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## Defective antipneumococcal polysaccharide antibody response in children with recurrent respiratory tract infections

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**Background:** Recurrent pyogenic infections are known to occur in patients with an impaired response to polysaccharide antigens. We investigated the occurrence of deficient responses to pneumococcal capsular polysaccharides in patients with recurrent respiratory tract and recurrent systemic infections.

**Methods:** Forty-five patients, 1.7 to 17.1 years of age, were immunized with 23-valent pneumococcal polysaccharide vaccine. Antibody levels to seven pneumococcal serotypes (3, 4, 6A, 9N, 14, 19F, 23F) were determined by ELISA before and after immunization. In addition, patients received a booster immunization with diphtheria toxoid, tetanus toxoid, and poliomyelitis virus vaccine.

**Results:** Thirty-five patients had normal serum immunoglobulin levels. Five of these patients (14%) had low antipneumococcal preimmunization antibody levels and failed to respond to pneumococcal vaccination, whereas the response to booster immunization with protein antigens was appropriate. Three patients were younger than 3 years old, and one had a family history of IgG2 deficiency. Low IgG developed in a fifth patient during follow-up. Ten patients had a humoral immunodeficiency. Seven of these patients failed to respond to pneumococcal vaccination.

**Conclusions:** We conclude that a defective immune response to polysaccharide antigens in patients requires long-term follow-up to distinguish transient maturational delay from a persistent selective impaired response to polysaccharide antigens, which on occasion may precede the development of humoral immunodeficiency disease. (*J ALLERGY CLIN IMMUNOL* 1993;91:110-9.)

**Key words:** *Pneumococci, antepolysaccharide antibodies, respiratory tract, Fallot, immunodeficiency, cell wall polysaccharide, otitis*

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The development of antibodies to polysaccharides of encapsulated bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type b in early life is much poorer than to proteins. For many polysaccharide antigens, adequate antibody titers are not attained until the age of 18 to 24 months or later after infection or vaccination with polysaccharide vaccines.<sup>1-6</sup> The key component of the host defense against encapsulated bacteria is opsonophagocytosis of the microorganism for which the presence of specific anticapsular antibodies, complement and phago-

*Abbreviations used*

|          |                                                                |
|----------|----------------------------------------------------------------|
| TI-2:    | Thymus independent type 2                                      |
| PRP:     | Polyribosylribitolphosphate                                    |
| Pvax:    | Pneumococcal vaccination (23 valent)                           |
| DTP:     | Diphtheria toxoid, tetanus toxoid, poliomyelitis virus vaccine |
| C-PS:    | Pneumococcal common cell wall polysaccharide                   |
| GMT:     | Geometric mean antibody titer                                  |
| SEM:     | Standard error of the mean                                     |
| max inc: | Maximal-fold increase                                          |
| CVI:     | Common variable immunodeficiency                               |

cytic cells, is required. Consequently, the frequent occurrence of infections with encapsulated bacteria occurring in neonates and infants reflects the ontogeny of the components of host defense.<sup>7, 8</sup>

Studies in experimental animals suggest that polysaccharides may behave as so-called thymus independent type 2 (TI-2) antigens. The major characteristics of an immune response against TI-2 antigens are delayed development of antibodies, restricted immunoglobulin isotype distribution of antibodies, and lack of memory formation.<sup>9</sup> Moreover, the definition of TI-2 antigens implies that T cells are not required for antibody formation, although they do play a role in the regulation of the response.<sup>10, 11</sup> In human beings the characteristics of the antibody response against polysaccharides strongly resemble those of the TI-2 antibody response as defined in animals.<sup>9, 12</sup> The TI-2 nature of a number of type-specific polysaccharides of *S. pneumoniae* and of polyribosylribitolphosphate (PRP), the polysaccharide of *H. influenzae* type b has been shown.<sup>12-16</sup>

Apart from that described in infancy, an impaired antibody response after infection with encapsulated bacteria or vaccination with polysaccharide vaccines is also seen in immunocompromised patients,<sup>17-19</sup> patients with immunoglobulin isotype deficiency such as selective IgA deficiency or IgG2 deficiency, and common variable immunodeficiency disease.<sup>20-26</sup> More recently, impaired anti-polysaccharide antibody formation has been found in patients with recurrent respiratory tract infections and without any sign of an underlying immunodeficiency.<sup>27-33</sup> After our preliminary reports,<sup>28, 33</sup> we now describe the occurrence of selective deficiency of anti-polysaccharide antibody formation in patients with recurrent respiratory tract infections more precisely.

