Immunogenicity and Tolerance of a 7-Valent Pneumococcal Conjugate Vaccine in Nonresponders to the 23-Valent Pneumococcal Vaccine

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There is still a lack of effective vaccination strategies for patients with a deficient antibody response to bacterial polysaccharide antigens. In an open trial, we evaluated the immunogenicity and tolerance of a new 7-valent pneumococcal conjugate vaccine in 22 infection-prone nonresponders to pneumococcal polysaccharide vaccine and 21 controls. In the patient group, nonresponsiveness was confirmed by repeated vaccination with a 23-valent pneumococcal polysaccharide vaccine. The study protocol provided two doses of the pneumococcal conjugate vaccine, given 4 to 6 weeks apart, for both groups. The antibody response was determined before each vaccination and on follow-up by an enzyme-linked immunosorbent assay and compared to the response in a functional opsonophagocytosis assay. Patients showed a significantly lower postvaccination immune response for all serotypes than did controls. The postvaccination response was serotype dependent. A median titer of $>1 \mu g/ml$ in patients was recorded only for serotypes 4, 9V, 14, and 19F, which are known to be more immunogenic than serotypes 6B, 18C, and 23F. In the patient group, 76% responded to serotype 19F (Pnc 19F), 65% responded to Pnc 14 and 4, 60% responded to Pnc 9V, 55% responded to Pnc 18C, 50% responded to Pnc 23F, and 25% responded to Pnc 6B. In the control group $>95\%$ of individuals showed a titer of $>1 \mu g/ml$ to every serotype. The vaccine was tolerated well, and no major side effects have been reported. The new pneumococcal conjugate vaccine is clearly more immunogenic in previous nonresponders than is the 23-valent pneumococcal vaccine. Immunization with a pneumococcal conjugate vaccine should be considered as a strategy to protect high-risk patients.

Streptococcus pneumoniae (pneumococcus) is the world’s leading cause of otitis media and is frequently isolated from patients with meningitis, pneumonia, and sinusitis. Despite modern antimicrobial therapy, morbidity and mortality, especially due to pneumococcal meningitis, remain high. In addition, the rapid emergence of multidrug-resistant pneumococcal strains throughout the world since the late 1970s has emphasized the importance of preventing pneumococcal infection (12). Therefore, vaccine strategies have become a major topic in clinical medicine and public health. However, the efficacy of the currently used 23-valent pneumococcal vaccine has raised much controversy. Some studies demonstrated that it failed to protect high-risk patients (13), whereas others presumed an efficacy of up to 70% (10, 22). Moreover, a major drawback of the 23-valent vaccine is its limited immunogenicity in immunocompromised patients and children younger than 2 years (8, 20).

Isolated nonresponsiveness to polysaccharide vaccines is characterized by an impaired immune response to polysaccharide antigens, such as the capsular polysaccharides of Haemophilus influenzae or pneumococci (3, 20, 27), but an intact antibody response to protein antigens. Recurrent infections are a common clinical phenomenon in patients suffering from a polysaccharide-specific immunodeficiency. The first description of such a patient was published in 1987 (3). A considerable number of other reports followed (4, 15, 21, 29). Therefore, several vaccines are being developed based on the expectation that the immunogenicity of the polysaccharide antigens is improved by linking them to a protein carrier (conjugate vaccine) (24). The enormous impact of conjugate vaccines has already been demonstrated for the H. influenzae type b (Hib) conjugate vaccine (18, 30), which was able to induce a rapid decline of Hib disease in areas with high vaccine coverage. However, this remains to be confirmed for the pneumococcal conjugate vaccines.

The pneumococcal conjugate vaccines evaluated so far consist of 5 to 11 carrier protein-linked serotypes. Their efficacy is currently being tested in field trials, and they have been shown to induce antipolysaccharide antibodies in young infants (1, 14, 26).

In the present study, we evaluated the immunogenicity and tolerance of a 7-valent conjugate vaccine in patients with recurrent pulmonary infections who were nonresponders to the 23-valent pneumococcal vaccine.

