ORIGINAL ARTICLE

Hib vaccination in infants born prematurely

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Aims: To document the immunogenicity and persistence of antibody to polyribosyl-ribitol phosphate (PRP) as well as the clinical protection against invasive *Haemophilus influenzae* type b (Hib) disease in premature infants immunised at the routine schedule.

Methods: Blood was obtained at 2, 5, 12, and 64 months of age from a cohort of prematurely born infants ($\leq$32 weeks gestation). Anti-PRP antibody concentrations were compared with those of a control cohort of infants born at full term and vaccinated at the same schedule. Hib vaccine failures occurring between October 1992 and October 2000 were reported by paediatricians through an active, prospective, national survey in the UK and Republic of Ireland. The number of prematurely born children with vaccine failure was compared with the corresponding number born at term.

Results: Twenty seven prematurely born infants were followed to 5 years of age. Compared with term infants they had a significantly lower geometric mean concentration of anti-PRP antibody and/or a significantly lower proportion above one or both of the conventional protective antibody concentrations (0.15 and 1.0 µg/ml) at all ages. A total of 165 cases of invasive Hib disease were identified over eight years of national surveillance. Eighteen were premature (<37 weeks); approximately 12 would be expected. The relative risk of UK premature infants developing disease compared with term infants was 1.5 (95% CI 0.9 to 2.6).

Conclusions: Premature infants develop lower antibody concentrations than term infants following Hib conjugate vaccination. Premature infants may also have an increased risk of clinical vaccine failure, but interpretation is limited by the small number of premature infants developing invasive Hib disease over eight years of national surveillance. Overall, vaccination with Hib conjugate vaccines affords a high level of protection to premature babies.

Infants born prematurely are immunised according to chronological age without taking account of their gestational age or birth weight. Among the reasons for this policy are that premature infants may be at increased risk of certain vaccine preventable diseases such as pertussis and that varying the schedule for certain groups may result in confusion and a lower rate of vaccine coverage. Studies with diphtheria, tetanus, and pertussis (DTP) vaccines also suggest that premature infants can achieve adequate antibody concentrations.[1–3]

For other vaccines the data are variable. D'Angio and colleagues[2] and O'Shea and colleagues,[4] for example, documented suboptimal antibody responses to doses of polio vaccines, while others have shown adequate responses.[5–8] A lower seroconversion rate following hepatitis B vaccine has also been documented,[9] but not universally.[10–13]

With regard to the *Haemophilus influenzae* type b (Hib) conjugate vaccines, Munoz *et al* quantified antibody responses to two doses of PRP-OMP given at 2 and 4 months of age in 36 premature infants and found only 53% developed a concentration >1 µg/ml compared with 92% in term infants.[14] Washburn *et al* reached a similar conclusion with 22 preterm infants who had chronic lung disease and received PRP-OMP (55% >1 µg/ml).[15] However, a study with the conjugate HbOC revealed little difference in responses between 16 preterm infants (all <29 weeks) and an unmatched group of term infants.[2] Using PRP-T at a schedule of 2, 4, and 12 months of age, Kristensen *et al* showed prematurely born infants (27–36 weeks gestation) to have a lower response after two but not after three doses of vaccine.[16] At a 2, 3, and 4 month schedule, Robinson *et al* found that infants <32 weeks gestation, who had not received dexamethasone, achieved a similar GMC to that of term infants.[17]

One group has addressed the long term persistence of antibody after Hib immunisation. Sixteen premature infants who had received four doses of HbOC in infancy were evaluated at 3 and 7 years of age. Compared with age matched term controls they had a lower Hib antibody concentration at both ages. It was concluded that continued clinical follow up of cohorts of preterm children might help clarify their susceptibility to Hib disease in later childhood.[18 19]

In this study we sought to follow to school age a cohort of prematurely born infants vaccinated with PRP-T (ActHib, Pasteur Merieux) according to the UK accelerated schedule of three doses at 2, 3, and 4 months of age. As part of a national surveillance study of invasive *Haemophilus influenzae* disease in childhood, we were also able to determine whether premature infants were more likely to present with clinical vaccine failure than their term counterparts.

