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Immune response to octavalent diphtheria- and tetanus-conjugated pneumococcal vaccines is serotype- and carrier-specific: the choice for a mixed carrier vaccine

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Background. **Development of protein-conjugated pneumococcal vaccines for infants has led to formulations that are immunogenic in the age group at highest risk for pneumococcal diseases. This study focuses on the search for an optimal formulation.**

Methods. **In a randomized trial Icelandic infants (n = 160) were immunized at age 3, 4 and 6 months with one of two octavalent pneumococcal conjugate vaccines (serotypes 3, 4, 6B, 9V, 14, 18C, 19F and 23F conjugated to diphtheria toxoid (PncD) or tetanus protein (PncT) followed with a booster of either the same conjugate or 23-valent polysaccharide vaccine at 13 months. Safety data were collected after each vaccination, and IgG responses (enzyme-linked immunosorbent assay) were measured at 3, 4, 6, 7, 13 and 14 months.**

Results. **Both conjugates were safe and caused fewer local reactions than the routine vaccines (P < 0.0001). At 7 months both groups had significant IgG response to all serotypes. The geometric mean concentration range was 0.35 to 4.09 and 0.65 to 3.38 μg/ml for PncD and PncT, respectively, with 88.2 to 100% and 92.4 to 100% of subjects reaching ≥0.15 μg/ml. The PncD gave better primary responses to serotypes 3, 9V and 18C, whereas PncT gave better response to serotype 4. Similar responses were induced to the other serotypes. Good booster IgG responses were obtained in all vaccine groups; 97.5 to 100% of subjects reached ≥1 μg/ml.**

Conclusions. **Both octavalent pneumococcal conjugates were safe and immunogenic in infants. Based on the results from this and similar trials, a mixed diphtheria and tetanus pneumococcal conjugate vaccine was designed to provide the optimal immune response to each serotype.**

INTRODUCTION

Streptococcus pneumoniae is a major cause of pneumonia, sepsis and meningitis in adults and children. More than 1 million children die from pneumococcal pneumonia each year, and it is a major pathogen in otitis media and sinusitis. Antibiotic resistance of pneumococci has been increasing, causing new concerns.[1]

A polysaccharide capsule surrounds the pneumococcus. Variations in the polysaccharide define >90 serotypes of *S. pneumoniae*. Serotype-specific polysaccharide antibodies provide protection against infection. Children <2 years of age are unable to produce protective antibodies to most of these polysaccharides, which are T cell-independent antigens.[2, 3] Protein-conjugated pneumococcal polysaccharide vaccines for pediatric use have been investigated for several years. These vaccines contain up to 11 serotypes and have different protein carriers that have been selected because of known safety and immunogenicity in infants. The immunogenicity of several pneumococcal conjugate vaccines has been demonstrated.[4–8] Conjugate vaccines have proven efficacious against *Haemophilus influenzae* type b infection.[9] Excellent efficacy of a 7-valent pneumococcal polysaccharide vaccine conju-

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gated to CRM_{197} has been demonstrated against invasive disease in infants[10] and moderate efficacy against middle ear infection and pneumonia.[11]

In this study the safety and immunogenicity of two octavalent pneumococcal conjugate vaccines were compared in 160 Icelandic infants. The eight serotypes, 3, 4, 6B, 9V, 14, 18C, 19F and 23F, were conjugated to either diphtheria toxoid (PncD vaccine) or tetanus protein (PncT vaccine). The booster responses elicited by the conjugate vaccines were compared with those elicited by the conventional polysaccharide vaccine. This work was part of a continuing search for the best formulation to induce protective immune response and memory in infants.

MATERIALS AND METHODS

Vaccines. The two octavalent pneumococcal conjugate vaccines contained capsular polysaccharides of serotypes 3, 4, 6B, 9V, 14, 18C, 19F and 23F conjugated with either diphtheria toxoid (3 μg of polysaccharide per serotype; PncD, Lot 940115) or tetanus protein (1 μg of polysaccharide per serotype; PncT, Lot S3004). The study vaccine was given intramuscularly in the right thigh concomitantly with a combined diphtheria-, tetanus-, whole cell pertussis- and diphtheria toxoid-conjugated *H. influenzae* vaccine (DTwP//PRP~D) administered intramuscularly in the left thigh. Inactivated polio vaccine (IPV; IMOVAX POLIO) was administered subcutaneously in one upper arm. Booster vaccinations were either with the same pneumococcal conjugate vaccine or a 23-valent pneumococcal polysaccharide vaccine containing 25 μg of polysaccharide from each of following serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F (PPS; Pneumo23). All vaccines were manufactured and supplied by Aventis Pasteur, Marcy l'Etoile, France.

