MAJOR ARTICLE

Predictors of Pneumococcal Conjugate Vaccine Immunogenicity among Infants and Toddlers in an American Indian PnCRM7 Efficacy Trial

Katherine L. O'Brien,[1] Jennifer Moïsi,[2] Lawrence H. Moulton,[2] Dace Madore,[3,a] Angelia Eick,[1] Ray Reid,[1] Robert Weatherholtz,[1] Eugene Millar,[1] Diana Hu,[4] Jill Hackell,[3] Robert Kohberger,[3,a] George Siber,[3] and Mathuram Santosham[1]

[1]Center for American Indian Health and [2]Departments of International Health and Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; [3]Wyeth Vaccines, Pearl River, New York; [4]Indian Health Service, Department of Health and Human Services, Tuba City, Arizona

Background. Pneumococcal conjugate vaccines are important for the prevention of serious illness and death among infants. Factors associated with pneumococcal conjugate vaccine immunogenicity have not been explored.

Methods. Children <24 months of age received 2, 3, or 4 doses of 7-valent pneumococcal conjugate vaccine (PnCRM7) or control vaccine depending on age at enrollment. Serum samples were tested for serotype-specific antibodies by enzyme-linked immunosorbant assay. Multiple linear regression was used to determine predictors of immunogenicity.

Results. Among 315 PnCRM7-vaccinated subjects and 295 control subjects enrolled at <7 months of age, geometric mean concentrations (GMCs) of antibodies were significantly higher after dose 3 than after dose 2 for all serotypes except type 4. The proportion of subjects with antibody concentrations ⩾5.0 μg/mL was higher for all serotypes, but the proportion with concentrations ⩾0.35 μg/mL was higher only for types 6B and 23F. Three-dose and 2-dose regimens for those 7–11 and 12–23 months of age, respectively, were highly immunogenic. Increased maternal antibody concentrations were associated with reduced responses to dose 1 and 3 but not to dose 4 of PnCRM7.

Conclusions. Maternal antibody is associated with a reduced infant response to PnCRM7 but does not interfere with immune memory. In infants, a third priming dose increases the antibody GMC and the proportion achieving an antibody concentration ⩾5.0 μg/mL but has little impact on the proportion achieving a concentration ⩾0.35 μg/mL.

Pneumococcal polysaccharide-protein conjugate vaccines (PCVs) were developed to overcome the inadequate immunogenicity of polysaccharide pneumococcal vaccines among infants, a group at high risk for invasive pneumococcal disease. The immunogenicity of PCVs varies by the polysaccharide, the carrier protein, concomitant vaccines, and the immunized population.

Seven-valent PCV (PnCRM7; serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F; Prevnar, Wyeth Vaccines) immunogenicity has been reported in the general US population [1, 2], in those with recurrent otitis media [3], HIV infection [4], and sickle cell disease [5]. Immunogenicity for the 9-valent (adding serotypes 1 and 5) PnCRM has been reported in The Gambia [6], South Africa [7, 8], and the United Kingdom [9]. Limited data have been published on the immunogenicity after each dose in a series or on the relative immunogenicity

Received 30 August 2006; accepted 21 December 2006; electronically published 24 May 2007.

Reprints or correspondence: Dr. Katherine L. O'Brien, 621 N. Washington St., Baltimore, MD 21205 (klobrien@jhsph.edu).

The Journal of Infectious Diseases 2007; 196:104–14

0022-1899/2007/19601-0017$15.00
DOI: 10.1086/518438

Potential conflicts of interest: participation in Wyeth Scientific advisory boards (K.L.O.B. and M.S.), receipt of research funding from Wyeth Vaccines (K.L.O.B., L.H.M., R.R., R.W., and M.S.), and stock ownership in Wyeth Vaccines (J.H., R.K., D.M., and G.S.).

Presented in part: 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, 16–19 December 2001 (abstract 81); 5th International Symposium on Pneumococci and Pneumococcal Diseases, Alice Springs, Australia, 2–6 April 2006 (abstract SY 4.07).