MATERIALS AND METHODS

The study was a prospective open trial carried out with children and adolescents with recurrent infections (more than three per year) who previously failed to respond to the 23-valent pneumococcal polysaccharide vaccine. To evaluate the efficacy of the 23-valent pneumococcal vaccine, we applied the definition recommended by Sanders et al. (21), which considers the vaccination successful if postvaccination titers of $>1 \mu g/ml$ are found in at least five out of seven measured serotypes.

For immunological characterization of patients and controls, immunoglobulin and immunoglobulin G (IgG) subclass levels were measured by nephelometry and specific antibodies to Clostridium tetani and Hib were measured by an enzyme-linked immunosorbent assay (11, 29, 32).

Clinically the patients suffered from recurrent otitis (18%), sinusitis (36%), or pneumonia (68%). Patients with severe immunodeficiency (failure to respond to protein antigens) were excluded from the study.

The protocol was reviewed and approved by the ethics committee of the Johann Wolfgang Goethe-University, Frankfurt, Germany. Signed consent was obtained from all parents, and all aspects of Good Clinical Practice were followed.

A total of 44 patients were recruited for the study, 22 of whom met the criteria of nonresponsiveness to the 23-valent pneumococcal vaccine (group A) and 22 of...
whom were healthy patients (group B). Out of the 44 patients, 41 completed the study (2 dropouts were recorded in group A, and 1 was recorded in group B).

The study protocol provided a repeated dose of pneumococcal polysaccharide vaccine (0.5 ml each, given intramuscularly into the lateral aspect of the mid thigh) to confirm nonresponsiveness in group A and two injections of the pneumococcal conjugate vaccine for both patients and controls, each at a time interval of 4 to 6 weeks. Possible side effects were recorded on diary cards and reviewed on each visit.

Blood samples from all patients and controls were obtained prior to each vaccination and on follow-up (4 to 6 weeks after vaccination) and were stored at −20°C so that serotype-specific antibodies could be measured for all of the sera at the same time.

Vaccines. The new 7-valent pneumococcal conjugate vaccine (Weyth/Leiderle, Münster, Germany) consists of seven pneumococcal serotypes (4, 9V, 14, 19F, and 23F) at 2 μg each and 6B and 18C at 4 μg each) linked to a nontoxic variant of diphtheria toxin (CRM 197). The 23-valent pneumococcal polysaccharide vaccine (Pneumovax; MSD Merieux) was used to confirm nonresponsiveness in patients (group A). Both vaccines were injected intramuscularly into the gluteal region.

Measurement of pneumococcal antibodies. Serotype-specific antibodies reactive with serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F were measured by a new ELISA technique as previously described (31). Briefly, Nunc Covalink NH microtiter plates were used for direct immobilization of polysaccharides to measure pneumococcal antibodies. All sera were preincubated with 10 μg of pneumococcal polysaccharide C (Statens Seruminstitut, Kopenhagen, Denmark) per ml for 60 min for blocking of nonspecific anti-polysaccharide C (anti-CPSn) antibodies.

The pneumococcal antibody reference serum (lot 89-SF), provided by Carl E. Frash (Center for Biologics, Rockville, Md.), was used for assay standardization. The minimum antibody detection level was 0.1 μg/ml.

Opsonophagocytosis assay. The opsonophagocytosis assay measures the complement-dependent phagocytic activity of antipneumococcal antibodies. A flow cytometric modification was used, as recently described by Martinez et al. (16). This assay uses HL-60 granulocytes as effector cells and nonviable 5,6-carboxyfluorescein succimidylester-labeled S. pneumoniae as bacterial targets. The uptake of bacteria by the granulocytes was detected by flow cytometry and used as a qualitative measure for the specific anti-pneumococcal antibody activity. A positive immune response was defined as a postimmunization antibody concentration of >1 μg/ml or an opsonic titer of >1:64. This level was chosen by reference to Hib data, although it has been shown that a titer of >0.39 μg/ml was protective in an animal model (11).

