Type-Specific Antibodies to Pneumococcal Capsular Polysaccharide Acquired either Naturally or after Vaccination with Prevenar in Children with Underlying Chronic or Recurrent Lung Diseases

David Navarro,1,2 Amaro Escribano,3,4 Laura Cebrián,5 Concepción Gimeno,1,2 Leonor García-Maset,3,4 Juan García-de-Lomas,1,2,5* and the Spanish Pneumococcal Infection Study Network†

Department of Microbiology, University Clinical Hospital, Valencia, Spain; Department of Microbiology, School of Medicine, University of Valencia, Valencia, Spain; Unit of Pneumology, Department of Pediatrics, University Clinical Hospital, Valencia, Spain; Department of Obstetrics, Gynecology, and Pediatrics, School of Medicine, University of Valencia, Valencia, Spain; and Instituto Valenciano de Microbiología, Valencia, Spain

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The antibody response to capsular polysaccharides of pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F elicited either naturally or after vaccination with Prevenar was investigated in a cohort of children (n = 163) with underlying chronic or recurrent lung diseases at risk of developing pneumococcal pneumonia and ultimately invasive disease. Serum concentrations of serotype-specific antibodies, as measured by enzyme-linked immunosorbent assay, in unvaccinated children (n = 88) were higher in nasopharyngeal carriers (n = 10) than in noncarriers (n = 78) both at baseline and during follow-up. However, the antibody levels depended on the serotype and age of the children. During the study period, 35% of unvaccinated noncarriers and 60% of unvaccinated carriers displayed serum antibodies to all serotypes above the reported WHO working group putative protective serum concentration against invasive disease (0.2 µg/ml). Overall, children vaccinated with Prevenar before enrollment (n = 61), irrespective of their carrier status, displayed significantly higher serum levels of antibodies to all serotypes than unvaccinated children. More than 85% of the vaccinated children had protective serum antibody concentrations at baseline; although antibody titers tended to decrease over time, the above-mentioned figure remained without change at the end of follow-up. The vaccine Prevenar elicited a significant rise in serum antibody concentrations against all serotypes in 14 children vaccinated at entry. All of these children acquired and maintained serum antibody levels of >0.2 µg/ml throughout the study (a mean of 13 months of follow-up). These data support the systematic use of the vaccine Prevenar in children with underlying chronic or recurrent lung diseases and stress the fact that a percentage of vaccinated children may need to be revaccinated in order to achieve protection against pneumococcal disease.

Streptococcus pneumoniae is one of the most common pathogens in children, causing acute otitis media, pneumonia, and, less frequently, systemic infections, such as sepsis and meningitis. A few pneumococcal serotypes (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) cause the majority of infections in Spain (3). The capsular oligosaccharides or polysaccharides (PS) of these serotypes, each conjugated to a protein carrier, the non-toxic diphtheria toxin mutant CRM197, constitute the immunogen of the vaccine Prevenar (Wyeth Lederle Vaccines), which has recently been licensed in both the United States and Europe due to its effectiveness in preventing pneumococcal invasive disease (17). Although children less than 2 years of age are the major target for vaccination with Prevenar, its use has been recommended for individuals at risk of developing invasive pneumococcal disease, such as those with underlying lung diseases or immunocompromised patients (3), although its effectiveness in these populations has not been extensively evaluated.

Experimental and clinical data indicate that antibodies to pneumococcal capsular PS protect against the development of acute otitis media, pneumonia, invasive disease, and colonization due to homologous serotypes and, to a lesser extent, heterologous serotypes by virtue of their capacity to mediate microbial opsonophagocytosis (2, 13, 22). These antibodies are thought to be induced specifically by colonizing or infecting pneumococcal strains or even nonspecifically by other oropharyngeal or enteric capsulated bacteria (10). Little is known, however, about the kinetics of the natural acquisition of such antibodies in children. In the present study, we longitudinally analyzed serum concentrations of serotype-specific antibodies to pneumococcal capsular PS in nonvaccinated children with underlying chronic or recurrent lung diseases, who are thus at increased risk of developing pneumococcal invasive disease and are therefore more frequently amenable to vaccination with Prevenar, in order to infer their level of natural protection against invasive pneumococcal disease and compare it to that achieved after vaccination with Prevenar. We expected this study to determine whether a selective use of Prevenar targeting those children at real risk of developing invasive disease or, rather, systematic vaccination would be more appropriate in our population group.

