Reduction of Antibody Response to an 11-Valent Pneumococcal Vaccine Coadministered with a Vaccine Containing Acellular Pertussis Components

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In pneumococcal conjugate vaccines (PCVs), polysaccharide antigens are often conjugated to protein carriers related to other common vaccines. It is therefore important to test PCV interaction with other pediatric vaccines when administered simultaneously. We assessed the immune response to an 11-valent PCV conjugated to diphtheria and tetanus carriers (PncD/T11), administered concomitantly, but in separate sites, with a combined vaccine containing epitopes related antigenically to the carriers: polyribosylribitol phosphate-tetanus toxoid (PRP-T), diphtheria toxoid (DT), and tetanus toxoid (TT). In addition, these combinations contained inactivated poliovirus vaccine (IPV) and either whole-cell pertussis (wP) or acellular pertussis (aP) components. After coadministration of PncD/T11 with the combined vaccine containing wP (DTwP/IPV/PRP-T), the responses to all polysaccharides in the PncD/T11 were satisfactory. In contrast, when coadministered with an aP-containing combination (DTaP/IPV/PRP-T), the response to all seven pneumococcal conjugates to TT was significantly reduced after primary and booster immunization. The pneumococcal conjugates to DT were not significantly reduced after the primary series, but were somewhat reduced after booster. It is likely that some suppression of the tetanus-mediated response occurred even when the PncD/T11 was coadministered with wP, but this suppression was masked by the adjuvant effect of wP. By replacing wP with aP, this adjuvant effect was removed, unmasking the suppression of the tetanus-mediated response. With the increasing use of multiple aP-containing vaccines in infancy, novel approaches to adjuvants and carrier protein technology are likely to be required.

The first pneumococcal conjugate vaccine (PCV) was recently licensed for use in infants and toddlers. Other PCVs are currently under development (14, 19, 26). To integrate the delivery of PCVs into existing infant immunization schedules, PCVs need to be simultaneously administered with other childhood vaccines: mostly with poliovirus, tetanus toxoid (TT), diphtheria toxoid (DT), pertussis, Haemophilus influenzae type b (Hib), hepatitis B virus, measles, mumps, rubella, and varicella vaccines.

The most widely used glycoconjugate vaccines utilize common infant vaccine proteins as carriers, and thus their use in infant immunization programs is leading to the delivery of increased amounts of these common antigenic epitopes. Examples of such vaccines are Hib conjugate vaccines, meningococcal C conjugate vaccines, and PCVs that may be conjugated to TT, DT, or derivatives of DT such as CRM197 (1–5, 11, 14, 27).

We have previously shown that in infants who simultaneously received DT, TT, whole-cell pertussis vaccine (DTwP), TT-conjugated Hib vaccine (polyribosylribitol phosphate-tetanus toxoid [PRP-T]), and a 4-valent TT-conjugated PCV, a reduced response to Hib and TT was observed, and the magnitude of the reduced response depended on the total dose of the TT (4). This phenomenon is analogous to that seen with carrier-induced epitope suppression (CIES).

This observation led to the development of a candidate 11-valent PCV in which the antigenic load of any single carrier is minimized by producing a bi-carrier glycoconjugate. This 11-valent vaccine (PncD/T11) contained seven polysaccharides conjugated to TT and four polysaccharides (those judged to need the largest carrier amounts) conjugated to DT. Initial studies showed it was safe and immunogenic for all 11 pneumococcal serotypes included in the vaccine (serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F) when administered alone or simultaneously with other childhood vaccines, including a combined vaccine consisting of DTwP, PRP-T, and inactivated trivalent poliovirus vaccine (DTwP/IPV/PRP-T) (18, 21, 27, 28; Dagan et al., 37th Annu. Infect. Dis. Soc. Am. Meet., abstr. 640, 1999). Furthermore, the responses to the other simultaneously administered antigens were not impaired (unpublished data).

