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The clinical syndrome of specific antibody deficiency in children

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Summary

Specific antibody deficiency (SAD) is an immune deficiency which has been reported in adults and children with recurrent respiratory tract infections; however, the clinical features of SAD are not well described. This study evaluated formally the clinical syndrome of SAD, by comparing the clinical features of children with SAD and those of children with recurrent infection but normal immune function tests. SAD was defined as an adequate IgG antibody response to less than 50% of 12 pneumococcal serotypes tested following 23-valent unconjugated pneumococcal immunization. An adequate IgG antibody response was defined as a post-immunization titre of ≥ 1·3 μg/ml or ≥ four times the preimmunization value. Seventy-four children with recurrent infection were evaluated where immune deficiencies other than SAD had been excluded. Eleven (14·9%) of these children had SAD. Clinical features differed between the group with SAD and the group with normal antibody responses. A history of otitis media, particularly in association with chronic otorrhoea was associated with SAD [relative risk (RR) of SAD in those with chronic otorrhoea 4·64 (*P* = 0·02)]. SAD was associated with allergic disease, particularly allergic rhinitis [RR of SAD in those with allergic rhinitis 3·77 (*P* = 0·04)]. These two clinical associations of SAD were independent in this study [RR of chronic otorrhoea in those with allergic rhinitis 0·85 (*P* = 0·28)]. SAD was not an age-related phenomenon in this population. SAD has a distinct clinical phenotype, presenting as recurrent infection associated with chronic otorrhoea and/or allergic disease, and the condition should be sought in children with these features.

Keywords: allergic rhinitis, immunodeficiency – primary, paediatric, pneumococcus

Introduction

Many primary immune deficiency disorders predispose the individual to infection with polysaccharide coated organisms, particularly those immune disorders which include a deficiency of the IgG2 subclass. It has also become recognized that a distinct group of patients have poor serological response to polysaccharide antigens but normal levels of immunoglobulins and IgG subclasses, and normal responses to protein antigens [1]. This pattern of immune dysfunction has been termed specific antibody deficiency (SAD) and has been reported by several authors in subjects with a clinical history of recurrent infection [1–5]. SAD has been reported more commonly in children, and this may be related to the age-dependent development of polysaccharide antibody responses, with deficient responses being normal in healthy children under 2 years of age. The clinical significance of SAD is not well understood, particularly in young children where there is an overlap between the immune findings of SAD and those of the healthy child. Moreover, the prevalence of SAD has been reported only in patients with recurrent respiratory infections, and its prevalence in other patient groups such as those with other forms of recurrent infection is not known. This is relevant, because polysaccharide-coated organisms are cultured commonly from children with otitis media and other non-respiratory infections that represent an important cause of morbidity and mortality in childhood. We therefore investigated the prevalence and clinical features of SAD in children who presented with recurrent infections and suspected antibody deficiency. We

reviewed the case notes and laboratory investigations of all children with recurrent infection assessed for the presence of SAD over an 18-month period, in order to characterize the clinical features of impaired polysaccharide antibody responses in this group of children.

Materials and methods

Subjects

Children evaluated by the Immunology Department of the Royal Children's Hospital, Melbourne between 1 January 2004 and 30 June 2005 were included in the study. This is the only paediatric immunology service for the region of Victoria, Australia, and serves a population of approximately 3 million people. Inclusion criteria for the study were age 2–18 years at the time of evaluation and the formal assessment of specific antibody response to unconjugated pneumococcal vaccine. Specific antibody response was assessed for all children with suspected antibody deficiency [6]. Exclusion criteria were an identified primary or secondary immune disorder, including isolated IgG, IgG2 or IgA deficiency, immunosuppressive medication and anatomical abnormalities that might account for the history of infection. All subjects underwent assessment of total IgG, IgA, IgM, IgE and IgG subclass levels, IgG responses to protein antigens, isohaemagglutinins, total and differential leucocyte count, lymphocyte markers, proliferative response to mitogen and complement CH100. Subjects also received one dose of 23-valent pneumococcal vaccine (Pneumovax 23, Merck, NJ, USA) and underwent assessment of pneumococcal antibody levels at the time of vaccination and 4–6 weeks later. Pre- and post-vaccination sera were analysed in pairs. An adequate IgG antibody response to a given serotype was defined as a post-immunization antibody concentration of 1·3 μg/ml or greater, or a fourfold increase over the preimmunization value [2]. SAD was defined as a satisfactory response to less than 50% of the serotypes tested. The study protocol was approved by the Royal Children's Hospital Human Research Ethics Committee.