* Corresponding author. Mailing address: Department of Microbiology, University Clinical Hospital, Av. Blasco Ibáñez 17, 46010 Valencia, Spain. Phone: 34-96 393 1317. Fax: 34-96 398 7836. E-mail: jglomas@retemail.es.
† Contributing participants in the Spanish Pneumococcal Infection Study Network are listed in Acknowledgments.
MATERIALS AND METHODS

Patients. The study involved 163 children (93 males) aged 7 months to 15 years (mean, 4.6 years) attended to in the Pediatric Department of the University Clinical Hospital of Valencia. The children had the following underlying pulmonary conditions: recurrent pneumonia, bronchiectasis and/or atelectasis (n = 62), bronchial hyperreactivity and asthma (n = 56), cystic fibrosis (n = 10), recurrent upper respiratory infections (n = 8), bronchial tree malformations (n = 9), pulmonary bronchiolitis (n = 7), ciliary dyskinesia (n = 6), and bronchiolitis obliterans (n = 5). The study protocol was approved by the Clinical Ethics Committee of the University Clinical Hospital. Written consent to participate was given by parents before initiation of the study.

Eighty-eight children had not been vaccinated with Prevenar before initiation of the study and were not vaccinated during follow-up. Sixty-one children had been vaccinated with Prevenar before recruitment. Seventeen patients were vaccinated at baseline. The criteria underlying this decision were the presence of chronic pulmonary disease (e.g., cystic fibrosis), a poor course (frequent exacerbations), or specific parent request. The vaccinal protocol used was that recommended by the Vaccination Advisory Committee of the Spanish Association of Pediatrics, according to age: in patients under 2 years of age, vaccination was carried out at 2, 4, and 6 months, with a recall dose at between 12 and 24 months; for children over 2 years of age, a single dose was administered (3).

Samples. Serum samples and nasopharyngeal (NP) swabs were obtained at baseline and sequentially during follow-up, as indicated below. Serum samples were stored at −20°C until use. NP swabs were cultured within 1 h of collection on nalidixic acid-supplemented tryptic soy agar containing 5% sheep blood and brain heart infusion (BHI) broth. The plates and the BHI broths were incubated at 37°C in 5% CO2 for 24 h. The BHI broths were subcultured at 24 h and incubated as described above. Streptococcus pneumoniae was identified on the basis of colony morphology, Gram stain characteristics, optochin sensitivity, and positive latex agglutination (Slides Pneumo-Kit; BioMerieux, SA). At least 10 colonies/plate were screened for pneumococcal identity. Pneumococcal serotyping was carried out by the Quellung reaction using reference sera from the Statens Serum Institut.

Antibody testing. Serotype-specific (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) pneumococcal serum immunoglobulin G (IgG) levels were measured by enzyme-linked immunosorbent assay (ELISA) according to the WHO consensus protocol (1), which includes preadsorption of sera with C polysaccharide and serotype 22F polysaccharide and uses 89-5F serum as a reference (see reference 21 for details). Sera were assayed in duplicate. For the purpose of this study, serum levels of antibodies that were >0.2 µg/ml were considered to provide protection against invasive pneumococcal disease (1, 12).

Statistical analysis. Mean serum concentrations of antibodies were statistically compared by the paired t test for independent or dependent samples, as required. Statistical significance of correlations between serum concentrations of antibodies against different serotypes was determined by the Spearman test. P values of ≤0.05 were considered statistically significant. The SPSS 10.0 statistical package was used throughout the study.

