Epidemiol. Infect. (1993), **111**, 109–119

Levels of anti-pneumococcal antibodies in young children in Papua New Guinea

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(*Accepted 24 January 1993*)

SUMMARY

Anti-pneumococcal polysaccharide antibody (anti-PPS) levels were measured in 153 serum samples collected from children aged between 2 and 47 months living in the highlands of Papua New Guinea (PNG). Fifty-seven of the samples were collected during acute episodes of lower respiratory tract infection (ALRI). Total IgA and IgG increased steadily with age; however, no association was found between the levels of these antibodies and the health status of the child. Total IgM levels showed little relationship to the age of the child but under 12 months of age levels were somewhat higher on average in children with pneumonia. For most of eight pneumococcal serotypes tested, specific IgG levels were found to decline rapidly in the first 6–8 months, reaching a minimum at approximately 12 months of age. Serotype 3 was exceptional in having very low titres in the youngest children. A separate analysis of 24 cord sera suggested that antibodies to this serotype do not usually cross the placenta in PNG. Children with pneumonia tended to have lower levels of specific IgG than healthy controls of the same age. Specific anti-PPS IgA levels were found to increase steadily with age, but were not associated with health status.

INTRODUCTION

Pneumonia is the commonest cause of hospital admission and death in both children and adults in Papua New Guinea (PNG) [1, 2]. The most commonly isolated bacterial agents are *Streptococcus pneumoniae* and *Haemophilus influenzae*, one or both of which can be isolated from lung aspirates and blood culture of more than 60% of children with moderate or severe pneumonia [1]. Pneumococcal vaccines have been shown to reduce pneumonia-associated mortality in the highlands of Papua New Guinea [3].

Antibody responses of children in developed countries have been well documented: pneumococcal vaccine, like other capsular polysaccharide vaccines,

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does not stimulate an antibody response in young children as efficiently as it does in adults [4, 5]. However, antibody responses to pneumococcal polysaccharides, both natural and vaccine-related, in children living in the highlands of Papua New Guinea are not well understood. These children face an environment very different from that encountered in developed nations, and these differences may be compounded by differences in genetic makeup.

A case-control study of children in the highlands of PNG has shown that those with moderate or severe pneumonia have lower levels of anti-pneumococcal polysaccharide IgG than age-matched healthy children [6, 7]. This report relates to a separate community-based study aimed to determine the ages at which children naturally acquire anti-pneumococcal polysaccharide antibody (anti-PPS) and the levels of anti-PPS in children in the community. We also consider whether there is any change in these levels when the child has an episode of mild pneumonia and whether the levels are related to carriage of the corresponding serotype in the upper respiratory tract.

MATERIALS AND METHODS

Subjects and samples

All subjects in this study were participants in a community study of respiratory morbidity in 156 children under 5 years of age in the Asaro Valley, Eastern Highlands Province. Details of the morbidity surveillance are reported elsewhere [8]. The definition of pneumonia used and the morbidity rates are reported by Smith and colleagues [9].

Briefly, after informed consent had been obtained from the mothers, blood samples and nasal swabs were collected from the children on entry to the study. Nasal swabs were transported to the bacteriology laboratories of the PNG Institute of Medical Research in Goroka and cultured using standard methods to detect and type *S. pneumoniae* [8]. Serum samples were aliquotted and stored at −50 °C. Further blood samples and nasal swabs were collected at approximately 6-monthly intervals from healthy children, and also whenever the child developed symptoms of pneumonia. The duration of the study was 18 months.

Pneumococcal antibody levels were measured in randomly selected samples from healthy children (i.e. those not currently suffering from pneumonia) in each age group. Samples from children with pneumonia were tested where available. In many cases this resulted in the selection of sequential samples from the same child.

