Archives of Disease in Childhood, 1989, **64**, 520–524

Haemophilus influenzae type b conjugate vaccine trial in Oxford: implications for the United Kingdom

G TUDOR-WILLIAMS,* J FRANKLAND,* D ISAACS,* R T MAYON-WHITE,†
J A MACFARLANE,‡ D G REES,§ AND E R MOXON*

**University Department of Paediatrics, †Department of Community Medicine, Manor House, ‡Department of Community Health, Radcliffe Infirmary, Oxford, and §Department of Computing and Mathematical Science, Oxford Polytechnic*

SUMMARY The safety and immunogenicity of a *Haemophilus influenzae* type b conjugate vaccine was investigated in 103 infants immunised at 3, 5, and 9 months of age; the infants also received diphtheria, pertussis, and tetanus and polio vaccines. Side effects were compared with 99 matched infants receiving diphtheria, pertussis, and tetanus and polio vaccines only. No serious side effects were observed and the incidence of minor side effects was no greater in the recipients of *H influenzae* type b conjugate vaccine. Two doses of the vaccine (standard and low) were compared: geometric mean titres of serum anticapsular antibody rose from 0·11 μg/ml before immunisation to 26·4 μg/ml after three immunisations with the standard dose and 14·6 μg/ml with the low dose. The geometric mean titre among 21 unimmunised infants at this age was 0·06 μg/ml. Both doses therefore generated antibody concentrations likely to be protective after three immunisations. There were no non-responders.

Incorporation of an *H influenzae* type b conjugate vaccine into the primary immunisation schedule has the potential for preventing over 1000 cases of systemic *H influenzae* type b disease and 50 deaths each year in the United Kingdom.

Haemophilus influenzae type b causes potentially fatal systemic disease such as meningitis and epiglottitis and other serious infections affecting soft tissues, joints, and lungs. Recent studies[1 2] suggest that the incidence of *H influenzae* type b disease in the United Kingdom is higher than previously reported.[3]

The polyribosyl-ribitol phosphate capsule, which is specific to serotype b *H influenzae* organisms, is a major virulence determinant.[4] Serum antibody to the capsule has been shown to be protective in infants.[5]

Immunocompetence to polysaccharides matures more slowly than to proteins and lipopolysaccharides. Active immunisation using purified polyribosyl-ribitol phosphate is ineffective in children less than 18 months of age[6]; they constitute a major proportion of the population at risk. A conjugate of a polysaccharide hapten and a carrier protein gives T cell dependent properties to the polysaccharide[7–9] with better immunogenicity in young children and predominantly IgG antibody responses that can be boosted.[10] Concentrations of antibody induced by immunisation can be higher than those after natural infection at the same age.[11] At least four different conjugates are undergoing clinical evaluation,[10 12–14] and one large efficacy study has been published.[14] It has been shown that a conjugate (HbO–C) of oligosaccharides derived from polyribosylribitol phosphate linked to a non-toxic mutant diphtheria toxin[10] is more immunogenic than the polyribosyl-ribitol phosphate-diphtheria toxoid conjugate used in the first efficacy trial (H Peltola, J Eskola, H Kayhty, PH Makela. Immunogenicity of *Haemophilus influenzae* oligosaccharide-protein conjugate vaccine (HbO–C) compared to polysaccharide-protein conjugate vaccine (PRP–D) in infancy. Personal communication, 1988). No conjugate has been tested in infants resident in the United Kingdom or according to the British schedule of primary immunisation at 3, 5, and 9 months of age and there is a need to define the optimal dose of polysaccharide.[15] Our study aimed to examine the serum antipolyribosyl-ribitol phosphate antibody state of unimmunised infants in the United Kingdom and to provide safety and immunogenicity data

comparing standard and low dose regimes using the HbO–C *H influenzae* type b conjugate vaccine.