Study population. During May through October, 1995, 160 healthy infants were recruited during routine child care visits at three health centers in Reykjavik and Hafnarfjordur, Iceland. The study was conducted according to Good Clinical Practice after having been approved by the ethics committees of the Landspitali-University Hospital in Reykjavik and the Icelandic Medical Society. Written informed consent was obtained from the parents of all infants before study enrollment. Infants were then randomized to receive a three dose primary vaccination with either the PncD (n = 80) or PncT (n = 80) pneumococcal conjugate at the same time as regular immunizations with DTwP//PRP~D at 3 and 4 months of age and DTwP//PRP~D and IPV at 6 months of age. At 13 months the infants were randomized again to receive a booster vaccination with either the same conjugate vaccine or PPS vaccine. Parents recorded local and systemic reactions in a safety diary for 3 consecutive days after each injection, and a study nurse made a home phone-call on Day 4. The diary was collected at next visit.

Samples. Blood samples were obtained from all infants before and after primary and booster vaccinations at ages 3, 7, 13 and 14 months of age. Additionally a random half of the infants had blood drawn at 4 months and blood was drawn from the other half at 6 months to evaluate the kinetics of the antibody response. For serotypes 6B, 19F and 23F antibody responses were measured at all time points. For the other serotypes, 3, 4, 9V, 14 and 18C, IgG antibodies were measured pre- and postpriming and postbooster.

Measurements. IgG antibodies to pneumococcal polysaccharides were measured by enzyme-linked immunosorbent assay (ELISA) at the Department of Immunology, Landspitali-University Hospital, according to the consensus ELISA protocol[12] with minor modifications. In brief ELISA plates (Maxisorp; Nunc, Roskilde, Denmark) were coated with 10 μg of the pneumococcal polysaccharide (American Type Culture Collection, Manassas, VA, except 6A from Aventis Pasteur) per ml for 5 h at 37°C. The international standard, 89-SF (kindly provided by Dr. Carl E. Frasch, Food and Drug Administration, Bethesda, MD) and test sera were diluted 1/50 and adsorbed with 10 μg/ml cell wall polysaccharide (Statens Seruminstitut, Copenhagen, Denmark), before incubation at four 2-fold dilutions for 2 h in the coated ELISA plates. Bound IgG was detected by 2 h of incubation with monoclonal antibody to human IgG, HP-6043-HRP (Hybridoma Reagent Laboratory, Baltimore, MD). The reaction was developed by tetramethylbenzidine (Kirkegaard & Perry Labs Inc., Gaithersburg, MD), and the reaction was stopped by addition of 0.18 M H_2SO_4. Optical density was measured at 450 nm in an ELISA spectrophotometer (Titertek Multiscan Plus MK II; Flow Laboratories, Irvine, UK). IgG antibody levels were calculated from the international standard 89-SF and expressed in μg/ml. Assignment of anti-6A IgG level in the 89-SF is currently not available and therefore 6A IgG levels were arbitrarily given the 6B IgG assigned levels but expressed in arbitrary units per ml (AU/ml).

Statistical analysis. Fisher's exact test was applied to compare the frequency of adverse events. The IgG antibody results are expressed as geometric mean concentration (GMC) with 95% confidence interval. A paired t test on log-transformed data was applied to evaluate the response within groups and t test for comparison between vaccine groups. Fisher's exact test was used to compare the number of infants in each group reaching $\geq$0.15 μg/ml and $\geq$1.0 μg/ml. Pearson's correlation coefficient was used to calculate correlation between IgG antibodies to serotypes 6A and 6B.

RESULTS

One hundred sixty healthy, full term infants were recruited and randomized to receive either PncD or PncT. One hundred fifty-seven infants (98%) completed the primary vaccination series and were analyzed for safety and immunogenicity at 7 months of age. Eighty received PncD, and 77 received PncT. At 13 months of age 152 (95%) infants received a booster injection with either the same conjugate or PPS, and 149 (93%) were evaluated for safety and immunogenicity at 14 months of age. For the analyses infants were grouped according to the vaccine received at priming and booster: PncD/PncD, 39 infants; PncD/PPS, 39 infants; PncT/PncT, 34 infants; and PncT/PPS, 40 infants (Tables 1 to 3).