Determination of antibody levels

Total IgA, IgG and IgM class antibody levels were measured by electro-immunoassay. IgA and IgG class antibody titres to eight serotypes of pneumococci (types 2, 3, 5, 6B, 7F, 14, 19F, and 23F) were measured by ELISA as described previously [6]. Briefly, 100 μl pneumococcal polysaccharide antigens diluted in PBS (phosphate-buffered saline) were incubated in ELISA plates at room temperature for 15 min. Serum samples, diluted at 1/25 in PBS/Tween + 10% skim milk, were added after five washes in PBS/Tween. After 90 min of incubation at room temperature, 100 μl of alkaline-phosphatase-conjugated goat anti-human IgG (Tago Inc., Burlingame, CA, USA) diluted 1/2000 in PBS/Tween + 5% skim

milk powder was added and incubated for a further 90 min at room temperature. After five washes with PBS/Tween, 100 μl of enzyme substrate (product no. 104-105, Sigma, St Louis, MO, USA) was added and the optical density was read after 30 min at 405 nm using a Titertek Multiscan II plate reader. Purified capsular polysaccharide antigens were kindly supplied by Institut Mérieux, Lyon, France.

Each ELISA run included a standard serum (pooled serum) of known titre which allowed the construction of standard curves of optical density against titre which were used to convert optical density to antibody titres.

Statistical analysis

Since levels of different antibodies in the same child are correlated, the results of separate statistical analyses for each serotype would not be independent.

We therefore aggregated the data for all serotypes and carried out overall tests which allowed for this correlation structure and for the differences between serotypes in the mean levels. This procedure enabled a more powerful statistical analysis to be carried out, and avoided the need for corrections for multiple testing which would be associated with a large number of significance tests.

The logarithmically transformed antibody titres were thus modelled using a multivariate normal regression model fitted by maximum likelihood using the program FISHER [10, 11]. Within this model, age effects were allowed for by polynomial regression. The correlations between repeated measurements of the same antibody in the same child, and between levels of different antibodies in the same sample were allowed for by modelling the variance–covariance of the residuals, using the approach suggested by Diggle [12] for analysing repeated measures data.

Significance testing was carried out by including extra terms in the model for (i) whether specific antibody levels are different in pneumonia cases, and (ii) whether specific antibody levels are different when the same serotype of *S. pneumoniae* is carried in the nose. Statistical significance was then assessed by likelihood ratio tests. Two-tailed tests were used.

A simplified model, which did not include terms which depended on the serotype, was used to analyse total antibody levels.

RESULTS

Specimens from a total of 71 children were analysed. Ages of the children when the specimens were collected varied from 2·6 to 46·6 months, with a median age of 20·4 months. The distribution of the number of specimens per child is shown in Table 1. Of the total 153 specimens, 57 (37·3%) were collected when the children had an episode of pneumonia.

Fig. 1(*a*, *b*) shows the levels of total antibody of each immunoglobulin class in healthy children and children with pneumonia, respectively. Total IgG and total IgA in healthy children show a steady increase with age up to 24 months and levels were almost the same in children with pneumonia and in healthy children for each class of immunoglobulin. Total IgM levels failed to show any relationship with age, but appeared to be higher in sick children under 9 months of age. This difference, however, may have been a chance effect associated with small sample numbers since it is not statistically significant.

Table 1. *Distribution of number of specimens per child*

Number of specimens	Frequency	Percentage of children
1	20	28·2
2	31	43·7
3	11	15·5
4	7	9·9
5	2	2·8
Total	71	

