SPECIFIC ANTIBODY DEFICIENCY IN CHILDREN SUFFERING RECURRENT INFECTIONS

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Abstract - Specific antibody deficiency has been recognized as an immunodeficiency. In order to investigate an antipolysaccharide antibody defect as a cause of reccurent infections, 30 children were studied. Patients who had been identified to have a major immunodeficiency or structural abnormality or a disease known to cause infection were excluded.

Thirty patints, aged 1 to 13.8 years (mean age, 5.8; male: female, 13:17) were chosen; all had normal IgG and IgG1. The level of IgA, IgG2 and IgG3 were of lower than normal in some cases. All were immunized with Hib conjugate (PRP-T) vaccine, and 26 with pneumococcal vaccine. Antibody responses were measured 4-6 weeks later. Twelve showed a poor response to immunization: 8 to Pneumovax, 3 to Hib and 1 to both. No correlation was observed between IgG2 levels and specific antibody responses to polysaccharide antigens. The infections were more severe and more frequent in children who responded poorly to polysaccharide antigens. Children who had infections in more than one site were most likely to have deficient antibody responses.

These results show that assessment of specific antibody responses to challenge immunization is an essential part of the investigation of children suffering from recurrent pyogenic infections. The study confirms that measurement of immunoglobulin isotypes and IgG subclasses alone does not exclude significant hormonal immune deficiency.

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Key Words: Antibody deficiency, recurrent infections, IgG2 - Pneumovax, PRP-T

INTRODUCTION

Antibodies to the capsular polysaccharides play a major role in host defence against *Haemophilus influenzae* type B [Hib], pneumococcal and meningococcal infections and low levels of serum anti-capsular polysaccharide antibodies increase the risk of recurrent respiratory tract and invasive infections. Defective antibody response to polysaccharides has been reported in a number of well defined immunodeficiency syndromes such as severe combined immunodeficiency, common variable immunodeficiency, ataxia telangectasia, Wiskott-Aldrich, complement C3 deficiency, AIDS and isolated IgA and IgG2 subclass deficiency (1) and more recently in patients with recurrent respiratory tract infections and normal immunoglobulins (2-7). Young children have poorer antibody responses to polysaccharide antigens than to protein antigens. An adequate response to polysaccahrides does not develop until two years of age or later, whether promoted by infection or active immunization (8,9).

Patients with recurrent infections and also healthy people may have subnormal serum concentrations of one or more immunoglobulin isotypes and several studies have shown that there is a very poor correlation between serum immunoglobulin concentrations and the severity of symptoms but a better correlation with poor specific antibacterial responses (10-12). Therefor assessment of specific antibody response to protein antigens and pneumococcal capsular polysaccharide has considerable practical value as a screening test for humoral immunity in patients suffering from recurrent infections (13). We have studied the mode of presentation of specific antibodies in patients with recurrent infections but normal serum immunoglobulins.

MATERIALS AND METHODS

Patients : All patients were seen between June 1989 and May 1994 as tertiary referrals in the pediatric immunology clinic for investigation of recurrent or major bacterial infections. A full history and examination were carried out and appropriate investigations were performed to exclude major immunodeficiency, structural abnormality or disease recognized to cause recurrent infection, such as cystic fibrosis, cilia dyskinesia or congenital asplenia. IgG and IgM levels were normal and IgA was present in all cases. The study group consisted of thirty patients aged between 1 to 13.8 years (mean age: 5.8); only two patients were younger than two (Table 1). All patients had been immunized routinely against diphtheria, tetanus, poliomyelitis and MMR. Antibody responses to these immunizations were assessed.

Five patients had been routinely immunized with Hib-Tetanus conjugate vaccine prior to referral to the

clinic. 28 patients were immunized as a test procedure with 0.5 ml of a standard dose of 25 μg of *H. influenzae* type b capsular polysaccharide conjugated to tetanus toxoid (PRP-T: a polymer of ribosylribophosphate covalently linked to tetanus toxoid, Pasteur - Mereux) intramuscularly. 26 were immunized with 0.5 ml of a polyvalent pneumococcal vaccine (Pneumovax II; Merck and Co. Inc., Rahway, NJ, USA) containing 25 μg of each of 23 pneumococcal capsular polysaccharide types intramuscularly. Antibody levels to these test immunogens were measured before and four to six weeks after immunization.