Collection of clinical and laboratory data

Clinical data were extracted from patient charts by an investigator (C. L.) who was blind to the laboratory records of the patients. Laboratory data were extracted by a different investigator (R. J. B.) who was blind to the clinical data, and who grouped the patients into those with SAD, those with normal immune function tests and those with another identified immune deficiency. After all clinical and laboratory data were obtained, categorized and entered into a database, the clinical features of those with SAD and those with normal immune function tests were compared. Data were analysed using statistical software SPSS 13·0 (SPSS Inc., Chicago, IL, USA). Binary outcomes were analysed using the χ^2 test, or where expected values were small using Fisher's exact test.

Enzyme-linked immunosorbent assay (ELISA) for anti-pneumococcal IgG

IgG anti-pneumococcal antibody titres were determined by a modified ELISA protocol developed from World Health Organization methodology [7]. This method involves a double preabsorption step using C-polysaccharide and serotype 22F. Optimal concentrations of individual pneumococcal serotype polysaccharides [American Type Culture Collection (ATCC), Rockville, MD, USA] were bound on the surface of microtitre plates (Nalge Nunc International, Rochester, NY, USA). Plates were incubated for 5 h at 37°C and stored overnight at 4°C. Controls and patient samples were preadsorbed overnight at 4°C, at an initial dilution of 1 : 100 in phosphate-buffered saline (PBS) buffer with *Streptococcus pneumoniae* C-polysaccharide (Statens Serum Institut, Copenhagen, Denmark) at 10 μg/ml and serotype 22F (ATCC) at 30 μg/ml. The Food and Drug Administration (FDA) 89-SF reference sample (CBER; US FDA, Rockville, MD, USA) was used as a standard, at eight twofold dilutions from 1 : 100 to 1 : 12800, and was also preadsorbed with *S. pneumoniae* C-polysaccharide at 10 μg/ml. This standard is a serum pool prepared from six healthy adults immunized with the 23-valent polysaccharide vaccine (Lederle-Praxis Biologicals, Pearl River, NY, USA). All subsequent dilutions of standard, control and serum samples were in PBS containing 10% fetal calf serum (FCS).

On the day of testing, the coated plates were washed four times with PBS containing 0·05% Tween 20 and blocked with PBS 10% FCS for 1 h at 37°C. Patient samples, controls and standard dilutions were then added in triplicate and the plates incubated for 2 h at 37°C. After the 2-h incubation, wells were washed four times with PBS containing 0·5% Tween 20 then 50 μl of a 1 : 5000 dilution of anti-human IgG (γ chain) affinity-isolated horseradish peroxidase-conjugated anti-serum (Chemicon International, Temecula, CA, USA) was added to each well, and the plates incubated for a further 2 h at 37°C. After another washing step, the bound enzyme was detected using tetramethyl benzidine microwell substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA). The reaction was stopped by addition of 2 M H_3PO_4 after 9 min and the optical density read at 620 nm (reference filter 450 nm). The serotype-specific IgG concentration in μg/ml was calculated from the standard curve obtained with twofold serial dilutions of 89SF.

Results

Clinical features associated with SAD

Seventy-four patients met the inclusion criteria, of whom 11 (14·9%) were found to have SAD. A further 15 patients were

Table 1. SAD risk according to age, sex, vaccination and infectious disease history.

Characteristic	SAD percentage (*n*)	RR (95% CI)	*P*-value
Males	15·6 (7/45)	1·13 (0·36–3·52)	0·26
Females	13·8 (4/29)	1·0	
Age under 5 years	15·8 (6/38)	1·14 (0·38–3·40)	0·25
Age 5 years or over	13·9 (5/36)	1·0	
Previous pneumococcal vaccine	11·1 (1/9)	0·82 (0·12–5·80)	0·41
No previous pneumococcal vaccine	13·6 (8/59)	1·0	
History of otitis media	18·9 (7/37)	4·73 (0·62–36·12)	0·08
No history of otitis media	4·0 (1/25)	1·0	
≥ Eight episodes of otitis media per year	20·0 (3/15)	2·30 (0·58–9·13)	0·17
< Eight episodes of otitis media per year	8·7 (4/46)	1·0	
Spontaneous tympanic membrane perforation	7·1 (1/14)	0·48 (0·06–3·57)	0·30
No spontaneous tympanic membrane perforation	14·9 (7/47)	1·0	
Chronic otorrhoea	36·4 (4/11)	4·64 (1·36–15·75)	0·02*
No chronic otorrhoea	7·8 (4/51)	1·0	
Recurrent bronchitis or pneumonia	15·6 (5/32)	1·56 (0·41–5·98)	0·24
No recurrent bronchitis or pneumonia	10·0 (3/30)	1·0	
Other significant infection	13·3 (2/15)	1·04 (0·24–4·64)	0·33
No other significant infection	12·8 (6/47)	1·0	