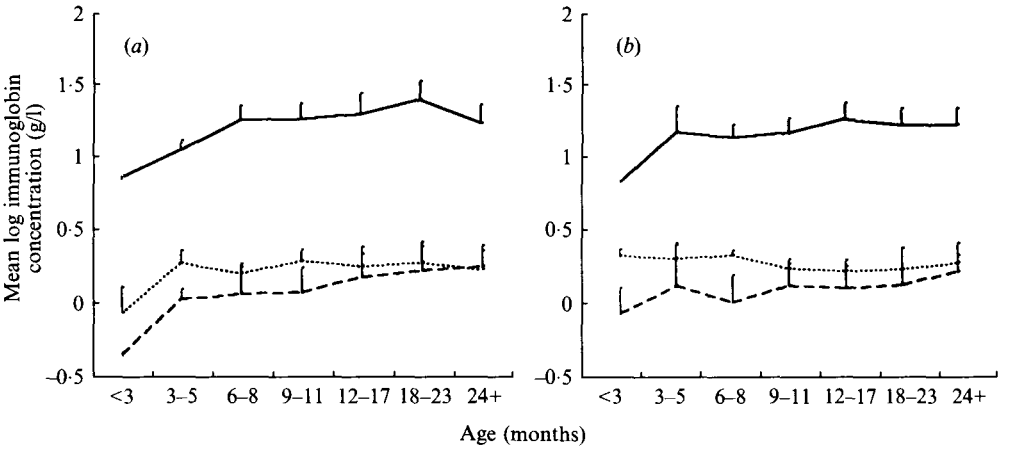

Fig. 1. Log-transformed immunoglobulin concentrations in children. (*a*) Total immunoglobulin levels in healthy children. Total IgG + 1 S.D. (——); total IgA + 1 S.D. (– – –); total IgM + 1 S.D. (------). (*b*) Total immunoglobulin levels in sick children. Total IgG + 1 S.D. (——); total IgA + 1 S.D. (– – –); total IgM + 1 S.D. (------).

Table 2 gives the means and standard deviations of the log-transformed titres of the IgG and IgA class antibodies directed against the eight pneumococcal serotypes selected, separately for both healthy and sick children. There is considerable variation between the serotypes in the mean levels, with standard deviations which are quite large in relation to the means. Fig. 2 shows the age dependence of geometric mean titres for the IgG antibodies. In 5 of the 8 serotypes specific IgG levels in healthy children show a rapid and dramatic decline over the first year, before rising again. Regression analysis and inspection of the raw data including exact ages indicated that the minimum levels were generally at approximately 9–11 months. Serotypes 6 and 23 show quite low levels of specific IgG in the youngest age group, but also low levels in older age groups, and the same general trend is evident of a fall at low ages and rise later.

IgG anti-serotype 3 was exceptional in that the lowest titres were in the specimens from the youngest children. In order to determine whether this was a chance finding, or whether it reflected an absence of transfer of antibodies to this serotype across the placenta, we tested 24 placental sera from individuals unrelated to those in the main sample. As shown in Table 3, only three of the placental sera had detectable levels of serotype-3-specific IgG. Levels of specific IgA in the placental sera were minimal for all eight serotypes tested.

Specific IgG antibody titres for children with pneumonia showed the same

Table 2. *Titres of specific anti-PPS antibodies in community study*

	Healthy children				Children with pneumonia			
			Log_e titre				Log_e titre	
Serotype	*N**	GMT†	Mean	S.D.	*N**	GMT†	Mean	S.D.
IgG 2	94	144·8	4·98	1·16	55	100·8	4·61	1·10
3	78	96·7	4·57	1·52	47	68·3	4·22	1·32
5	94	309·4	5·73	1·08	57	224·2	5·41	0·97
6B	80	27·1	3·30	1·24	49	26·3	3·27	1·40
7F	80	164·4	5·10	1·19	47	110·3	4·70	1·21
14	83	113·1	4·73	1·23	47	107·1	4·67	1·42
19F	92	286·5	5·66	1·39	54	203·6	5·32	1·52
23F	95	46·8	3·85	1·33	55	25·1	3·22	1·22
IgA 2	96	39·7	3·68	1·16	56	39·7	3·68	1·22
3	89	29·3	3·38	1·45	49	29·2	3·37	1·21
5	78	14·4	2·67	0·88	45	12·7	2·54	1·06
6B	87	23·1	3·14	0·68	53	20·6	3·02	0·77
7F	82	18·8	2·93	0·75	46	22·2	3·10	0·81
14	70	25·2	3·23	0·85	48	24·1	3·18	0·85
19F	94	38·9	3·66	1·20	56	33·1	3·50	1·30
23F	89	23·7	3·16	0·91	53	30·9	3·43	0·86

* Numbers of samples tested varied because in some cases the volume of serum was insufficient to carry out all tests.