RESULTS

Pneumococcal serotype-specific antibodies in unvaccinated children. Of the 88 unvaccinated children, 78 (mean age, 5.3 years at the start of the study; range, 7 months to 14 years) had negative NP cultures at enrollment (group 1). The remaining 10 children (mean age, 4.5 years; range, 18 months to 14 years) were found to be colonized (group 2). Four of the colonizing strains could not be serotyped. Three of the remaining six strains were vaccine serotypes (19F [n = 2] and 9V [n = 1]). Of the 61 children vaccinated with Prevenar before entry into the study, 44 (group 3) were not pneumococcal carriers (mean age, 3.7 years; range, 8 months to 15 years), and 17 (mean age, 3.8 years; range, 18 months to 11 years) were carriers (group 4). Thirteen of the 17 colonizing strains were serotyped; of these strains, four were vaccine serotypes (6B [n = 2] and 19F [n = 2]). The children included in the study groups were age and sex matched (P ≥ 0.5 for both variables).

Table 1 shows the mean serum concentrations of antcapsular pneumococcal PS IgG antibodies at enrollment.

Table 2 shows the percentages of children with baseline serum levels of specific IgG antibodies below the putative protective level against pneumococcal invasive disease caused by any serotype (1, 12), we found that the number of children displaying serum antibody levels above the protective threshold in groups 1 and 2 varied according to the serotype considered (Table 2). Most unvaccinated noncarriers had baseline protective antibody levels against serotypes 6B, 14, and 19F but not against the remaining serotypes. Only 28 (35.8%) children of this group were protected against all vaccine serotypes. Likewise, most unvaccinated carriers had serum antibodies to serotypes 6B, 14, and 19F that were above the threshold of protection. In addition, a significantly (P = 0.01) greater number of children in this group than in the former group had protective levels of antibody against the rest of the serotypes. In fact, 60% of the children had serum antibody levels of >0.2 µg/ml against all of the vaccine serotypes.
heterologous serotypes; the differences, however, did not reach statistical significance ($P \geq 0.1$ for all serotypes).

At a second time point, serum and NP samples were available from 20 children in group 1. These samples were obtained an average of 12 months after the initiation of the study. Four of these children became colonized during follow-up (colonizing strains belonged to serotypes 2, 6B, and 14 or were non-typeable). For those who remained noncolonized, serum concentrations of antibodies against all serotypes in follow-up samples did not vary significantly ($P \geq 0.1$ for all serotypes) with respect to those measured at baseline (Table 4). In contrast, a statistically significant rise ($P \leq 0.05$ for all serotypes) in serum antibody levels against both homologous and heterologous serotypes was observed in children who became colonized during follow-up (mean serum concentrations before and during colonization, respectively, were as follows: serotype 4, 1.0 and 14.8 $\mu$g/ml; serotype 6B, 1.6 and 30.0 $\mu$g/ml; serotype 9V, 1.2 and 25.2 $\mu$g/ml; serotype 14, 3.8 and 100.2 $\mu$g/ml; serotype 18C, 0.5 and 10.0 $\mu$g/ml; serotype 19F, 2.3 and 12.0 $\mu$g/ml; serotype 23F, 0.9 and 13.7 $\mu$g/ml). These children achieved statistical significance in serum antibodies against both homologous and heterologous serotypes.

