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Antipolysaccharide antibodies in 450 children with otitis media

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SUMMARY

We have measured antibodies to pneumococcal and Haemophilus polysaccharides in a prospective study of 450 children aged 2–16 years with otitis media requiring grommets (ear tubes). Pneumococcal antibody levels were significantly higher in the 2–6 year ($P < 0{\cdot}004$) and 7–10 year ($P < 0{\cdot}04$) study groups in comparison with age-matched controls. There was no difference in Haemophilus antibody levels between the study and control group children for the age groups 2–6 years and 11–16 years. Haemophilus antibody levels were significantly lower in the 7–10 year ($P < 0{\cdot}003$) group in comparison with age-matched controls. Eighty-eight out of 450 (19·6%) children had pneumococcal antibody levels below the 25th percentile. Nineteen out of 88 (21·6%) children with pneumococcal antibody levels below the 25th centile were test immunized with 23 valent Pneumococcal polysaccharide and unconjugated Haemophilus type b capsular polysaccharide. Of these 19 children (aged 4–11 years), five mounted suboptimal responses to both polysaccharide antigens, whilst one child failed to respond to Haemophilus polysaccharide alone. There was no significant difference in the prevalence of IgG subclass deficiency between the normal responders and poor responders to immunization ($P = 0{\cdot}12$). We found no evidence of specific polysaccharide antibody deficiency in the vast majority of the 450 children studied. However, the significance of poor antibody responses to test immunization in a small minority of children with otitis media is unclear. Long-term follow up of these children is required to determine whether poor immunization responses herald the development of frank antibody deficiency.

Keywords polysaccharide antibodies otitis media grommets (ear tubes) test immunization

INTRODUCTION

Otitis media has long been recognized as a common childhood affliction. Prospective epidemiological studies have highlighted the extent of the problem, with 62% of children in Boston, USA, suffering at least one attack and 17% at least three attacks of otitis media within their first year [1]. Amongst the microorganisms responsible for causing otitis media, *Streptococcus pneumoniae* serotypes 6, 14, 19, 23 are responsible for approximately 30% of all paediatric cases [2,3]. Multiple risk factors influence the predisposition to middle-ear infection in children, including the shorter eustachian tube in infants [4], exposure to parental smoking [5] and underlying immunodeficiency [6]. It is generally assumed that the vast majority of such children do not have a persistent major defect in immunocompetence, but minor humoral immunodeficiencies cannot be ruled out. Nevertheless, in a significant number of children proven to have primary antibody deficiency, recurrent otitis media is an early presenting feature [7,8].

Several screening studies have been performed for antibody deficiency in children with otitis media. In 1968, Buckley *et al*. reported low total immunoglobulin levels affecting at least one or more isotypes in a third of patients (199/600) with recurrent infections presenting to a large teaching hospital in the USA [9]. These findings are unlikely to be representative of the general population in view of the highly selective group of patients and the frequent finding of radiologically proven bronchiectasis and pansinusitis in the study group, leading to the expectation of a high incidence of underlying primary antibody deficiency. Indeed, Buckley found five cases of agammaglobulinaemia and 10 cases of hypogammaglobulinaemia in her study group. Subsequent studies from the UK, Sweden, USA and Japan performed largely on small numbers of very young children aged 6–36 months with recurrent otitis media as their main clinical problem have produced conflicting results, ranging from low serum IgG2 subclass levels, accompanied by reduced spontaneous antibody levels, to *S. pneumoniae* type 6 to increased serum IgG2 levels [10–16]. These studies are difficult to interpret in view of the physiological delay in achieving normal IgG2 subclass levels in this age group (6–32 months) and the accompanying failure to mount an adequate polysaccharide antibody response [18]. In the absence of test immunization, low spontaneous antibody levels to *S. pneumoniae*

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type 6 cannot be equated with significant immunodeficiency in view of its poor immunogenicity [19]. It is noteworthy that in the only study where test immunization with pneumococcal polysaccharide (14 valent 'Pneumovax') was carried out in children aged 1–3 years, both study and control group children failed to respond [13].

We report the results of a prospective study of polysaccharide antibodies in 450 children with recurrent otitis media requiring grommet insertion and/or adenotonsillectomy.

