ORIGINAL ARTICLE

Responses to a fourth dose of *Haemophilus influenzae* type B conjugate vaccine in early life

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Objective: To describe the immune response of preterm infants, with a reduced response to primary *Haemophilus influenzae* type B (Hib) immunisation, to a fourth dose of Hib conjugate vaccine given in early life.
Design: Prospective observational study.
Setting: Five Wessex Neonatal Units.
Patients: Infants born at < 32 weeks and immunised with three doses of combined acellular pertussis-Hib vaccine, with a Hib IgG geometric mean concentration (GMC) < 1.0 μg/ml after these primary immunisations.
Interventions: An additional fourth dose of Hib conjugate vaccine given before 1 year of age. Blood taken to assess Hib IgG concentration and avidity after immunisation.
Main outcome measures: Hib IgG GMC and avidity index.
Results: Ninety six infants (mean gestational age at birth 29.1 weeks) received a fourth dose of Hib at a mean age of 7.8 months. Hib IgG GMC after the primary immunisations was 0.17 μg/ml (95% confidence interval (CI) 0.14 to 0.20) rising to 4.68 μg/ml (95% CI 3.36 to 6.57) after the fourth dose ($p < 0.0001$). The IgG response to the fourth dose correlated positively with the response after the primary immunisations ($p < 0.001$). Hib IgG geometric mean avidity index (GMAI) after the primary immunisations was 30.87 (95% CI 20.40 to 46.73). This increased to 124.73 (95% CI 109.93 to 141.51) after the fourth dose ($p < 0.0001$).
Conclusion: Preterm infants with very low IgG responses to Hib after primary immunisations with a combined acellular pertussis-Hib vaccine mount a good response to a fourth dose of Hib. This study suggests that all infants will benefit from a fourth dose of Hib, regardless of the age at which it is given.

Since 1998 invasive *Haemophilus influenzae* type B (Hib) disease in the United Kingdom has been increasing despite high levels of immunisation. This may in part be due to a failure of a large number of infants to achieve protective Hib IgG titres after accelerated immunisation at 2, 3, and 4 months with acellular pertussis containing Hib combination vaccines.[1] In response, the UK Department of Health has offered all children aged 6 months to 4 years a Hib booster.[2] There are, however, few data available on the use of an early (< 1 year of age) booster dose of Hib in infants with low IgG titres after accelerated primary immunisation and no data generated on Hib boosting when primary immunisation has been given at the same time as a meningococcal serogroup C conjugate vaccine, introduced into the United Kingdom schedule in 1999.

We have previously shown that the IgG response of preterm and term infants to Hib combined with an acellular pertussis vaccine is very low.[3] In this study we investigate the response of preterm infants with low levels of Hib IgG after primary immunisation to an early fourth dose of Hib. We believe the results provide the evidence base for the recent Hib booster campaign, particularly as the group studied (preterm infants) represents the ''worst case scenario'' in relation to Hib response.

METHODS

Subjects and immunisations

Preterm infants born at less than 32 weeks gestation were recruited from five neonatal units in one UK region. The study had ethical approval from the four local research ethics committees serving the five centres, and informed consent was obtained from the parents before enrolment. All infants received three doses of a combined diphtheria/tetanus/acellular pertussis (DTaP)-Hib conjugate vaccine (Infanrix-Hib; GlaxoSmithKline, Uxbridge, Middlesex, UK; 0.5 ml dose containing 30 IU diphtheria toxoid, 40 IU tetanus toxoid, 25 μg pertussis toxin, 25 μg filamentous haemagglutinin, 8 μg pertactin, and 10 μg purified capsular polysaccharide of Hib covalently bound to about 30 μg tetanus toxoid) and meningococcal serogroup C conjugate vaccine (Meningitec; Wyeth, Collegeville, Pennsylvania, USA; 0.5 ml dose containing 10 μg meningococcal serogroup C oligosaccharide conjugated to about 15 μg CRM_{197} protein) at 2, 3, and 4 months of age.[3] Preterm infants with a Hib IgG < 1.0 μg/ml after the three primary immunisations were offered a fourth dose of Hib conjugate vaccine (Act-HIB; Aventis Pasteur MSD, Lyon, France; 0.5 ml dose containing 10 μg purified Hib capsular polysaccharide covalently bound to 24 μg tetanus protein).