*$P < 0·05$ Fisher's exact test.

excluded from the study due to the presence of IgA deficiency ($n = 5$), low total IgG level ($n = 3$), common variable immune deficiency (CVID; $n = 3$), immunosuppressive medication ($n = 2$), persistent neutropenia ($n = 1$) and Wiscott Aldrich syndrome ($n = 1$). The prevalence of SAD among all patients evaluated for recurrent infection was 11 of 89 (12·4%), which equalled the prevalence of all other antibody deficiencies identified in this population. Tables 1 and 2 show the clinical features which distinguished those with and without SAD. A history of chronic otorrhoea and a history of physician-diagnosed allergic disease (particularly allergic rhinitis) were significantly associated with the presence of SAD [relative risk (RR) 4·64 ($P = 0·02$) and 3·77 ($P = 0·04$)], respectively. A history of otitis media and a history of eczema were also associated with an increased risk of SAD [RR 4·73 ($P = 0·08$) and 3·25 ($P = 0·06$)], respectively. However, these latter associations did not reach conventional levels of statistical significance. SAD was seen exclusively in those with some form of allergic disease – SAD was identified in eight of 41 subjects with at least one allergic disease, and none of 21 subjects with no allergic disease ($P = 0·03$). There were no associations between other clinical features of antibody deficiency disorders and the laboratory finding of SAD. In particular, no association was noted between recurrent bronchitis, pneumonia, perforating otitis media or other significant infections and SAD. Although clinical data were missing for several study participants, the clinical associations of SAD were not altered by making assumptions about the presence or absence of specific features in these participants (data not shown).

Table 2. SAD risk according to history of allergic disease and laboratory abnormalities.

Characteristic	SAD percentage (*n*)	RR (95% CI)	*P*-value
Asthma	13·8 (4/29)	1·14 (0·31–4·15)	0·29
No asthma	12·1 (4/33)	1·0	
Eczema	23·8 (5/21)	3·25 (0·86–12·32)	0·06
No eczema	7·3 (3/41)	1·0	
Allergic rhinitis	26·3 (5/19)	3·77 (1·00–14·20)	0·04*
No allergic rhinitis	7·0 (3/43)	1·0	
Food allergy	22·2 (2/9)	1·96 (0·47–8·25)	0·24
No food allergy	11·3 (6/53)	1·0	
Any allergic disease	19·5 (8/41)	n.a.	0·03*
No allergic disease	0·0 (0/21)	1·0	
Raised IgE	11·1 (2/18)	0·78 (0·18–3·40)	0·31
Normal IgE	14·3 (7/49)	1·0	
Eosinophilia	20·0 (2/10)	1·66 (0·40–6·86)	0·28
No eosinophilia	12·1 (7/58)	1·0	

*$P < 0·05$ Fisher's exact test; n.a.: not available.

Table 3. Risk of adequate response to individual serotypes according to age at assessment.