Increasingly, in developed countries, the use of acellular pertussis (aP) vaccines is replacing that of whole-cell pertussis (wP) vaccines to improve the safety and tolerability of pertussis vaccines (8). Previous experience with combinations of aP and Hib conjugates has demonstrated the potential for reduced immunogenicity of the Hib component in such vaccines (6). It
TABLE 1. Design of studies 1 to 3a

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>Study vaccine (PncD/T11)</th>
<th>Blood sample</th>
<th>Concomitant vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Study 1</td>
<td>Study 2</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>DTwP/IPV/PRP-T</td>
<td>DTwP/IPV/PRP-T</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>DTwP/IPV/PRP-T</td>
<td>DTwP/IPV/PRP-T, OPV</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>DTwP/IPV/PRP-T</td>
<td>DTwP/PRP-T, OPV</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>OPV</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>DTwP/IPV/PRP-T</td>
<td>DTwP/IPV/PRP-T, OPV, MMR</td>
</tr>
<tr>
<td>13</td>
<td>+</td>
<td>OPV, MMR</td>
<td></td>
</tr>
</tbody>
</table>

a See the text for detailed explanation. Study 1 was conducted in 1998 to 1999, study 2 was conducted in 1999 to 2000, and study 3 was conducted in 2000 to 2001.

was thus essential to determine the immune response to PncD/T11 when coadministered with a p-containing vaccines. We therefore examined the response to PncD/T11, PRP-T, DT, and TT vaccines after coadministration of PncD/T11 with either DTwP/IPV/PRP-T or DTaP/IPV/PRP-T.

MATERIALS AND METHODS

Study design. From 1998 to 2001, three successive phase II studies were conducted in southern Israel, as part of the clinical development of the PncD/T11 (Table 1). In each study, randomization was carried out either between an adjuvanted and a nonadjuvanted formulation and a placebo (study 3). Thus, a nonadjuvanted PncD/T11 arm was common in all studies, and only results with this formulation are discussed in the present report. In each case, the vaccine was given as a four-dose regimen (at ages 2, 4, 6, and 12 months).

In all three studies, the concomitant vaccines at 2, 4, 5, and 12 months included DT, TT, Hib, and poliovirus (DPV, IPV, or both). The one exception was in study 1, when oral polio vaccine (OPV) was given at 7 months of age and no poliovirus vaccine was given at 6 months of age (Table 1). In studies 1 and 2, the concomitant vaccines included wP, and in study 3, they included aP. In study 1, on all four occasions when PncD/T11 was injected, the concomitant combination vaccine was DTwP/IPV/PRP-T. In all studies, the two vaccines were given in separate limbs (left and right thighs). In studies 2 and 3, to comply with the Israeli program of immunization, IPV was omitted from the third dose administered at 6 months of age: thus, DTwP/IPV/PRP-T and DTaP/IPV/PRP-T were used for the third dose in studies 2 and 3, respectively. In study 1, OPV was given at ages 7 and 13 months and MMR was given at 13 months, while in studies 2 and 3, OPV was given at ages 4, 6, and 12 months, and in study 2, MMR was given at age 12 months. In study 3, MMR was given at age 13 months. In study 3, the randomization included an additional placebo arm with concomitant vaccines only (no PncD/T11) (Table 1). All infants also received hepatitis B vaccine at 0, 1, and 7 months of age.

All three studies were approved by the Local and National Ethics Committee, and written informed consent was obtained from the parents or legal guardians of each subject before enrollment. All of the study subjects were Jewish infants recruited from six Mother and Child Health Centers in Beer-Sheva, located in southern Israel.

Healthy infants were recruited at age 2 months (±2 weeks). The presence of any of the following precluded enrollment in the study: known or suspected impairment of immunologic function, acute illness or fever, history of invasive pneumococcal disease, recent vaccination or treatment with immunoglobulin (Ig) or corticosteroids, and suspected or known hypersensitivity to any vaccine component.

Venous blood was obtained in all three studies at identical time points: 2 months (baseline), 7 months (postprimary), 12 months (prebooster), and 13 months (postbooster) of age. Sera were stored at −70°C until analyzed.