Statistical analyses. For each serotype, the median and range of antibody concentrations are given. Undetectable antibody concentrations were assigned the value of the minimum detection level. Statistical analysis was performed by the two-sided t test and the Mann-Whitney U test.

RESULTS

A total of 24 children with recurrent infections (group A) and previous failure to respond to the 23-valent pneumococcal vaccine were recruited for the study. Two of these patients withdrew informed consent after the first study visit. A repeated dose of the 23-valent pneumococcal vaccine was given to 22 patients. No significant increase in the level of pneumococcal antibodies was confirmed in 20 patients. Two patients were excluded from further evaluation because of a significant antibody response (>1 μg/ml in five out of seven serotypes). Nonresponsiveness to polysaccharide vaccines is associated with a variety of other immunological defects (20); accordingly, we found the following characteristics in our patients (group A): isolated IgG2 deficiency was found in nine, IgG2 deficiency combined with IgA deficiency was found in three, and isolated IgA deficiency was found in one. Asthma was found in nine patients, and five of these also had allergies. Two patients with asthma had IgG2 deficiency without allergy, and one had asthma, IgG2 deficiency, and allergy. A detailed patient description, including immunoglobulin, IgG subclass, and specific antibody levels in comparison to the control group, is given in Table 1.

Adverse reactions. Adverse events were reported according to the World Health Organization body system preferred term codes affected, and counting multiple events per preferred term at its maximum severity resulted in the numbers given in Table 2.

The most frequent adverse reaction was injection site pain; 56% group A patients and 36% group B patients were affected after the first conjugate vaccination, and 39% of group A patients and 43% of group B patients were affected after the second conjugate vaccination. Reduced movements of the extremity vaccinated were registered for four patients in group A and four patients in group B after the first conjugate vaccination and for two patients in group A and two patients in group B after the second conjugate vaccination (Table 2). Swelling at the injection site was documented in six patients in group A after the first conjugate vaccination but only in 1 patient in group B at the same time. After the second conjugate vaccination this reaction was recorded in four patients in group A and again in one patient in group B. Bodily discomfort was observed in four group A patients and one group B patient after the first conjugate vaccination and in three group A patients and two group B patients after the second conjugate vaccination. All reactions resolved spontaneously. In general, patients who had adverse reactions to the first dose were not always the same patients who had adverse reactions to the second dose.

Fever of >38°C was reported in four recipients during the 5-day follow up after any of the injections (4.7% of the vaccinated).

Antipneumococcal antibody concentrations before vaccination. Although patients with recurrent infections (group A) had already received at least two doses of the 23-valent pneumococcal vaccine before being vaccinated with the new conjugate vaccine, antibody levels for most investigated serotypes were significantly lower than in unvaccinated controls (Table 3). The most evident differences in median preimmunization levels between groups were found for types 6B, 19F, and 23F (median, 1, 1.5, and 0.7 μg/ml in controls, compared to 0.1, 0.3, and 0.1 μg/ml in patients [P < 0.001]). Higher preimmunization levels for serotypes 6B, 19F, and 23F are thought to reflect frequent natural exposure to these serotypes in Germany (19).

Antipneumococcal antibody concentrations after the first dose of conjugate vaccine. In the patient group, the antibody response to the first dose of conjugate vaccine was very poor (Table 3). For only one serotype could a median concentration of >1 μg/ml be achieved (serotype 19F; median antibody concentration, 2.3 μg/ml). However, for serotypes 4 and 18C, 8.0- and 6.0-fold rises of the median antibody concentration after the first dose of the conjugate vaccine were observed in this group. In the control group, median antibody concentrations were significantly higher for all serotypes after the first dose (P < 0.05), associated with a 6.3- to 30-fold rise of the median antibody concentration (Table 3).
Antipneumococcal antibody concentrations after the second dose conjugate vaccine. In the patient group, the antibody response to the second dose of the conjugate vaccine was more pronounced (Table 3). For four out of the seven vaccine serotypes (serotypes 4, 9V, 14, and 19F), a median concentration of $>1 \mu g/ml$ was induced. Nevertheless, antibody levels for all serotypes were significantly lower in patients than in controls after the second dose (Table 3). If only responders ($>1 \mu g/ml$) in group A were considered, however, the results for group A and group B subjects were similar, except for serotype 4 (median, 2.2 $\mu g/ml$ in group A and 3.9 $\mu g/ml$ in group B).