Immunological investigations

Total serum IgG, IgA and IgM concentrations were measured by rate nephelometry on a Beckman Array. IgG subclasses were measured by radial immunodiffusion, using an in-house standard calibrated against the WHO standard (67/97). Values within 2 SD of the mean for age were considered normal.

Levels of total IgG antibodies to diphtheria, tetanus toxoid, *Haemophilus influenza* type b capsular polysaccharide and pneumococcal capsular polysaccharides were measured using an ELISA technique (14). Post-immunization antibody response to Hib-polysaccharide of above 1 μg/ml and to pneumococcal capsular polysaccharide, using pneumovax II without absorption of cell wall polysaccharide, above 20 U/ml, with at least a two - fold increase over the preimmunization level. Antibody responses to tetanus toxoid and diphtheria were considered normal, if a level greater than 0.1 IU/ml was observed. Isohaemagglutinins, anti-A and anti-B as appropriate to blood group, were measured by a standard assay.

Statistics

The relationship between antibody responses to pneumococcal and Hib capsular polysaccharide antigens with IgG2 levels were assessed by chi-square test. The relationship between age and antibody response to Hib-Tet and Pneumovax was assessed by regression analysis anus.

RESULTS

The clinical characteristics of patients are presented in Table 1. The ages of the responders and non - responders to peumococcal capsular polysaccharide vaccine were similar at the time of first immunization. For responders the range was 1.1-13.8 years with a mean of 6.24 years; only one patient was below 2 years of age. For the nonresponders to Pneumovax the range was 2.8-12.5 years with a mean of 5.8 years. Patients in group IIb (non responders to Hib- Tetanus conjugate vaccine) were much younger: (age range : 1.1-4.4 years, mean of 2.4 years). Individual responses to Pneumovax and group means in the responder and non-responder populations are shown in Figure 1.

Table 1. Characteristics and clinical presentation of the study group with recurrent infections

	Group I Normal antibody levels n = 18	Group IIa No response to pneumovax n = 8+1*	Group IIb No response to Hib-Tet conjugate n = 3+1*
male : female	6 : 12	6 : 3	2 : 2
Age range years (mean)	1.1-13.8 (6.24)	2.8-12.5 (5.8)	1.1-4.4 (2.4)
Recurrent URTI	12 (66%)	9 (100%)	4 (100%)
Recurrent otitis	5 (27%)	5(56%)	4 (100%)
Media pneumonia	8 (44%)	5 (56%)	2 (50%)
Meningitis/septicaemia	1(5.5%)	3 (33%)	1 (25%)
Osteomyelitis/arthritis	0	1 (11%)	1 (25%)
Recurrent abscesses/cellulitis	3(17%)	3(33%)	0
Atopic tendency(asthma/ eczema)	3(17%)	49(44%)	1 (25%)
FTT, gastroenteritis	1(5%)	1 (11%)	2 (50%)
Urinary infections	1 (5%)	0	1 (25%)

URTI = upper respiratory tract infections; FTT : failure to thrive

* Patients No. 1 did not respond to both pneumovax and Hib-conjugate vaccine

Patients in both groups presented with recurrent infections such as upper respiratory tract infection, otitis media, pneumonia and recurrent abscesses / cellulitis. However patients with infection at several sites were more likely to have specific antibody deficiency (Fig. 2). Of four patients whose major presenting symptoms were recurrent skin abscesses, two did not produce antibodies to Pneumovax. In both of these cases, other primary imunodeficiencies which may give rise to abscess formation, such as chronic granulomatous disease and the hyper-IgE syndrome, were excluded by normal IgE levels and nitroblue tetrazolium reduction.

Patients were divided into two groups according to their responsiveness to polysaccharide antigens (table 1). Group I contained 18 patients who responded to both Hib - tetanus conjugate vaccine and Pneumovax. Group II were divided into two subgroups: group IIa contained 8 patients who failed to respond to Pneumovax and group IIb consisted of 3 patients who failed to respond to Hib - Tetanus conjugate vaccine. One patient did not respond to either pneumovax or Hib - Tetanus conjugate vaccine. For analysis of the results this patient was included in both, IIa and IIb subgroups. This patient did not produce a protective anibody response to tetanus and diphtheria toxoids, and showed a very low isohaemagglutinin titer (1:1). One other patient who failed to respond to pneumovax had a low isohaemagglutinin titer (anti-B=1:2).