† Geometric mean titre.

general trends as for healthy children (Fig. 2). There is a general tendency for the levels in sick children to be somewhat lower than those in healthy children, but it is not obvious from the figure whether this might be simply a chance effect. The likelihood ratio test and examination of parameter estimates showed that the specific IgG titres were statistically significantly lower among children with pneumonia ($\chi^2_1 = 5{\cdot}6$, $P = 0{\cdot}018$) but the magnitude of the differences is much less than that previously observed between community controls and inpatients by Witt and colleagues [6, 7]. This is consistent with the milder degree of pneumonia in the children of the present study than in the hospitalized patients of the earlier one.

The age dependence of the specific IgA class antibody levels is shown in Fig. 3. Serotypes 2, 3 and 19 show steep increases in antibody level with age, whilst the other serotypes show only small age trends. There is no clear association with health status ($\chi^2_1 = 0{\cdot}4$, $P = 0{\cdot}53$).

In 218/2185 of the specific antibody assays, the serotype of the assay was cultured from the nasal swab collected at the same time as the blood sample. Specific IgG and IgA antibody levels in the serum were slightly higher when the same serotype was carried in the nose (data not shown). However, these differences were not statistically significant (IgA: $\chi^2_1 = 2{\cdot}2$, $P = 0{\cdot}13$; IgG: $\chi^2_1 = 1{\cdot}1$, $P = 0{\cdot}29$).

DISCUSSION

This report describes the natural acquisition of anti-pneumococcal antibodies in children in the highlands of Papua New Guinea. As in many other developing countries, levels of exposure to respiratory pathogens, especially *S. pneumoniae*,