Safety. No severe adverse events that were related to the vaccines occurred and no immediate reactions (appearing within 15 min of vaccination) were the result of the trial vaccines. Local reactions (erythema, swelling, induration or pain) appeared during the 3 days after each vaccination. No differences were apparent between the two conjugate vaccine groups after the three primary injections (Table 1). Compared with previous injection, increased total local reactions were recorded ($P \leq 0.05$) after the third dose of both trial vaccines but only significant for pain in the PncD group which was the most commonly observed reaction. After each vaccination with PncD or PncT, concomitantly with DTwP//PRP~D, more local reactions were observed at the DTwP//PRP~D vaccination site than at the site of the study vaccines ($P < 0.0001$ for all parameters). After the booster dose fewer PncD recipients experienced local reactions than did PncT recipients ($P = 0.0153$), mainly because of a difference in the occurrence of redness between the two groups ($P = 0.0215$). The differences between the PncD/PncD and PncT/PncT groups for the other local reactions after the booster were not significant. When the conjugate vaccines were compared with the polysaccharide vaccine, PncD induced significantly fewer local reactions than PPS for all parameters. Although PncT induced fewer reactions than did PPS, differences were significant for pain only ($P = 0.0006$).

Systemic reactions including fever, irritability, drowsiness, crying anorexia, diarrhea and vomiting were recorded (Table 2). Systemic reactogenicity appeared similar for the conjugate vaccine groups at all time points. The PncD booster caused fewer febrile reactions that the PPS booster ($P = 0.026$). No other differences between vaccines were significant. Compared with previous injection, less systemic reactions were recorded after the second dose (any reaction, $P < 0.0001$; fever, $P < 0.001$ for both groups), but subsequently increased after the third dose (any reaction, $P < 0.01$; fever, $P < 0.0001$). Booster dose with both conjugate vaccines at 13 months resulted in less systemic reactions than the third dose at 6 months of age (any reaction, $P < 0.0001$; fever, $P < 0.0001$).

Antibody responses. The three primary vaccinations at 3, 4 and 6 months of age with the PncD and PncT pneumococcal conjugates induced a significant

TABLE 1. Proportion of infants presenting local reactions during the 3 days after three doses of two octavalent pneumococcal conjugate vaccines (PncT and PncD), and the concomitantly administered DTwP//PRP~D vaccine, and after a booster dose of either the same conjugate vaccine or PPS

	Proportion of Infants (%)			
	PncD	DTwP//PRP~D	PncT	DTwP//PRP~D
First dose (3 mo of age)		$N = 80$		$N = 80$
Any reaction	12.5	72.5	20	66.3
Redness	1.3	50.0	5.0	43.8
Induration	3.8	35.0	3.8	36.3
Swelling	3.8	47.5	7.5	45.0
Pain	12.5	55.0	18.8	48.8
Second dose (4 mo of age)		$N = 80$		$N = 78$
Any reaction	13.8	53.8	14.1	62.8
Redness	7.5	43.8	5.1	41.0
Induration	5.0	27.5	2.6	25.6
Swelling	5.0	28.8	3.8	39.7
Pain	7.5	21.3	9.0	35.9
Third dose (6 mo of age)		$N = 77$		$N = 80$
Any reaction	27.5	70	28.6	81.8
Redness	10.0	45.0	7.8	55.8
Induration	5.1	26.3	1.3	27.3
Swelling	3.8	43.8	6.5	46.8
Pain	20.0	45.0	19.5	55.8
	PncD	PPS	PncT	PPS
Booster dose (13 mo of age)	N=39	N=39	N=34	N=40
Any reaction	12.8	69.2	38.2	55.0
Redness	2.6	41.0	20.6	30.0
Induration	5.1	25.6	11.8	20.0
Swelling	7.7	30.8	11.8	27.5
Pain	7.7	56.4	14.7	55.0

TABLE 2. Proportion of infants presenting systemic adverse events during the 3 days after three doses of two octavalent pneumococcal conjugate vaccines (PncD and PncT) administered concomitantly with DTwP//PRP~D vaccine at 3, 4 and 6 months and after a booster dose of either the conjugate vaccine or a 23-valent polysaccharide vaccine (PPS)