In the patient group (group A), the median antibody concentration after the second dose increased by a factor of 1.3 (serotype 19F) to 12.0 (serotype 9V), except for serotypes 6B where there was no further increase in the antibody concentration. In the control group (group B), there was no further increase in the median antibody concentration for most serotypes detected; however, for serotype 6B the median antibody concentration increased from 6.3 to 12.0 $\mu g/ml$ (19-fold rise).

A minimum protective antibody level against pneumococcal disease has not been defined yet. As an arbitrary estimate, many studies used a cutoff value of $>1 \mu g/ml$, by analogy to the experience with Hib. The percentage of patients with an antibody concentration of $>1 \mu g/ml$ after two doses of conjugate vaccine varied among serotypes, indicating different immunogenicities: 19F (70%), 14 and 4 (65%), 9V (60%), 18C (55%), 23F (50%), and 6B (25%). The individual response to two doses of pneumococcal conjugate vaccine is displayed in Table 1 for 6B, 23F, and 19F (serotypes with poor, moderate, and strong immunogenicity, respectively).

A successful vaccination is defined, according to Sanders et al. (21), if postvaccination titers of $>1 \mu g/ml$ are found in at least five of seven measured serotypes. A significant response in five serotypes was demonstrated in 50% of group A patients. In this group, 80% responded to at least two serotypes and 20% (4 patients) did not respond at all. In the four nonresponding patients, a more pronounced immunodeficiency was suspected. Although three of these four patients had protective levels of antibodies to tetanus ($>0.1 IE/ml$), at enrollment all four developed hypogammaglobulinemia (low IgG and IgA levels according to the criteria of the World Health Organization [28]) on follow-up. Two similar cases have already been described, which led the previous authors to suggest that in some patients a defective response to polysaccharide vaccination may precede the development of a generalized immunodeficiency (21).

In contrast, 100% of control subjects showed a favorable response to serotypes 4, 6B, 18C, and 19F and 95% responded to serotypes 14 and 9V.

Opsonophagocytic titers. An opsonophagocytosis assay was performed for serotype 23F to confirm antibody functionality (Fig. 2). This moderately immunogenic serotype was chosen for its great variation of the individual IgG antibody response observed for both vaccinee groups. Median titers were 1:4 for group A and group B before vaccination; 1:4 and 1:4,096, respectively, after the first dose; and 1:128 and 1:4,096, respectively, after the second dose. We found that 65% of patients in group A had a titer of $>1:64$ after the second dose, compared to 100% in group B. In general, patients with an antibody concentration of $>1 \mu g/ml$ (17 out of 20 patients) had opsonophagocytosis results of $>1:64$. However, 3 out of 20 patients had a positive opsonophagocytic titer ($>1:64$) but an antibody concentration less than 1.0 $\mu g/ml$. Nevertheless, opsonic titers varied among individuals, and correlation of opsonic titers with antibody concentration was low ($r = 0.4$).

**DISCUSSION**

Our data confirm the findings of others on the safety and the enhanced immunogenicity of the new pneumococcal conjugate vaccine in patients and controls.