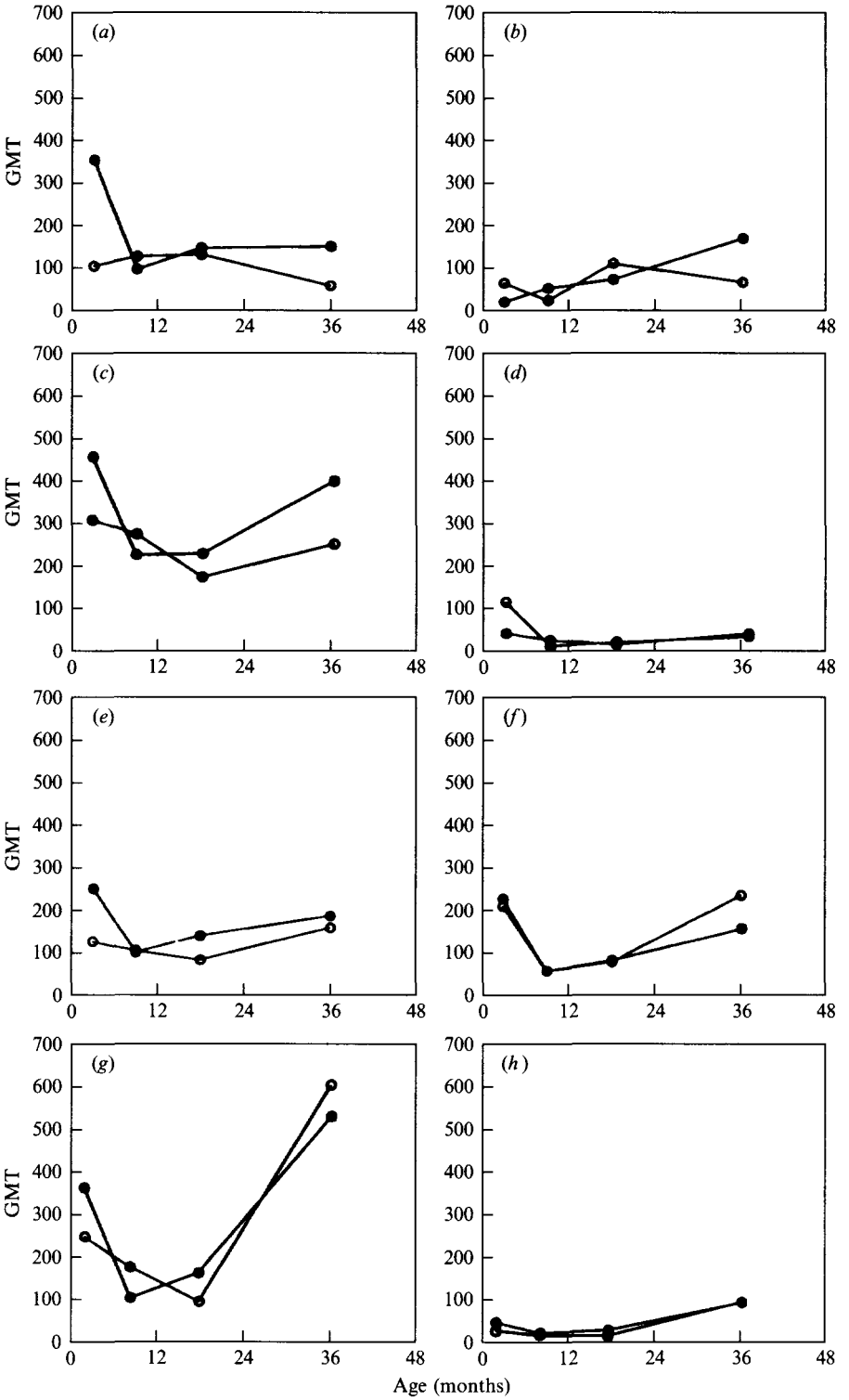

Fig. 2. For legend see opposite.

Table 3. *Mean $\log_e$ titres of specific anti-PPS IgG in 24 cord blood samples*

Serotype	Mean of positives	S.D.	No. with detectable antibody
2	3·10	0·78	24
3	2·79	0·33	3
5	3·75	0·61	24
6B	2·85	0·80	24
7F	3·17	1·00	24
14	3·35	0·62	24
19F	3·63	0·78	24
23F	2·82	0·52	24

are very high [8]. This has important implications for the development of immunity in young children.

Studies in developed countries have shown that young children respond poorly to pneumococcal polysaccharide antigens, and the response is serotype dependent with a general tendency to increase with age. An exception to this trend occurs in children 18–23 months of age who show significantly lower levels of anti-pneumococcal antibodies than children 12–17 months [13, 14]. Total serum IgG levels in these children are high at birth but decline to a minimum level at 3 months before beginning to rise again [15]. Total IgA and IgM levels show no initial decline, but increase progressively as the child grows older. The decline in total IgG presumably indicates a decline in maternally derived antibodies as the immune system of the child begins to mature [15].

In this study, analysis of both total and specific anti-PPS IgA titres did show an increase with age; however, antibodies of the IgG and IgM classes showed different patterns of age-dependence from those found in industrialized countries. Total IgM levels showed no tendency to increase with age, and were highest in children under 12 months of age who were sick with pneumonia. In contrast to the situation in developed countries, where the infant is faced with only occasional infections and the IgM response gradually builds up, the Papua New Guinean child is faced with a barrage of infections from birth onwards. Much of the total IgM observed presumably represents the primary immune response to a range of pathogens, including respiratory pathogens such as *S. pneumoniae*.

Raised total serum IgG concentrations in PNG highlands children compared to expatriate controls have been demonstrated elsewhere [6]. Total IgG levels in the present study showed no initial decline and gradually rose over the first 2 years of life. The absence of an initial decline may reflect the small sample size in the youngest age group but the high levels of total IgG would be expected to lead to rapid turnover and hence to hasten catabolism of maternally derived IgG and the early onset of the child's own IgG synthesis. The same factors probably contribute to the rapid initial decline of IgG anti-PPS antibody and the rapid decay observed on serial correlations in antibody levels. Specific anti-PPS IgG to most serotypes shows a decline over the first 6–8 months reaching minimal levels at 9–11 months

Fig. 2. Geometric mean titres of serotype-specific IgG by age group and health status. Age groups are 0– < 6 months, 6– < 12 months, 12– < 24 months and 24 months+. (*a*), Serotype 2, (*b*) serotype 3, (*c*) serotype 5, (*d*) serotype 6B, (*e*) serotype 7F, (*f*) serotype 14, (*g*) serotype 19F, (*h*) serotype 23F. –○–, Sick; –●–, healthy.

