Antibody persistence and *Haemophilus influenzae* type b carriage after infant immunisation with PRP-T

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**Abstract**

*Objectives*—To assess the persistence of serum *Haemophilus influenzae* type b antibodies and the prevalence of *H. influenzae* type b carriage in a group of preschool age children previously vaccinated in infancy.

*Design*—Names were randomly selected from immunisation records. Families were visited on five occasions over a period of 12 months and throat swabs were taken from all family members present, with blood obtained from children at the first and last visits.

*Results*—One hundred and fifty three children at a median age of 3.6 years had a geometric mean titre (GMT) of 1.06 µg/ml (95% CI 0.80 to 1.38). Eight per cent had an undetectable antibody concentration, received a booster dose of plain PRP vaccine, and responded with concentrations > 2 µg/ml. GMT at 4.5 years of age was 0.89 µg/ml (0.69 to 1.16). Twelve children who had been exposed to *H. influenzae* had a GMT of 4.7 v 0.8 µg/ml for those without exposure.

*Conclusions*—Accelerated immunisation against *H. influenzae* without a second year booster results in persistence of satisfactory serum concentrations of antibody to 4.5 years of age. In those with undetectable antibody, immunological memory may still be present.

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**Keywords:** *Haemophilus influenzae* type b; conjugate vaccine; carriage; immunity
Subjects and methods

Using the Oxfordshire child health computer records, 309 names of children born between March and May 1991 who had received three doses of PRP-T, DTP, and oral polio vaccine by 5 months of age were randomly selected. After approval by their general practitioner (GP), families were sent a letter inviting them to participate in the study. Those who consented were visited at home on five occasions over the period of one year.

**SERUM ANTIBODIES AND *H INFLUENZAE* TYPE B BOOSTER**

At the first and last visits (at approximately 3.5 and 4.5 years of age) a specimen of blood was obtained after application of a local anaesthetic cream (EMLA, Astra Pharmaceuticals). The blood was centrifuged on return to the laboratory and serum stored at −20°C until serological tests were done. Anti-PRP antibodies were quantified using an enzyme linked immunosorbent assay technique described previously. A non-protective concentration was defined as < 0.15 µg/ml. Children with such a concentration at the first visit were offered a booster dose of the plain PRP vaccine (batch S3031, donated by Pasteur Merieux, Marnes La Coquette). A further specimen of blood was then obtained three to six weeks later. The PRP vaccine was chosen in preference to the conjugate vaccine better to assess the natural immune response on exposure to *H influenzae* type b. Extrapolating from studies in which previously vaccinated children were compared with unvaccinated children, it also seemed likely that the magnitude of the antibody response to the PRP vaccine might allow differentiation of those children primed by vaccination and those in whom immunological memory had been lost. Finally, as children of 3.5 years of age generally have a good antibody response to the plain PRP vaccine, it would also provide them with a protective concentration of anti-PRP antibody.

**THROAT SWABS**

Swabs were taken from the subjects and any other family member older than 12 months of age present at the time of the home visits. All swabs were performed by the same investigator (JBM) in a standardised manner: a cotton tipped wooden swab was passed firmly over the tonsillar region, including the crypts and the posterior pharyngeal wall. The swab was immediately placed into a vial of transport media (tryptone soy broth enriched with X and V factors, each to a concentration of 15 µg/ml) and kept at room temperature until plated onto enriched Columbia antisem agar plates on the same day as collection. Plates were inspected for colonies exhibiting iridescence or producing antigen antibody precipitation halos at 18–24 and 48 hours respectively after incubation at 37°C in an atmosphere of 5% carbon dioxide. The plates were then kept at 4°C and inspected daily for precipitation halos for a further three days. Three suspect colonies were subcultured onto media containing X, V, and X+V growth factors for identification of species. Strains identified as *H influenzae* were further analysed by conventional slide agglutination and polymerase chain reaction.

**STATISTICS**

As there was no unvaccinated control group available (> 90% of Oxford children have received the *H influenzae* type b vaccine), this was designed as a descriptive study. In a previous Oxford study, it was determined that 5% of vaccinated children at 4 years of age had an anti-PRP antibody concentration < 0.15 µg/ml. It was therefore calculated that a sample size of 150 would provide a 95% confidence interval (CI) of 1.5 to 8.5% assuming 7500 children of this age in Oxfordshire (Statcalc Epiinfo version 6). With an expected uptake of 50–60% from previous studies, 300 children were to be approached.

Statistical analyses were performed using SPSS (SPSS Inc, Chicago). Ages are reported as median (range) and anti-PRP antibodies converted by logarithmic transformation and reported as geometric mean titre (GMT) (95% CI). Antibody concentrations were compared using the Mann-Whitney U test or Wilcoxon’s matched pairs signed ranks test and carriage rates by Fisher’s exact test.

The study was approved by the Central Oxford Research Ethics Committee.