Patients and methods

Two hundred and thirty two mothers living in and around the city of Oxford, with full term healthy babies born consecutively during a two month period, were invited to participate in the study of the *H influenzae* type b conjugate vaccine. Of these, 103 mothers (including one with twins) agreed to take part; thus 104 infants were enrolled into the 'index' group. The infants were randomised using batched envelopes to receive a 0·5 ml dose containing 10 μg or 2 μg oligosaccharide (*H influenzae* type b HbO–C conjugate vaccine lot No A2K61/22 without adjuvant (Praxis Biologics); 0·9% sodium chloride was used as a diluent). *H influenzae* type b vaccine was given intramuscularly on the same occasion as diphtheria, pertussis, and tetanus vaccine but in a different site (opposing anterolateral thighs) at 3, 5, and 9 months of age. Parents were given a standard form and asked to record any local or systemic reactions over the next five days and measure daily axillary temperatures. The families were routinely visited the next day. Venepuncture for serology was performed before immunisation at each visit and at 10 months. All venepunctures and immunisations were performed in the child's home by one of us (GT–W) and only one attempt at venepuncture was made on any one visit.

Local reactions to *H influenzae* type b conjugate and diphtheria, pertussis, and tetanus vaccines could be compared for each index subject. To obtain comparative data for systemic reactions, 192 mothers from the same area giving birth to full term healthy infants in the month on either side of the recruiting period for the *H influenzae* type b conjugate vaccine were approached. Of these, 97 families including three with twins (100 infants) agreed to participate. This 'comparison' group received vaccine for diphtheria, pertussis, and tetanus (and oral polio) at home but were not given *H influenzae* type b conjugate vaccine or placebo. The child was visited the next day and the parents were requested to record reactions and measure temperatures as in the index group. The same batch of diphtheria, pertussis, and tetanus vaccine (A0726A, Wellcome) was used for both the index and comparison groups. Blood was taken from a random sample of 21 of these infants only once, at 10 months, to examine concentrations of antibody in United Kingdom children unimmunised with *H influenzae* type b conjugate vaccine.

One family from each group moved and were lost to follow up; therefore our results relate to 103 infants in the index group (52 received the 10 μg dose of vaccine) and 99 infants in the comparison group.

Serum samples were separated on the day of venepuncture and stored in 0·5 ml aliquots at −70°C. The Farr type radioantigen binding assay for total serum antipolyribosyl-ribitol phosphate antibody,[16 17] using the recommended criteria of the Office of Biologics Research and Review,[18] was performed in duplicate at three initial dilutions in both our own laboratories and those of Praxis Biologics. For neat sera with low concentrations of antibody, we interpreted the standard curves down to 0·05 μg/ml (7–8% binding in our assays). End point dilutions up to 1:1000 were performed to give estimates of high concentrations of antibody. Polyribosyl-ribitol phosphate intrinsically labelled with tritium radioantigen was supplied by Dr Porter Anderson, University of Rochester, USA, and Office of Biologics Research and Review lyophilised standard reference serum (70 μg/ml) was supplied by Dr Carl Frasch, Bureau of Biologics, Federal Drug Administration, Bethesda, USA.

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

STATISTICAL METHODS

The proportion of children in the index and comparison groups were compared for all adverse reactions by χ^2 tests using Yates's correction (or by Fisher's exact test if expected values were <5). The proportion of unimmunised infants at 3 and 10 months of age whose antibody concentrations were above 0·05 μg/ml were compared by χ^2 test using Yates's correction. Mann-Whitney U tests were used to compare antibody responses to the two doses of *H influenzae* type b conjugate vaccine at a given age. For each dose Wilcoxon signed rank tests were used to assess the responses to first, second, and third immunisations.

Results

The vaccine was well tolerated and highly immunogenic. After three immunisations, the 10 μg doses resulted in a rise in geometric mean serum antipolyribosyl-ribitol phosphate antibody titre from 0·11 at 3 months to 26·4 μg/ml at 10 months of age. The 2 μg doses generated lower responses at 10 months ($p=0·007$) although the geometric mean titre rose from 0·11 to 14·6 μg/ml, and at both doses all infants achieved greater than four fold increases in antibody titre and 98% achieved values above 1·0 μg/ml (figs 1 and 2).