Vaccines. (i) Pneumococcal conjugate vaccines. Each dose of PncD/T11 (Aventis Pasteur, Lyon, France) contained 11 pneumococcal capsular polysaccharide conjugates, in a dose of 0.5 ml: 1 µg of polysaccharide/dose for serotypes 1, 4, 5, 7F, 9V, 19F, and 23F conjugated to tetanus protein; 3 µg of polysaccharide/dose for types 3, 14, and 18C conjugated to DT; and 10 µg of polysaccharide/dose for type 6B conjugated to DT. Separate lots were used for each study. Release testing ensured that each vaccine lot was similar in conjugate quantification, pH, and animal immunogenicity. Each conjugate lot used to produce the vaccine was shown to be similar for each serotype based on the proportion of unconjugated polysaccharide, proportion of free carrier, and protein/polysaccharide ratio for each conjugate. None of these criteria changed over the course of the clinical trial as monitored by stability protocols. The lots released for the studies were not only compared to lots stored at the manufacturing facility but also compared to similar lots released for other clinical trials. Conjugate intermediates and bulk vaccine also met standard criteria for pyrogens, sterility, identity, and residuals.

The liquid vaccine was presented in a prefilled syringe with a 25G 5/8 needle for intramuscular injection. The PncD/T11 vaccine was administered to the upper right thigh at 2, 4, 6, and 12 months of age. The per-protocol window for each dose was ±2 weeks.

(ii) Concomitant vaccines. In studies 1 and 2, DTwP/IPV/PRP-T (Pentacoq; Aventis Pasteur, Lyon, France) was used. In study 3, DTaP/IPV/PRP-T (Pentacel, Aventis Pasteur, Toronto, Canada) and DTaP/PRP-T (Actacea; Aventis Pasteur, Toronto, Canada) were used. Apart from the differences in the pertussis antigen content, the DTwP and DTaP combinations differed also in the DT and TT content. The wP combination contained ~30 limes flocculation (Lf) of DT

TABLE 2. DT and TT protein content administered at each visit a

<table>
<thead>
<tr>
<th>Study</th>
<th>Protein content (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DTwP/T11</td>
</tr>
<tr>
<td>Study 1</td>
<td>64</td>
</tr>
<tr>
<td>Study 2</td>
<td>59</td>
</tr>
</tbody>
</table>

a Ages 2, 4, 6, and 12 months.

b In studies 1 and 2, DTwP; in study 3, DTaP.

c —, not relevant.
TABLE 3. Postprimary GMC of serotype-specific pneumococcal antipolysaccharide IgG in studies 1 to 3

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Receiving PncD/T11</th>
<th>Not receiving PncD/T11</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study 1 (n = 59)</td>
<td>Study 2 (n = 64-69)</td>
</tr>
<tr>
<td>TT conjugates</td>
<td>Study 3 (n = 51)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.72 (1.41–2.11)</td>
<td>4.15 (3.42–5.04)</td>
</tr>
<tr>
<td>4</td>
<td>3.30 (2.49–4.37)</td>
<td>5.23 (4.32–6.35)</td>
</tr>
<tr>
<td>5</td>
<td>1.92 (1.51–2.44)</td>
<td>2.23 (1.78–2.80)</td>
</tr>
<tr>
<td>7F</td>
<td>4.24 (3.56–5.06)</td>
<td>3.65 (3.06–4.35)</td>
</tr>
<tr>
<td>9V</td>
<td>1.55 (1.24–1.94)</td>
<td>1.85 (1.46–2.33)</td>
</tr>
<tr>
<td>19F</td>
<td>5.07 (3.63–7.08)</td>
<td>6.63 (4.99–8.81)</td>
</tr>
<tr>
<td>23F</td>
<td>1.48 (1.05–2.09)</td>
<td>1.82 (1.37–2.42)</td>
</tr>
<tr>
<td>DT conjugates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.58 (1.25–2.00)</td>
<td>2.61 (2.20–3.11)</td>
</tr>
<tr>
<td>6B</td>
<td>1.08 (0.76–1.53)</td>
<td>1.28 (0.91–1.81)</td>
</tr>
<tr>
<td>14</td>
<td>1.86 (1.29–2.69)</td>
<td>2.13 (1.58–2.88)</td>
</tr>
<tr>
<td>18C</td>
<td>0.78 (0.58–1.04)</td>
<td>1.17 (0.95–1.45)</td>
</tr>
</tbody>
</table>