**Results**

Of 309 names obtained from the immunisation records, 274 families were approached (permission to approach families was refused by the GP in 22 cases, and 13 families had moved out of the area) and 162 (59%) of the 274 initially consented to participate.

**SERUM ANTIBODIES**

Blood was obtained from 153 out of 162 (94%) at the first visit (median age 3.6 years, range 3.5–3.7). The geometric mean antibody concentration (GMT) was 1.06 µg/ml (95% CI 0.80 to 1.38); 92% > 0.15 µg/ml, 51% > 1 µg/ml. The 12 individuals whose antibody concentration was < 0.15 µg/ml were given a dose of PRP vaccine and a further specimen of blood taken a median of 23 days later (range 17–35). The GMT was 8.8 µg/ml with all > 2 µg/ml. The majority (10 out of 12) had postbooster antibody concentrations between 2–10 µg/ml. In two individuals, however, concentrations of 41 and 59 µg/ml were recorded. This represents at least a 290-fold increase on concentrations before the booster.

These 12 children were otherwise well, with no history suggestive of immunodeficiency. All had immunoglobulins measured and only one had a mild deficiency (of total IgA, 0.3 g/l; lower limit for age, 0.4 g/l).

At a median of 4.5 years (4.4–4.6), 151 were available for a further blood sample (five refused a further test, four because they had already had a postbooster blood test; three withdrew during the course of the study, two moved out of the area, and one died). Blood was obtained in 147 (97%). The GMT was 0.93 µg/ml (0.73 to 1.19); 92% > 0.15 µg/ml, 48% > 1 µg/ml. Excluding the individuals who...
received a booster dose of PRP, the remaining 139 children had a GMT of 0.89 µg/ml (0.69 to 1.16); 91% > 0.15 and 45% > 1 µg/ml.

Paired specimens were available for 132 children (excluding those who received the PRP booster). The GMT dropped from 1.29 µg/ml (0.99 to 1.68) at 3.6 years to 0.91 µg/ml (0.69 to 1.19) at 4.5 years (p=0.001).

**Table 1 Influence of *H. influenzae* type b exposure on serum anti-PRP antibody concentrations.** At age 3.6 years *H. influenzae* type b status reflects carriage detected over the previous 12 months.

<table>
<thead>
<tr>
<th>Type of Exposure</th>
<th>At age 3.6 years</th>
<th>At age 4.5 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject a carrier</td>
<td>25.7 (1.1 to 579.4)</td>
<td>15.3 (1.3 to 178.7)</td>
</tr>
<tr>
<td>Subject not a carrier</td>
<td>0.9 (0.8 to 1.3)</td>
<td>0.8 (0.6 to 1.0)</td>
</tr>
<tr>
<td>Family member a carrier</td>
<td>1.4 (0.7 to 2.3)</td>
<td>1.3 (0.3 to 5.8)</td>
</tr>
<tr>
<td>Family member not a carrier</td>
<td>1.0 (0.8 to 1.3)</td>
<td>0.9 (0.7 to 1.1)</td>
</tr>
<tr>
<td>Subject +/- family member a carrier</td>
<td>22.7 (4.6 to 112.4)</td>
<td>4.7 (1.0 to 20.8)</td>
</tr>
</tbody>
</table>

- Anti-PRP antibody given in µg/ml with GMT (95% CI). All comparisons significantly different at p < 0.05, except for * where p = 0.5.

**Discussion**

Two observations appear to have been the basis of the US recommendations for a *H. influenzae* type b booster dose in the second year of life: the observed decline in serum anti-PRP antibody after primary vaccination and a clinical vaccine failure at 15 months of age reported in a trial of PRP-OMP conjugate vaccine. Studies with the conjugate vaccine PRP-T given at 2, 3, and 4 months of age have also shown a decline in serum antibody with age, from a GMT of 5.0 µg/ml at 5 months of age to 0.8 µg/ml at 12 months of age. We have now documented the persistence of a satisfactory concentration of antibody to 3.6 (GMT 1.1 µg/ml) and 4.5 years of age (0.9 µg/ml).