The incidence and frequency of infections in group II were higher compared with group I. In addition, invasive bacterial infections such as meningitis, septicemia, osteomyelitis and arthritis were only found in group II patients. However, one patient in group I presented on two occasions with a septic shock-like illness but no organisms were identified. She had a high pre-immunization titer to pneumococcal polysaccharide but did not achieve a two fold- rise after immunization with Pneumovax.

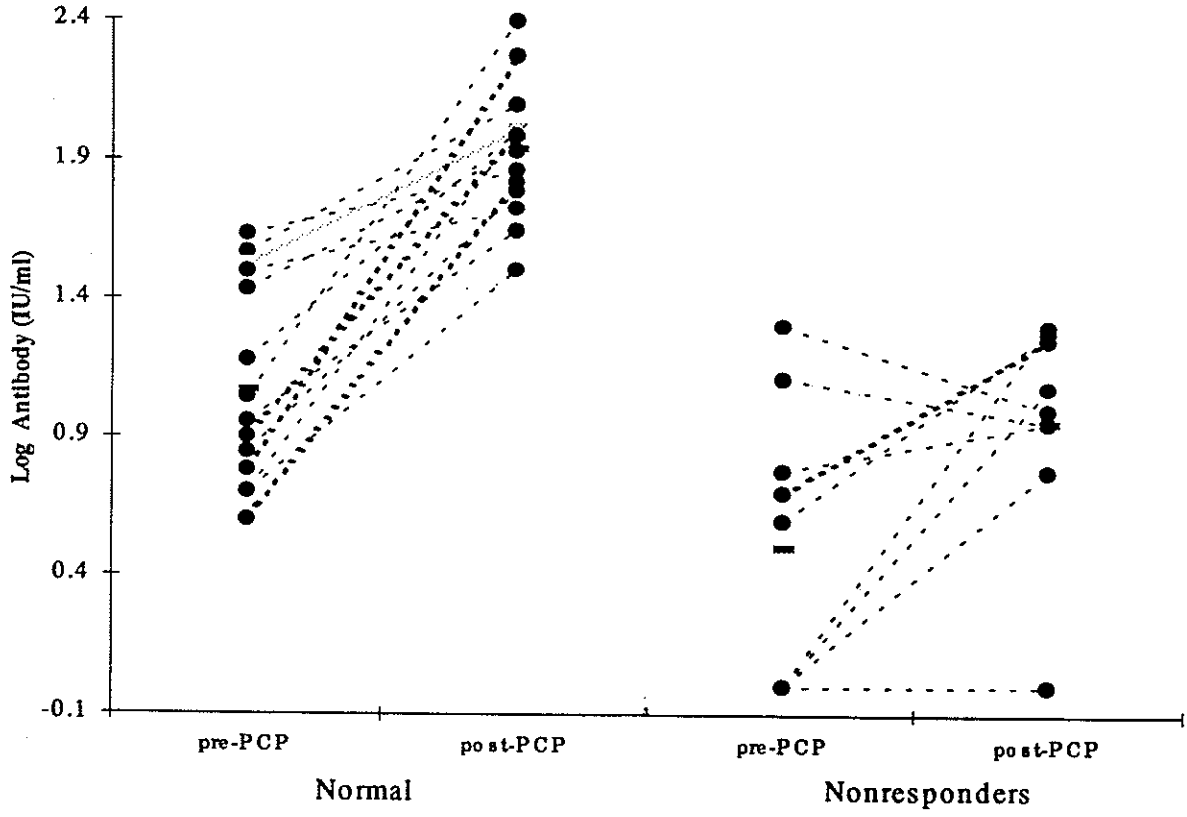

Fig. 1- Individual pre - and posti-immunization pneumococcal antibody levels [log IU/ml] in the responder and non-responder populations. o.....o = individual response, pre - and post; o.....o = group means pre - and post.