*The 95% CI is given in parentheses. P < 0.001 for all serotypes for the groups that did not receive PncD/T11 compared to each of the three studies in children to whom PncD/T11 was administered; P < 0.001 for serotypes 1, 4, 7F, 9V, 23F in study 3 versus study 1 and versus study 2 in children receiving PncD/T11; P < 0.001 for serotypes 1, 4, 7F, 9V, 23F in study 3 versus study 1 and versus study 2 in children receiving PncD/T11; 0.001 < P < 0.05 for serotypes 3, 4, and 18C between study 1 and study 2 in children receiving PncD/T11; 0.001 < P < 0.05 for serotypes 5 and 19F between study 1 and study 2 in children receiving PncD/T11; 0.001 < P < 0.05 for serotypes 1, 4, 7F, 9V, 23F in study 3 versus study 1 and versus study 2 in children receiving PncD/T11; 0.001 < P < 0.05 for serotypes 5 and 19F between study 1 and study 2 in children receiving PncD/T11.

TABLE 4. Postbooster GMC of serotype-specific pneumococcal antipolysaccharide IgG in studies 1 to 3

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Receiving PncD/T11</th>
<th>Not receiving PncD/T11</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study 1 (n = 57)</td>
<td>Study 2 (n = 64-67)</td>
</tr>
<tr>
<td>TT conjugates</td>
<td>Study 3 (n = 49)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.18 (3.23–5.42)</td>
<td>6.86 (5.41–8.69)</td>
</tr>
<tr>
<td>4</td>
<td>5.85 (4.47–7.67)</td>
<td>9.14 (7.23–11.5)</td>
</tr>
<tr>
<td>5</td>
<td>4.14 (3.20–5.35)</td>
<td>6.25 (5.07–7.70)</td>
</tr>
<tr>
<td>7F</td>
<td>6.40 (5.02–8.17)</td>
<td>7.61 (6.26–9.25)</td>
</tr>
<tr>
<td>9V</td>
<td>2.73 (2.08–3.58)</td>
<td>4.39 (3.46–5.56)</td>
</tr>
<tr>
<td>19F</td>
<td>15.9 (11.9–21.3)</td>
<td>16.7 (12.8–21.7)</td>
</tr>
<tr>
<td>23F</td>
<td>3.56 (2.68–4.72)</td>
<td>6.22 (4.88–7.95)</td>
</tr>
</tbody>
</table>

*The 95% CI is given in parentheses. P < 0.001 for all serotypes for the groups that did not receive PncD/T11 compared to each of the three studies in children to whom PncD/T11 was administered; P < 0.001 for serotypes 1, 4, 5, 7F, 9V, 19F, and 23F in study 3 versus study 1 and versus study 2 in children receiving PncD/T11; P < 0.001 for serotypes 14 and 18C in study 1 versus study 2 in children receiving PncD/T11; 0.001 < P < 0.05 for serotypes 1, 4, 5, 7F, 9V, 18C, and 23F in study 1 versus study 2 in children receiving PncD/T11; 0.001 < P < 0.05 for serotypes 14 in study 1 versus study 3 in children receiving PncD/T11.

(ii) Antibodies to concomitant vaccines. In study 3, anti-PRP, anti-TT and anti-DT were performed by the Aventis Pasteur USA Laboratory, Swiftwater, Pa. PRP was measured by radioimmunoassay (RIA). The assay was a modification of that described by Norden et al. (17). Anti-TT was measured by the EIA (7). Anti-DT was performed by measuring neutralizing antibodies by the Vero cell assay (9).

(iii) Total DT and TT dose at each injection. The different DT and TT loads at each dose in studies 1, 2, and 3 are presented in Table 2.