	Proportion of Infants (%)									
	First Dose* (3 mo of Age)		Second Dose (4 mo of Age)		Third Dose (6 mo of Age)		Booster Dose (13 mo of Age)			
	PncD (N = 80)	PncT (N = 80)	PncD (N = 80)	PncT (N = 78)	PncD (N = 80)	PncT (N = 77)	PncD (N = 39)	PPS (N = 39)	PncT (N = 34)	PPS (N = 40)
Any systemic side effect	95	96.3	71.3	74.4	88.8	93.5	51.3	64.1	58.8	75.0
Fever ≥38°C	58.2	68.4	31.3	39.0	68.8	79.2	17.9	43.6	29.4	50.0
Irritability	87.5	81.3	47.5	52.6	58.8	70.1	25.6	38.5	38.2	45.0
Drowsiness	46.3	45.0	33.8	33.3	32.5	24.7	12.8	23.1	17.6	17.5
Crying	68.8	63.8	31.3	38.5	48.8	50.6	17.9	30.8	26.5	35.0
Anorexia	26.3	31.3	8.8	19.2	30.0	31.2	17.9	33.3	14.7	30.0
Diarrhea	17.5	16.3	15.0	12.8	16.3	15.6	28.2	20.5	8.8	17.5
Vomiting	8.8	17.5	8.8	9.0	16.3	10.4	2.6	2.6	14.7	0

* First and second doses were given concomitantly with DTwP//PRP~D, third dose was given concomitantly with DTwP//PRP~T and IPV.

TABLE 3. Geometric mean serotype-specific IgG levels to each of the eight serotypes of two octavalent pneumococcal conjugate vaccines (PncT and PncD) before and 4 weeks after the three primary series injections (3 and 7 months of age) and 4 weeks after booster vaccination with a conjugate vaccine (PncD or PncT) or 23-valent pneumococcal polysaccharide vaccine (PPS) at 13 months of age (14 months)

Serotype	Geometric Mean Serotype-specific IgG Level							
	3 mo		7 mo (postprimary)*		14 mo (postbooster; primary vaccine/booster vaccine)†			
	PncD (N = 80)	PncT (N = 80)	PncD (N = 80)	PncT (N = 77)	PncD/PncD (N = 39)	PncD/PPS (N = 38)	PncT/PncT (N = 33)	PncT/PPS (N = 40)
3	0.36 (0.29; 0.45)	0.32 (0.27; 0.38)	3.40 (2.88; 4.01)	2.04 (1.78; 2.33)	3.93 (2.88; 5.36)	7.59 (5.55; 10.4)	1.99 (1.59; 2.49)	6.44 (4.79; 8.65)
4	0.27 (0.21; 0.34)	0.2 (0.16; 0.25)	0.351 (0.31; 0.4)	1.44 (1.22; 1.70)	0.75 (0.56; 1.01−)	3.30 (2.48; 4.39)	2.38 (1.87; 3.03)	7.67 (4.69; 12.6)
6B	0.57 (0.45; 0.71)	0.40 (0.31; 0.52)	1.01 (0.83; 1.22)	1.24 (0.96; 1.59)	2.51 (1.69; 3.73)	2.13 (1.44; 3.16)	2.65 (1.78; 3.93)	3.62 (2.26; 5.8)
9V	0.318 (0.25; 0.40)	0.24 (0.19; 0.29)	0.888 (0.79; 1.00)	0.646 (0.53; 0.79)	2.05 (1.51; 2.78)	2.47 (1.82; 3.36)	1.52 (1.15; 2.01)	4.24 (3.10; 5.79)
14	1.69 (1.34; 2.13)	1.08 (0.85; 1.38)	3.58 (2.99; 4.28)	3.02 (2.47; 3.70)	6.54 (4.70; 9.11)	4.29 (2.93; 6.29)	5.65 (4.02; 7.94)	10.4 (7.14; 15.2)
18C	0.28 (0.23; 0.35)	0.22 (0.18; 0.26)	0.85 (0.74; 0.98)	0.49 (0.40; 0.59)	1.30 (1.06; 1.60)	2.19 (1.61; 2.98)	0.81 (0.62; 1.04)	3.00 (2.24; 4.02)
19F	0.68 (0.56; 0.82)	0.59 (0.47; 0.74)	4.09 (3.49; 4.79)	3.38 (2.8; 4.07)	5.6 (3.93; 7.98)	8.54 (5.75; 12.7)	9.01 (7.01; 11.6)	19.4 (13.0; 29.0)
23F	0.53 (0.42; 0.66)	0.44 (0.35; 0.55)	1 (0.86; 1.17)	1.08 (0.9; 1.3)	1.9 (1.37; 2.63)	1.64 (1.23; 2.18)	1.9 (1.46; 2.49)	3.01 (2.1; 4.32)