METHODS

Immunogenicity studies

Parents of premature infants ($\leq$32 weeks gestation) who were inpatients of the Special Care Baby Unit of the John Radcliffe Hospital, Oxford were approached between February 1991 and April 1992. They were provided with verbal and written information about the study and formal consent was obtained. The first immunisation was usually given while in hospital, and

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subsequent immunisations were given at home. PRP-T was given by separate injection from DTP at 2, 3, and 4 months of age. OPV was given concurrently. There were no further doses of Hib vaccine given.

Blood was obtained by venepuncture at 2 months of age (prior to the first dose of Hib vaccine), at 5 months of age (one month after the third dose), at 12 months of age, and again at 5 years of age. A local anaesthetic cream (EMLA, Astra Pharmaceuticals) was applied prior to venesection on all occasions except at 2 months of age.

The blood was centrifuged on return to the laboratory and serum stored at −20°C until serological tests were performed. Anti-PRP antibodies were quantified using an enzyme linked immunosorbent assay (ELISA) technique described previously.[20]

For comparison, data are presented from healthy term infants who were born at the same hospital and were enrolled in parallel studies of the immunogenicity and persistence of antibody to PRP-T. One hundred and seven infants were enrolled in 1990 and received three doses at 2, 3, and 4 months of age. Blood was obtained at 2, 5, 12, and 72 months of age; the results have been reported previously.[21]

Invasive *Haemophilus influenzae* disease in childhood study

This study commenced in October 1992, coincident with the introduction of the Hib conjugate vaccine into the routine immunisation schedule in the UK and the Republic of Ireland (ROI). In the UK, infants <12 months of age were offered PRP-T at 2, 3, and 4 months of age and in the ROI, HbOC was offered at 2, 4, and 6 months of age. No booster dose of Hib vaccine was included in either schedule. Surveillance for invasive *Haemophilus influenzae* disease in children was performed under the auspices of the British Paediatric Surveillance Unit (BPSU) of the Royal College of Paediatrics and Child Health in association with microbiologists and public health physicians. The BPSU has a programme of active surveillance for rare paediatric conditions. More than 90% of paediatricians routinely report to the BPSU on the report card sent to them on a monthly basis.[22] The methodology has been detailed previously.[21] Briefly, consultant paediatricians were requested to report any child with invasive *H influenzae* disease (isolation of *H influenzae* from a normally sterile site or a positive Hib antigen test combined with a clinical picture compatible with invasive Hib disease). The paediatrician was then sent a questionnaire requesting clinical, demographic, and laboratory information. Vaccination details were obtained from the general practitioner or if necessary from an administrator at the child health immunisation computer record centre. The local consultant microbiologist was contacted and asked to send the isolate to the Haemophilus Reference Unit in Oxford for verification and typing by standard slide agglutination and PCR techniques.[23]

In the ROI an investigator telephoned all laboratories serving paediatric hospitals every two weeks to maximise ascertainment. Isolates were sent to Oxford via the pathology department at the Waterford Regional Hospital.

For the purposes of this report a true vaccine failure (TVF) was defined as invasive Hib disease occurring more than one week after three doses had been given to a child when younger than 1 year of age.

Statistics

Statistical analyses were performed using SPSS (SPSS Inc., Chicago) and Epi Info version 5. Age and birth weights are reported as median (range). Anti-PRP antibodies were converted by logarithmic transformation and reported as geometric mean concentration (GMC) with 95% confidence intervals. Concentrations <0.15 µg/ml were allocated a value of 0.08 for the purposes of calculations. Antibody concentrations of term and preterm infants were compared using the t

test for independent samples. All proportions were compared using the χ^2 test (Yates's corrected) or Fisher's exact test. Analysis of the influence of various neonatal factors on antibody concentrations in premature infants was performed using stepwise multiple regression analysis. The following factors were included: gestational age, breast feeding, transfusion with blood or plasma, and treatment with corticosteroids or supplemental oxygen.