## MATERIAL AND METHODS

### Patient population

Forty-five patients, 1.7 to 17.1 years of age, all white, were included in this study. The patients came to the out-

patient clinic with the primary complaint of recurrent respiratory tract infections. Inclusion criteria for the study are indicated in Table I. Patients with recurrent infections caused by anatomic abnormalities of the respiratory tract or recognized disease entities such as cystic fibrosis, granulocytopenia, or complement deficiency, were excluded from the study. Parental consent was obtained before immunization intramuscularly with a 23-valent pneumococcal vaccine (Pneumovax [Pvax]; Merck and Co., Inc., Rahway, N.J.) containing 25 µg of purified type-specific capsular polysaccharides of 23 pneumococcal serotypes (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, 33F). Serum was drawn before and 1, 2, and 4 weeks after immunization. All samples were stored at -80° C until use.

Patients who had low antidipteria and antitetanus antibody titers were also immunized with boosters of diphtheria toxoid, tetanus toxoid, and poliomyelitis virus vaccine (DTP, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands). Antibody levels were determined in serum drawn before and after 2 weeks of vaccination.

### Determination of serum immunoglobulins and antidipteria and antitetanus antibodies

Serum IgM, IgG, IgA, and IgG subclasses were determined by radial immunodiffusion as described.<sup>34, 35</sup> Normal values for age represent a range of 2 SD above and below the mean for age.<sup>34, 35</sup> Isohemagglutinins anti-A and anti-B were determined by standard methods. Antidipteria and antitetanus antibody titers were determined as described.<sup>36</sup> A twofold or greater rise in serum antidipteria and antitetanus antibody levels was defined as a normal response, as long as the postimmunization titer was at least 0.01 U/ml.

### Determination of type-specific antipolysaccharide antibodies

Serum antibody levels to seven common pneumococcal serotypes (3, 4, 6A, 9N, 14, 19F, and 23F) were measured by ELISA in preimmunization and postimmunization serum samples. All sera were analyzed in duplicate, and sera from a single subject were analyzed in the same ELISA run. Microtiter plates (Greiner Labortechnik, Langerthal, Germany) were coated with capsular polysaccharides (10 µg/ml, American Type Culture Collection [ATCC], Rockville, Md.) in saline solution (NaCl 0.9%) at 37° C overnight. Subsequently the plates were washed with phosphate-buffered saline, 0.05% Tween 20 (vol/vol) and incubated for 2 hours at 37° C with serial dilutions of serum samples in diluting buffer (phosphate-buffered saline, 0.05% vol/vol Tween-20, 1% bovine serum albumin). Thereafter the plates were washed again and incubated with peroxidase labeled affinity-purified goat antihuman Ig (Cappel, Durham, N.C.). The plates were washed again and finally incubated with 0.1 mg/ml 3,3',5,5'-tetramethylbenzidine dihydrochloride (Sigma Chemical Co., St. Louis, Mo.) in 0.05 mol/L phosphate-citrate buffer with 0.03% sodium perborate (Sigma). After an incubation of 10 to 20 minutes,

**TABLE I.** Inclusion criteria for the study

| Infectious manifestations                       |                                                                                                                   |
|-------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|
| Acute otitis media/otorrhea                     | Fever $>38.5^{\circ}\text{C}$ during at least 24 hours                                                            |
| Acute purulent rhinitis/sinusitis with coughing | Fever $>38.5^{\circ}\text{C}$ during at least 24 hours                                                            |
| Bacterial pneumonia                             | Fever $>38.5^{\circ}\text{C}$ during at least 24 hours; radiography of thorax showing bacterial (lobar) pneumonia |
| Bacteremia/sepsis                               | Blood culture positive for <i>S. pneumoniae</i> , <i>Neisseria meningitidis</i> , or <i>H. influenzae</i> type b  |
| Bacterial meningitis                            | Liquor culture positive for <i>S. pneumoniae</i> , <i>N. meningitidis</i> , or <i>H. influenzae</i> type b        |

**General criteria:** older than 1.5 years of age, and more than six bacterial upper respiratory tract infections during the previous year or recurrent invasive infections with encapsulated bacteria.

the reaction was stopped by adding 1 mol/L  $\text{H}_2\text{SO}_4$ , and absorbancy at 450 nm was read on a spectrophotometer (Titertek Multiscan; Flow Labs, Irvine, Calif.). Because the various purified serotype-specific polysaccharide antigens of ATCC may be contaminated with the species-specific pneumococcal common cell wall polysaccharide (C-PS), all serum samples were preincubated with excess free C-PS (50  $\mu\text{g}/\text{ml}$ ) overnight at  $4^{\circ}\text{C}$  before analysis to block anti-C-PS antibodies.<sup>37-41</sup> Antibody levels were compared with a hyperimmune plasma pool standard.<sup>33, 42</sup> This plasma pool contains 2648 ng of antibody nitrogen per milliliter (ng Ab N/ml) for pneumococcal serotype 3; 1196 ng Ab N/ml for serotype 4; 539 ng Ab N/ml for group 6; 927 ng Ab N/ml for group 9; 1778 ng Ab N/ml for serotype 14; 440 ng Ab N/ml for group 19; and 2689 ng Ab N/ml for group 23, as determined by the RIA technique.<sup>42</sup> Antibody concentrations were expressed in units per milliliter where the reference plasma pool represents 100 U/ml for each serotype.