PATIENTS AND METHODS

Recruitment of children

Children aged 2–15 years presenting to the Ear, Nose and Throat (ENT) surgeons at the Radcliffe Infirmary for insertion of grommets and/or adenotonsillectomy were recruited after informed consent was obtained from parents. The study was approved by the Central Oxford Research Ethics Committee. In order to allow for seasonal variations, recruitment was performed at random over a period of 32 months from November 1990 to July 1993. Details of previous ENT operations were obtained from each child's notes. Clotted blood samples were obtained at the time of venepuncture for induction of anaesthesia for measurement of antibodies to pneumococcal and Haemophilus type B capsular polysaccharides. None of the children in the study had received Haemophilus conjugate vaccine for two reasons: first, because Haemophilus conjugate vaccine was only introduced nation-wide into the UK primary schedule of immunization (at 2, 3 and 4 months) on 1 October 1992, and second, because children under the age of 2 years were not included in this study, for which recruitment ended in July 1993. This ensured that none of the children receiving Haemophilus conjugate vaccine from October 1992 was included. Since immunization against pneumococcal infection is not routinely performed in children aged 2–15 years, it was assumed that none of the children had received 'Pneumovax'.

Assays

IgG subclasses were measured by radial immunodiffusion using commercial plates containing agarose and monospecific antisera against individual subclasses (The Binding Site, Birmingham, UK). Interassay coefficients of variation were < 7%.

Specific antibodies to pneumococcal and Haemophilus polysaccharides were measured by an ELISA. 23 valent Pneumovax II (Pasteur Mérieux MSD Ltd, Maidenhead, UK) was used as the coating antigen [20]. Antibodies to Haemophilus capsular polysaccharide were measured by ELISA using Haemophilus capsular polysaccharide conjugated to poly L-lysine as the coating antigen [21]. Optical densities were read using a Uniskan ELISA reader at 405 nm for pneumococcal antibodies and 490 nm for Haemophilus antibodies. A log/lin standard curve was plotted from which antibody concentrations of the test samples were calculated. In house standards were prepared for pneumococcal antibodies using a commercial immunoglobulin preparation (Gammagard; Baxter Healthcare, Glendale, CA) diluted in a negative serum obtained from a healthy blood donor and assigned arbitrary units/ml. The Haemophilus antibody assay was calibrated against the US Food and Drugs Administration standard for anti-*Haemophilus influenzae* type B polysaccharide antibodies (lot no. 1983) and results were expressed in μg/ml.

Absorption studies with pneumococcal cell wall capsular polysaccharide

In view of the contamination of 'Pneumovax' with cell wall capsular polysaccharide (CWCP), the effect of pre-absorbing sera with CWCP prior to assay was investigated according to the method of Musher *et al.* [22]. In brief, serum samples diluted 1:50 in PBS–T were incubated at room temperature with 10 μg CWCP for 1 h before assay. CWCP isolated from a capsule deficient mutant of *S. pneumoniae* serotype 2 was obtained from the Statens Serum Institute (Copenhagen, Denmark).

Immunization protocol

Children with spontaneous pneumococcal antibody levels (unabsorbed) below the 25th centile for age were eligible for inclusion in the immunization study. Parents of eligible children were sent an explanatory letter offering test immunization with 23 valent 'Pneumovax' and unconjugated Haemophilus capsular polysaccharide (Hib PRP) vaccine. Immunization with unconjugated Hib PRP was used as an additional marker of the response to polysaccharide antigens [23]. Intramuscular immunization with Pneumovax and unconjugated Hib PRP was performed simultaneously in opposite limbs (antero-lateral thighs). Post-immunization samples were obtained 3–4 weeks later for measurement of antibody responses.

Controls

Stored serum samples from 149 healthy children aged 2–16 years taking part in sibship studies of insulin-dependent diabetes and asthma (Oxford–Barts–Windsor study) were used to define a normal range for pneumococcal and Haemophilus antibodies. Children with a history of recurrent infection were excluded from these control groups.

Since the children who formed the control group for spontaneous antibody levels were bled some years ago, a prospective group of healthy controls was sought to study immunization responses to Pneumovax. A control group of 13 normal healthy children aged 6–10 years with no history of ear or systemic infection was recruited from a cohort of children who had previously taken part in immunogenicity studies of Haemophilus conjugate vaccine in Oxford [24]. Control children taking part in the immunization study were selected on the basis of spontaneous pneumococcal antibody levels below the 25th centile.

RESULTS

Of the 471 children enrolled in the study, 21 were excluded from analysis either due to incomplete results and/or missing clinical histories.

Statistical analysis

In view of the skewed distribution of antibody levels, results are expressed as median values with interquartile ranges. The difference in antibody levels between the study and control groups was analysed by the Mann–Whitney test. Fisher's exact test was used to analyse the distribution of IgG subclass deficiencies between poor responders and normal responders to immunization.