Statistical analysis. The data analyses were performed by Aventis Pasteur Biometry Department (Lyon, France). For each immunogenicity variable, the 95% confidence interval (95% CI) was calculated using either the exact binomial distribution (from percentage) or the normal approximation (from the geometric mean concentrations [GMC]).
antibody concentration ≥0.5 µg/ml and 1.0 µg/ml between the study groups were done by chi-square or Fisher exact test. Since multiple comparisons were performed for each serogroup and for each study, only *P < 0.001* was considered significant. However, all comparisons with 0.001 < *P < 0.05* are shown in the tables.

**RESULTS**

The numbers of children enrolled to receive PncD/T11 in studies 1, 2, and 3 were 63, 70 and 54 children, respectively. The number of recruited children to the group that did not receive pneumococcal vaccine (from study 3) was 58. There was no significant difference in the ages at vaccination. The first, second, and third doses in study 1 were given at mean ages (± standard deviation) of 2.0 ± 0.2, 3.8 ± 0.2, and 5.8 ± 0.2 months, respectively. There was no significant difference between the groups for the first dose. Although for the second and third doses there was a statistically significant difference between the groups, the mean ages did not differ by more than 2 weeks at each visit. The proportion of males was 49%, with no significant differences between the groups.

**Geometric mean pneumococcal anticapsular IgG concentrations.** When comparing between studies 1 and study 2, no significant differences were found for any of the serotypes after the primary or the booster immunization. One exception was the postprimary sample for serotype 1 (1.72 versus 4.15 µg/ml in study 1 versus study 2, respectively; *P < 0.001*) (Tables 3 and 4 and Fig. 1).

Study 3 was remarkable for significantly lower GMCs than those of studies 1 and 2 for all serotypes conjugated to TT (TT conjugates), namely serotypes 1, 4, 5, 7F, 9V, 19F, and 23F. This difference was significant at the level of *P < 0.001* compared to at least one of the two other studies (studies 1 and 2) after primary immunization and for all comparisons after boosting (Tables 3 and 4 and Fig. 1). Serotypes 1, 9V, and 23F showed the greatest difference in GMC following both postprimary and postbooster visits.

When compared to the antibody responses to the TT conjugates, the responses to the DT conjugates (serotypes 3, 6B, 14, and 18C) were less affected. The concentrations of antibodies against serotypes conjugated to DT were similar in study 3 to those in studies 1 and 2 with two exceptions: the GMCs of serotypes 14 and 18C following boosting were significantly higher in study 2 than in study 3 (*P < 0.001*) (Table 4 and Fig. 1).

Proportion of children with pneumococcal anticapsular IgG antibody concentrations ≥0.5 and ≥1.0 µg/ml. When compar-
ing antibodies for each pneumococcal serotype in children in study 1 and study 2 who received PncD/T11, proportions of infants achieving GMCs of $\geq 0.5$ µg/ml (not shown) or $\geq 1.0$ µg/ml for both postprimary and postbooster visits did not differ significantly. The one exception was proportions with IgG $\geq 1.0$ µg/ml specific for serotype 1 at the postprimary visit, for which the proportion in study 2 was statistically higher than that of study 1 (97% versus 78%, respectively; $P = 0.001$) (Table 5 and Fig. 2). However, the proportion of children with serotype-specific anticapsular IgG of $\geq 0.5$ µg/ml was lower in children in study 3 than the proportion observed in studies 1 and 2 for all TT conjugates. The difference was significant at $P < 0.001$ when compared to study 1, study 2, or both for serotypes 1, 9V, and 23F at the postprimary visit. The difference was more accentuated when the proportions of antibodies with concentrations $\geq 1.0$ µg/ml was compared between PncD/T11 recipients in study 3 versus those in studies 1 and 2 (Table 5 and Fig. 2). The proportion was lower at the postprimary visit for all TT conjugates, and reached $P < 0.001$ when compared with studies 1 and 2 for serotypes 1, 4, 7F, 9V, and 23F. Even at the postbooster visit, there was still a difference in proportion of children with antibody concentration $\geq 1.0$ µg/ml. This reached the level of $P < 0.001$ for serotypes 1, 9V, and 23F.

No significant differences between PncD/T11 recipients in study 3 when compared to study 1 and 2 were observed at any point in the study for the DT conjugates.