### Evaluation of antibody responses to pneumococcal polysaccharides

Antibody levels were determined before and at 1, 2, and 4 weeks after immunization. In 90% of the cases, maximal antibody levels were attained at 2 weeks after vaccination. The maximal-fold increase (max inc) was calculated for each patient by dividing the highest postvaccination titer achieved by the preimmunization titer. A response to a capsular polysaccharide was based on two criteria: a twofold or higher increase in serum antibody titer and a postimmunization antibody concentration of at least 20% (20 U/ml) of the hyperimmune plasma pool of healthy volunteers immunized with Pvax. A postimmunization antibody titer of more than 20 U/ml was defined as a response.

### Statistical analysis

For comparison of various data between patient groups a Kruskal-Wallis test was performed. Differences associated with probability values of  $p < 0.05$  were considered significant. Linear regression analysis was used to assess relationships between age and preimmunization and postimmunization antibody titers and between age and max inc.

## RESULTS

### Patients

The patients were divided into two separate groups depending on their serum immunoglobulin levels. Group I contained 35 patients with serum immunoglobulin (Ig) levels within 2 SD of the mean for their age. Group II contained 10 patients who had a deficiency of one or more immunoglobulin isotypes such as selective IgA deficiency ( $n = 3$ ), IgG2 deficiency ( $n = 4$ ), IgA-IgG2 deficiency ( $n = 1$ ), low IgG1 ( $n = 1$ ), or hypogammaglobulinemia ( $[n = 1]$ ), Table II). The clinical characteristics of both groups show a similar high frequency of recurrent sinopulmonary problems (90% to 100%) and recurrent otitis media acuta or otorrhea ([80% to 90%], Table III). However, the patients of group II suffered more frequently from lower respiratory tract infections and recurrent invasive infections than those of group I (Table III).

### Antipolysaccharide antibody levels

Determination of serum antibody titers was limited to seven serotypes, including both strong (types 3, 4, and 9N), intermediate (types 14 and 19F), and weak immunogens (types 6A and 23F).<sup>1-5, 43</sup> No statistically significant correlation occurred between age and preimmunization and postimmunization antibody levels or between age and the max inc in either group (results not shown).

The results in Table IV show that, apart from antibodies to polysaccharide 3 (37.1 U/ml), geometric mean antibody titers (GMTs) before immunization of the children of group I are below 20 U/ml. After immunization the GMTs increased and the differences between preimmunization and postimmunization titers were statistically significant for all serotypes ( $p < 0.05$ ). In the patients from group I a more than twofold increase in antibody titer was observed for the three pneumococcal serotypes 3, 4, and 9N, and a twofold rise was observed for serotypes 14 and 23F.