Assays

Blood was taken by needle venepuncture into Microtainer Serum Separator tubes four weeks after the additional fourth Hib vaccine was given. Antibody concentrations to Hib were assayed at the Centre for Applied Microbiology and Research (CAMR), Salisbury, Wiltshire, UK by enzyme linked immunosorbent assay using a standardised protocol as previously described.[4] The lower limit of detection of the assay was 0.15 μg/ml. Samples with undetectable Hib IgG were assigned a value of half the lower limit of detection (0.08 μg/ml). Hib IgG avidity was determined by the

Abbreviations: 95% CI, 95% confidence intervals; GMAI, geometric mean avidity index; GMC, geometric mean concentration; Hib, *Haemophilus influenzae* type B

Table 1 Geometric mean concentration (GMC) of Hib IgG after both primary immunisations and additional fourth dose of Hib and geometric mean avidity index (GMAI) after the fourth dose in preterm infants, stratified according to response to primary immunisations

Response to primary immunisations (μg/ml)	GMC after primary immunisations	GMC after fourth dose	GMAI after fourth dose
< 0.15	0.08	2.59	86.58
95% CI	Assigned	1.64 to 4.10	68.53 to 109.40
Number	51	51	42
0.15–1.0	0.40	9.51	127.00
95% CI	0.34 to 0.46	6.08 to 13.76	100.60 to 160.33
Number	45	45	38
p Value	< 0.0001	0.0001	0.022

95% CI, 95% confidence interval.

Immunobiology Unit, Institute of Child Health, London, UK as previously described.[5] Avidity results in this study are multiplied by 100 compared with previous reports for computational ease. Avidity could only be measured where Hib IgG was > 0.5 μg/ml. The lower limit of detection of the avidity assay was 50 avidity units. Sera with unrecordable avidity were assigned a value equal to half the lower limit of detection (25 avidity units).

Statistical analysis

Hib IgG concentrations after the third and fourth doses were $\log_{10}$ transformed to achieve normality. Hib IgG geometric mean concentrations (GMCs) and avidity index (GMAI) were calculated with 95% confidence intervals (95% CI) and compared using Student's *t* test. The effect of patient variables on antibody responses was assessed using linear regression. Variables with $p < 0.2$ were entered into a multivariable regression model.

RESULTS

A total of 122 preterm infants (mean gestational age at birth 29.1 weeks (range 24.2–31.9); mean birth weight 1266 g (range 490–2100)) were immunised at 2, 3, and 4 months of age. The Hib IgG response in 105 of these infants after primary immunisations has been reported previously.[3] The Hib IgG GMC after the primary immunisation for all the preterm infants was 0.29 μg/ml. Ninety six (79%) had Hib IgG < 1.0 μg/ml after primary immunisations and received a fourth immunisation; in 51 (42%) Hib IgG was < 0.15 μg/ml (historical level for short term protection). The mean age at receipt of the additional dose was 7.8 months (range 6.2–11.5).

Table 2 Proportion of infants achieving Hib IgG concentrations ⩾ 0.15 and ⩾ 1.0 μg/ml after primary immunisations and after additional fourth dose of Hib conjugate vaccine

Hib IgG (μg/ml)	After primary immunisations (n = 122)	After fourth dose (n = 96)
⩾ 0.15	58%	95%
⩾ 1.0	21%	85%

In these 96 infants, the Hib IgG GMC after the primary immunisation was 0.17 μg/ml (95% CI 0.14 to 0.20). This rose to 4.68 μg/ml (95% CI 3.36 to 6.57) after the fourth dose ($p < 0.0001$). Table 1 shows GMCs for infants with Hib IgG concentration < 0.15 μg/ml and ⩾ 0.15 μg/ml after the primary and booster immunisations. Table 2 shows the proportions of infants achieving Hib IgG concentrations ⩾ 0.15 and 1.0 μg/ml after the primary and additional fourth immunisations. Five infants failed to achieve a Hib IgG ⩾ 0.15 μg/ml after the fourth dose. There was no difference between these infants and those who did achieve concentrations ⩾ 0.15 μg/ml in mean age at receipt of the fourth dose (8.1 and 7.8 months respectively, $p = 0.52$).