It was assumed that 7.1% of infants are born at <37 weeks gestation, 3.2% at <35 weeks, and 1.7% at ≤32 weeks (for comparison with the immunogenicity study),[24] that the coverage for vaccination of premature infants was 93% (that is, the same as term infants), and that the age specific attack rate was the same for both term and preterm infants. The latter was assumed, as there are no published data comparing the attack rates of Hib disease in term infants with those born prematurely. Data from the Immunisation Division, Communicable Diseases Surveillance Centre, London indicate that the proportion of prematurely born infants completing three doses of vaccine by 6 months of age was not lower than the proportion of term infants completing three doses by 6 months of age (personal communication, Marie Rush). The annual national birth rates were provided by the Office of National Statistics, London. The annual UK birth cohort varied from 700 200 to 781 000 over the period of the study.

All studies were approved by the Central Oxford Research Ethics Committee.

RESULTS

Immunogenicity studies

Forty one premature infants were recruited. The median gestational age (GA) was 30 weeks (range 23–32) and 16 (39%) were <28 weeks gestation. The median birth weight was 1.26 kg (range 0.62–2.17); 49% were male. Fifty nine per cent received one or more blood transfusions, 46% plasma (63% blood or plasma), and 12% received corticosteroids (all were <25 weeks gestation at birth). Culture proven sepsis was documented in 24% of infants, most commonly due to coagulase negative staphylococci.

Data were available on 40 premature infants at 2 and 5 months, 26 at 12 months, and 27 at 64 months of age. Reasons for the drop in numbers included children moving out of the area, insufficient blood available for testing, and refusal to have a blood test.

Risk factors for a lower Hib antibody concentration at 2 months of age were lower gestational age, prior blood transfusion, and sepsis. When these were included in a stepwise linear regression model only gestational age was significantly related to antibody concentration ($r = 0.38$, p = 0.03). There was no statistically significant influence of any factor (including gestational age) on antibody concentrations at 5, 12, and 64 months of age.

Table 1 details the Hib antibody concentrations and proportions of infants with concentrations <0.15 and <1.0 µg/ml. For comparison, the results are also shown for infants born at term who were followed in a parallel study.

Clinical vaccine failure study

During the eight year period 1 October 1992 to 1 October 2000, 165 true vaccine failures were reported to the BPSU study. Twelve of these occurred in the ROI.

Eighteen infants (16 UK, two ROI) were born prematurely (<37 weeks gestation); 10 were less than 35 weeks gestation. The median gestational age of premature infants was 34 weeks (range 29–36). The median age at Hib disease was 20 months (range 5–68) and there was a median time period of 15 months (range 0.6–63) between the third dose of Hib vaccine and disease. Ten premature infants (56%) presented with meningitis, three (17%) with epiglottitis, two (11%) with bacteraemia, and one each with pneumonia, septic arthritis,

Table 1 Serum Hib antibody concentrations and proportions with concentrations <0.15 μg/ml and 1.0 μg/ml, by age, for premature infants and term infants

Median age (months)	Premature GMT μg/ml (95% CI) (number)	Term GMT μg/ml (95% CI) (number)	Premature % <0.15 μg/ml (number)	Term % <0.15 μg/ml (number)	Premature % <1.0 μg/ml (number)	Term % <1.0 μg/ml (number)	Proportions <0.15 μg/ml Ratio prem:term	Proportions <1.0 μg/ml Ratio: prem:term
2	0.21* (0.16 to 0.27) (40)	0.37 (0.31 to 0.44) (90)	35* (40)	10 (90)	95 (40)	89 (90)	3.5	1.1
5	2.73† (1.63 to 4.58) (40)	4.60 (3.51 to 6.04) (105)	5 (40)	1 (105)	33* (40)	12 (105)	5.3	2.6
12	0.34* (0.20 to 0.56) (26)	0.88 (0.66 to 1.17) (95)	23* (26)	6 (95)	77 (26)	57 (95)	3.7	1.4
64	0.24‡ (0.16 to 0.37) (27)		41 (27)		81 (27)			
72		0.51 (0.31 to 0.85) (59)		32 (59)		71 (59)	1.3	1.1