Serotype	Age group	Serotype response rate percentage (*n*)	RR for serotype response	*P*-value
1	Under 5 years	65·8 (25/38)	0·79 (0·60–1·04)	0·08
	5–16 years	83·3 (30/36)	1·0	
3	Under 5 years	81·6 (31/38)	1·22 (0·93–1·61)	0·14
	5–16 years	66·7 (24/36)	1·0	
4	Under 5 years	81·6 (31/38)	0·98 (0·79–1·21)	0·84
	5–16 years	83·3 (30/36)	1·0	
5	Under 5 years	55·3 (21/38)	0·80 (0·56–1·14)	0·21
	5–16 years	69·4 (25/36)	1·0	
6B	Under 5 years	18·4 (7/38)	0·44 (0·20–0·96)	0·03*
	5–16 years	41·7 (15/36)	1·0	
7	Under 5 years	86·8 (33/38)	0·98 (0·82–1·16)	0·27
	5–16 years	88·9 (32/36)	1·0	
9V	Under 5 years	68·4 (26/38)	0·95 (0·70–1·27)	0·13
	5–16 years	72·2 (26/36)	1·0	
14	Under 5 years	55·3 (21/38)	0·62 (0·46–0·85)	< 0·01**
	5–16 years	88·9 (32/36)	1·0	
15	Under 5 years	73·7 (28/38)	0·88 (0·70–1·24)	0·31
	5–16 years	83·3 (30/36)	1·0	
18C	Under 5 years	89·5 (34/38)	1·11 (0·91–1·35)	0·28
	5–16 years	80·6 (29/36)	1·0	
19F	Under 5 years	50·0 (19/38)	0·95 (0·61–1·48)	0·81
	5–16 years	52·8 (19/36)	1·0	
23F	Under 5 years	42·1 (16/38)	0·76 (0·47–1·22)	0·25
	5–16 years	55·6 (20/36)	1·0	

*$P < 0·05$; **$P < 0·01$. χ^2 test, or where expected values are below 5, Fisher's exact test was used.

Age-dependent serotype-specific responses to polysaccharide antigens

Table 3 shows the proportion of subjects with a satisfactory antibody response to each serotype when grouped by age: < 5 years age at the time of assessment, and ≥ 5 years at the time of assessment. Inadequate responses to serotypes 6B and 14 were significantly more likely in the younger age group [RR 2·27 ($P = 0·03$) and 1·61 ($P < 0·01$)], respectively. This suggests that in this population IgG antibody responses to these two serotypes in the 23-valent pneumococcal vaccine were age-related. However, IgG responses to the other 10 serotypes assessed were not age-related in this study, and SAD was not over-represented in the younger age group. An adequate response to serotype 6B was seen in only 22 of 74 (29·7%) children, suggesting that serotype 6B was not strongly immunogenic in this population as a whole.

Deficient responses to serotypes 4, 9V, 15 and 23F are predictive for SAD

We also examined whether an inadequate response to any individual pneumococcal serotype would be predictive for the diagnosis of SAD. Table 4 shows the risk of SAD in those who failed to respond to each of the 12 serotypes assessed. Failure to respond to any single serotype was associated with increased risk of SAD. The risk was greatest for those who failed to respond to serotypes 4, 9V, 15 or 23F. A poor response to one of these serotypes increased the risk of SAD by a ratio of 8·21 ($P < 0·01$), 6·30 ($P < 0·01$) and 9·67 ($P < 0·01$) for serotypes 4, 9V and 15, respectively. None of the children with SAD mounted an adequate response to serotype 23F.

Discussion

We have found SAD to be common in this cohort of children, who were evaluated at a tertiary paediatric immunology service for suspected antibody deficiency. In this series of 74 children without another identified immune deficiency, 11 (14·9%) had SAD as defined by published criteria [2]. SAD has been found in 6–14% of children evaluated for recurrent infection in other studies, with prevalence varying according to the referral population, their age and the serological definition of SAD used in the study [4,5,8,9]. A chart review from one centre found SAD to be the single most commonly diagnosed form of primary immunodeficiency, accounting for 21 of 91 (23·1%) cases of primary immunodeficiency [10]. Our study confirms that SAD is the most commonly identified immune deficiency in children evaluated for possible antibody deficiency. In this study SAD accounted for the same number of cases (11 of 89; 12·4%) as all other antibody deficiencies combined.

Table 4. Risk of SAD associated with an inadequate response to individual serotypes.