### Pneumococcal anticapsular IgG antibody concentration distribution

The antibody concentration distribution at the postprimary visit (age 7 months) is presented as reverse cumulative frequency (RCF) curves (Fig. 3). Three observations were made when examining the RCF curves from the three studies. (i) The distribution of the antibody concentrations of the group not receiving PncD/T11 was distinct from the other three groups, which received PncD/T11, for each serotype, demonstrating that PncD/T11 was immunogenic in all three studies. (ii) For the PncD/T11 recipients, study 1 and study 2 had similar antibody concentration distributions, except for serotype 1, for which study 2 had a distribution-demonstrating pattern with a higher value than that of study 1. (iii) For all TT conjugates, the antibody concentration distribution in study 3 was distinctly lower, with little overlap with those in studies 1 and 2. Again, for DT conjugates as for the GMC and analyses of the proportion of concentrations $\geq 1.0$ µg/ml, the RCF curves were practically identical among PncD/T11 recipients in all three studies.

### Immune response to concomitant vaccines

The products used for the concomitant vaccines in studies 1 and 2 (namely DTwP/IPV/PRP-T) were not similar and not produced at the same site as the concomitant DTaP/IPV/PRP-T and DTaP/PRP-T combinations that were used for study 3, which precluded comparisons. However, the comparison between the groups not receiving PncD/T11 in study 3 could be used as a comparison for those receiving PncD/T11 in study 3 to determine whether additional TT load by the PncD/T11 caused any interference with other concomitant vaccines, especially anti-TT and -PRP antibody production after receiving the combination vaccines concomitantly with PncD/T11.

In study 3, no significant differences were found between the two groups in the postprimary antibodies against diphtheria, pertussis components, and polioviruses 1, 2, and 3. After the primary vaccination series at 2, 4, and 6 months, the values for the group receiving PncD/T11 versus the group receiving the concomitant vaccine only was anti-diphtheria GMC (IU/milliliter), 0.48 (95% CI, 0.33 to 0.68) and 0.40 (95% CI, 0.28 to 0.58), respectively; anti-pertussis toxin GMC (ELISA units [EU] per milliliter), 48.2 (95% CI, 42.1 to 55.3) and 50.2 (95% CI, 43.6 to 57.7), respectively; anti-FHA GMC (EU per milliliter), 35.4 (95% CI, 30.0 to 40.7) and 34.1 (95% CI, 29.1 to 40.0), respectively; antipoliovirus geometric mean titer (GMT; 1/dilution), 435 (95% CI, 259 to 730) and 597 (95% CI, 385 to 926), respectively; antipoliovirus 2 GMT (1/dilution), 102 (95% CI, 1,496-2,954) and 2,467 (95% CI, 1,874 to 3,247), respectively; and antipoliovirus 3 GMT (1/dilution), 547 (95% CI, 325 to 921) and 960 (95% CI, 625 to 1,473), respectively. Similarly,
after a booster dose at 12 months of age, no difference between the groups was seen (data not shown).

For the tetanus-containing concomitant vaccine, no significant difference was observed in the anti-TT or anti-PRP antibody response between the two groups: for anti-TT antibody, the postprimary GMC was 1.50 IU/ml (95% CI, 1.24 to 1.82) in the PncD/T11 recipients versus 1.40 IU/ml (95% CI, 1.62 to 1.75) in the controls. The respective figures for postbooster visit were 4.21 IU/ml (95% CI, 3.44 to 5.14) versus 3.84 (95% CI, 3.17 to 4.66). The respective numbers for anti-PRP GMC at postprimary and postbooster visits were 3.38 μg/ml (95% CI, 2.21 to 5.16) versus 4.07 (95% CI, 2.68 to 6.18) and 12.9 μg/ml (95% CI, 9.45 to 17.6) versus 18.4 μg/ml (95% CI, 13.1 to 26.0).

The proportions of children with anti-TT antibody ≥1.0 IU/ml and anti-PRP antibodies ≥1.0 μg/ml after the primary series were similar (results not shown). The RCF curves showed no difference of distribution after the primary vaccination series at age 7 months for both anti-TT and anti-PRP antibodies between the PncD/T11 recipients and controls in study 3 (Fig. 4). Similarly, no difference was found in the postbooster RCF curves (not shown).