Financial support: Wyeth Vaccines; National Institutes of Health; World Health Organization; National Vaccine Program Office; Centers for Disease Control and Prevention.

The opinions expressed here are those of the authors and do not necessarily reflect the views of the US Indian Health Service.

[a] Present affiliations: retired (D.M.); independent consultant (R.K.).

at various ages in the same population [8–10]. Most immunogenicity studies have evaluated the response to some of the doses [1–7]. No studies have evaluated predictors of the immune response to PCV. Given that PCVs are now being seriously considered for global introduction, it is critical to understand the determinants of immunogenicity.

Some Native American populations—notably Alaska Natives, Navajo, and White Mountain Apaches—have higher rates of pneumococcal nasopharyngeal colonization and invasive pneumococcal disease than the general US population [11–13], epidemiologic characteristics similar to those found in many developing countries. The basis for this increased risk is unknown; hypotheses include household crowding, indoor air pollution, infection pressure from viral respiratory illnesses, and host factors (i.e., reduced immune response to polysaccharide antigens). We conducted an efficacy trial of PnCRM7 among Navajo and White Mountain Apache Indians [14] and demonstrated efficacy against vaccine-serotype invasive pneumococcal disease of 76.8% (95% confidence interval [CI], −9.4 to 95.1), similar to that found among children in The Gambia (77% [95% CI, 51 to 90]) and among HIV-negative children in South Africa (83% [95% CI, 39 to 97]). We report here the immunogenicity results of the American Indian PnCRM7 trial.

METHODS

Study description. This was a group-randomized efficacy trial conducted between April 1997 and May 2000 on the Navajo and White Mountain Apache reservations in the southwestern United States. All children in a defined community who were 6 weeks to 23 months of age and whose parents or guardians provided consent for participation were assigned to receive the same vaccine [15]. The intervention vaccine was PnCRM7, and the control vaccine was *Neisseria meningitidis* group C protein conjugate vaccine (MnCC; Wyeth Vaccines) [14].

Subjects enrolled between 6 weeks and 6 months of age (primary efficacy [PE] group) received 3 doses of vaccine 2 months apart (4 weeks minimum) and a booster dose at 12–15 months of age (at least 2 months after dose 3). Subjects enrolled between 7 and 11 months of age (infant catch-up [ICU] group) received 2 doses of vaccine 2 months apart (4 weeks minimum) and a booster dose at 12–15 months of age (at least 2 months after dose 2). Subjects enrolled between 12 and 23 months of age (toddler catch-up [TCU] group) received 2 doses of vaccine at least 2 months apart. Children enrolled in the study also received routine childhood vaccines (table 1).

A subset of the efficacy trial participants were enrolled in a nested immunogenicity evaluation. Their blood was drawn at predetermined times according to their vaccine schedule (table 1).

Antibody determination by ELISA. All serum samples were tested at Wyeth Vaccines in a blinded fashion for serotype-specific anti-pneumococcal IgG antibodies by a standard ELISA without 22F absorption [16] and for *Haemophilus influenzae* type b (Hib) polyribosylribitolphosphate polysaccharide (PRP) IgG [17]. The pneumococcal antibody concentration limit of quantitation was 0.01 μg/mL; samples with a value below this were assigned a value of 0.005 μg/mL.

Statistical analysis. Only participants' observations meeting per-protocol definitions (available from authors) were included in the analyses, up to the point of the first protocol violation. For any particular sampling time, all eligible observations were included.

At each sampling time and for each vaccine serotype, the geometric mean concentration (GMC) of antibodies (with a standard 95% confidence interval [CI]) and reverse cumulative distribution curves were calculated. Comparisons of responses over time and across serotypes were made using the Spearman rank correlation coefficients for the logarithms of antibody concentrations. Proportions of children achieving various antibody concentrations are reported with 95% CIs. We used 0.35 μg/mL (the proposed correlate of protection against invasive disease derived by combining results of the American Indian, northern California, and South African PCV efficacy trials [18, 19]), 1.0 μg/mL (the correlate against invasive disease efficacy in the American Indian PnCRM7 efficacy trial [20]), and 5.0 μg/mL (the proposed correlate of protection against nasopharyngeal colonization [21]) as antibody concentrations of interest in our analysis of response to PnCRM7.