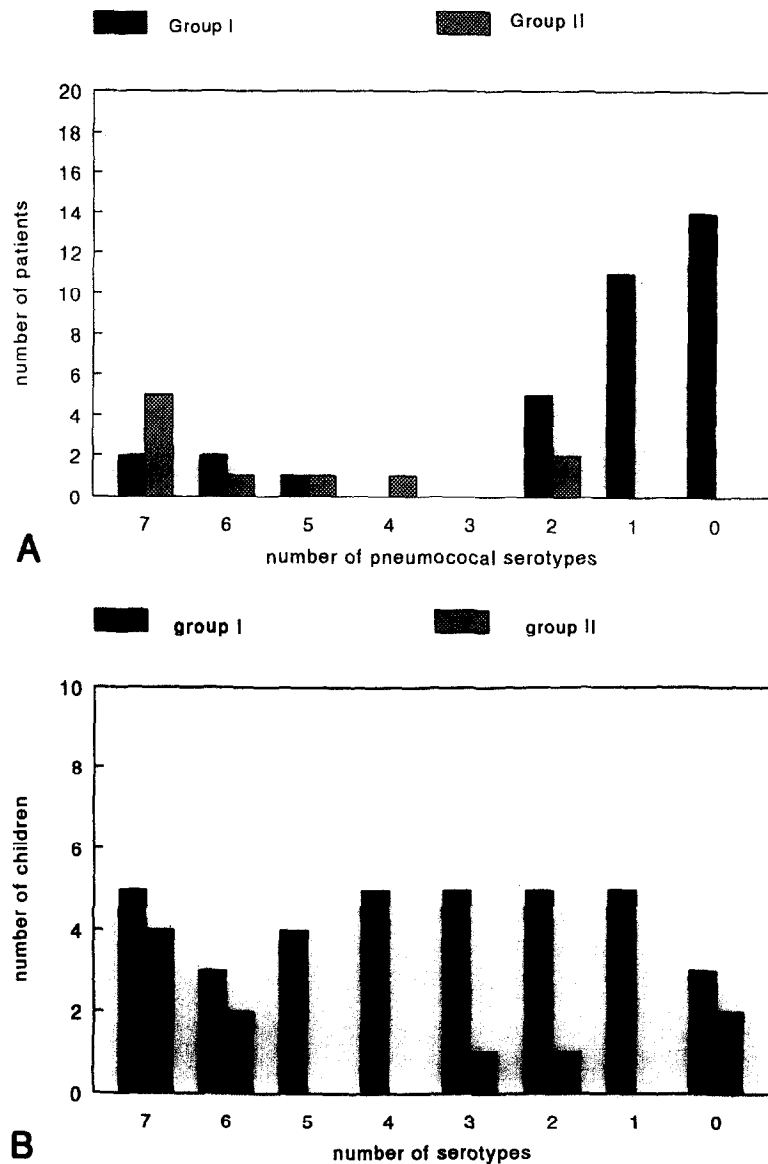


**FIG. 1. A,** Numbers of patients of group I and group II (y-axis), who are immunized with Pwax and failed to achieve specific antipolysaccharide antibody titers above 20 U/ml to 7 to 0 pneumococcal capsular polysaccharides (x-axis). **B,** Numbers of patients of group I and group II (y-axis) who failed to show a twofold rise in specific antipolysaccharide antibody levels to 7 to 0 pneumococcal capsular polysaccharides after Pwax immunization (x-axis).

A less than twofold increase was seen for serotypes 6A and 19F. This pattern of response reflects the different immunogenic properties of the seven pneumococcal serotypes. In patient group II the GMTs increased after immunization, but the differences are not statistically significant for all serotypes. Comparison of the GMTs to the various pneumococcal serotypes in patients of group I and II shows that the mean titers in group I are higher than those of patients of group II both before and after immunization (Table IV;  $p < 0.05$  for all serotypes).

### Nonresponders to pneumococcal vaccination

Fig. 1, A shows that five of the 35 patients of group I showed postimmunization antibody titers below 20 U/ml for five to seven of the seven pneumococcal serotypes tested, whereas the remaining patients of group I (30/35) all had postimmunization antibody levels above 20 U/ml for five or more serotypes. In addition, these five patients showed a less than twofold antibody increase to at least five of the seven pneu-

**TABLE II.** Serum immunoglobulin levels and diagnosis of 10 patients with various types of immunodeficiency disease

| No.   | Age at time of<br>Pvax (yrs) | Sex    | IgM<br>(gm/L) | IgG<br>(gm/L) | IgA<br>(gm/L) | IgG1<br>(gm/L) | IgG2<br>(gm/L) | IgG3<br>(gm/L) |
|-------|------------------------------|--------|---------------|---------------|---------------|----------------|----------------|----------------|
| II.1  | 2.8                          | Female | 1.8           | 6.7           | <i>0.04</i>   | 3.7            | 1.3            | 0.4            |
| II.2  | 3.1                          | Female | 2.0           | 10.1          | 0.2           | 7.6            | <i>nd</i>      | 0.5            |
| II.3  | 6.5                          | Male   | <i>0.3</i>    | 8.2           | <i>nd</i>     | 5.5            | 1.1            | 0.2            |
| II.4  | 6.5                          | Male   | 2.1           | 9.1           | 0.3           | 7.8            | <i>nd</i>      | 0.4            |
| II.5  | 7.2                          | Male   | 2.1           | 8.4           | 0.3           | 7.7            | <i>0.1</i>     | 0.2            |
| II.6  | 8.9                          | Female | 1.0           | 11.8          | 1.6           | 7.9            | <i>0.2</i>     | 0.1            |
| II.7  | 9.5                          | Female | 1.1           | 2.3           | <i>nd</i>     | 3.0            | <i>nd</i>      | 0.1            |
| II.8  | 10.4                         | Female | <i>0.2</i>    | 7.5           | <i>nd</i>     | 8.3            | <i>0.5</i>     | 0.5            |
| II.9  | 11.0                         | Male   | 0.9           | 9.4           | <i>nd</i>     | 9.7            | 4.2            | 1.0            |
| II.10 | 17.1                         | Male   | 1.2           | 6.2           | 1.2           | 2.9            | 3.1            | 0.3            |

*nd*, Not detectable; *anti A and anti B*, antiisohemagglutinins A and B; – = bloodgroup AB; *Def*, deficiency. Patients 2 and 4 are siblings; patients 5 and 6 are siblings of patient I.4 (nonresponder patient of group I). Serum immunoglobulin levels of the patients were compared with those of age-matched controls of the same laboratory. Ig concentrations below 2 SD of the mean for age *in italics*. Nonresponder patients for Pvax *in italics*.

