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Impaired IgG2 anti-pneumococcal antibody responses in patients with recurrent infection and normal IgG2 levels but no IgA

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SUMMARY

Combined IgA and IgG2 deficiency is well recognized as being associated with an increased susceptibility to pyogenic infection. A similar syndrome is described in four patients with IgA deficiency who were unable to produce IgG2 subclass antibodies to pneumococcal capsular polysaccharides but whose total IgG2 levels were in the normal or near normal range. This emphasizes the importance of measuring class and subclass responses to different types of antigen when investigating patients with suspected deficiencies of antibody production.

Keywords Pneumococcal infections IgG subclasses

INTRODUCTION

A hundred years ago, acquired immunity to pneumococcal infection was shown to be type specific (Fraenkel, 1886), and some 50 years later the central protective role of specific antibody was demonstrated (White, 1938; Heffron, 1939). Immunization with pneumococcal polysaccharides evokes antibodies of the IgA and IgM class, and IgG1 and IgG2 subclasses in healthy adults (Siber *et al.*, 1980; Freijd *et al.*, 1984; Oldfield *et al.*, 1985). It is not clear whether each of these heavy chain isotypes is equally protective. There is some evidence in mice that they are not (Briles *et al.*, 1981). IgG anti-pneumococcal capsular polysaccharide antibodies were found to be 40 times more protective on a weight for weight basis than IgM antibodies. IgA antibodies were not found to protect against systemic infection with pneumococci. Consequently, total anti-pneumococcal antibody activity is likely to be an inaccurate measure of protective immunity against this group of bacteria.

Patients with IgA and IgG2 deficiency are particularly susceptible to recurrent infections (Oxelius, 1974; Oxelius *et al.*, 1981, 1982; Stanley, Corbo & Cole, 1984; Matter *et al.*, 1985), and they are often found to have impaired lung function (Bjorkander *et al.*, 1985). We have identified four patients with recurrent infections and IgA deficiency who had normal or near normal serum IgG2 levels. This paper reports the investigation of their immunological defect with particular emphasis on their immunoglobulin class and subclass responses against pneumococcal capsular polysaccharides.

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MATERIALS AND METHODS

Serum specimens

Serum was obtained from healthy volunteers, aged 20 to 55 years, and the four patients, before and 14 days after immunization with Pneumovax (Merck, Sharp and Dohme); a polyvalent pneumococcal vaccine containing 50 μg of capsular polysaccharide from each of 14 pneumococcal serotypes: types, 1, 2, 3, 4, 6, 8, 9, 12, 14, 19, 23, 25, 51 and 57 (American Nomenclature).

Measurement of serum immunoglobulins and IgG subclasses

Levels of immunoglobulin G, A and M were determined by nephelometry.

IgG subclasses were measured by radial immunodiffusion using mouse monoclonal antibodies specific for each of the subclasses (Lowe *et al.*, 1982). The monoclonals were prepared in our laboratory and are available from Unipath (Bedford).

Determination of anti-pneumococcal and anti-tetanus toxoid antibody

Anti-capsular polysaccharide antibody titres of each class and subclass were determined by a four layer solid phase radioimmunoassay (RIA) as described previously (Oldfield *et al.*, 1985). Briefly, polyvinyl chloride microtitre plates (Linbro, Flow laboratories) were coated with Pneumovax, and then successively incubated with human serum, mouse monoclonal antibodies to human immunoglobulin class and subclasses, with a final radiolabelled polyclonal sheep-anti-mouse antibody. Antibody titres were calculated from a plot of counts per minute bound to wells versus $\log_2$ of serum dilution. Positive and negative control sera were included in each assay. The positive control serum was taken from a healthy adult serum 14 days after immunization with Pneumovax; the negative control serum was from one of the patients.

Tetanus toxoid antibody levels were detected by a similar assay except that plates were coated overnight at 4°C with tetanus toxoid in simple solution (Wellcome) diluted 1 in 40 with isotonic phosphate buffered saline, pH 7·6.