Among subjects in the PE group, we explored the effects of maternally derived antibody, age at dose 1, sex, dosing interval, and breast-feeding status on the antibody response to dose 1, 3, and 4. We fitted multiple linear regression (MLR) models using log-transformed antibody concentrations. For the dose 1 analysis, we also adjusted for the expected deterioration in maternally derived antibody, to separate out the parameter of interest, infant response to vaccine, from the remaining maternal antibody. We used MLR to model the reduction in maternally derived pneumococcal antibody from before to after dose 1 among the children receiving MnCC. Using this model, we predicted the post–dose 1 serotype-specific pneumococcal antibody concentration for each PnCRM7-vaccinated subject on the basis of their pre–dose 1 antibody concentration and other covariates. Using the logarithmic values, the predicted post–dose 1 value was subtracted from the observed post–dose 1 value to obtain the infant response to vaccine. Test results were considered to be statistically significant if the 2-tailed P value was $<.05$.

Ethics and consent. The study was approved by the institutional review boards of the Johns Hopkins School of Medicine, the Navajo Nation, the Phoenix Area Indian Health Service, and the US Indian Health Service. Tribal approval was given by the Navajo Nation and the White Mountain Apache

Table 1. Schedule of study vaccine, routine concomitant vaccine, and blood draws, by age and study group.

	Age														
Item	Birth	6 weeks	4 months	6 months	7 months	7–11 months	10–13 months	11–14 months	12 months	15 months	13–16 months	12–23 months	13–24 months	14–25 months	15–26 months	21–27 months
Hepatitis B	X	X[a]							X[a]							
DTaP		X	X	X												
IPV/OPV		X	X						X							
Hib-OMP		X[a]	X						X[a]							
MMR									X							
Varicella										X						
PnCRM7 or MnCC (dose no.)		PE1	PE2	PE3		ICU1	ICU2		PE4, ICU3			TCU1		TCU2		
Blood sample		IPE[b]	IPE[b,c]	IPE[b]	PE[b,c]			ICU[b]	IPE[b]		PE,[b] ICU[b]		TCU[b]		TCU[b]	PE[b]

NOTE. DTaP, diphtheria-tetanus toxoids–pertussis vaccine; Hib, *Haemophilus influenzae* type b; ICU, infant catch-up group; IPE, intensely monitored primary efficacy subjects only; IPV, injected polio vaccine; OMP, meningococcal outer membrane protein complex; OPV, oral polio vaccine; MMR, measles, mumps, and rubella vaccine; MnCC, *Neisseria meningitidis* group C protein conjugate vaccine; PE, primary efficacy group; PnCRM7, 7-valent pneumococcal conjugate vaccine; TCU, toddler catch-up group.

[a] Administered as Comvax (Merck Laboratories).

[b] Sample assayed for IgG antibodies to *Streptococcus pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F to *N. meningitidis* group C.

[c] Sample assayed for IgG antibodies to Hib polyribosylribitolphosphate polysaccharide.

Tribe. Parents or guardians of all enrolled children provided written, informed consent.

RESULTS

A total of 315 PnCRM7- and 295 MnCC-immunized subjects were included in the PE analysis (mean age at dose 1, 2.8 months; 49.1% male). There were 91 (49 PnCRM7 and 43 MnCC immunized; mean age at dose 1, 9 months; 52.9% male) and 307 (155 PnCRM7 and 152 MnCC immunized; mean age at dose 1, 16.4 months; 55.7% male) subjects included in the ICU and TCU groups, respectively (tables 1 and 2). Observations after dose 1 include smaller numbers of subjects because of protocol violations.