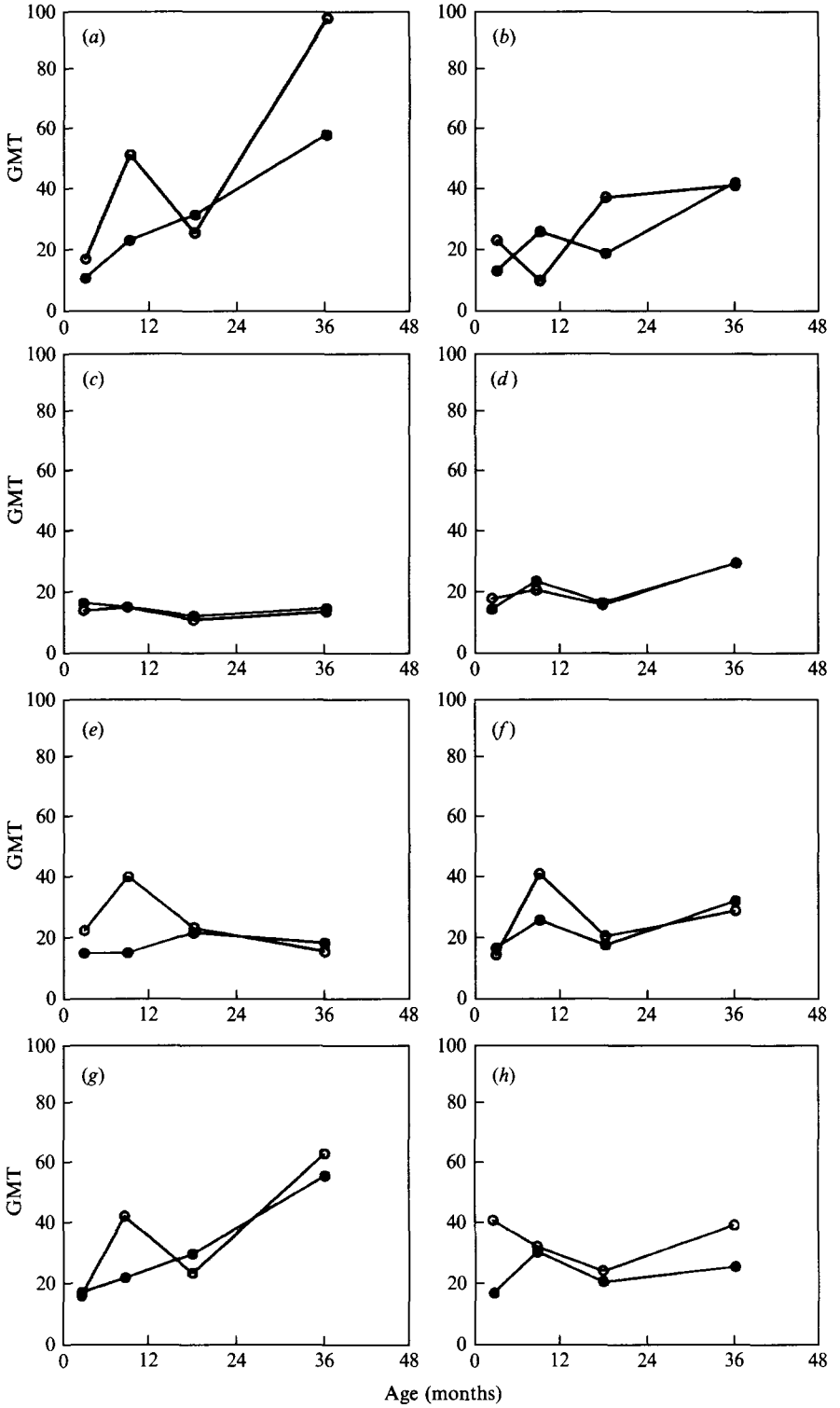

Fig. 3. For legend see opposite.

but with detectable antibody levels in all age groups. A similar study done in English children less than 10 years of age shows a decline in IgG anti-PPS levels in the first 3 months reaching minimum levels at 4–8 months of age, and remaining low until after 2 years of age [16]. A rapid decline in maternal antibodies has also been shown to occur with measles antibodies [17] and is likely to occur for antibody to other encapsulated bacteria such as *H. influenzae* type b.