### Table 3: Statistical correlations between serum concentrations of antibodies against the different pneumococcal serotypes included in the Prevenar vaccine and between them and the ages of the nonvaccinated noncarrier children (study group 1)

<table>
<thead>
<tr>
<th>Serotype</th>
<th>4</th>
<th>6B</th>
<th>9V</th>
<th>14</th>
<th>18C</th>
<th>19F</th>
<th>23F</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.52*</td>
<td>0.08</td>
<td>0.15</td>
<td>0.48*</td>
<td>0.16</td>
<td>0.43*</td>
<td>−0.02</td>
<td></td>
</tr>
<tr>
<td>6B</td>
<td>0.49*</td>
<td>0.34*</td>
<td>0.27*</td>
<td>0.48*</td>
<td>0.40*</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9V</td>
<td>0.56*</td>
<td>0.12</td>
<td>0.76*</td>
<td>0.21</td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0.03</td>
<td>0.54*</td>
<td>0.16</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18C</td>
<td>0.13</td>
<td>0.18</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19F</td>
<td>0.18</td>
<td>0.35*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23F</td>
<td>−0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* *, statistically significant ($P \leq 0.05$).

As expected, a trend toward a direct correlation among serum concentrations of antibodies against all serotypes, except serotypes 4 and 23F, and the age of children was found in individuals in group 1 (Table 3). However, statistical significance was achieved only for antibodies against serotype 19F.

Statistically significant correlations were observed among serum concentrations of antibodies against different serotypes (Table 3), most notably between antibodies to serotype 6B and those against the remaining serotypes.

With respect to children included in group 2, age and serum antibody concentrations correlated significantly only for antibodies against serotype 14 ($P = 0.03$). Likewise, a number of statistically significant correlations were found among serum levels of antibodies against the different serotypes (data not shown). However, these data should be viewed with caution, due to the limited number of patients in this group.

For unvaccinated carriers, serum levels of antibodies against the homologous serotypes were higher than those against the heterologous serotypes; the differences, however, did not reach statistical significance ($P \geq 0.1$ for all serotypes).

### Table 4: Mean serum concentrations of type-specific antibodies at different time points in children followed up longitudinally

<table>
<thead>
<tr>
<th>Group (no. of subjects) and sampling time point*</th>
<th>Type-specific antibodies [µg/ml (SD)] to serotype:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 0</td>
<td>Time 1</td>
</tr>
<tr>
<td>1 (20)</td>
<td>4</td>
</tr>
<tr>
<td>Time 0</td>
<td>0.5 (0.5)</td>
</tr>
<tr>
<td>Time 1*</td>
<td>1.8 (3.1)</td>
</tr>
<tr>
<td>3 (24)</td>
<td>4.9 (6.1)</td>
</tr>
<tr>
<td>Time 0</td>
<td>4.7 (7.9)</td>
</tr>
<tr>
<td>Time 1*</td>
<td>3.8 (8.1)</td>
</tr>
<tr>
<td>4 (10)</td>
<td>7.9 (17.0)</td>
</tr>
<tr>
<td>Time 0</td>
<td>6.4 (12.1)</td>
</tr>
<tr>
<td>Time 1*</td>
<td>0.7 (0.3)</td>
</tr>
<tr>
<td>5 (14)</td>
<td>12.3 (12.2)</td>
</tr>
<tr>
<td>Time 0</td>
<td>2.3 (2.2)</td>
</tr>
</tbody>
</table>

* Group 1, nonvaccinated non-pneumococcal carriers; group 3, noncarrier children vaccinated with Prevenar before enrollment; group 4, carrier children vaccinated with Prevenar before enrollment; group 5, children vaccinated with Prevenar at entry.

* The children who became colonized ($n = 4$) are excluded.

* Serum samples were available for seven children.
any case, the percentage of children displaying serum antibody
statistically significant difference (P < 0.05). In the time after
vaccination (6.8 and 14.1 months, respectively) may account
for this finding.

An inverse correlation between the time lag from vaccination
and serum levels of antibodies against all serotypes except
serotypes 19F and 23F was found (Table 5). The majority of
children, carriers (88.7%) as well as noncarriers (86.3%),
displayed concentrations of protective antibody against all sero-
types (Table 2).

No significant correlations were found between the levels of
antibodies against all serotypes except serotype 23F and
the age of children included in group 3.