RESULTS

Clinical evidence of immunodeficiency

All four patients suffered from infections with encapsulated bacteria (Table 1), and in Patients 1 and

Table 1. Clinical history of the patients correlated with total levels of immunoglubulin classes (normal adult range shown in parentheses)

Patient	Age	Sex	Disease	Isolates	IgG (g/l) (6–16)*	IgA (g/l) (0·75–4)*	IgM (g/l) (0·25–2)*
1	43	M	Pneumonia and meningitis	Pneumococci	7	<0·1	0·3
2	30	F	Recurrent upper respiratory tract infections	No pathogen isolated when assessed	18·0	<0·1	1·3
3	19	F	Otitis media. Pneumonia and meningitis	Pneumococci	9·2	<0·1	0·9
4	17/12	F	Otitis media. Recurrent upper respiratory tract infections	Pneumococci Haemophilus Influenzae	11·5	<0·1	0·6

* Based on healthy adult range in UK protein reference laboratories.

Table 2. IgG subclass levels in patients and healthy adult controls

Patient	IgG1 (g/l) 5·4 (4·0–8·0)* 2·86–6·8†	IgG2 (g/l) 3·0 (1·6–5·0)* 0·3–3·27†	IgG3 (g/l) 0·44 (0·23–0·88)* 0·13–0·82†	IgG4 (g/l) 0·6 (0–1·0)* Not evaluated†
1	4·3	1·7	1·0	0·2
2	13·1	1·8	1·0	0·15
3	8·0	1·0	1·0	0·05
4	8·8	1·2	0·3	0·06

* Healthy adult median values (range in parentheses) obtained for sera from 12 healthy adults assessed at the same time as the patients' serum.

† Normal values in children (9 months–2 years); see Oxelius (1979).

Table 3. Log 2 class and subclass antibody titres against pneumococcal capsular polysaccharide before and 14 days after immunization with Pneumovax

	IgG1		IgG2		IgA		IgM	
Patient	Pre 5 (4–6·3)*	Post 9 (7·5–10·5)*	Pre 5·5 (4–7·2)*	Post 9·5 (7·5–12)*	Pre 4 (3·5–6)*	Post 10·5 (8·7–12)*	Pre 5·8 (5–7·5)*	Post 8·5 (7·5–10·75)*
1	<3	<3	<3	<3	<3	<3	4	4
2	5·3	6·8	4·8	5	<3	<3	6·5	7·5
3	6	8	<3	<3	<3	<3	7	7·8
4	4	7·8	<3	4	<3	<3	6	8
	NS	$P<0·05$	$P<0·01$	$P<0·01$	$P<0·01$	$P<0·01$	NS	$P<0·05$

* Median from eight normal healthy adults (range in parentheses).

Significance of difference between healthy adults and patients was calculated using a Wilcoxon sum-of-ranks test.

There was no detectable anti-pneumococcal capsular polysaccharide antibody of the IgG3 or IgG4 subclass before or after immunization in patients or controls.

3 these infections were life threatening. They all had normal neutrophil counts and complement levels. Patients 1 and 3 were shown to have spleens by scanning.

Immunoglobulin class and subclass levels

All four patients had absent serum IgA but normal or elevated levels of serum IgG and IgM (see Table 1). Serum IgG subclasses were within the normal range except in Patient 2 who had an elevated serum IgG1 level (Table 2) and patient 3 who had a low serum IgG2 level.

Pneumococcal antibody levels and response to vaccination with Pneumovax

The response to vaccination with pneumococcal capsular polysaccharides (Pneumovax) is shown in Table 3. Post-immunization titres of IgG1, IgG2, IgA and IgM were significantly reduced in patients compared with controls ($P<0·05$). Their levels of IgG2 specific antibody were particularly low compared with those of the control group ($P<0·01$) both before and after immunization. Despite this, they had normal or near normal total IgG2 levels in their serum (see Fig. 1). There is some overlap of specific antibody of IgG1 and IgM classes between patients and controls.