### TABLE 3. Immune response to the 7-valent pneumococcal vaccine in patients and controls

<table>
<thead>
<tr>
<th>Pneumococcal serotype and patient group</th>
<th>Before 1st conjugate</th>
<th>Before 2nd conjugate</th>
<th>After 2nd conjugate</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonresponders</td>
<td>0.1 (0.1–4.4)</td>
<td>0.8 (0.1–13)</td>
<td>2.2 (0.1–13)</td>
</tr>
<tr>
<td>Controls</td>
<td>0.1 (0.1–5.6)</td>
<td>3.8** (1.3–24)</td>
<td>3.9** (1.6–23)</td>
</tr>
<tr>
<td>6B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonresponders</td>
<td>0.1 (0.1–0.7)</td>
<td>0.1 (0.1–1.7)</td>
<td>0.1 (0.1–6.7)</td>
</tr>
<tr>
<td>Controls</td>
<td>1.0*** (0.1–11)</td>
<td>6.3*** (0.1–279)</td>
<td>12.0*** (1.3–438)</td>
</tr>
<tr>
<td>9V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonresponders</td>
<td>0.1 (0.1–2.0)</td>
<td>0.1 (0.1–2.0)</td>
<td>1.2 (0.1–17)</td>
</tr>
<tr>
<td>Controls</td>
<td>0.3 (0.1–3.3)</td>
<td>3.6** (0.7–40)</td>
<td>4.6*** (0.9–46)</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonresponders</td>
<td>0.3 (0.3–2.1)</td>
<td>0.3 (0.3–18.7)</td>
<td>1.7 (0.3–19)</td>
</tr>
<tr>
<td>Controls</td>
<td>0.3 (0.3–4.8)</td>
<td>8.3*** (0.3–995)</td>
<td>10*** (0.3–999)</td>
</tr>
<tr>
<td>18C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonresponders</td>
<td>0.1 (0.1–6.6)</td>
<td>0.6 (0.1–8.7)</td>
<td>0.6 (0.1–8.4)</td>
</tr>
<tr>
<td>Controls</td>
<td>0.3 (0.1–9.49)</td>
<td>7.8** (0.8–105)</td>
<td>7.8** (2.2–80.8)</td>
</tr>
<tr>
<td>19F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonresponders</td>
<td>0.3 (0.1–14.8)</td>
<td>2.3 (0.1–139)</td>
<td>3.1 (0.1–165)</td>
</tr>
<tr>
<td>Controls</td>
<td>1.5*** (0.1–17)</td>
<td>8.2** (1.1–186)</td>
<td>9.3* (2.3–190)</td>
</tr>
<tr>
<td>23F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonresponders</td>
<td>0.1 (0.1–0.2)</td>
<td>0.1 (0.1–10.2)</td>
<td>0.7 (0.1–14.3)</td>
</tr>
<tr>
<td>Controls</td>
<td>0.7** (0.1–6.3)</td>
<td>21*** (0.5–157)</td>
<td>17** (1–248)</td>
</tr>
</tbody>
</table>

* Medians and ranges (in parentheses) are shown. Statistical differences between patients and controls were calculated by the Mann-Whitney U test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$).
vaccines (7, 9). Moreover, our study specifically investigated children with recurrent infections who are also unresponsive to the 23-valent polysaccharide vaccine. These children were able to make measurable, and probably frequently protective, responses to the conjugate vaccine. Regarding side effects, no significant difference could be established between patients and controls. The pneumococcal conjugate vaccine was well tolerated, and most of the adverse reactions observed were only mild or moderate.

Conjugate vaccines are a new generation of vaccines conjugating the poorly immunogenic capsular polysaccharide components to a carrier protein. This process is thought to work throughout the recruitment of T-cell help, which transforms the antipolysaccharide immune response from a T-cell-independent to a T-cell-dependent response (24). However, it seems that the immunogenicity of pneumococcal conjugates is lower than that of Hib vaccines (26). In particular, immune responses to serotype 6B have repeatedly been shown to be poor. In addition, the conjugate vaccines are unlikely to elicit protective antibody levels in all patients without the administration of a second dose (17, 26). Nevertheless, the results of our studies with the 7-valent vaccine in the control group and recent data from the efficacy trial from Nelson et al. (2) suggest that vaccine efficacy is very high after a single dose only.