**TABLE III.** Clinical characteristics of patients of group I and group II

|                                                         | Group I        |       | Group II       |        |
|---------------------------------------------------------|----------------|-------|----------------|--------|
| No. of children                                         | 35             |       | 10             |        |
| Sex male:female                                         | 20:15          |       | 5:5            |        |
| GM age at time of<br>Pvax immunization in years (range) | 4.1 (1.7-14.7) |       | 7.2 (2.8-17.1) |        |
| No. of children                                         |                |       |                |        |
| <2 years of age                                         | 2              |       | 0              |        |
| 2-3 years of age                                        | 7              |       | 1              |        |
| 3-4 years of age                                        | 9              |       | 1              |        |
| >4 years of age                                         | 17             |       | 8              |        |
| Recurrent otitis media/otorrhea                         | 30/35          | (86%) | 9/10           | (90%)  |
| Recurrent purulent rhinitis/sinusitis with coughing     | 32/35          | (91%) | 10/10          | (100%) |
| Lobar pneumonia                                         | 14/35          | (40%) | 7/10           | (70%)  |
| Bacteremia/sepsis                                       | 3/35           | (9%)  | 0/10           | (0%)   |
| Bacterial meningitis                                    | 5/35           | (14%) | 1/10           | (10%)  |
| Osteomyelitis and arthritis                             | 1/35*          | (3%)  | 0/10           | (0%)   |
| Recurrent invasive infections                           | 3/35**         | (9%)  | 7/10           | (70%)  |
| Severe hearing loss caused by infections                | 5/35           | (20%) | 2/10           | (20%)  |

GM, Geometric mean; patients of group I (35 children with normal serum Ig) and group II (10 patients with immunodeficiency, see also Table II) both suffer from recurrent upper respiratory tract infections and invasive bacterial infections. Invasive infections comprise lobar pneumonia, bacteremia, meningitis, osteomyelitis and arthritis; \*nonresponder patient I.5; \*\*nonresponder patients I.3, I.4, I.5 (see text).

mococcal antigens (Fig. 1, B). These five patients were designated as nonresponders. Seven additional patients of group I failed to show any antibody increase to five to seven polysaccharides on Pvax immunization, but showed prevaccination antibody levels of more than 20 U/ml for at least five antigens (compare Fig. 1, B with A). These patients were not designated as nonresponders because of their already good preimmunization antibody levels.

The five nonresponders with normal serum Ig

showed the following results: a 1.7-year-old girl (patient I.1) failed to show a twofold rise in antibody titer to all seven antigens, and she had preimmunization antibody titers above 20 U/ml only for the strong immunogenic serotypes 3 and 4. The second patient (patient I.2), a 3.2-year-old boy, failed to have a twofold rise to five serotypes and remained below 20 U/ml for all seven antigens except for polysaccharide 3. Both patients suffer from recurrent upper respiratory tract infections. Patient I.3, a 3.4-year-old

| IgG4<br>(gm/L) | Anti A/anti B | Diagnosis         |
|----------------|---------------|-------------------|
| 0.2            | 1:8           | IgA def           |
| <0.1           | 1:128         | IgG2 def          |
| 0.2            | 1:64          | IgA def           |
| 0.1            | 1:128         | IgG2 def          |
| 0.1            | nd            | low IgG2          |
| 0.1            | 1:2           | low IgG2          |
| 0.1            | 1:8           | CVI               |
| <0.1           | —             | IgA def, low IgG2 |
| <0.1           | —             | IgA def           |
| <0.1           | 1:2           | low IgG1          |

girl with Fallot's tetralogy, showed very low antibody levels for all pneumococcal antigens except polysaccharide 3 and failed to have any increase in polysaccharide antibody levels on immunization. She has recurrent upper and lower respiratory tract infections and recurrent pneumococcal bacteremia (3 times in a 4-year period; unfortunately, serotyping was performed only once: pneumococcal group 19). The remaining two patients (I.4 and I.5) showed very low antibody levels for all polysaccharide types and failed to have any increase in antibody level for any of the seven antigens on immunization. Patient I.4 (4.4 years old) has an older brother and sister, both known with an IgG2 deficiency (Patients II.5 and II.6 in Table II) and absent responses to P<sub>vax</sub> vaccination. All three siblings show recurrent pyogenic upper respiratory tract infections and recurrent lobar pneumonia. Patient I.5, 14.7-years old at the time of P<sub>vax</sub> immunization, has chronic recurrent sinopulmonary problems and recurrent invasive pneumococcal infections like pneumococcal osteomyelitis at 12 years of age (group 9) and pneumococcal arthritis at the age of 13 years (group 10). Low serum IgG (4 to 5 gm/L) developed during follow-up.