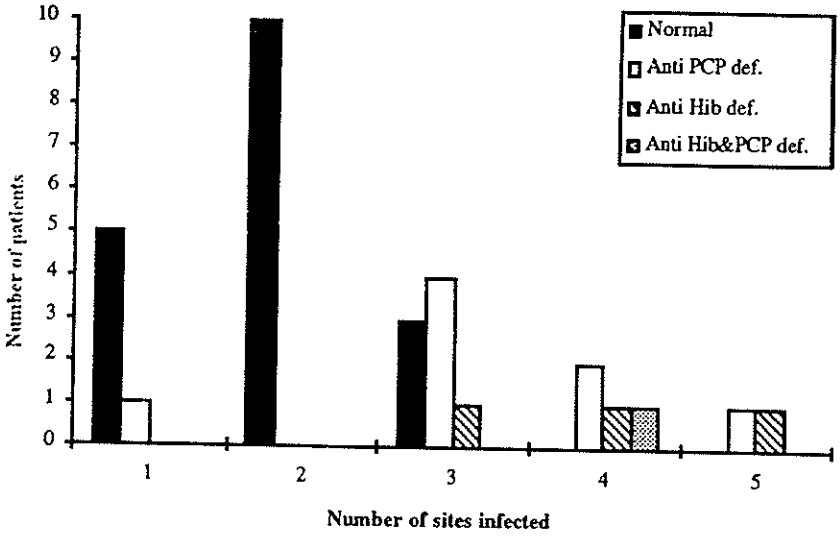

Fig. 2- Number of sites involved in recurrent infections broken down by antibody response to immunisation.

Total IgG and IgG1 were normal in all children and IgA, IgM and other IgG subclasses were present in all cases. No patient was found to be completely IgA deficient. In group I, six patients had normal immunoglobulins while 13 patients had low levels of IgA and/or IgG2 + IgG3. In group IIa: 3 patients had normal level of immunoglobulins, 6 had low levels of IgA and/or IgG2 + IgG3. In group IIb 3 patients had normal immunoglobulins and 1 had low level of IgG2 (Table 2).

There was no association between specific antibody response to Pneumovax or Hib-Tetanus vaccine and IgG2 levels ($p = 0.683$ and $p = 0.634$;). Also there was no correlation between levels of antibody to Pneumovax and Hib - Tet conjugate vaccines. Thus some patients who produced low antibody response to Pneumovax, made a variable level of antibody to Hib and vice versa (Table III). No relation was found between age and antibody response to either Pneumovax or Hib conjugate vaccine (data not shown).

Table 2. Serum immunoglobulins and IgG subclasses in patients with recurrent infections. A reduction is defined as < 2 SD below mean for age

Immunoglobulin and subclass pattern	Normal immunisation responses	Absent pneumovax responses	Absent Hib conjugate responses
	n = 18	n = 8+1*	n = 3+1*
Low IgG2 & 2, IgA	2	1	0
Low Low IgG2 & 3	2	0	0
Low IgG2, IgA	1	1	0
Low IgG2	4	4	1
Low IgG3	2	0	0
Low IgA	1	0	0
Normal IgG & subclasses	6	3	3

Patients No. 1 did not respond to both pneumovax and Hib-conjugate vaccine

Table 3. Lack of correlation between IgG2 level and response to Pneumovax. Chi-square = 0.454 (df = 1); p = 0.683 (Fisher's exact text). b) Lack of correlation between IgG2 level and response to Hib-tetanus conjugate vaccine. Chi-square = 1.54 (df = 1); p = 0.598 (Fisher's exact test)

	No response to pneumovax	Normal response to pneumovax	Total number
Low IgG2	6 (23.08%)	9 (34.62%)	15 (57.69%)
Normal IgG2	3 (11.54%)	8 (30.77%)	11 (42.31%)
Total	9 (34.62%)	17 (65.38%)	26 (100%)

	No response to Hib	Normal response to Hib	Total number
Low IgG2	1 (3.33%)	14 (46.67%)	15 (50%)
Normal IgG2	3(10.0%)	12 (40%)	15 (50%)
Total	4 (13.33%)	26 (86.67%)	30 (100%)

DISCUSSION

Our results confirm those of previous studies, indicating that patients with recurrent respiratory tract infections may have defective antibody response to capsular polysaccharide antigens irrespective of immunoglobulin concentrations (15). Most of these patients produce normal antibodies to protein antigens, so using polysaccharide conjugate vaccines may overcome this defect on many occasions even in some primary immunodeficiency syndromes such as Wiskott - Aldrich syndrome (16). However a proportion of young children, patients with primary immunodeficiency and those with recurrent pyogenic infections may require booster doses (10,16,17) or may fail to responsd to these vaccinations; these patients will require treatment with antibiotic prophylaxis or intravenous immunoglobulin, according to the frequency and severity of their infections (4,18).