Serum anti-PRP antibody has been shown to correlate with protection against invasive *H. influenzae* type b disease, but the interpretation of this relationship has several limitations. For a non-vaccinated population the concentration of 0.15 µg/ml was a good cut off for disease susceptibility, but in a population vaccinated with an unconjugated PRP vaccine a higher concentration of 1 µg/ml was necessary. Reasons proposed for this difference include the additional presence in natural immunity of antibodies to *H. influenzae* type b components other than the polysaccharide and the lower avidity of antibodies induced by the unconjugated vaccine. In considering the correlation with antibody after use of a *H. influenzae* type b conjugate vaccine, account must be made of its capacity to induce immunological memory. This will not be reflected through simple assays.
of serum antibody. Its presence, however, might be inferred by the magnitude of the antibody response to a dose of the unconjugated PRP vaccine. Weinberg et al, for example, studied 30 children vaccinated at 2–17 months of age with PRP-OMP and revaccinated them 10–14 months later with the plain PRP vaccine. The resulting anti-PRP IgG concentration was 20-fold higher than that of 13 control children immunised with PRP for the first time. In our study, after receiving a PRP booster we observed two subgroups among those who initially undetectable anti-PRP antibody concentrations. Two individuals achieved antibody concentrations 290-fold and 420-fold higher than values before the booster. Neither child had documented H influenzae type b carriage nor exposure to a H influenzae type b carrier (although this cannot be excluded), and thus had clearly been primed. The remaining 10 individuals who achieved much lower anti-PRP concentrations appeared to have had a lesser degree, or possibly lacked immunological memory, for PRP. An impressive rise in antibody concentration (>300-fold) seen in two other individuals who were exposed to PRP through pharyngeal carriage of H influenzae type b is also consistent with priming. The adequacy of a lower anti-PRP antibody concentration after conjugate vaccines can also be inferred from the high protective efficacy shown in the Finish and Icelandic populations with the least immunogenic conjugate vaccine PRP-D.16 17

In the prevaccine era, susceptibility to H influenzae type b disease was relatively much lower by the age of 5 years. A study of unvaccinated children in this age group showed them to have a GMT anti-PRP antibody of 0.4 µg/ml (0.2 to 0.6) with 25% <0.15 and 20% >1 µg/ml.18 In a separate group of 70 3–6 year old unvaccinated Oxford children, the GMT was also 0.4 µg/ml (0.3–0.6) (personal communication, Dr Helen Griffiths). The figure of 0.9 µg/ml achieved at 4.5 years of age in this study therefore suggests maintenance of at least equivalent concentrations of antibody after vaccination at 2, 3, and 4 months of age in the absence of a booster dose. Even if those with documented exposure to H influenzae type b are excluded, the resulting GMT of 0.7 µg/ml compares favourably. A study performed in Oxford children aged 4 years old in 1991 showed a higher anti-PRP concentration in those previously immunised with HBoc at 3, 5, and 9 months of age than in unvaccinated children (GMT 1.4 (0.8 to 2.2) v 0.4 (0.2 to 0.6) µg/ml).12 Using the Swedish schedule of 3, 5, and 12 months and a different PRP-tetanus toxoid conjugate, Claesson et al have also showed persistence of higher anti-PRP antibody concentrations at 6 years of age when compared with unvaccinated children (GMT 2.06 v 1.32 µg/ml, total anti-PRP antibody).14

The capacity of the H influenzae type b conjugate vaccines to reduce pharyngeal carriage of H influenzae type b has been documented in several countries,15 16 and we have also shown it in our study. To do so, however, we used an historical control group. The absence of a difference in isolation rates of non-H influenzae type b among these cohorts provides support for the validity of this comparison.

The role of H influenzae type b carriage in boosting serum anti-PRP antibody is indicated by the higher concentrations in those with documented carriage. At the first antibody assessment at 3.6 years of age, it also appeared that exposure to a family member influenced antibody concentrations, but this was not the case at 4.5 years of age. Since it is likely that self carriage of H influenzae type b is required to boost serum antibody, this difference may be accounted for by the lack of background data on subjects at the first visit. It is conceivable that they were carriers before the first samples were taken and thus had already been boosted.

A previous study gave circumspect evidence for an association between higher antibody concentrations and concurrent H influenzae type b carriage.19 We have been able to strengthen this association by describing two individuals who had serum anti-PRP antibody measured at the beginning and end of the study period, and who showed dramatic rises in antibody in the presence of H influenzae type b carriage. We also documented spontaneous, but lesser, increases in a further 28 individuals. Two explanations may be put forward for this. H influenzae type b exposure may have taken place, but was undetected. For example, colonisation might have occurred between three monthly swabs, at a concentration below the limits of detection of our culture, or in another contact who was not swabbed. Another possibility is that these individuals were boosted by cross reactive antigens. What is striking is that large boosts in anti-PRP concentrations require pharyngeal colonisation with H influenzae type b.

Will this concentration of antibody continue to decline as these children get older, particularly as boosting by H influenzae type b carriage diminishes? An implication of this is that a pool of susceptibles will be created in later childhood. This phenomenon is now well described with other childhood vaccine programmes and has necessitated booster doses being introduced at older ages. Of particular relevance to protection against H influenzae type b disease, however, is the potential that these individuals have immunological memory for H influenzae type b. In addition, it is possible that exposure to other natural antigens might help elicit anti-PRP antibodies. Cross reacting bacteria of the respiratory and intestinal tracts have been described, the best studied example being Escherichia coli K100.20 The age related acquisition of these natural antibodies may therefore lessen the decline in anti-PRP antibody as children grow older. Continued surveillance of invasive disease and the prevalence of serum antibody in vaccinated children as they grow older is needed to answer this question.

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