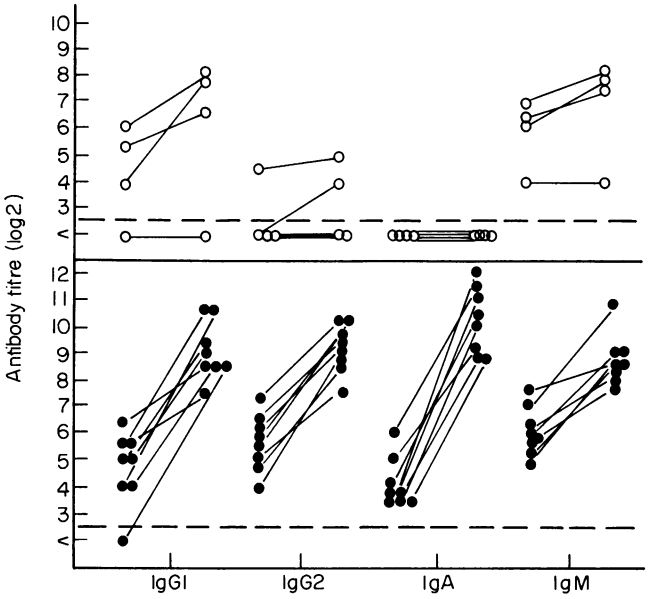

Fig. 1. Anti-pneumococcal capsular polysaccharide titres before and 14 days after immunization with Pneumovax in healthy controls (●) and patients (○). Preimmunization titres are shown on the left of each block and post-immunization titres on the right.

Table 4. Log 2 IgG subclass anti-tetanus toxoid antibody levels in patients and controls

Patients	IgG1 4 (<2–8)	IgG2 <2 (<2–8)*	IgG3 4 (<2–>8)*	IgG4 4 (<2–8)*
1	3	<2	<2	<2
2	3	<2	<2	4
3† Pre-immunization	6	6	<2	6
Post-immunization	>8	8	<2	8
4	7	<2	<2	<2

* Median values for sera from eight healthy adults assessed at the same time as the patients' sera (range in parentheses).

† Patient 3 was immunized with tetanus toxoid (Wellcome).

There was no significant difference between patients and controls by a Wilcoxon sum-of-ranks test.

Tetanus toxoid antibody levels in patients and controls

Whilst these four patients had impaired anti-pneumococcal antibody production, they had normal levels of tetanus toxoid antibody compared with controls (see Table 4). The antibodies to tetanus toxoid are predominantly IgG1 although antibodies of all subclasses are evoked by immunization with this antigen (Feehally *et al.*, 1986). This indicates that these patients were able to make normal antibody responses to this thymus-dependent antigen.

Low levels of anti-pneumococcal antibody is associated with infection in IgA deficient patients

In order to clarify the association of IgA deficiency with diminished capacity to respond to

Table 5. IgG specific anti-pneumococcal activity in unselected IgA deficient patients who were seen at hospital, correlated with clinical history

	Age	Sex	Log 2 IgG2 anti-pneumococcal ab titre	Clinical history
1	31	M	<3	Coeliac disease. Recurrent episodes of bacterial meningitis
2	22	M	<3	Bronchiectasis. Died of overwhelming pulmonary infection.
3	28	M	3·5	Diabetes mellitus. Recent chest infection. Haemophilus influenzae and pneumococcus isolated
4	66	F	3·5	Rheumatoid arthritis. IgA deficiency probably due to penicillamine therapy
5	34	F	5	Coeliac disease
6	77	F	5	Upper respiratory tract infection
7	35	M	7	Sarcoidosis
8	42	M	7	Coeliac disease
9	31	M	7·5	Recent return from abroad Investigation for tropical disease
10	65	F	7·5	Abdominal pain
11	30	F	8·0	Crohn's disease

pneumococcal capsular polysaccharides, we looked at baseline anti-Pneumovax antibodies in 11 other patients with selective IgA deficiency and correlated the IgG2 level of specific anti-pneumococcal antibody with clinical history. These results are summarized in Table 5. Three of the four patients with IgG2 anti-Pneumovax titres of less than 4 had had serious infections. However, four of the seven patients with higher levels of IgG2 anti-pneumococcal antibody presented with gastrointestinal disease and only one of these seven patients presented with infection.