*p<0.05 versus term infants at same age; †p=0.08 versus term infants at 5 months of age, ‡p<0.05 versus term infants at 72 months of age.

and cellulitis. Sixty seven per cent were male. Two children died (11%). In contrast, for true vaccine failures born at term (n = 147), the median age of disease was 27 months (range 4–70) (p > 0.1 versus premature infants) and the time period between third vaccine dose and disease was 22 months (range 0.4–65 months) (p > 0.1 versus premature infants). The major clinical presentations of term infants included meningitis (54%), epiglottitis (21%), bacteraemia (12%) and pneumonia (5%). Fifty seven per cent were male. There were three deaths (2%, p = 0.09 versus premature infants).

The distribution of premature to term infants among TVF over the study period was as follows: 1 *v* 3 (1993), 1 *v* 13 (1994), 3 *v* 21 (1995), 3 *v* 20 (1996), 1 *v* 20 (1997), 3 *v* 12 (1998), 4 *v* 26 (1999), and 2 *v* 32 (2000).

Subdividing the premature TVF by the age of a hypothetical Hib booster dose (given at 12–15 months of age) and adding one week to develop a protective antibody response, reveals that 14 cases (78%) (12 in UK) were ≥12.25 months of age and 13 cases (72%) ≥15.25 months of age at the time of disease. One of the two deaths was in a child older than the age of a hypothetical booster dose.

The proportion of TVF among vaccinated premature infants in the UK was not statistically significantly greater than the proportion of TVF among vaccinated term infants: <37 weeks, relative risk (RR) 1.5 (95% CI 0.9 to 2.6; p = 0.14); <35 weeks, RR 1.7 (95% CI 0.8 to 3.5; p = 0.12); or ≤32 weeks, RR 1.6 (95% CI 0.5 to 4.3; p = 0.2).

DISCUSSION

In this study we have shown that premature infants born at ≤32 weeks gestation achieve lower anti-PRP antibody concentrations following Hib vaccination and lower proportions above one or both of the conventional protective concentrations than infants who are born at term. In the absence of clinical data it may be reasonable to conclude therefore, that these infants are at a significantly greater risk of invasive Hib disease and should be offered a booster dose. A booster dose for premature infants might be considered more important in the UK and Ireland, where a booster dose is not routinely administered to any child, in contrast to the practice in most other developed countries. Through a unique national, active, prospective, enhanced surveillance study we have been able to examine the clinical significance of these lower antibody concentrations. This is the first published study to address this issue. The surveillance data indicate a trend to a higher relative risk (1.5) of clinical vaccine failure in premature infants (<37 weeks) compared with term infants, but with a 95% confidence interval which extends from 0.9 to 2.6. For premature infants ≤32 weeks gestation (the group investigated in the immunogenicity study) the relative risk is 1.6 (0.5–4.3). Neither figure reaches statistical significance, perhaps reflecting the small number of cases. These data have been generated from eight years of national surveillance in which approximately 5.5 million infants have received three doses of Hib conjugate vaccine and been followed for clinical vaccine failure. It is unlikely that similar data could be or will be generated from any other population.

Our immunogenicity data are generally consistent with those of other published studies. Kristensen *et al* measured the response to two doses of PRP-T at 2 and 4 months of age and showed a lower concentration in premature infants, but concentrations comparable to term infants after a third dose at 12 months of age.[16] Using PRP-T at the UK schedule, Robinson *et al* measured a GMC of 4.63 μg/ml in premature infants who had not received dexamethasone therapy and 0.51 μg/ml in those who had received dexamethasone.[17] There was no group of term infants for comparison. This GMC is higher than that found in our study (2.73 at 5 months of age) which may be accounted for by the later age of vaccination in the study of Robinson *et al*, and the fact that 12% of our cohort also received dexamethasone during their neonatal course. With a different Hib conjugate vaccine (HbOC) and schedule (four doses at 2, 4, 6, and 12–19 months of age), 16 preterm infants were followed to 3 and 7 years of age.[2 18 19] After three doses of vaccine the GMC of preterm and term infants was equivalent, but on follow up the GMC for premature infants was significantly lower than that of term infants: 0.99 versus 3.06 μg/ml at 3 years of age, and 1.41 versus 3.21 at 7 years of age. The fourth dose of vaccine may account for the persistence of higher antibody concentrations of antibody in this study when compared with ours. The difference in antibody concentrations between preterm and term infants is consistent with our study.