The 10 μg dose resulted in significant increases in

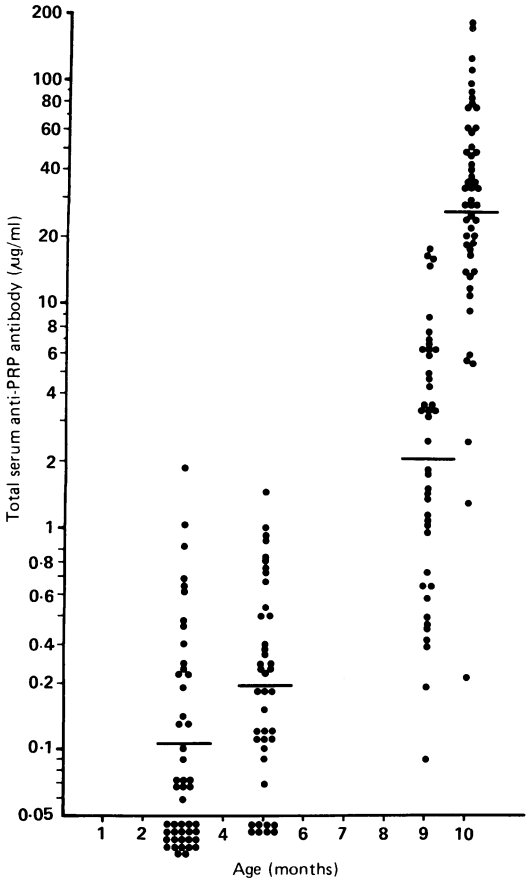

Fig 1 *Antipolyribosyl-ribitol phosphate (PRP) antibody concentrations in infants immunised at 3, 5, and 9 months with a 10 μg dose of H influenzae type b conjugate vaccine. Horizontal bars=geometric mean titres. (Results less than the lower limit of sensitivity of the assay were assigned a value of 0·05 μg/ml for calculation of the geometric mean titre.)*

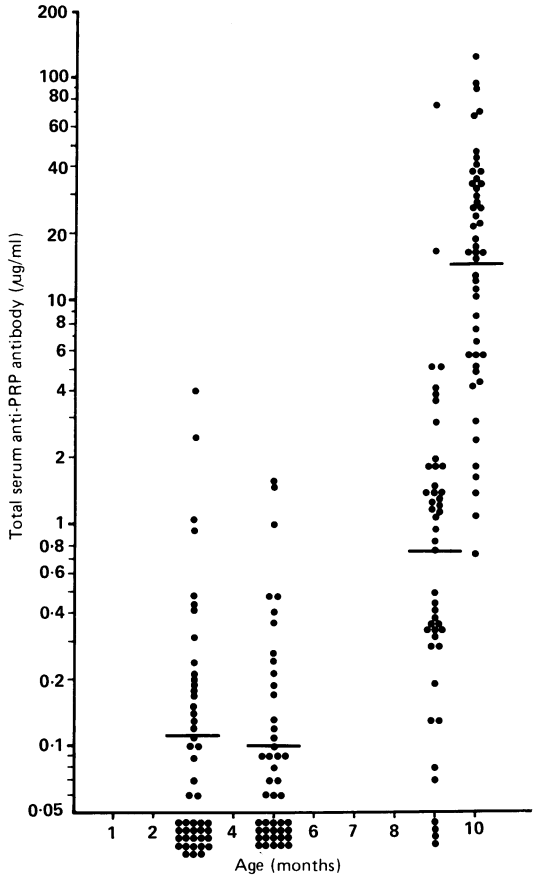

Fig 2 *Antipolyribosyl-ribitol phosphate (PRP) antibody concentrations in infants immunised at 3, 5, and 9 months with a 2 μg dose of H influenzae type b conjugate vaccine. Horizontal bars=geometric mean titres.*

antibody titre (p=0·0006) after one immunisation. No increase was observed after the first 2 μg dose (p=0·23). Geometric mean titres at age 5 months were 0·19 and 0·10 μg/ml respectively. After the second immunisation booster responses were noted with both dosage regimes: geometric mean titres at 9 months rose to 2·05 and 0·75 μg/ml respectively. The 2 μg dose produced a wider scatter of results, however, and four individuals had antibody concentrations below the limit of sensitivity of our assay.