In group II only three patients responded to three or more pneumococcal serotypes (patients II.1, II.7, II.9, Fig. 1, A and B and Table II), including patient II.7 with hypogammaglobulinemia. Of the nonresponder patients, a 3.1-year-old girl with IgG2 deficiency (patient II.2), showed a twofold rise to the strong immunogenic serotypes 3, 4, and 9N, but only the antibody titers for serotypes 3 and 9N increased above 20 U/ml. Her older brother, deficient in IgG2 (II.4, Table II), showed very low antibody levels for all types except polysaccharide 3 and failed to show any antibody increase on P<sub>vax</sub> immunization. Both brother and sister have severe recurrent upper respiratory tract infections. The remaining five patients of

group II had low antibody levels and failed to respond to any of the seven pneumococcal serotypes tested (patients II.3, II.5, II.6, II.8, II.10). Patient II.5 and II.6 are the older relatives of patient I.4. Hypogammaglobulinemia developed in patient II.8 during follow-up. All five patients have recurrent upper and lower respiratory tract infections. Patient II.10 also had pneumococcal meningitis at the age of 15 years (serotype 14).

### Antibody responses to diphtheria and tetanus toxoid

All patients in both groups produced normal antibody responses to diphtheria and tetanus toxoid booster immunizations (Table V). In addition, patient I.5 responded normally to hepatitis B virus immunization (results not shown).

### DISCUSSION

The results of our study confirm and extend previous reports that patients with recurrent upper and lower respiratory infections may have a defective antibody response to polysaccharide antigens while having normal serum immunoglobulins and normal antibody responses to protein antigens. However, as a result of the lack of standardization of antipolysaccharide antibody assays, including the possible interference of anti-C-PS antibodies, the different reports are presumably not fully comparable.

The population of patients in this study consisted of a highly selected group of patients, referred to our hospital by either their own pediatrician or otolaryngologist. The clinical symptoms comprise severe recurrent pyogenic sinopulmonary infections and otitis media acuta, and some of the patients also had a history of one or more invasive infections with encapsulated bacteria. We measured the antibody response after pneumococcal vaccination to seven different pneumococcal capsular polysaccharides, known to be frequently involved in respiratory tract infections.<sup>8, 43-45</sup> We corrected for anti-C-PS antibodies by overnight incubation of sera with free C-PS.

Special attention was given to the definition of a response to P<sub>vax</sub>. Because of lack of healthy and age-matched P<sub>vax</sub> vaccinated controls, we choose to define a response to pneumococcal vaccination using a combination of two criteria, namely a twofold increase in antibody titer and a certain minimal postvaccination antibody titer for five or more of the seven pneumococcal serotypes. Of the group of 35 patients with normal serum Ig, five children (14%) could be distinguished as nonresponders. Had a twofold rise in serum antibody titer been our only criterion, the number of nonresponder patients in group I would have

**TABLE IV.** GMT to seven pneumococcal serotypes before and after pneumococcal vaccine and max incr in patients of group I and group II