DISCUSSION

In the present study, children who received PncD/T11 at the same time as a combination of DTaP and PRP-T showed a lower-than-expected antibody response to all the pneumococcal polysaccharide antigens presented as TT conjugates. In contrast, responses to the DT-conjugated antigens were relatively spared and simultaneously administered vaccines, including PRP (given in combination with DTaP with or without IPV) and TT, were not affected. The present findings are of great importance for the understanding and development of new vaccines.

We have previously shown (4) that the simultaneous administration of TT-conjugated pneumococcal polysaccharides with PRP-T and TT antigens given simultaneously with combination containing wP can cause a phenomenon similar to CIES, resulting in lower antibody response to TT or polysaccharides conjugated to it. We have also demonstrated that this phenomenon was dose dependent. To overcome this effect, a modified 11-valent pneumococcal conjugate vaccine was developed. The new candidate vaccine was formulated with two carrier proteins, and to further reduce the amount of tetanus in the...
vaccine, reduced amounts of carrier and polysaccharide were used. Seven capsular antigens (formulated in a reduced dose of $1 \mu g$ of polysaccharide for each serotype) were conjugated to TT (28). The remaining four polysaccharides that needed to be administered at higher doses to stimulate immunogenicity were conjugated to DT. The administration of this mixed-carrier conjugate (PncD/T11) simultaneously with a variety of concomitant antigens, including TT and PRP-T to adults, toddlers, and infants, in the presence of wP resulted in a safe and immunogenic formulation (18, 21, 27, 28) (Dagan et al., 37th Annu. IDSA Meet., abstr. 640). However, as we have demonstrated, when aP rather than wP was present in the concomitantly administered vaccine, a reduction in the primary immunogenicity of the pneumococcal polysaccharides conjugated to TT was observed.

We have considered various physicochemical possibilities that may have affected the integrity of the vaccine, either during the preparation of the lots or following the release of the lots from the manufacturing facility. All release criteria for the study lots were met, and the same vaccine lot as used in the study 3 was also used in other studies with good immunogenicity. Quality control analyses did not show any cold chain failure during shipping or storage of the clinical material (unpublished data). A number of tests were thus performed to confirm the integrity of the lots used in the study. The techniques included: (i) rabbit immunogenicity studies; (ii) size analysis by the multangle laser light scattering method; (iii) antigenicity analysis using EIA and nephelometry; and (iv) proton nuclear magnetic resonance analysis of the monovalent conjugates. The vials from the lot used in study 3 were retrieved from the clinical site and compared, using the techniques outlined above, to lots stored at the company site, monovalent conjugates and the results of the original testing. No significant differences could be detected. There was thus no reason to suspect that the lower immunogenicity in study 3 was due to the manufacturing failures of the specific lot used in this study or to changes in vaccine integrity following release and transport of the vaccine.

Previous descriptions of reduced immunogenicity of the glycoconjugate component of aP-containing combination vaccines have invoked the phenomenon of CIES as an explanation (10). CIES refers to the expansion of carrier-specific B cells and the consequent intramolecular antigenic competition between B cells for the same antigen, but typically it results in an increase in antibody response to the carrier with a decrease in the response to the conjugate partner (polysaccharide), which was not noted in this study. Carrier competition for T-cell help has also been suggested (4; Fattom et al., 36th Intersci. Conf. Antimicrob. Agents Chemother., abstr. G93, 1996) despite the
fact that the classic experiments of Leclerc et al. (15) demonstrated little role for CD4 T cells in CIES.

The reduced response to polysaccharide was only seen when aP replaced wP in the combination, suggesting that either the loss of the general adjuvanticity of wP (19, 21) or a potentially suppressive component of aP, such as FHA (16), may explain the observed phenomenon (20, 23). The latter is unlikely as the reduction in polysaccharide response following primary immunization is seen only in the TT-conjugated serotypes, suggesting a degree of antigen specificity for this mechanism.