DISCUSSION

IgA deficiency occurs in approximately 1:700 individuals in Great Britain (Holt, Tandy & Anstee, 1977). However, only a proportion of these patients have an abnormal history of infection (Amman & Hong, 1971). Antibodies of the IgG class can compensate in most individuals as can be seen by the protective effect of passively transferred IgG in patients with panhypogammaglobulinaemia (Bruton, 1952; Janeway, 1970). A close association between infection, reduced IgA and low serum IgG2 subclass has been noted by several authors (Oxelius, 1974; Oxelius *et al.* 1981, 1982; Stanley, Corbo & Cole, 1984; Matter *et al.*, 1985). Although asymptomatic IgA deficiency is associated with HLA B8, DR3, there is no such association in patients with IgA deficiency and infection (Hammarstrom *et al.*, 1984). The current study identifies a further group of susceptible patients on the basis of impaired IgG2 subclass responses to pneumococcal capsular polysaccharides.

The cellular basis of this immunodeficiency is not well understood, but there is indirect evidence that different populations of B lymphocytes and antigen presenting cells respond to thymus-dependent and independent antigens (MacLennan *et al.*, 1982). Pneumococcal polysaccharides evoke good antibody responses of all immunoglobulin classes in congenitally athymic rodents. On

this basis they are defined as thymus-independent antigens (Mosier & Subbarao, 1982). The antibody response to a hapten such as dinitrophenol (DNP) is dependent on the carrier to which it is conjugated. DNP conjugated to protein is a thymus-dependent antigen whereas DNP conjugated to polysaccharide elicits a thymus-independent response (Gray *et al.*, 1985; Scott & Fleishman, 1982). Although antigens based upon a polysaccharide carrier evoke a predominant IgG2 response in humans, there is a significant amount of IgG2 produced after immunization with thymus-dependent antigens such as tetanus toxoid (Feehally, 1986). Therefore a deficient IgG2 response to thymus-independent antigens may not be reflected in abnormal total levels of this subclass.

Whilst few patients present in adult life with this immunodeficiency, it is clear that young children have impaired antibody responses to encapsulated pyogenic bacteria such as pneumococci and *Haemophilus influenzae*. It has been estimated that each year in Third World countries as many as 5 million children die from respiratory infections with these bacterial pathogens (Shann *et al.*, 1984). Children do not develop the capacity to make mature immune responses to capsular polysaccharide antigens until around two years of age (Borgono *et al.*, 1978; Cowan *et al.*, 1978; Schur, Rosen & Norman, 1979; Granoff, 1980). Their susceptibility to infection is greatest between 6 months and 2 years of age when antibody levels against these bacteria are very low.

Because the antibody response to any particular hapten is dependent on the carrier, attempts have been made to conjugate polysaccharides to proteins in order to make responses thymus-dependent (Goebel, 1940; Eskola *et al.*, 1985; Anderson *et al.*, 1985). Antibody is made by children under 1 year of age when they are immunized with these preparations (Eskola *et al.*, 1985; Anderson *et al.*, 1985). However, it is not entirely clear whether the antibody response is sustained, or whether the fine specificity, class and subclasses of the antibody evoked are as protective as those produced by older individuals to the native polysaccharide. The results of trials assessing the clinical efficacy of immunization with these vaccines is awaited.

This study illustrates that it is not wise to exclude selective antibody production defects on the basis of total levels of immunoglobulin classes and sub-classes, and total specific antibody levels. Certain clinically significant defects will only be detected if specific immunoglobulin class and subclass responses to both thymus-dependent and thymus-independent antigens are measured. The patients described in this study had serious infections. Identification of their immuno-deficiency allows a more rational approach to prevention and treatment of future episodes of infection.

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