| Serotype | Pvax   | Group I         |                   | Group II        |                   |
|----------|--------|-----------------|-------------------|-----------------|-------------------|
|          |        | GMT $\pm$ SEM   | max inc $\pm$ SEM | GMT $\pm$ SEM   | mac inc $\pm$ SEM |
| 3        | Before | 37.1 $\pm$ 0.29 | 2.4 $\pm$ 0.30    | 13.3 $\pm$ 0.71 | 1.9 $\times$ 0.61 |
|          | After  | 88.0 $\pm$ 0.34 |                   | 25.8 $\pm$ 0.60 |                   |
| 4        | Before | 13.8 $\pm$ 0.43 | 3.3 $\pm$ 0.45    | 3.2 $\pm$ 0.36  | 2.5 $\pm$ 0.90    |
|          | After  | 44.8 $\pm$ 0.44 |                   | 7.9 $\pm$ 0.9   |                   |
| 6A       | Before | 18.4 $\pm$ 0.44 | 1.7 $\pm$ 0.38    | 3.4 $\pm$ 0.37  | 1.8 $\pm$ 0.74    |
|          | After  | 32.0 $\pm$ 0.50 |                   | 6.1 $\pm$ 0.90  |                   |
| 9N       | Before | 18.0 $\pm$ 0.41 | 3.2 $\pm$ 0.40    | 3.5 $\pm$ 0.48  | 2.2 $\pm$ 0.84    |
|          | After  | 57.9 $\pm$ 0.48 |                   | 7.7 $\pm$ 0.87  |                   |
| 14       | Before | 8.4 $\pm$ 0.32  | 2.0 $\pm$ 0.31    | 4.4 $\pm$ 0.44  | 1.4 $\pm$ 0.56    |
|          | After  | 16.9 $\pm$ 0.40 |                   | 6.2 $\pm$ 0.69  |                   |
| 19F      | Before | 19.7 $\pm$ 0.38 | 1.7 $\pm$ 0.31    | 4.0 $\pm$ 0.48  | 1.6 $\pm$ 0.61    |
|          | After  | 34.4 $\pm$ 0.46 |                   | 6.2 $\pm$ 0.86  |                   |
| 23F      | Before | 16.0 $\pm$ 0.36 | 2.0 $\pm$ 0.31    | 6.0 $\pm$ 0.63  | 1.4 $\pm$ 0.47    |
|          | After  | 31.3 $\pm$ 0.41 |                   | 8.6 $\pm$ 0.85  |                   |

Serotype, Specific pneumococcal capsular serotype; max inc, maximal fold increase, i.e., maximal titer after Pvax divided by the titer before Pvax.

Group I contained 35 patients with normal serum immunoglobulins, group II contained 10 patients with a humoral immunodeficiency. Antibody titers are determined by ELISA and are expressed in units per milliliter with use of standard serum pool as a reference.

been much higher, namely 12 of 35 patients (34%). In group II, 7 of the 10 patients with a humoral immunodeficiency were nonresponders. Of these, the 3.1-year-old girl deficient in IgG2 (patient II.2), would have been called a responder in case a twofold rise had been the only criterion.

The concept of a "minimal antibody level" above which there is adequate protection against pneumococcal disease is still controversial and not readily predictable for the various pneumococcal serotypes, but 200 to 300 ng Ab N/ml has been suggested as a protective level against invasive infections in adults.<sup>46</sup> Since the assignment of weight-based antibody concentrations (nanograms of antibody nitrogen per milliliter) to ELISA units are inevitably approximate,<sup>37, 41, 47</sup> we have not converted ELISA units to nanograms of antibody nitrogen per milliliter. However, when we converted weight-based antibody concentrations to our ELISA units, assuming that 100 U/ml correspond with the described amount of nanograms of antibody nitrogen per milliliter of the reference serum,<sup>42</sup> the geometric mean postimmunization levels of serotypes 6A and 19F in patients of group I remained below 200 ng Ab N/ml. The geometric mean postimmunization titers of the patients of group II remained below 200 ng Ab N/ml for all serotypes except for polysaccharide 3 and polysaccharide 23F.

In addition, the same five patients of group I could be distinguished in failing to reach 200 ng for 5 to 7 serotypes, whereas the 30 remaining patients of group I showed antibody levels above 200 ng Ab N/ml for 4 to 7 serotypes (results not shown). Of the 10 patients with humoral immunodeficiency disease, the same patients would have been designated as nonresponder, apart from patient II.2 who showed antibody titers above 200 ng Ab N/ml for 3 (polysaccharide 3, 9N, 23F) rather than two serotypes (polysaccharide 3, 9N).

The frequency of nonresponder patients with normal serum Ig has only been studied by Herrod et al.<sup>31</sup> who reported 10 out of 55 patients (18%) with recurrent respiratory tract infections and a poor response to *Haemophilus influenzae* type b (PRP) capsular polysaccharide vaccine. Our results appear to be similar, despite differences in methodology as well as polysaccharide antigens studied. We did not study the antibody response to PRP in our patient group, although prevaccination levels were extremely low in all nonresponder patients with the exception of patient II.4 (data not shown).

Some features of our nonresponding patients with normal serum immunoglobulins require discussion. The first two patients are young enough for their impaired response to be physiologic, and therefore this may be of transient nature. Nonresponding children

in other studies are usually younger than six years, and in some they are even younger than 3 years.<sup>30-32, 48</sup> The third patient, a 3.5-year-old girl with Fallot's tetralogy, is also young. In a recent report 57% of the children with Fallot's tetralogy are reported to have defects in components of host defense such as T cells, complement, or Ig levels.<sup>49</sup> Impaired responses to polysaccharide antigens may be yet another example of a host defense defect in these patients. We found slight disturbances in *in vitro* T-cell functions in this patient, although the relation with defective antibody formation to polysaccharide antigens remains to be established.<sup>50</sup> Nonresponder patient I.4 has two siblings with low IgG2 and low-to-normal IgG3 (patients II.5 and II.6), and like his older brother and sister, he did not respond to any of the seven pneumococcal antigens. It is possible that IgG subclass deficiency will also develop in him during follow-up. Low IgG levels did develop in patient I.5 during follow-up.