The adjuvant effect of wP is likely to be important in activating carrier-specific CD4+ T cells. In this study, the ratio of tetanus-specific T cells relative to polysaccharide-specific B cells may have been reduced or cells suppressed by the relatively large amount of carrier protein, but masked by the adjuvant effect of wP in the wP-containing formulation. DT-conjugated polysaccharides were relatively spared (reduced responses to two of the DT-conjugated polysaccharides were only seen at the booster phase) because less diphtheria was incorporated in the aP vaccine formulation and more of each pneumococcal serotype was linked to DT (3 μg) compared to tetanus-linked polysaccharides (1 μg). This too may explain why the Hib response was spared as 10 μg of this polysaccharide was linked to the tetanus carrier. Other aP combinations in which suppression of the Hib response was seen (despite a dose of 10 μg of PRP) have generally contained larger absolute amounts of tetanus compared to the formulation we have studied and have also contained Al(OH)3, which in itself is thought to interfere with the integrity of the PRP (22), thus functionally reducing the amount of PRP available for the immune response.

It is thus likely that a delicate balance in the amount of the carrier protein(s) and polysaccharide present in the combined vaccine formulations finely regulates responses, particularly in the absence of an adjuvant such as wP. This hypothesis is further backed up by our findings that responses to the TT conjugates were improved in the alum-adjuvanted formulation studied in studies 1 and 2 (Dagan et al., 37th Annu. IDSA Meet., abstr. 640).

This study has revealed the increasing complexity associated with the development of combination vaccines using glycoconjugate vaccines and common carrier proteins. A pneumococcal conjugate vaccine formulation optimized on the basis of studies conducted with concomitant wP administration was found to perform suboptimally when a component of the concomitant vaccine was changed to aP. The interaction between the amount of carrier protein and the general adjuvanticity of wP seems critical to the response to conjugated polysaccharides, but both factors appear critical at different thresholds. Increased amounts of carrier protein can be tolerated when the vaccine is delivered together with a powerful adjuvant such as wP, but the tolerable threshold for carrier protein is reduced in the absence of such an additional stimulus.

Whether our finding has clinical implication remains somewhat unclear. A longstanding debate exists with regard to the relative importance of the concentration of circulating antipolysaccharide IgG versus the priming effect of previous doses in protection against invasive infections after administration of conjugate vaccines. Most authorities believe that the most important mechanism in prevention of invasive Hib or pneumococcal infections is priming and that an excellent protection against invasive infection can be achieved even in the presence of low circulating antibody levels (6). With regard to carriage and mucosal infections, such as acute otitis media, studies have demonstrated that protection may be correlated not only with priming, but also with specific humoral antipolysaccharide antibody concentrations (13). (Kilpi et al., 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 282, 2001; Dagan et al., 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. G-1526. 2001). This is supported by Väkeväinen et al. (24), who demonstrated the need for 2 to 6 times more anti-6B antibodies from 50% opsonophagocytic killing of serotype 6A than for serotype 6B. Thus, a vaccine with higher antibody concentrations may be more protective against acute otitis media or other mucosal infections caused by the serotypes in the vaccine on one side and play a more important role in herd protection than a vaccine that elicits a lower antibody response.

We have observed a significant reduced antibody response to S. pneumoniae serotype 1 after the primary vaccination series in study 1 compared to study 2. Furthermore, a tendency (0.001 < P < 0.05) for reduced response in study 1 compared to study 2 was observed for serotypes 3, 4, and 18C after the primary vaccination series and for serotypes 1, 4, 5, 9V, 18C, and 23F after the booster administration. We could not find a plausible explanation for this tendency for different responses in the two studies. However, except for serotype 1, the differences between the two studies were not in the same order of
magnitude as those between study 3 (with concomitant aP) and either study 1 or study 2 (with concomitant WP).

This study illustrates the importance of evaluating new vaccines together with concomitantly administered vaccines that are likely to be administered together when licensed. With the increasing use of aP vaccines in developed countries and the need to deliver more and more vaccines in the first year of life, new and novel approaches to adjuvants and carrier protein technology are likely to be required.

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