In 21 infants from the comparison group who had not received the *H influenzae* type b conjugate vaccine, the geometric mean titre at 10 months was 0·06 μg/ml. There was a fall in antibody concentrations ($p<0·01$) when compared with the combined results for the index infants at 3 months of age in samples taken before their first immunisation with *H influenzae* type b conjugate vaccine (fig 3).

No major adverse reactions occurred in either group and no complications from venepuncture were observed. Adverse reactions did not differ significantly by dose of *H influenzae* type b conjugate vaccine and their results have been combined. A comparison of side effects is shown in the table. Local reactions defined as redness or swelling or warmth >2 cm occurred in 2% of index infants after administration of *H influenzae* type b conjugate vaccine and 19% after diphtheria, pertussis, and tetanus vaccine. Local reactions to diphtheria, pertussis, and tetanus vaccine in the comparison infants were of similar frequency. Systemic reactions

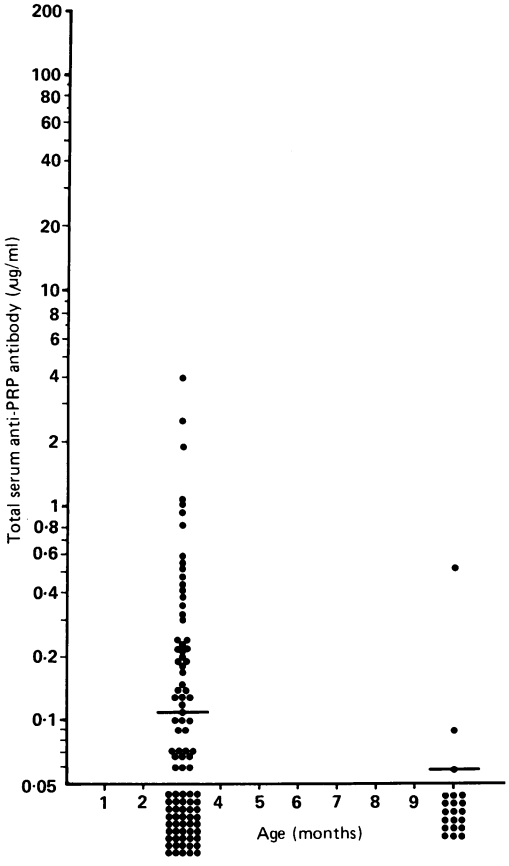

Fig 3 *Antipolyribosyl-ribitol phosphate (PRP) antibody concentrations in unimmunised infants in the United Kingdom. Horizontal bars=geometric mean titres.*

Table *Side effects of immunisation with H influenzae type b conjugate and diphtheria, pertussis, and tetanus vaccines compared with immunisation with diphtheria, pertussis, and tetanus alone*

	Index infants (n=103)	*Comparison infants (n=99)*
Local reactions (redness, swelling, or warmth >2 cm)		
Site of *H influenzae* type b conjugate:		
First immunisation	1	
Second immunisation	2	
Third immunisation	2	
Site of diphtheria, pertussis, and tetanus:		
First immunisation	24	21
Second immunisation	18	15
Third immunisation	18	21
Systemic reactions		
Axillary temperature >38°C		
First immunisation	5	7
Second immunisation	10	4
Third immunisation	9	19
Irritability >4 hours total		
First immunisation	25	27
Second immunisation	18	30*
Third immunisation	27	37
Persistent crying >1 hour		
First immunisation	25	41*
Second immunisation	11	26**
Third immunisation	11	19

*p<0·05; **p<0·01.

were similar or less frequent in the index children compared with those in the comparison group.