Serotype	Adequate response	No. with SAD % (*n*)	RR for SAD	*P*-value
1	No	26·3 (5/19)	2·41 (0·83–7·01)	0·08
	Yes	10·9 (6/55)	1·0	
3	No	31·6 (6/19)	3·47 (1·20–10·09)	0·02*
	Yes	9·1 (5/55)	1·0	
4	No	53·8 (7/13)	8·21 (2·81–24·00)	< 0·01**
	Yes	6·6 (4/61)	1·0	
5	No	25·0 (7/28)	2·88 (0·92–8·95)	0·05
	Yes	8·7 (4/46)	1·0	
6B	No	19·2 (10/52)	4·23 (0·58–31·08)	0·08
	Yes	4·5 (1/22)	1·0	
7	No	33·3 (3/9)	2·71 (0·88–8·38)	0·10
	Yes	12·3 (8/65)	1·0	
9V	No	36·4 (8/22)	6·30 (1·84–21·56)	< 0·01**
	Yes	5·8 (3/52)	1·0	
14	No	28·6 (6/21)	3·03 (1·03–8·86)	0·04*
	Yes	9·4 (5/53)	1·0	
15	No	50·0 (8/16)	9·67 (2·89–32·29)	< 0·01**
	Yes	5·2 (3/58)	1·0	
18C	No	45·5 (5/11)	4·77 (1·76–12·96)	< 0·01**
	Yes	9·5 (6/63)	1·0	
19F	No	25·0 (9/36)	4·75 (1·10–20·51)	0·04*
	Yes	5·3 (2/38)	1·0	
23F	No	28·9 (11/38)	n.a.	< 0·01**
	Yes	0·0 (0/36)	n.a.	

$^*P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$ Fisher's exact test; n.a.: not available.

The polysaccharide-coated organism *S. pneumoniae* accounts for up to 55% of bacterial otitis media in children, which may make children with SAD at particular risk for severe or persistent otitis media [11]. In particular, those with impaired host defence to capsular polysaccharides may be predisposed to chronic otorrhoea related to persistent infection with *S. pneumoniae* or other encapsulated organisms. Consistent with this, one small study of children with SAD suggested previously that recurrent otitis media may be a common feature of this condition, with nine of nine children affected [12]. In contrast, a large series of children undergoing surgery for chronic serous otitis media (CSOM) found that preimmunization pneumococcal antibody levels were similar to or higher than those of a control group [13]. This latter finding may, however, be influenced by differential levels of exposure to *S. pneumoniae*, as only 19 of these children with CSOM were evaluated using a 23-valent pneumococcal vaccine, and five of these 19 children had a poor serological response. The prevalence of SAD may also differ in children with forms of otitis media other than CSOM. In our study there was a trend to increased risk of SAD in those with a history of acute otitis media [RR 4·73 ($P = 0{\cdot}08$)] and a significantly increased risk of SAD in those with persistent otorrhoea in the absence of tympanostomy tube placement [RR 4·64 ($P = 0{\cdot}02$)]. Those with a history of tympanic membrane perforation or of frequent otitis media (at least eight episodes per year) without persistent otorrhoea did not appear to have an increased risk of SAD. Nevertheless, the overall results suggest an association between chronic ear infection and SAD, which emphasizes the importance of encapsulated organisms in childhood otitis media and suggests that children with persistent otorrhoea should be screened for SAD.

SAD has been reported previously in populations of children with recurrent respiratory infection [4,5]. However, in this study a history of severe or recurrent respiratory infections was not associated with increased risk of SAD, in comparison with those evaluated for possible antibody deficiency in the absence of significant respiratory infection. This may reflect the predominance of viruses as aetiological agents in childhood respiratory infections. While this small study cannot rule out a significant association between respiratory infections and SAD, those with a history of chronic discharging otitis media appear to be the group with highest prevalence of SAD in this population.

Antibody deficiencies including CVID and IgA deficiency are known to be associated with increased risk of allergic disease. One small series of children with SAD found just one of six to have positive skin prick tests [14]. However, the prevalence of allergic disease in SAD has not been reported otherwise. We found that all children with SAD had at least one form of allergic disease, most commonly allergic rhinitis. Within the population assessed, there was a significant increase in risk of SAD in those with any form of allergic

disease (19·5% versus 0%, $P = 0·03$), particularly those with allergic rhinitis [RR 3·77 ($P = 0·04$)]. Although allergic rhinitis may predispose to otitis media and CSOM, in this study there was no association seen between allergic rhinitis and chronic otorrhoea – 15·7% of those with allergic rhinitis presented with chronic otorrhoea, compared to 18·6% without allergic rhinitis [RR 0·85 ($P = 0·28$)]. Therefore the associations of SAD with chronic otorrhoea and with allergic rhinitis appeared to be independent of each other in this study. The finding that allergic disease is associated with SAD suggests that this disorder may represent a form of immune dysregulation, with impaired immune responses to both pathogens and to harmless environmental antigens. An association between multiple food allergies and both cellular and humoral immune abnormalities has been reported previously, although immune responses to polysaccharide antigens were not assessed in this study [15]. This new recognition of an association between SAD and allergic disease may help in defining the molecular basis of SAD. Recently some forms of antibody deficiency with associated autoimmune/allergic disease have been found to be caused by mutations in the transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI), which mediates isotype switching in B cells [16,17]. Dysregulated B cell function in these patients leads to impaired antibody responses to polysaccharide antigens as well as to the production of autoreactive antibodies. It will be of interest to explore the role of TACI mutations in children with SAD. Other investigators have found an increase in circulating CD5 positive B cells in subjects with SAD but not in those with CVID, and this suggests an alternative pathogenesis for SAD [18]. Although a single case of SAD has been reported due to a mutation in the Bruton's tyrosine kinase gene, this is unlikely to underlie the majority of cases of this common condition [19]. In our study there was an equal male : female ratio in cases of SAD, and in no case was there a reduction in the percentage or number of circulating B cells. The molecular basis of SAD remains a subject for ongoing investigation.