The effect of a number of patient variables (gestational age at birth, birth weight, age at third dose, Hib IgG after third dose, age at fourth dose, number of doses of antenatal steroids, and number of courses of postnatal steroids) on IgG response to the fourth dose were assessed using linear regression. Variables with $p < 0.2$ in linear regression (age at third dose, $p = 0.063$; IgG after third dose, $p < 0.001$; age at fourth dose, $p = 0.034$) were analysed further using multivariable regression. Table 3 shows the results of the multivariable regression analysis. Of note, IgG response to a fourth dose correlated positively with age at fourth dose in linear regression, but this ceased to be significant when controlled for age at completion of the primary immunisations.

The Hib GMAI was determined after the third dose, and after the fourth dose when given. As the response to the primary immunisations was so low in this group, it was only possible to measure GMAI after the third dose in 11 infants. GMAI after the third dose in infants not requiring an additional dose was also measured. Table 4 shows the results. Table 1 shows Hib GMAIs after the fourth dose for infants with Hib IgG < 0.15 μg/ml or ⩾ 0.15 μg/ml after the primary immunisations.

Table 3 Multivariable analysis: influence of age in days at completion of primary immunisations, IgG response (μg/ml) to primary immunisations, and age (days) at receipt of fourth dose on IgG response to a fourth dose of Hib

	Age at third dose	IgG after third dose	Age at fourth dose
% Change in IgG titre per unit change in variable	+0.1%	+71.6%	+0.4%
95% CI	−0.8% to +1.0%	+35.2% to +100%	−0.02% to +0.9%
p Value	0.77	< 0.001	0.061

95% CI, 95% confidence interval.

Table 4 Hib geometric mean antibody avidities (GMAIs) after the third dose of Hib for infants who did not require a fourth dose and after the third and fourth dose for those who did

	Hib IgG >1.0 μg/ml after primary immunisations (no 4th dose given)	Hib IgG <1.0 μg/ml after primary immunisations (4th dose given)
Hib GMAI after 3rd dose	76.87	30.87
95% CI	67.15 to 88.00	20.40 to 46.73
Number	26	11
Hib GMAI after 4th dose	NA	103.86
95% CI	NA	87.87 to 122.77
Number	NA	80
p Value	NA	< 0.0001

95% CI, 95% confidence interval; NA, not applicable.

Of those infants who were given an additional dose of Hib, 10 had received at least one course of postnatal steroids for chronic lung disease. The Hib GMC for these infants after the primary immunisations was 0.16 μg/ml. This rose to 3.68 μg/ml after the fourth dose ($p = 0.002$).

DISCUSSION

We have shown that preterm infants with very low IgG responses to Hib conjugate vaccine mount an adequate response to an early additional fourth dose of Hib. The GMC after the booster, even in infants who were < 0.15 μg/ml after the primary immunisations, compares favourably with the concentrations achieved after the three dose primary course in term infants receiving a separate Hib vaccine at 2/3/4 months (Hib IgG GMC 4.50 μg/ml, 95% CI 3.21 to 6.32).[6] These findings, in preterm infants with IgG concentrations after the primary immunisations lower than those of term infants[3] indicate that good responses can be expected in term infants boosted soon after primary immunisation, as occurred recently in the United Kingdom.

We are unable to say with certainty whether the response to the additional dose represents a primary or a memory response, as there are no comparable data on the response expected in naïve and primed infants to a booster dose given at this early age. However, as the GMAI after the booster was higher in the group with Hib IgG concentrations ⩾ 0.15 μg/ml after the primary immunisations than in those with concentrations of < 0.15 μg/ml, this suggests some degree of memory in the former group. The GMAI after the booster in those with concentrations < 0.15 μg/ml was similar to that seen in the 26 infants with concentrations >1.0 μg/ml, suggesting a primary rather than a memory response.

This study strongly suggests that all infants will benefit from an additional fourth Hib dose, irrespective of the timing of its delivery or the antibody concentrations achieved after the third dose. For children in whom the fourth dose may be a priming rather than a boosting dose, there is the question of whether a subsequent booster would be needed. We intend to follow up this cohort of preterm infants to compare antibody persistence in those for whom the fourth dose was acting as a booster compared with those in whom it was probably a priming immunisation.

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