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

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Abstract

***Ob jectives*—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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Routine immunisation with *H influenzae* type b conjugate vaccines was introduced in the UK in October 1992. Primary immunisation with PRP-T (polyribosyl-ribitol phosphate (PRP) conjugated to tetanus toxoid, Pasteur-Merieux) given simultaneously, but by separate injection from diphtheria, tetanus, pertussis (DTP) vaccine was offered at 2, 3, and 4 months of age. For the first year a 'catch up' programme offered one dose of HbOC (oligosaccharides of PRP conjugated to a mutant diphtheria toxin CRM197, Lederle Praxis) to older children aged between 12 and 48 months.

This 'accelerated' immunisation schedule was instituted in 1990 to improve uptake rates and achieve earlier protection against pertussis than the previous 3, 5, and 9 months schedule.[1] It differs from schedules used in the United States and most other European countries where primary vaccination is completed at an older age (usually 6 months) and a *H influenzae* type b booster is provided in the second year of life.

Although the highest incidence of invasive *H influenzae* type b infection in the era before the use of vaccine was recorded in children less than 2 years of age, considerable morbidity was also seen until 5 years of age and older.[2] This age related susceptibility to *H influenzae* type b disease correlates with absent or low serum antibody concentrations to PRP.[3] The natural acquisition of anti-PRP antibody is thought to be due to exposure to *H influenzae* type b through asymptomatic oropharyngeal carriage or through exposure to other cross reactive antigens.[4] The relative contribution to natural immunity of each of these is not known. An unexpected effect of widespread vaccination with *H influenzae* type b conjugate vaccines has been a reduction of *H influenzae* type b carriage.[5] In a vaccinated population therefore, boosting of serum antibody through carriage of *H influenzae* type b may not be relied upon, and vaccine induced protection may need to endure until at least school age. This raises the possibility that without a booster dose, vaccine protection may wane prematurely.

The best means of identifying loss of protection is through active surveillance for cases occurring in vaccinated children. Surveillance for vaccine failures is currently being performed through collaboration of the Oxford Vaccine Group, the British Paediatric Surveillance Unit, and the Public Health Laboratory Service Communicable Disease Surveillance Centre. Maintenance of high vaccine efficacy into the fourth year of life has been shown.[6]

As serum anti-PRP antibody correlates with protection against *H influenzae* type b disease,[7] this may also be used to assess the duration of protection. In Oxford, routine vaccination began 18 months before the national programme, giving us the opportunity to assess the persistence of serum anti-PRP antibody in a group of children not yet of school age. Other objectives of the study included assessment of: (1) the antibody response to a booster dose of PRP vaccine in those with unprotective anti-PRP antibody concentrations at the first visit; (2) the prevalence of oropharyngeal carriage of *H influenzae* type b in these children and their families over the period of a year; and (3) the influence of such carriage on the children's antibody concentrations.

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.[8] A non-protective concentration was defined as < 0.15 μg/ml.[7] 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,[9] 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,[10] 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 antiserum 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.[11]

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,[12] 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).

PHARYNGEAL CARRIAGE OF *H INFLUENZAE* TYPE B

Throat swabs were obtained from 160 children on one or more occasions over the course of the year. The point prevalence of *H influenzae* type b carriage at each of the five visits was 1.9% (three out of 158) at the first visit, 0.6% (one out of 159) at the second, 0% (out of 158) at the third, 0% (out of 157) at the fourth, and 1.3% (two out of 156) at the final visit. The first visit took place over the winter, the second and third over the spring and summer, and the fourth and fifth over the autumn and winter. Overall 3.1% (five out of 160) of the subjects carried *H influenzae* type b on one or more occasions, one child carried on two consecutive visits.

Any other family members over the age of 12 months who were present at the time of the home visit were also swabbed. Overall 3.1% (five out of 160) of mothers, 0.5% (one out of 89) of fathers, and 3.2% (five out 156) of siblings had *H influenzae* type b detected on one or more occasions over the 12 months. A seasonal variation was also evident. Of the 16 isolations of *H influenzae* type b over the course of the study, only six involved two members of the same family.

H INFLUENZAE TYPE B CARRIAGE AND SERUM ANTIBODY

Table 1 presents the relationship between serum anti-PRP antibody concentrations and carriage or contact with a carrier of *H influenzae* type b. At the first visit (median 3.6 years of age), the seven children who were either carriers of *H influenzae* type b (3), or were exposed to a carrier in their family (4), had a higher GMT (22.7 μg/ml) than those without recognised exposure to *H influenzae* type b (0.9 μg/ml, p<0.001). At the final visit, the 12 individuals who had been exposed to *H influenzae* type b over the previous 12 months had a higher GMT than the 127 without such exposure (excluding those who received a booster): 4.7 μg/ml *v* 0.8 μg/ml, p=0.008.

Thirty of the 132 children with paired blood specimens from the first and final visits showed an increase in antibody concentrations. The most dramatic increases were seen in two individuals who had documented *H influenzae* type b carriage during the year. The antibody concentrations (μg/ml) at the first visit were 1.12 and 0.2 and rose to 550 and 61.8 respectively. Excluding these two individuals, the GMT for the remaining 28 went from 0.64 (0.43 to 0.96) to 1.48 μg/ml (1.00 to 2.18) (p<0.001). A greater than twofold rise in antibody concentration was seen in 50% and a greater than fourfold rise in 21%.

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 indicates carriage at this visit; at age 4.5 years H influenzae type b status reflects carriage detected over the previous 12 months

H influenzae type b status	*At age 3.6 years*	*At age 4.5 years*
Subject a carrier	25.7 (1.1 to 579.4) n=3	15.3 (1.3 to 178.7) n=5
Subject not a carrier	0.9 (0.8 to 1.3) n=150	0.8 (0.6 to 1.0) n=134
Family member a carrier	20.6 (2.7 to 156.3) n=4	1.3 (0.3 to 5.8) n=7*
Family member not a carrier	1.0 (0.8 to 1.3) n=149	0.9 (0.7 to 1.1) n=132*
Subject +/- family member a carrier	22.7 (4.6 to 112.4) n=7	4.7 (1.0 to 20.8) n=12
No subject or family carriers	0.9 (0.7 to 1.2) n=146	0.8 (0.6 to 1.0) n=127

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.

H INFLUENZAE CARRIAGE BEFORE AND AFTER VACCINE PERIODS

Finally, we compared the carriage rates of *H influenzae* among children in this study with those of a previous Oxford study.[12] Children were of a similar median age (54 *v* 52 months), throat swabs and microbiological techniques were performed in the same way, and the specimens were taken at the same time of year (October/November 1995 *v* November 1991). The carriage rate in the current study was significantly lower than that seen in 1991: 1.3% (two out of 156) *v* 6.7% (eight out of 120), p=0.02. Interestingly, there was no significant difference in carriage rates of *H influenzae* type f (3.2% *v* 4.2% p=0.8); or *H influenzae* type e (3.8% *v* 5.8% p=0.6).

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.[13] 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.[8 14] 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.[7] 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.[9] 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 with 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.[12] 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).[18]

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,[5 19 20] 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 circumstantial evidence for an association between higher antibody concentrations and concurrent *H influenzae* type b carriage.[12] 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.[21] 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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