The age-related maturation in antibody responses to pneumococcal serotypes after 23-valent pneumococcal vaccination has been noted in previous studies of adults and children with recurrent respiratory tract infections [2]. This relationship with age is also true for the healthy population, at least until the age of 5 years [20]. The implications of this age-related deficit in pneumococcal polysaccharide antibody responses for the diagnosis of SAD are not clear. This is particularly the case in view of the great individual variation in antibody responses to pneumococcal vaccine shown in both adult and paediatric studies [21]. Some previous studies have found serotype 3 to be strongly immunogenic in the under-5s, although that was not the case in a large study of SAD in children with recurrent respiratory tract infection [2,4]. In our study serotype 3 was strongly immunogenic in over 80% of under-5s, and was one of the most immunogenic serotypes in this age group. Conversely adequate responses to serotypes 6B and 14 were seen less commonly in the under-5s in this study (RR 0·44 and 0·62, respectively), which is in keeping with previous reports [2,20]. However, for many serotypes there was little difference in response rates between those under 5 and those over 5 years old, which is in contrast to some previous population-based reports [20]. Moreover, the overall risk of SAD was not increased in the under-5s in this study (data not shown). This suggests that the age-related maturation of antibody responses to polysaccharide antigens is not a major factor leading to misdiagnosis of SAD in children aged 2–5 years.

Overall response rates to individual serotypes present in the 23-valent pneumococcal vaccine were also assessed in this population. For the group as a whole, the percentage responding to each of 12 serotypes evaluated varied from 29·7% for serotype 6B to 87·8% for serotype 7. The poor immunogenicity of serotype 6B has been noted previously in studies of children and adolescents with recurrent infection, and this appears to be the case using both unconjugated and conjugated pneumococcal vaccines [2,22]. This may be related to the restricted nature of the human B cell response to serotype 6B [23]. The power of individual serotype responses to predict the presence or absence of SAD was also evaluated. In this study responses to serotypes 4, 9V, 15 and 23F best discriminated between those with SAD and those without SAD. However, the positive predictive value of failure to respond to one of these four serotypes for the presence of SAD was low, ranging between 28·9% and 53·8% for individual serotype responses. The negative predictive value of an adequate response to one of these four serotypes for the absence of SAD was high, ranging between 93·4% and 100% for individual serotype responses. Adequate response to ≥ 2 of these four serotypes carried a high negative predictive value (98%) and response to < 2 of the four serotypes carried a high positive predictive value (100%) for the presence of SAD. These data have implications for the rationalization of laboratory services. The data suggest that in a population of children with recurrent infection, SAD may be diagnosed by the evaluation of serological responses to just four pneumococcal serotypes (4, 9V, 15 and 23F) where response to less than two of the serotypes is classified as SAD. However, the predictive value of this approach would first need to be confirmed in a second prospective study.

In summary, our data suggest that SAD is common in children with recurrent infection and no other defined immune deficiency, and that children with SAD are at increased risk of otitis media and chronic otorrhoea. SAD is also associated with allergic disease; however, in this population the association between SAD and chronic otorrhoea was not explained by the presence of allergic disease. The association with allergic disease does suggest that SAD may be part of a broader disorder of immune regulation. In this study the age-related increase in immune responses to polysaccharide antigens did not appear to impact on the diagnosis of SAD. We suggest that children with persistent

otorrhoea in association with otitis media should be evaluated for SAD, as antibody replacement therapy can be effective [12,14]. Future studies should address the long-term prognosis of SAD, and molecular analysis of these patients will also be of great interest.

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