An important implication of this study is that occurrence of infection with pyogenic bacterial or even viral infection is better correlated with impaired antibody response to polysaccharide antigens after immunization or natural infection with these encapsulated bacteria than with IgG2 subclass deficiency (the most frequent finding in children with recurrent upper and lower respiratory trract infections) or other immunoglubulin isotype deficiency. The non-responder patients showed more recurrent respiratory and major invasive infections than responders, irrespective of other immunoglubulin abnormalities. Sanders and coworkers (7) reported 45 patients with recurrent infections of whom 10 had a deficiency of one or more immunoglobulin isotype and 7 did not respond to 5 out of 7 pneumococcal serotypes. There was no difference in frequency of sinopulmonary infection among these immunodeficient patients compared to the other patients but they suffered more frequently from lower respiratory tract infection and recurrent invasive infections. Five out of 35 patients with normal immunoglobulins did not respond to immunization and showed similar frequency and severity of infections as non-responder patients with immunoglobulin deficiency. Our results conform with those of Sanders and coworkers, in that patients with a selective deficiency of antibodies to polysaccharide antigens have more invasive and frequent infections than patients with a normal response to polysaccharide antigens. However, concomitant lack of immunoglobulin isotypes and specific antibody deficiency has occasionally been reported in healthy blood donors (12).

The Hib-conjugate vaccine commonly used is highly immunogenic and evaluation of Hib antibody titers may not seem helpful in identifying children with polysaccharide antibody deficiency. It is important to note that failure to respond to this vaccine appears to be associated with a high risk of infection. The unconjugated Hib-PRP vaccine is no longer available so it is not possible to measure the antibody response of patients to this pure polysaccharide antigen, although it would have been of interest to know whether the use of this pure polysaccharide as a test immunogen would have any additional discriminatory value in children with recurrent infections. An impaired antibody response to polysaccharide may be selective to each different antigen, since three non-responder patients to Hib-Tet conjugate vaccine made high antibody responses to Pneumovax as well as to tetanus and diphtheria toxoids. Only the non-responder patient to both Hib-Tet and Pneumovax did not make a protective level of antibodies to tetanus and diphtheria.

It is likely that, in some patients under 5 years of age, a poor response represents only a delay in the maturation of immune response, but in older children it is more likely to be a permanent defect. Patients who

did not generate protective levels of antibodies to Pneumovax or Hib-Tet conjugate vaccines were re-challenged on at least two occasions with those vaccines at an interval of six months apart, and despite this boosting, antibody levels declined (unpublished results). Therefore re-immunization of patients with recurrent infections over a short period of time has no protective effect but serves to confirm the persistence of the immune defect. No adverse effects were observed from repeated challenge with Pneumovax in this group.

Antibiotic prophylaxis alone may be sufficient in some patients, particularly if delayed maturation of the immune system is suspected. However, Silk and co-workers (18) and Zora and co-workers (4) have suggested the effectiveness of IVIG in specific antibody deficient patients. Our experience in three non-responder patients, who continued to have frequent respiratory tract infections despite antibiotic prophylaxis, in whom treatment with IVIG was commenced, supports this. Furthermore our follow-up data in these patients and also one patient with chronic mucocutaneous candidiasis who had absent antipolysaccharide responses shows that some patients with specific antibody deficiency may gradually progress towards a more severe antibody deficiency (unpublished results). Early commencement of IVIG may prevent the development of bronchiectasis or invasive bacterial infections in these patients.

We suggest that in vivo evaluation of B cell function can be performed by measuring specific antibody levels before and after the administration of the test antigens. This should include a pure polysaccharide antigen, such as Pneumovax. A total antibody level above 20 U/ml and at least a two-fold increase should be achieved in children older than two years. Failure to achieve this level indicates a significant immunological defect in those over the age of 2 years. This may be transient but in older children is likely to be permanent and may be a harbinger of a progressive humoral immune deficiency. Measurement and IgG subclasses alone are not sufficient to identify patients such as these who have a significant immunological defect and increased susceptibility to invasive infection.

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