Specific antibody levels

Specific antibody levels to Pneumococcal polysaccharide (PnPs) and *H. influenzae* type b capsular polysaccharide (Hib) were obtained in 450 and 432 children, respectively.

Table 1.

a. Pneumococcal polysaccharide antibody levels (unabsorbed) ELISA U/ml—median (interquartile range)

	Nos	2–6 years	Nos	7–10 years	Nos	11–16 years
Study group	183	16 (8–31)	196	28 (16–54)	71	43 (28–54)
Controls	36	8·5 (5–17·5)	53	23 (14–43)	50	34 (20–49)

b. Pneumococcal antibody levels (absorbed) ELISA U/ml—median (interquartile range)

	Nos	2–6 years	Nos	7–10 years	Nos	11–16 years
Study group	175	7 (2–15)	177	15 (7–34)	67	24 (14–46)

Antibodies to Pneumococcal polysaccharide (see Tables 1a, 1b)
There was a significant rise in levels of pneumococcal antibodies in relation to age; this was found in both study and control groups (χ^2 = 64·9; $P < 0·001$). Comparisons of antibody levels between the study and control groups using the Mann–Whitney test revealed higher antibody levels in the 2–6 year and 7–10 year study groups (2–6 year group, $P < 0·004$; 7–10 year group, $P < 0·04$; 11–16 year group, $P = 0·11$).

Following absorption with cell wall polysaccharide there was a 43–56% fall in the median level of pneumococcal antibodies in the three age groups (see Table 1b).

Antibodies to Haemophilus type b capsular polysaccharide
The distribution of Haemophilus antibody levels in each age group is summarized in Table 2. As with pneumococcal antibodies, Haemophilus antibody levels rose with age and were significantly different across all three age groups (χ^2 = 21·68, $P < 0·001$). In comparison with the controls, Haemophilus antibody levels were significantly lower in the 7–10 year group (Mann–Whitney test, $P < 0·003$). There was no difference in Haemophilus antibody levels in the other age groups between study and control children.

Number of children with antibody levels below the 25th centile

Pneumococcal antibodies. Eight-eight out of 450 (19·6%) children in the study group had pneumococcal antibodies (unabsorbed) below the 25th centile (40 in 2–6 year group, 43 in 7–10 year group and five in 11–16 year group). A similar proportion (32/128) (25%) of children in the control group had pneumococcal antibody levels below the 25th centile (six in 2–6 year group, 14 in 7–10 year group, 12 in 11–16 year group). Following absorption with cell wall polysaccharide, this number rose to 190 in the study group. There were insufficient sera to study the effect of absorption in the control group of children.

Haemophilus antibodies. One hundred and two out of 432 (23·6%) children in the study group had Haemophilus antibody levels below the 25th centile (43 in the 2–6 year group, 43 in the 7–10 year group and 16 in the 11–16 year group). When analysed in relation to the levels of Haemophilus antibodies that are deemed to be protective (0·15 μg/ml for short-term protection, 1·0 μg/ml for long-term protection), there was, as expected, an inverse relationship between age and the number of children with antibody levels < 0·15 μg/ml: 53/182 (29·1%) of children in the 2–6 year group, 32/183 (17·5%) in the 7–10 year group and 4/67 (6%) in the 11–16 year group had antibody levels < 0·15 μg/ml.

Responses to immunization. Children with unabsorbed pneumococcal antibody levels below the 25th centile were selected for immunization with 23 valent Pneumococcal polysaccharide ('Pneumovax') and unconjugated Haemophilus capsular polysaccharide vaccine, Hib PRP (a kind gift from Dr Richard Insel, University of Rochester Medical Centre, New York). Of the 88 children who were eligible, parental permission to immunize 19 children was obtained. The parents of the remaining 69 children declined to take part despite a second invitation. A normal response to immunization was defined as at least a two-fold rise in antibody levels, with the post-immunization level falling within the normal range.

Of the 19 children (aged 4–11 years), five mounted suboptimal responses to both polysaccharide antigens (Figs 1–3). Of the five poor responders to both polysaccharide antigens, low IgG2 subclass levels were noted in three. Conversely, IgG2 plus IgG4 subclass deficiencies were also present in five out of 13 other children in the study group, all of whom made satisfactory responses to test immunization. The distribution of IgG subclass deficiencies amongst poor responders and normal responders was not statistically significant (Fisher's exact test, $P = 0·1174$).