* Significant rises in specific IgG to all serotypes were induced by both vaccines after primary and booster vaccination ($P < 0.0001$).
† Groups according to primary/booster vaccination.
‡ Numbers in parentheses, 95% confidence interval.

rise in serotype-specific IgG to all eight serotypes ($P < 0.0001$) (Table 3; Fig. 1). No differences in GMC levels were observed between the PncD and PncT groups for serotypes 6B, 19F and 23F before, during or 1 month after the primary vaccination series. The proportion of infants reaching IgG level ≥0.15 μg/ml 1 month after the third dose of PncD and PncT, respectively, was 100 and 97% for serotype 6B, 99 and 100% for serotype 19F and 99 and 99% for serotype 23F. The conjugates of serotype 14 were equally immunogenic, and 100% reached 0.15 μg/ml in both groups. The PncD vaccine induced a higher primary response to serotypes 3 ($P < 0.0001$), 9V ($P = 0.0077$) and 18C ($P < 0.0001$); 100% in both groups reached 0.15 μg/ml against serotype 3, 100% *vs.* 94.7% against serotype 9V and 100% *vs.* 88% against serotype 18C, for PncD and PncT recipients, respectively. In contrast PncT induced a higher primary response to serotype 4, ($P < 0.0001$); 100% reached 0.15 μg/ml *vs.* 92% in the PncD group (data not shown).

Good booster responses (Table 3; Fig. 1) were observed in all four groups ($P < 0.0001$) 1 month after booster immunization at 13 months with either the same conjugate vaccine as used for the primary series or the polysaccharide vaccine. In the PncT-primed groups, the PPS booster induced higher IgG concentrations than did the PncT booster against five of the eight serotypes, namely serotypes 3, 4, 9V, 18C and19F. The same was observed for serotypes 3, 4 and 18C in the PncD group. One month after the booster all infants in all groups had IgG concentrations ≥1 μg/ml for all serotypes, except for serotype 6B in the PncT/PPS