Discussion

The serum antibody concentrations observed in unimmunised infants at 3 months and 10 months are consistent with the decline in serum antibody observed in North American infants.[19] Both are consistent with the relative protection from *H influenzae* type b infection observed during the early months of life.

The *H influenzae* type b conjugate vaccine was well tolerated with a low frequency of local reactions and no increase in systemic side effects when given with diphtheria, pertussis, and tetanus vaccine as compared with infants receiving diphtheria, pertussis, and tetanus vaccine alone. It has been shown that another conjugate (polyribosyl-ribitol phosphate-D) can be mixed with diphtheria, pertussis, and tetanus vaccine and given as a single injection without adverse effects on safety or immunogenicity.[20]

The conventional dose of polysaccharide in polyribosyl-ribitol phosphate is 25 μg. A variety of doses from 10–25 μg have been employed in trials of *H influenzae* type b conjugate vaccines. We found that 2 μg of oligosaccharide in the *H influenzae* type b conjugate induces booster responses after a second immunisation at 5 months. The 2 μg dose, however, did not affect the decline in antibody concentrations after immunisation at 3 months, whereas a significant rise in antibody concentrations was observed after a 10 μg dose at this age. Given the pattern of susceptibility in young infants it is desirable to achieve immunological priming at as young an age as possible and our results suggest that a 10 μg oligosaccharide dose would be more appropriate than a 2 μg dose for the immunisation schedule in the United Kingdom.

There has been much debate as to what constitutes a 'protective' concentration of antibody[21]: titres >1·0 μg/ml are widely accepted as correlating with long term protection after immunisation with unconjugated polyribosyl-ribitol phosphate. The Finnish trial of polyribosyl-ribitol phosphate-D, however, disclosed a higher protective efficacy than would have been predicted from the immunogenicity data.[14] In view of the T cell dependent characteristics of responses to the conjugate vaccines it seems likely that the priming effect of one dose may be more important than achieving a designated post-immunisation antibody titre that correlates with protection but was based on experience with the unconjugated polysaccharide vaccine.

Our data and the results of studies of other candidate vaccines suggest that incorporation of immunisation for *H influenzae* type b into the schedule in the United Kingdom has the potential for preventing over 85% of systemic infection with *H influenzae* type b in children.[12–14] Extrapolating from the incidence of systemic *H influenzae* type b disease in the Oxford region, this would represent 1105 cases with their attendant long term sequelae and 55 deaths each year.

This and even higher estimates[1] of attack rates from other regions in this country make a compelling case for considering how active immunisation might be successfully implemented. The availability of safe, immunogenic, and protective conjugate vaccines strengthens the argument that wider intervention studies should proceed in the United Kingdom.

We thank all the families who took part in this study. We thank Dr WOC Cookson and Mrs Lin Barnetson for providing computing expertise and Miss Gail Davies for typing the manuscript. We thank Dr Porter Anderson for supplying the tritiated polyribosyl-ribitol phosphate and for his constructive criticism, and Praxis Biologics for materials.