From the clinical data, there was no difference in the frequency of ENT intervention between the immunized and unimmunized groups of children. Furthermore, none of the children in either group had had infection outside the upper respiratory tract. The

Table 2. Antibodies to Haemophilus type b polysaccharide μg/ml: median (interquartile range)

	Nos	2–6 years	Nos	7–10 years	Nos	11–16 years
Study group	182	0·28 (0·14–0·74)	183	0·52 (0·20–2·8)	67	1·0 (0·32–4·4)
Controls	15	0·3 (0·2–0·9)	47	1·0 (0·4–4·6)	51	1·1 (0·6–2·0)

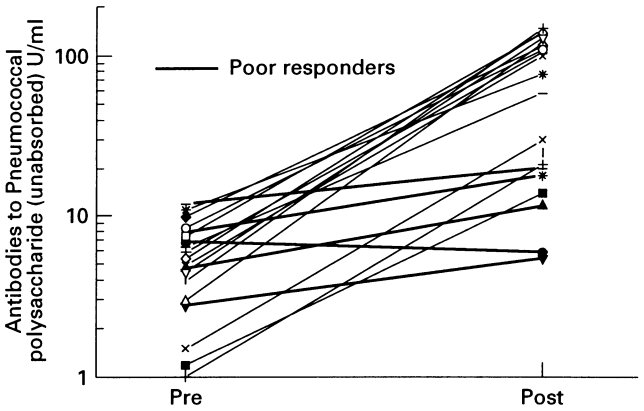

Fig. 1. Immunization responses to 23 valent Pneumovax (study group).

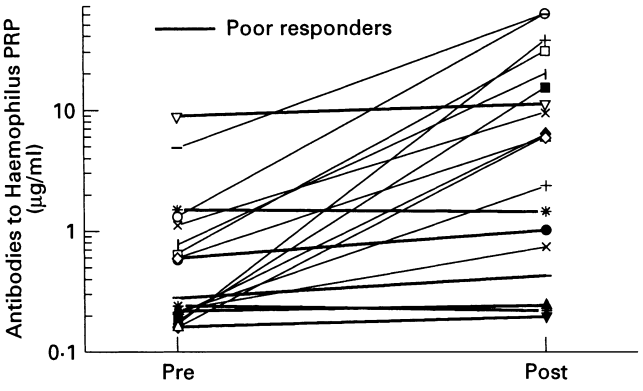

Fig. 3. Immunization responses to unconjugated Haemophilus polysaccharide (study group).

majority of children (372) underwent a single procedure, either insertion of grommets and/or adenotonsillectomy. Seventeen children had recurrent ENT intervention on three to six occasions, while 61 children had two procedures.

DISCUSSION

We have found no evidence of specific polysaccharide antibody deficiency in the vast majority of 450 children with otitis media requiring minor ENT surgery. On the contrary, pneumococcal antibody levels were significantly higher in the 2–6 year and 7–10 year study groups in comparison with age-matched controls. There was no difference in Haemophilus antibody levels between the study and control group children for the age groups 2–6 years and 11–16 years, although Haemophilus antibody levels were significantly lower in the 7–10 year group. This differential antibody response to polysaccharides in the 7–10 year group with higher pneumococcal antibody levels but lower Haemophilus antibody levels is unusual, and may reflect the predominance of *S. pneumoniae* as a pathogen in otitis media in contrast to capsulated *H. influenzae*. Overall, our results substantiate the report by Jorgensen *et al*. of high serum IgG2 subclass levels accompanied by high levels of anti-phosphorylcholine antibodies in a much smaller group of children with recurrent otitis media [14]. In the USA, Berman and colleagues too failed to find a significant difference in total IgG and IgG subclass levels in 89 children with recurrent otitis media, although they excluded children requiring grommets from the study [15].

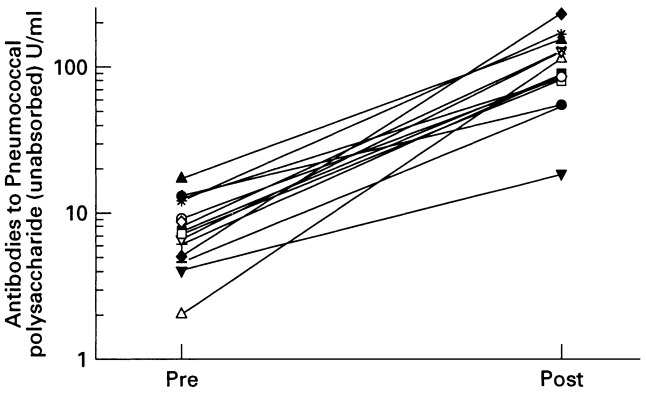

Fig. 2. Immunization responses to 23 valent Pneumovax (control group).