A statistically significant inverse correlation was observed
between serum levels of antibodies against pneumococcal se-
rotypes 4, 9V, and 18C in noncarriers and the elapsed time
since vaccination (Table 5). In addition, statistically significant
correlations were found among serum levels of antibodies
against several serotypes (Table 5). Comparable data were
obtained when children in group 4 were analyzed (data not
shown).

For vaccinated carriers, serum levels of antibodies against
the homologous serotypes were higher than those against the
heterologous serotypes, although the differences were not sta-
tistically significant (P < 0.1 for all serotypes).

Samples from a second time point were available from 24
children in group 3, which were collected an average of 9.6
months after the start of the study. Samples from a third time
point were also available from seven children (obtained an
average of 12.8 months after enrollment).

Serum concentrations of antibodies against most pneumo-
coccal serotypes tended to decrease over time. However, the
differences were statistically significant only for antibodies
against serotype 14 between time points 2 and 3 (P = 0.03). In
any case, the percentage of children displaying serum antibody
concentrations of >0.2 µg/ml remained without change during
follow-up.

NP and serum samples from a second time point were also
available from 10 children in group 4; these samples were
collected an average of 10.9 months after enrollment. No sta-
tistically significant variation in mean serum antibody concen-
trations against any serotype was observed (P > 0.9 for all
serotypes). The percentage of children with serum antibody
levels of 0.2 µg/ml at the end of follow-up remained unchanged
with respect to that found at baseline (89%).

Response to Prevenar in children vaccinated at enrollment.
Fourteen children (mean age at entry, 3.1 years; range, 1 to 6
years) were vaccinated with Prevenar at baseline (group 5).
Five of these children were pneumococcal carriers at baseline
(only one carried a vaccine serotype [6B]). None of the re-
main nine children became colonized during the follow-up
period. Measurements of serum concentrations of serotype-
specific antibodies were made at entry within 1 month before
vaccination and, on average, 1 and 12 months after vaccination.
Data are summarized in Table 4. A significant rise in serum
concentrations of antibodies against all serotypes was verified
after vaccination (P ≤ 0.021 for all serotypes), particularly
those targeted to serotype 14. No significant correlations be-
tween postvaccination antibody titers against all serotypes
and the age of children at the time of vaccination were found (P ≥
0.2 for all serotypes).

Antibody levels tended to decrease over time. However,
those against most serotypes were still significantly higher than
those measured before vaccination after an average of 13
months of follow-up (P ≤ 0.001 for serotype 4; P ≤ 0.001 for
serotype 6B; P = 0.15 for serotype 9V; P = 0.06 for serotype
14; P = 0.17 for serotype 18C; P = 0.03 for serotype 19F; P =
0.003 for serotype 23F). Seven children had serum concentra-
tions of PS antibodies against one or more serotypes of <0.2
µg/ml before vaccination; all of them acquired and maintained
antibody levels of >0.2 µg/ml against all serotypes at the end
of follow-up after vaccination.

Pneumococcal invasive disease during follow-up. A single
case of pneumococcal invasive disease (sepsis) occurred during
the follow-up period. The disease was caused by a pneumo-
coccal strain of serotype 6B. The patient had not been vacci-
nated with Prevenar and was colonized by a homologous se-
rotyte at the time of enrollment. The serum concentration of
antibodies against serotype 6B was 0.1 µg/ml.

DISCUSSION

In the present study, the level of protection against pneumo-
coccal invasive disease acquired either naturally or after
vaccination with Prevenar was assessed longitudinally in a
number of children with underlying chronic or recurrent lung
diseases. Studies comparable to our own and targeting both
healthy children and adults have been published (4, 6–9, 11, 18,
19). However, to our knowledge, this is the first study involving
children with subjacent pulmonary pathology that would pre-
dispose them to pneumococcal pneumonia and ultimately in-
vasive pneumococcal disease. Type-specific capsular PS se-
rum antibodies were quantitated by a specific and consensus
ELISA method (1, 21). Antibody concentrations measured by this
procedure correlated reasonably well with serotype-specific op-
onophagocytic titers of sera (18, 20); the former may therefore
be used, with certain limitations, to infer the level of protection
against invasive disease. In particular, antibodies to serotypes 4,
6B, 9V, 14, 18C, 19F, and 23F were analyzed, since these sero-
types cause the majority of invasive infections in Spain and con-

TABLE 5. Statistical correlations between serum concentrations of antibodies against the different pneumococcal serotypes included in Prevenar and between them and the ages of the children and the time elapsed from vaccination for noncarrier children vaccinated with Prevenar before enrollment (study group 3).