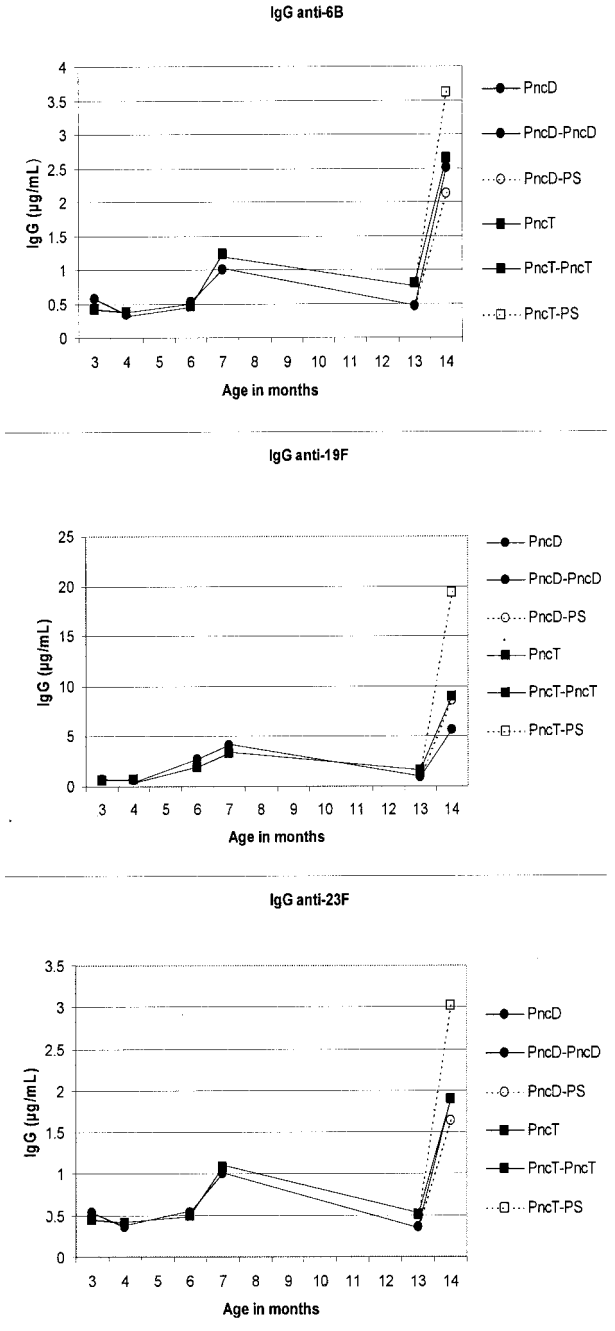

FIG. 1. IgG geometric mean concentrations for serotype 6B, 19F and 23F in each pneumococcal conjugate group, PncD (●) and PncT (■), before, during and after primary injections at 3, 4 and 6 months and before and after booster at 13 months either with the same conjugate (*closed symbols*) or the 23-valent vaccine pneumococcal polysaccharide (*PS*) vaccine (*open symbols*).

group and serotype 9V in the PncD/PncD group in which 97.5% of the infants reached 1 μg/ml (data not shown).

The IgG response to serotype 6A, which is cross-reactive with 6B, was measured after primary and booster immunization at 7 and 14 months of age, in a subgroup of 82 infants who received either same conjugate or PPS as a booster dose (Table 4). The levels of anti-6A IgG (in AU) and anti-6B IgG (in micrograms per ml) at 7 and 14 months of age were almost comparable and correlated significantly at 14 months ($P < 0.001$). This demonstrates that the 6B-tetanus and 6B-diphtheria conjugates elicit antibodies that cross-react with serotype 6A.

DISCUSSION

We demonstrated the safety and immunogenicity of two monocarrier octavalent pneumococcal conjugate vaccines, PncD and PncT, in infants. In agreement with the reports of other investigators for these and similar vaccines,[13, 14] the trial vaccines caused only mild local reactions and overall were better tolerated than DTwP//PRP~D given concomitantly. The collectively fewer local reactions after the PncD *vs.* PncT may be a result of a lower reactogenicity of the carrier protein.

Systemic reactions to the trial vaccines during the primary series could not be assessed as all infants received concomitant vaccines. However, after the booster dose given alone at 13 months of age, fewer infants experienced systemic events than during the primary series. Possible explanations for this lower reactogenicity include a lower reactogenicity of the conjugate vaccines *vs.* the coadministered DTwP// PRP~D vaccine or a lower reactogenicity in older infants. This is in concordance with safety results for the heptavalent pneumococcal CRM_{197} conjugate vaccine where a much lower reactogenicity was observed after a separate conjugate booster dose.[15] The polysaccharide booster caused more local reactions than the conjugate vaccine booster did. This may have been because of the higher polysaccharide dose for each serotype (25 μg compared with 3 and 1 in PncD and PncT, respectively) or the higher total polysaccharide dose (575 μg *vs.* 24 and 8 μg, respectively). The systemic safety profiles in the conjugate-booster groups and the polysaccharide-booster groups were similar.

Primary vaccination with both trial vaccines elicited significant IgG responses to all serotypes in the vaccine with >88% of 7-month-old infants reaching IgG antibody concentrations >0.15 μg/ml and a significant proportion reaching 1 μg/ml. The value of 0.15 μg/ml has been shown to reflect functional seroconversion measured by opsonophagocytosis and to discriminate vaccinated subjects from unvaccinated controls.[16] In the Kaiser Permanente Vaccine Study, the 7-valent CRM_{197} conjugate induced antibody concentrations to $\geq$0.15 μg/ml to all serotypes in 97% of the infants which correlated well with the 97% efficacy against invasive pneumococcal disease. Their data indicated also that this concentration might vary between serotypes.[17] It is also likely that the protective values may vary depending on the site of infection. Thus higher antibody concentration may be needed to protect at the mucosal sites as indicated by much lower efficacy of CRM_{197} against otitis media

TABLE 4. Specific IgG to the cross-reactive serotypes 6B and 6A at 7 and 14 months