We have also shown that most children with pneumococcal antibody levels below the 25th centile are able to mount satisfactory responses to test immunization with pneumococcal and unconjugated Haemophilus polysaccharides. This confirms their ability to handle polysaccharide antigens satisfactorily. The true clinical significance of poor antibody responses to test immunization in a minority of children with otitis media is unclear at present. Long-term follow up of these children is required to answer this question. It is possible that poor immunization responses may herald the development of frank immunodeficiency later in life [17].

The limited diagnostic value of IgG subclass measurements in the assessment of otitis media is reflected by the inability of IgG2 subclass measurements to discriminate between those children who mounted satisfactory antibody responses to test immunization and those who did not. This accords with the widely accepted view amongst clinical immunologists that IgG subclass measurements are of little value in the assessment of immunodeficiency, unless accompanied by the assessment of responses to test immunization [25].

We recognize that our study may be criticised on several points. The assumption that the insertion of grommets implies a preceding history of recurrent otitis media in all children in this study may not necessarily be correct. Although this is likely to be true for many of the children, the lack of uniform criteria for grommet insertion and wide variations in surgical practice suggest that a significant number of children may have had grommets inserted following a few episodes of otitis media [26]. In the absence of microbiological analysis of middle ear fluid, we were unable to estimate the precise number of children with non-suppurative otitis media (glue ear) who went on to have grommets. The etiology of glue ear is multifactorial and includes passive exposure to parental smoking in addition to bacterial and viral infection. In view of the marked increase in the number of operations for glue ear in recent years, with 36% of otorhinolaryngologists opting for surgery at the first visit [27], it is likely that a significant number of children in the present study would have had grommets inserted for glue ear.

The use of whole ‘Pneumovax’ containing 23 polysaccharides as a coating antigen in ELISA assays to assay pneumococcal antibodies may be criticised on the grounds that responses to strongly immunogenic individual serotypes such as type 3 may mask poor responses to other serotypes [28]. Whilst this is an

important consideration in the assessment of protective levels of serotype-specific pneumococcal antibodies in epidemiological studies, it is unlikely to be an important confounding factor when test immunization is used as an investigatory tool in the assessment of possible immunodeficiency.

The need to absorb sera with Pneumococcal CWCP before assaying pneumococcal antibody levels is well recognized, since failure to do so obscures the true level of protective antibodies to *S. pneumoniae* [22]. This is clearly relevant in the assessment of immunization responses in certain groups of patients in whom immunization is used for the sole purpose of protection, e.g. post-splenectomy, the elderly, nephrotic syndrome. In contrast, the case for absorption with CWCP when assessing antibody responses in patients with suspected primary immunodeficiency is less clear cut. The use of test immunization in this situation is primarily as an investigatory tool to determine immunocompetence. In this context, response to polysaccharides, irrespective of whether it is CWCP or capsular pneumococcal polysaccharide *per se*, is indicative of a functioning B cell pathway. To date we are unaware of any reports of patients with proven humoral immunodeficiency mounting a differential response to immunization with 'Pneumovax', i.e. responding to CWCP and not to pneumococcal capsular polysaccharide. This is not surprising given that CWCP is generally considered to be less immunogenic than pneumococcal capsular polysaccharide [29]. As expected, absorption of sera with CWCP led to a substantial rise in the number of children with pneumococcal antibody levels below the 25th centile (88 to 190). Although this difference is of interest, it is arguable whether the use of absorbed Pneumococcal antibody levels below the 25th centile for recruitment into the immunization study would have significantly altered the number of responders to test immunization. The choice of the 25th centile as a cut-off level for Pneumococcal antibodies was arbitrary, since there are no data to define a protective level of this antibody, and moreover, exposure to the pathogen before 16 years of age is variable. The use of this cut-off level gave a reasonable sample size for immunization. The results for this sample are no different if the 10th centile is used as a cut off for Pneumococcal antibodies. This is not surprising, since 15 of the 19 children immunized had base line antibody levels below the 10th centile.

Our results suggest that routine screening for antibody deficiency is not indicated in the management of the vast majority of children requiring grommets. We would recommend, however, that clinicians retain a high index of suspicion for underlying immunodeficiency in those children in whom recurrent otitis media is accompanied by systemic infection.

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