In comparison to the controls, our patients showed a rather low antibody response to the first dose and a moderate response to the second dose. Nevertheless, median titers were significantly lower for all serotypes analyzed. A successful vaccination for at least five out of seven serotypes could be demonstrated in 50% of patients, and 80% responded to at least

FIG. 1. Antibody response to two doses of pneumococcal conjugate vaccine. Individual response to serotypes with poor (6B), moderate (23F), and strong (19F) immunogenicity before the first dose and after the second dose of conjugate vaccine are displayed; the percentage of patients with antibody levels of ≥1 μg/ml was 25%, 50%, and 70%, respectively. Medians and statistical differences between patients and controls are indicated.
two serotypes. The opsonophagocytic assay confirmed functional activity of the antibodies.

It is interesting that in patients a median titer of $>1 \mu g/ml$ was recorded only for serotypes 4, 9V, 14, and 19F, which are known to be more immunogenic than serotypes 6B, 18C, and 23F. The poor response to these serotypes may be related to their physicochemical characteristics. It is well known that the structure of 6B resembles polyribosylribitolphosphate, the outer capsule of Hib (31). On the other hand, the poor response to polysaccharide 6B is related to the impaired host defense of our patients. However, our findings contrast with a recent report that one dose of a 5-valent pneumococcal conjugate vaccine was capable of inducing an IgG response in patients who are unresponsive to the polysaccharide vaccine (25). This difference may be explained by the reduced immunocompetence of the children studied. The study by Sorensen et al. (25) included only children with normal immunoglobulin and IgG subclass levels, whereas 9 of 20 patients in our study suffered from IgG subclass deficiency. Indeed, only 1 of 9 patients with IgG subclass deficiency responded to five of seven serotypes studied. It seems that the more highly impaired immune system of our patients required at least two doses of vaccine for priming B cells before antibody secretion was induced, although the efficacy of immunization varied widely among serotypes (from 70% in serotype 19 to 25% in serotype 6). It is tempting to speculate that a third dose of the conjugate might be beneficial in immunodeficient patients.

The origin of a deficient antibody response to bacterial polysaccharide is not yet clearly understood. Several mechanisms have been suggested, such as a genetic predisposition, like the G2m(n) allotype, or a defective expression of the complement receptor 2 on B cells, but none of them could consistently be demonstrated in all patients (20, 22). Most authors propose that there is a functional immaturity of the B-cell system which is unable to respond to stimulation with polysaccharide antigens (20). Irrespective of the underlying condition responsible for polysaccharide-specific immunodeficiency, specific immunodeficiency studies like ours provide an excellent model for analysis of immunogenicity and tolerance of the new pneumococcal conjugate vaccines.

The economic benefits of successful vaccination against pneumococcal infections in the general population, particularly the elderly, have been well documented. However, the overall efficacy rate of pneumococcal polysaccharide vaccine was only 57% and differed among high-risk groups for each group (5). No efficacy has been reported for the vaccine in patients with chronic diseases such as lymphoma, leukemia, Hodgkin's disease, multiple myeloma, sickle cell disease, and liver cirrhosis, in which the B-cell immune system is altered.

For these medical conditions, immunization with pneumococcal conjugate vaccines, requiring a T-cell-dependent response, seems a promising approach. Indeed, it was recently shown that priming with a pneumococcal conjugate vaccine and boosting with the 23-valent vaccine could decrease the number of vaccine failures among Hodgkin's disease patients (6).

However, in human immunodeficiency virus-infected patients, a 5-valent pneumococcal conjugate vaccine elicited similar antibody levels to those elicited by the common 23-valent vaccine (2). This finding emphasizes the importance of evaluating newer pneumococcal vaccines in all high-risk groups for whom pneumococcal immunization is recommended.

In conclusion, we found that although the pneumococcal conjugate vaccine elicited only low responses in the nonresponder (to the 23-valent vaccine) patients, the levels of antibody elicited by the conjugate vaccine were superior to those elicited by the 23-valent vaccine. Thus, a pneumococcal conjugate vaccine should seriously be considered as an important strategy to protect high-risk patients.

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