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Serotype-specific antibodies</th>
<th>Age</th>
<th>Time a</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>6B</td>
<td>0.25</td>
<td>−0.40</td>
</tr>
<tr>
<td>9V</td>
<td>0.02</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0.01</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>18C</td>
<td>0.33*</td>
<td>0.34*</td>
<td></td>
</tr>
<tr>
<td>19F</td>
<td>0.42*</td>
<td>0.51*</td>
<td></td>
</tr>
<tr>
<td>23F</td>
<td>0.42*</td>
<td>0.51*</td>
<td></td>
</tr>
</tbody>
</table>

a Time elapsed from vaccination.

b*, statistically significant (P ≤ 0.05).
stitute the immunogen of the Prevenar vaccine, whose use has been recommended on a systematic basis for children such as those selected for this study (3).

Among unvaccinated children, serum concentrations of capsular PS-specific antibodies against both homologous and heterologous pneumococcal serotypes (except serotype 9V) in baseline and follow-up samples were higher in NP carriers than in noncarriers. The differences, however, did not reach statistical significance. Our data coincide with those previously published by Goldblatt et al. (6) but not with those of Soininen et al. (19). In the latter work, only serum concentrations of antibodies against serotype 14 appeared to be significantly higher in carriers than in noncarriers. It must be pointed out, however, that the age ranges of individuals in those studies were notably different from those in our study. In addition, healthy rather than at-risk individuals were involved in both studies.

Pneumococcal NP colonization has been shown to elicit serotype-specific antibodies (14). Our data seem to support this affirmation, since children in group 1 who became colonized during follow-up experienced a significant rise in serum concentrations of antibody against the homologous and heterologous serotypes. However, it is often difficult to establish a relationship between NP colonization and development of type-specific antibodies, since the carrier state may go undetected as a result of insufficiently frequent sampling. On the other hand, it has been shown that the carrier state in adults may be as brief as 19 days (6); therefore, it cannot be ruled out that transient carriage in some of our patients was missed.

The highest serum concentrations of type-specific antibodies in unvaccinated children (both carriers and noncarriers) were those against serotype 14, while the lowest concentrations corresponded to serotypes 4 and 23F. This is likely related to the well-known different immunogenicities of these capsular PSSs (6, 19).

In unvaccinated children, a significant correlation among serum antibody concentrations against different serotypes, particularly between antibodies to serotype 6B and those to the remaining serotypes, was found. Considering the specificity of the ELISA employed in this work, the most likely explanation for this finding is the existence of cross-reactive capsular epitopes among the different serotypes. This phenomenon, previously reported by Soininen et al. (19), would explain the development of heterologous antibodies as a result of pneumococcal colonization, infection, or vaccination.

As expected on the basis of previous reports (7–9, 19), a direct correlation was observed between serum levels of antibodies targeted to the majority of pneumococcal serotypes and the age of children. However, statistical significance was reached only for antibodies to serotype 19B in unvaccinated noncarriers and for serotype 14 in unvaccinated carriers.