References

[1] Howard AJ. Systemic disease produced by Haemophilus influenzae. In: Cole PJ, ed. *The pathogenesis of Haemophilus influenzae*. MCI International Ltd, 1987:55–66.
[2] Tudor-Williams G, Frankland J, Isaacs D, *et al.* Haemophilus influenzae type b disease in the Oxford region. *Arch Dis Child* 1989;**64:**517–9.
[3] Broughton SJ, Warren RE. A review of Haemophilus influenzae infections in Cambridge 1975–81. *J Infect* 1984;**9:**30–42.
[4] Moxon ER. The molecular basis of Haemophilus influenzae virulence. *J R Coll Physicians Lond* 1985;**19:**174–8.
[5] Santosham M, Reid R, Ambrosino DM, *et al.* Prevention of Haemophilus influenzae type b infections in high risk infants treated with bacterial polysaccharide immune globulin. *N Engl J Med* 1987;**317:**923–9.
[6] Peltola H, Kayhty H, Virtanen M, *et al.* Prevention of Haemophilus influenzae type b bacteremic infections with the capsular polysaccharide vaccine. *N Engl J Med* 1984;**310:** 1561–6.
[7] Avery OT, Goebel WF. Chemo-immunological studies on conjugated carbohydrate-proteins. II. Immunological specificity of synthetic sugar-protein antigens. *J Exp Med* 1929;**50:**533–50.
[8] Paul WE. *New approaches for inducing natural immunity to pyogenic organisms*. Washington DC: US Government Printing Office, 1973:157–66. (DHEW Publication No (NIH) 74–553.)
[9] Schneerson R, Barrera O, Sutton A, Robbins JB. Preparation, characterization and immunogenicity of Haemophilus influenzae type b polysaccharide-protein conjugates. *J Exp Med* 1980;**152:**361–76.
[10] Anderson P, Pichichero ME, Insel RA. Immunisation of 2-month-old infants with protein-coupled oligosaccharides derived from the capsule of Haemophilus influenzae type b. *J Pediatr* 1985;**107:**346–51.
[11] Kayhty H, Jousimies-Somer H, Peltola H, *et al.* Antibody response to capsular polysaccharides of group A and C Neisseria meningitidis and Haemophilus influenzae type b during bacteremic disease. *J Infect Dis* 1981;**143:**32–41.
[12] Claesson BA, Trollfors B, Lagergard T, *et al.* Clinical and immunologic responses to the capsular polysaccharide of Haemophilus influenzae type b alone or conjugated to tetanus toxoid in 18–23 month old children. *J Pediatr* 1988;**112:**695–702.
[13] Lenoir AA, Granoff PD, Granoff DM. Immunogenicity of Haemophilus influenzae type b polysaccharide—Neisseria meningitis outer membrane protein conjugate vaccine in 2- to 6-month old infants. *Pediatrics* 1987;**80:**283–7.
[14] Eskola J, Peltola H, Takala AK, *et al.* Efficacy of Haemophilus influenzae type b polysaccharide-diphtheria toxoid conjugate vaccine in infancy. *N Engl J Med* 1987;**317:**717–22.
[15] Black SB, Shinefield HR, Hialt RA, *et al.* Efficacy of Haemophilus influenzae type b capsular polysaccharide vaccine. *Pediatr Infect Dis* 1988;**7:**149–56.
[16] Farr RS. A quantitative immunochemical measure of the primary interaction between I*BSA and antibody. *J Infect Dis* 1958;**103:**239–62.
[17] Anderson P. Intrinsic tritium labeling of the capsular polysaccharide antigen of Haemophilus influenzae type b. *J Immunol* 1978;**120:**866–70.
[18] Frasch CE. Office of Biologics Research and Review. *Haemophilus influenzae type b radioimmunoassay, 4-6-87 revision.* Bethesda MD: Department of Health and Human Services, 1987.
[19] Granoff DM, Munson RS. Prospects for prevention of Haemophilus influenzae type b disease by immunisation. *J Infect Dis* 1986;**153:**448–61.
[20] Eskola J, Kayhty H, Gordon LK, *et al.* Simultaneous administration of Haemophilus influenzae type b capsular polysaccharide-diphtheria toxoid conjugate vaccine with routine DPT + inactivated poliovirus vaccination of childhood. *Pediatr Inf Dis* 1988;**7:**480–4.
[21] Kayhty H, Peltola H, Karanko V, Makela PH. The protective level of serum antibodies to the capsular polysaccharide of Haemophilus influenzae type b. *J Infect Dis* 1983;**147:**1100.

Correspondence to Professor ER Moxon, University Department of Paediatrics, John Radcliffe Hospital, Headington, Oxford OX3 9DU.

Accepted 3 January 1989