There are differences between serotypes in the age-dependence of the response. IgG antibodies raised against some pneumococcal serotypes do not cross the placenta. In North American children specific antibodies to serotype 7 showed reduced transfer across the placenta [18]. In our study, we found that serotype 3 was exceptional both in showing the lowest levels in very young children and in its absence from most placental sera. Antibodies to serotype 7 were found in all placental sera tested. Possible explanations for this contrast between Melanesians and North Americans include differences between the two populations either in immunoglobulin isotype switching, or in immunoglobulin allotype polymorphisms. The immune system does not respond uniformly to all serotypes of pneumococcus. Serotype 6, for example, does not elicit a good response even when the children are vaccinated against this serotype [4, 5, 7, 14]. However, in the present study children who had high levels of IgG anti-PPS antibody for a particular serotype tended to show a relatively high response to other serotypes and high IgA for that serotype. This phenomenon may result from high early exposure to a range of bacteria in the environment. In addition, heterotypic or non-specific cross-reactivity may be established. *S. pneumoniae* type 6 is known to cross-react with *H. influenzae* type b [19, 20]. Cross-reactions between K30, K42 and K85 antigens of *Escherichia coli* and antisera to types 6, 25 and 5, respectively, have been reported by Heidelberger and his colleagues [21]. Similar cross-reaction has been reported for *Mycoplasma pneumoniae* [22].

The present analyses do not address directly the question of whether the antibodies are protective. However, the finding that pneumonia patients had significantly lower levels of anti-PPS IgG than healthy children of the same age is consistent with the concept of specific IgG offering protection. The data suggest that this aggregated effect operated at all ages if not all serotypes (see Fig. 2) but the number of samples by age was too small for conclusive evidence. Most of the cases were classed as mild pneumonia, and were not eligible for admission to hospital. Witt and colleagues [6] found a much larger difference among children under 6 months between hospitalized children with moderate and severe pneumonia and healthy controls in anti-PPS IgG levels than was found in this study. This is consistent with the finding of Lehmann and colleagues [23] that pneumococcal vaccination was associated with a reduced incidence of moderate and severe but not mild pneumonia. We could not detect any relationship between morbidity and levels of total IgG, so the effect is associated with specific rather than non-specific IgG activity.

Our results suggest that a natural immune response to *S. pneumoniae* develops in Papua New Guinean children well before their second birthday. However,

Fig. 3. Geometric mean titres of serotype-specific IgA by age group and health status. Age groups are 0– < 6 months, 6– < 12 months, 12– < 24 months and 24 months+. (*a*), Serotype 2, (*b*) serotype 3, (*c*) serotype 5, (*d*) serotype 6B, (*e*) serotype 7F, (*f*) serotype 14, (*g*) serotype 19F, (*h*) serotype 23F. –○–, Sick; –●–, healthy.

maternal antibody is lost early, exposing children under 18 months of age to high rates of infection. To be effective, vaccination strategies must aim to establish immunity during this critical period of susceptibility. The success of such vaccination strategies might be limited by a high rate of catabolism of antibody resulting from high total antibody levels. We are now using the ELISA system to examine the antibody response to a 23-valent pneumococcal vaccine (Institut Mérieux) in children from 4 months of age.

ACKNOWLEDGEMENTS

We thank Christine Coakley, Dr Veronica Spooner, Bronwyn Best and Makit Kajoi for their assistance in specimen collection. We also thank Audrey Michael, Tony Lupiwa and Benetty Kadivaion for technical assistance with bacteriological results and George Anian for the photographs. We gratefully acknowledge the generosity of Dr John Robbins and Institut Mérieux for the kind gift of pneumococcal polysaccharides and the National Institute of Biological Standardization for reference serum. This study was supported in part by the US National Academy of Science/National Research Council by means of a grant from the Office of the Science Advisor, Agency for International Development.

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