Impaired responses to polysaccharide antigens have been reported in patients with IgA or IgG2 deficiency and in patients with common variable immunodeficiency disease. Of the nonresponding patients of group II, patient II.8, having low IgM and IgG2 and absent IgA at the time of P<sub>v</sub>ax, had hypogammaglobulinemia during follow-up. These results, combined with the histories of patient I.5 and the siblings I.4, II.5, and II.6, suggest that in some patients a defective response to polysaccharide antigens may precede the development of a more generalized immunodeficiency. Although IgA deficiency may be associated with IgG2 deficiency and may even be associated with or precede common variable immunodeficiency disease as stated in this study and others,<sup>25, 26, 51-54</sup> the phenomenon of a defective antipolysaccharide antibody response preceding the development of hypogammaglobulinemia has as yet not been reported and merits further investigation.

The cellular and molecular basis of a defective response to polysaccharide antigens is unknown and is probably not the same for all patients. Furthermore, several mechanisms related to B or T lymphocytes could be responsible. In young patients a physiologic delay of the responsiveness caused by slow maturation may be the basis of the defect. In this respect, patients may resemble normal infants up to 2 years of age. It has recently been suggested that impairment of antibody formation to TI-2 antigens in early life is related to expression of type 2 complement receptor (CD21) on B lymphocytes.<sup>55-57</sup>

Apart from the possibility of B-lymphocyte defects, disturbed T-cell regulation of the TI-2 response may

**TABLE V.** Antidiphtheria and antitetanus antibody titers before and after booster immunization with DTP-vaccine

| Patient | Diphtheria toxoid |       | Tetanus toxoid |       |
|---------|-------------------|-------|----------------|-------|
|         | Before            | After | Before         | After |
| I.1     | 0.2               | 2.8   | 1.0            | 8.0   |
| I.2     | —                 | 3.2   | 0.9            | 5.2   |
| I.3     | 0.4               | >3.2  | 5.2            | 6.4   |
| I.4     | 2.0               | —     | 32.0           | —     |
| I.5     | 0.045             | 0.250 | 0.18           | 0.70  |
| II.1    | 2.0               | 4.5   | 1.0            | >5.2  |
| II.2    | 0.4               | 3.2   | 0.9            | 32.0  |
| II.3    | 0.1               | 8.0   | 0.9            | >5.2  |
| II.4    | 0.1               | >3.2  | 0.18           | 11.2  |
| II.5    | 2.0               | —     | 5.2            | —     |
| II.6    | 2.0               | —     | 5.2            | —     |
| II.7    | 0.1               | 11.2  | 1.0            | 11.2  |
| II.8    | 4.5               | 6.4   | 18.1           | 25.6  |
| II.9    | 0.1               | 11.2  | 1.0            | 11.2  |
| II.10   | 0.6               | >3.2  | 0.9            | 18.0  |

Antidiphtheria and antitetanus antibody titers before and after immunization with DTP-vaccine are shown. Titers are expressed in arbitrary units per milliliter. The five nonresponder patients of group I are shown. The nonresponder patients of group II are *in italics*.

also play a role.<sup>9-11</sup> The defective antibody response can be overcome by administration of polysaccharide-protein conjugate vaccines in young children and in nonresponder patients.<sup>23, 24, 31, 48, 58</sup> It is interesting to note that patients deficient in IgG2, although responding to protein-conjugate vaccine, cannot be boosted by the polysaccharide vaccine.<sup>24</sup> This difference between infants and patients deficient in IgG2 strongly suggests differences in the immunoregulatory defect underlying the deficient antibody responses. The high frequency of defective antibody responses to TI-2 antigens in patients with common variable immunodeficiency (CVI), and IgA and IgG2 deficiency makes it likely that these patients share a common immunoregulatory defect. Recent data indicate that CVI disease and IgA deficiency could be related disorders caused by either a common susceptibility gene or the lack of a recessive gene located in the major histocompatibility complex class III region of chromosome 6.<sup>51-53</sup>

We conclude that defective response to polysaccharide antigens in patients with recurrent respiratory tract infections is indicative of either a transient maturational delay or a persistent selective impaired response to polysaccharide antigens. Progressive immunoglobulin deficiency may develop in some of these patients and must be considered during follow-up.

Moreover polysaccharide-protein conjugate vaccines, if available, may overcome the defective response in the absence of severe immunoglobulin deficiency.

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