A concentration in the range of 0.2 to 0.4 μg/ml has been estimated by a WHO working group to afford protection against pneumococcal invasive disease (12). Our limited experience supports the above-mentioned threshold: one child in our cohort developed septicemia caused by a pneumococcal strain of serotype 6B. The patient had not been vaccinated, was colonized at baseline by a homologous serotype, and displayed serum levels of antibodies to serotype 6B of 0.1 μg/ml. However, it is accepted that serum concentrations providing protection against invasive disease may depend, among other factors, upon the age and risk group of the patients, the pneumococcal serotype considered, and the type of disease involved (15, 16). For the purpose of this study, 0.2 μg/ml was taken as a putative protective level. In accordance with this criterion, we found that the level of protection against invasive disease was markedly dependent upon the serotype considered and, to a lesser extent, on the NP carriage state. In this sense, the majority of unvaccinated noncarriers displayed protective levels of antibodies to serotypes 6B, 14, and 19F but not to the remaining serotypes. Only 35.8% of these children had concentrations of antibodies that were >0.2 μg/ml against all vaccine serotypes. This figure remained without change during follow-up. Comparable data were obtained from unvaccinated carriers, although the number of children susceptible to serotypes 4, 9V, 18C, and 23F was significantly lower than that of noncarriers. Six out of 10 children in this group had protective concentrations of antibody against all vaccine serotypes during the study period. Thus, our data indicate that a significant number of unvaccinated children from our cohort displayed low serum antibody titers against most pneumococcal serotypes included in the vaccine Prevenar, a situation that resembles that seen in healthy children of comparable ages (1).

Seventy-five children vaccinated with Prevenar were enrolled in this study. Sixty-one children had been vaccinated prior to the initiation of the study, and 14 were vaccinated at baseline. Data obtained from children vaccinated prior to enrollment indicated that overall, serum concentrations of type-specific antibodies were significantly higher in these children than in unvaccinated children, regardless of their carrier condition, both at baseline and at the end of the study period. Antibody levels in these children were found to decrease over time, which was most notable in noncarriers. Despite this fact, however, the percentage of children displaying antibody concentrations above the protective threshold remained around 85% at the end of follow-up. At this time point, the time elapsed from vaccination for most children was almost 2 years. As was expected on the basis of previous work (1), a number of statistically significant correlations were found among antibody levels against different serotypes.

Vaccination with Prevenar elicited a strong antibody response to all serotypes in all 14 children vaccinated at enrollment. A significant rise in antibody concentrations against all vaccine serotypes, particularly serotype 14, was measurable an average of 2.3 months after immunization. All children acquired antibody levels of >0.2 μg/ml against all serotypes (50% were presumably susceptible before vaccination) that persisted to the end of follow-up (an average of 13 months later), despite an overall decrease in antibody titers over time. The present study was not aimed at evaluating the efficacy of the Prevenar vaccine in our population. However, the data suggest that it may be similar to that achieved in healthy children of comparable ages (1). Definitive conclusions on this matter must await larger and controlled studies.

In summary, our data indicate that a small percentage of unvaccinated children displayed serum concentrations of type-specific PS antibodies above the putative protective level against invasive disease. The percentage of unvaccinated children actually protected against invasive disease might be even lower if relative avidities of naturally acquired antibodies are of a smaller magnitude than those of vaccine-induced antibod-
ies, as recently suggested (5). Our data thus favor the system-
atic use of the Prevenar vaccine in children with underlying
chronic or recurrent lung diseases that predispose them to
pneumococcal pneumonia and secondarily to invasive
chronic, irrespective of their pneumococcal NP carrier status.
Our data also stress the fact that a variable percentage (around
15% in our study) of children vaccinated with Prevenar may be
susceptible to invasive disease quite soon after immunization,
either due to a lack of response to the vaccine or as a conse-
quence of a marked decrease in serum antibody titers over
time, and may thus need to be revaccinated. Whether type-
specific antibody testing should be performed after vaccination
or whether systematic revaccination should be implemented
and what the right timing would be in both instances are
critical issues that remain to be elucidated.

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Javier Martinez-Lacasa (Hospital Mútua de Terrassa, Barcelona,
Spain); Cristina Latorre and Carmen Muñoz (Hospital Sant Joan de
Déu, Barcelona, Spain); Emilio Pérez-Trallero and Jose M. Marimon
(Hospital Donostia, San Sebastián, Spain); Juan García-de-Lomas
(Hospital Clínico, Valencia, Spain); and Ana Fleites (Hospital Central
de Asturias, Spain).