	N	GMC		*P**	*R*†
		6B (μg/ml)	6A (AU)		
Postprimary at 7 mo					
PncD	39	1.02 (0.29; 4.76)‡	0.78 (0.21; 3.1)‡	0.058	0.370 (0.022)§
PncT	41	1.23 (0.1; 4.33)	1.00 (0.33; 6.83)	0.113	0.317 (0.0046)
P¶		0.397	0.149		
Postbooster at 14 mo					
PncD	37	2.5 (0.3; 8.58)	2.27 (0.62; 10.69)	0.52	0.517 (0.001)
PncT	40	3.63 (0.39; 19.25)	2.56 (0.46; 21.08)	0.0458	0.551 (0.0002)
P		0.14	0.597		

* Comparison of IgG antibodies to serotypes 6A and 6B with paired *t* test.
† Pearson's correlation between IgG anti-6A and anti-6B.
‡ Numbers in parentheses, 95% confidence interval.
§ Numbers in parentheses, *P*.
¶ Comparison of IgG levels between PncD and PncT groups with *t* test.

compared with invasive disease.[11, 17] In our study not only did immunogenicity vary between the serotypes; it also varied between conjugates of the same serotype. Conjugation with diphtheria toxoid resulted in a higher primary response to serotypes 3, 9V and 18C but a lower response to serotype 4. The two conjugates appeared equally immunogenic for serotype 6B, 14, 19F and 23F. Concomitant administration of PRP-D did not result in lower pneumococcal antibodies in the PncD group, but PRP antibodies were lower than in the PncT group.[18] Similarly a decreased PRP response has been reported when *H. influenzae* type b vaccine shares tetanus protein carrier with the pneumococcal conjugate vaccine.[19] This influence of the protein carrier can be in both directions as an increased PRP response has been reported for *H. influenzae* type b vaccine when sharing CRM_{197} carrier with the Pnc conjugates.[20, 21] The significant antibody concentrations at 7 months of age strongly suggests that memory B cells have been generated during the primary vaccination with the conjugate vaccines that have provided the necessary T cell help. This is also reflected in the strong responses to the PPS at 13 months, an age when children would normally not respond to native polysaccharides.[2] At 14 months almost all the infants reached an IgG concentration of ≥ 1 μg/ml for all serotypes. For five serotypes, namely serotype 3, 4, 9V, 18C and 19F, the PPS booster caused a higher booster response than the PncT conjugate vaccine. The same was seen for serotypes 3, 4 and 18C in the PncD group. This may be caused by the 8- to 25-fold higher dose of polysaccharide in the PPS vaccine (25 μg/serotype) than in the conjugate vaccines. Based on our study and similar studies with either the same two octavalent vaccines[14] or other formulations, it was concluded that diphtheria toxoid conjugates are the best choice for serotype 3, 6B, 14 and 18C and tetanus protein conjugates are the best choice for serotypes 4, 9V, 19F and 23F. An 11-valent mixed diphtheria toxoid and tetanus protein pneumococcal conjugate vaccine has been developed and found to be safe and immunogenic in infants.[22–24]

The dosage of each polysaccharide is also critical as shown by comparing 1, 3 and 10 μg of each serotype polysaccharide in tetravalent D- and T-conjugated pneumococcal vaccines (serotype 6B, 14, 19F and 23F) in Finnish infants.[5] In that study the10 μg of D conjugate induced the highest primary responses, although the response was not much stronger than that induced by the 3 μg D conjugates. In children receiving the T conjugate, no dose response was observed in the primary series, and the best booster response induced by a PPS vaccine at 14 months was observed in the group who had received the lowest primary dose, suggesting the lower dose as optimal priming dose for memory induction.[6]

We have previously reported the strong correlation between the IgG concentrations with opsonic activity in these infants.[25] Persistence of antibodies and long term memory remain to be demonstrated. The conjugate vaccine induced IgG antibodies to serotype 6A to almost same degree as to serotype 6B. This cross-reactivity was further demonstrated with a subset of postbooster sera from this study that were tested for passive immunization against type 6A and 6B pneumococcal bacteremia and lung infection in mice. Cross-protection against serotype 6A was demonstrated, and the protection was shown to be strongly related to IgG antibody levels and opsonic activity.[26]

We conclude that both octavalent diphtheria- and tetanus-conjugated pneumococcal vaccines were safe and immunogenic in infants, but neither was optimal for all eight serotypes. An improved multivalent pneumococcal conjugate vaccine can be obtained by selecting the better of the two carriers for each serotype and combining them into a mixed diphtheria and tetanus carrier, multivalent conjugate vaccine.

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