Our study allows us to consider the risk of vaccine failure in premature infants in several ways: relative to conventional antibody surrogates of protection, relative to the antibody concentrations in term infants and relative to the clinical protection achieved in term infants. As judged by conventional surrogates of protection, premature infants are at greatly

increased risk of disease: 33%, 77%, and 81% of them had antibody concentrations less than 1.0 µg/ml at 5, 12, and 64 months of age respectively. Despite this, over an eight year period only 16 UK infants (out of more than 380 000 vaccinated premature infants) actually developed invasive Hib disease. When related to infants born at term, preterm infants also appear to be at greater risk of disease. For example, proportions less than 1.0 µg/ml were 33 versus 12% at 5 months (ratio 2.6), 77 versus 57% at 12 months (1.4), and 81% at 64 months versus 71% at 72 months (1.1). The latter comparison actually underestimates the difference between preterm and term infants as term infants were bled a median of eight months after preterm infants. Finally, relative to the clinical protection achieved in term infants premature infants have an increased risk of vaccine failure but one that does not achieve statistical significance. It is noteworthy that the age of disease presentation is also earlier in preterm infants than in term infants. This is consistent with the hypothesis that the antibody response of preterm infants is lower and the duration of protection may therefore be shorter.

It is not surprising that the conventional serological correlates of protection are poorly predictive of clinical protection after conjugate vaccination. These concentrations were derived from a non-vaccinated population (0.15 µg/ml) and from a population vaccinated with an unconjugated PRP vaccine (1.0 µg/ml).[25] In contrast to unconjugated PRP vaccination, immunological memory is induced following conjugate vaccination. This has been documented in term as well as preterm infants.[16 26] Low anti-PRP antibody concentrations following Hib conjugate vaccine may therefore be associated with higher than expected clinical protection. This can be inferred from the high protective efficacy shown in the Finnish and Icelandic populations where the least immunogenic conjugate vaccine, PRP-D, was used.[27 28] Indeed, we have shown that in the UK population where the absence of a booster dose results in relatively low antibody concentration through childhood, clinical protection actually remains high.[21]

Another possible mechanism for the apparent clinical protection of premature infants despite low anti-PRP antibody concentrations is herd immunity—that is, these infants are less exposed to Hib as a result of widespread vaccination in the childhood population. A reduction in pharyngeal carriage of Hib in UK children has been documented.[29] Herd immunity in the UK may also be inferred from the reduction in Hib disease in infants too young to be vaccinated or only partly vaccinated.[30]

It is conceivable that ascertainment of vaccine failures in this study was incomplete despite the fact that multiple sources were employed.[30] However, for the purposes of this particular analysis one would need to postulate that premature infants with Hib vaccine failure are specifically underreported. This seems unlikely. Consideration must also be given to the possibility that in practice, premature infants might actually be vaccinated at an older chronological age than term infants and therefore have better antibody responses than those achieved in this study which strictly followed the 2, 3, and 4 month schedule. However, data from the Immunisation Division, Communicable Diseases Surveillance Centre, London show that the proportion of prematurely born infants was not lower than the proportion of term infants completing three doses of vaccine by 6 months of age (personal communication, Marie Rush). The assumption was also made that the pre-vaccine attack rates in premature and term infants are similar. A review of the published literature did not find any data on the risk of Hib disease in premature infants. A recent analysis of the risk of pneumococcal disease in premature infants suggests they have an increased susceptibility.[31] It is conceivable therefore, that the small increased risk of Hib disease seen in our study simply reflects an increased background risk of premature infants to Hib disease per se rather than an increased risk of vaccine failure. It