REFERENCES
1. Black, S., H. Shinefield, B. Fireman, E. Lewis, P. Ray, J. R. Hanson, L. Elvin,
K. M. Enser, J. Hackell, G. Sibb, F. Malinoski, D. Madore, I. H. Cqang, R.
and immunogenicity of heptavalent pneumococcal conjugate vaccine in chil-
2. Brown, E. J., S. W. Hosea, and M. M. Frank. 1983. The role of antibody and
complement in the reticuloendothelial clearance of pneumococci from the
3. Comité asesor de Vacunas de la Asociación Española de Pediatría. 2002. La
enfermedad neumocócica y su prevención. Vacuna neumocócica conjugada
4. Coughlin, R. T., A. C. White, C. A. Anderson, G. M. Carlone, D. L. Klein, and
J. Trenor. 1998. Characterization of pneumococcal specific antibodies in
5. Ekström, E., A. Ahman, J. Verho, J. Jokinen, M. Väkeväinen, T. Kilpi, H.
avidity of antibodies evoked by heptavalent pneumococcal conjugate vac-
cines PncCRM and PncCMPC in the Finnish Otitis Media Vaccine Trial.
Pedoby, R. George, A. Soininen, J. Edmunds, N. Gay, H. Käyhty, and E.
Miller. 2005. Antibody responses to nasopharyngeal carriage of Streptococ-
192:387–393.
Shiftman, and H. C. Dillon. 1981. Epidemiologic studies of Streptococcus
pneumoniae in infants: antibody response to nasopharyngeal carriage of types
pneumoniae in infants: antibody to types 3, 6, 14, and 23 in the first two years
of Streptococcus pneumoniae in families. II. Relation of transfer of S. pne-
11. His, Z., O. Spencer, J. Miles, J. Johnson, R. Holliman, J. Sheldon, and P.
Riches. 2004. Antibody response to pneumolysin and to pneumococcal caps-
ular polysaccharide in healthy individuals and Streptococcus pneumoniae
2003. Serological criteria for evaluation and licensure of new pneumococcal
E. Ades, and G. M. Carline. 1999. Correlation of opsonophagocytosis and
passive protection assays using human antipneumococcal antibodies in an
infant mouse model of bacteremia for Streptococcus pneumoniae. J. Infect.
Dis. 180:139–140.
Jonsdottir. 2001. Serum samples from infants vaccinated with a pneumococ-
cal conjugate vaccine, PncT, protect mice against invasive infection caused
by Streptococcus pneumoniae serotypes 6A and 6B. J. Infect. Dis. 185:233–
244.
nia and bacteremia model in mice for the analysis of protective antibodies.
2002. Specificities and opsonophagocytic activities of antibodies to pneumo-
opment of antibodies to pneumococcal capsular polysaccharides depends on
the serotype: association with pneumococcal carriage and acute otitis media
and H. Käyhty. 2001. Are the opsonophagocytic activities of antibodies in
infant sera measured by different pneumococcal phagocytosis assays compa-
21. Wernette, C. M., C. E. Frasch, D. Madore, G. Carlone, D. Goldblatt,
B. Plikaytis, W. Benjamin, S. A. Quataert, S. Hildreth, D. J. Sikkema, H.
Käyhty, I. Jonsdottir, and M. H. Nahm. 2003. Enzyme-linked immunosor-
bent assay for quantitation of human antibodies to pneumococcal polysac-
22. Whitney, C. G., M. M. Farley, J. Hadler, L. H. Harrison, N. M. Bennett,
R. Lynfield, D. A. Reingold, P. R. Cieslak, T. Pilishvili, D. Jackson, R. R.
pneumococcal disease after the introduction of protein-poly saccharide con-