifestation of Hib infection. For example, specific outer-membrane-protein subtypes of Hib have been shown to be particularly associated with naso-pharyngeal carriage [21], with spread within a day care centre [22] and with early age of disease onset and meningitis [23]. Despite these associations, it has not been possible to demonstrate a clear relationship between Hib subtype and epiglottitis or meningitis, either in our own patients [9] or elsewhere [2].

Whisnant and colleagues in the 1970s showed that the frequencies of certain HLA and MNS red cell phenotypes were different in children with epiglottitis compared with children with meningitis and that convalescent-phase age-adjusted capsular antibody levels were higher in children with epiglottitis [7, 8]. However, the two groups of children in these studies were drawn from different populations and the interval between illness and children of convalescent sera ranged from weeks to several years. In the 1980s, a study of antibody responses in 121 children with Hib disease showed a comparatively increased convalescent response in a small group with epiglottitis [24]. In the 1990s, Trollfors and colleagues found a difference in mean capsular antibody levels between children with epiglottitis and meningitis, which became less apparent with time and was no longer detectable in late convalescence (6–12 months) [25].

Our findings confirm these previous reports and again demonstrate that, on average, children with epiglottitis respond more vigorously in convalescence than those with meningitis even when allowance is made for the confounding effect of age. However, it appears that this relatively poor antibody response is not a general feature of children convalescing from Hib meningitis. On the contrary, children with

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meningitis aged over 18 months fell into two approximately equal groups with differing levels of convalescent-phase antibody. The difference in age-adjusted mean antibody levels between children with epiglottitis and meningitis in this study was at least partly attributable to a sub-group of poorly responding children with meningitis who may have had a more severe illness than the others, as indicated by lower CSF glucose [26–29] and a longer duration of fever. Alternatively, the reduction in convalescent-phase antibody levels in this group may reflect complexing of antibody within immune complexes. Hib antigen may persist for some weeks after an episode of invasive disease, and children with meningitis may have higher levels of circulating antigen than those with epiglottitis [30].

No differences were observed in acute-phase sera, but the significant variation in serum collection time made it difficult to interpret these results.

In conclusion, we have again demonstrated a significant difference in levels of convalescent-phase antibody to Hib capsular polysaccharide between children with epiglottitis or meningitis that cannot be explained by age alone. However, in this study, we have shown that the poor convalescent-phase response was not a general feature of children with Hib meningitis but was instead attributable to a sub-group of poor responders. Overall, the majority of children with Hib meningitis had a convalescent-phase antibody response to Hib capsule that was indistinguishable from that of similarly aged children with epiglottitis.

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