should also be noted that the immunogenicity data presented derives from a relatively small group of very premature infants with a median gestational age of 30 weeks (all ≤32 weeks gestation) who may not be representative of all premature babies. It is also likely that less premature infants will have a good antibody response to Hib vaccination that may not be significantly different from that of term infants. As indicated above, despite these antibody concentrations, even this very premature group are not significantly over represented among the vaccine failures (RR 1.6 (0.5–4.3), p = 0.2).

Further information on the apparent protection derived from Hib conjugate vaccination in premature infants despite their lower antibody concentrations might be derived from analysis of antibody avidity or responses to booster doses. This was not done in our study and has not yet been assessed in UK premature infants.

If a booster were to be introduced in the UK at 12–15 months of age (to coincide with the measles/mumps/rubella dose) with the intention of preventing disease in these older, prematurely born infants, then over 380 000 extra doses would need to have been administered to prematurely born infants during this eight year period in order to prevent disease in 12 infants. This assumes that a booster dose would have prevented disease in these individuals.

Conclusions

This study provides both laboratory and clinical data on the current relative risk of vaccine failure of premature infants when vaccinated with the Hib conjugate vaccine at the UK schedule. The study indicates that premature infants (≤32 weeks gestation) have significantly lower antibody concentrations than term infants but that despite this, they achieve very high levels of clinical protection. The numbers of cases of vaccine failure are small and the confidence intervals for the relative risks indicate that the risk for premature infants may extend from 0.9 to 2.6. It is possible that with changes in neonatal practice, such as the increased use of steroids,[17] or of national recommendations, such as the introduction of acellular pertussis/Hib combinations,[32] that Hib antibody responses in infants born prematurely may be further suppressed and their risk of clinical disease may increase. Continued surveillance is vital in addressing the clinical relevance of such issues to Hib immunisation in the UK. The findings of this study also have implications for the vaccination and surveillance of premature infants following the introduction of the newer conjugate vaccines.

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Diagnosis of Prader-Willi syndrome

The relationship between genotype and phenotype in Prader-Willi syndrome has not been completely delineated. Clinical diagnosis is based on a list of major or minor criteria present in the neonatal period and in later childhood, adolescence, and adult life. Genetic diagnosis is dependent on methylation analysis at the *SNURF/SNRPN* locus. A study based on the former Anglia and Oxford Health Region (J Whittington and colleagues. (*J Med Genet* 2002;**39**:926–32) has provided more data about the relationship between clinical features and genetic diagnosis.

The study included 103 people with positive genetic tests for Prader-Willi syndrome. Sixty-one of these were from a population based sample within the region and 42 from other regions). There were 19 with negative genetic tests but positive clinical criteria, and 10 from the population study with neither appropriate clinical criteria nor positive genetic tests plus 22 controls with learning disorders of other aetiologies.

Four neonatal criteria were invariably present when genetic testing was positive; they were poor suck, feeding problems, floppiness at birth, and weak cry or inactivity. Hypogonadism was also an invariable accompaniment of the genetic diagnosis. When all learning-disabled subjects were considered the combination of poor suck at birth, weak cry or inactivity in infancy, decreased vomiting, and thick saliva correctly distinguished between Prader-Willi syndrome and other diagnoses in 92% of cases. No combination of clinical features, however, predicted a positive genetic diagnosis with certainty. When there is no information about neonatal features the most discriminatory clinical features are hypogonadism, small hands and feet, thick saliva, more than usual stubbornness, and insensitivity to hot and cold.

The authors of this paper suggest use of the core neonatal criteria to decide about genetic testing in infancy. In later life, if neonatal data are unavailable, indications for genetic testing include eating disturbance and learning disabilities together with hypogonadism (including infrequent (<5 per year) and sparse menstruation in postpubertal girls or women).