Pneumococcal immunogenicity in the PE group. PnCRM7 resulted in significantly increased antibody concentrations after each dose for all serotypes, compared with the control vaccine (table 2). The kinetics of response varied by serotype; the greatest increase in antibody concentration was observed for serotypes 6B and 14, depending on the dose (table 2 and figure 1). The proportion of PnCRM7-vaccinated subjects achieving an antibody concentration $\geq$0.35 μg/mL after dose 1 ranged by serotype from 20.4% to 78.7% (figure 1). The proportion of subjects achieving an antibody concentration $\geq$0.35 μg/mL after dose 3 was greater than that after dose 2 only for serotypes 6B ($P = .01$) and 23F ($P < .001$). The proportion of subjects achieving an antibody concentration $\geq$1.0 μg/mL after dose 3 was greater than that after dose 2 for all serotypes except type 4 (for serotype 14, $P = .02$; for all others, $P < .001$). The proportion of subjects who achieved a serotype-specific antibody concentration $\geq$5.0 μg/mL increased from after dose 2 to after dose 3 for all serotypes (for 4, $P = .009$; for all others, $P < .001$) and from after dose 3 to after dose 4 for all serotypes except type 14 ($P < .001$, for all). Dose 3 stimulated a large antibody response, suggestive of a booster or memory response, for serotype 6B only (figure 2). An antibody response to a fourth dose of PnCRM7 large enough to suggest a memory response was seen for all serotypes (table 2).

Although the proportion of subjects in the PE group achieving an antibody concentration $\geq$0.35 μg/mL did not increase after dose 3 compared with that after dose 2 for most serotypes, the antibody GMCs achieved after dose 3 were significantly higher than those achieved after dose 2 for all serotypes except type 4 (figure 2). By 9–12 months after dose 4, there was up to a 10-fold reduction in serotype-specific antibody concentrations, compared with those achieved immediately after dose 4, for all serotypes except type 19F; the antibody concentrations were higher for all serotypes among those who received PnCRM7 than among those who received MnCC.

Pneumococcal immunogenicity among subjects immunized between 7 and 23 months of age. Significant antibody responses were achieved for all serotypes after 2 doses of PnCRM7 among subjects initiating vaccination between 7 and 11 months (the ICU group) and after 1 dose for subjects initiating between 12 and 23 months (the TCU group), compared with the responses in subjects receiving MnCC (table 3). The proportion of subjects achieving an antibody concentration $\geq$0.35 μg/mL ranged by serotype from 82% to 94% after dose 2 in the ICU group and from 64% to 95% after dose 1 in the TCU group (figure 3). The last dose administered in each vaccine series elicited an increase in the antibody GMC of sufficient magnitude to suggest a booster response for serotypes 6B, 14, and 23F in the ICU group and for serotypes 6B, 14, 19F, and 23F in the TCU group. For the remaining serotypes, the magnitude of the increase in antibody concentration relative to that from the preceding dose was not substantial enough to unequivocally indicate a booster response.

Effects of maternally derived antibody, breast-feeding, age at first dose, sex, and dosing interval on the response to PnCRM7 in the first year of life. We evaluated the effects of maternally derived antibody, breast-feeding, age at first dose, sex, and dosing interval on the pneumococcal antibody response after dose 1, 3, and 4. In a univariate analysis, each variable was associated with the vaccine response for at least 1 serotype and dose (data not shown). We retained all variables in MLR models created for each serotype and each dose.

Increasing maternal pneumococcal antibody concentrations were associated with a lower response to dose 1 of PnCRM7 for all serotypes ($\beta = -0.19$ to $\beta = -0.78$; $P \leq .001$) except types 18C and 23F ($\beta = -0.08$ and $\beta = 0.03$, respectively; $P > .05$) and a lower response to dose 3 for all serotypes ($\beta = -0.09$ to $\beta = -0.21$; $P < .05$) except type 18C ($\beta = 0.03$; $P > .05$). There was no association between high maternal antibody concentrations and the response to dose 4 for any serotype. For some combinations of serotype and dose, there were associations between other variables in the model and the response to vaccine, but these were not consistently observed (data not shown).

The proportion of subjects who achieved an antibody concentration $\geq$0.35 μg/mL after dose 1 varied by maternal antibody tertile; the middle tertile had the lowest proportion for serotypes 9V, 14, 18C, 19F, and 23F, whereas the highest tertile had the lowest proportion for serotype 4 and the highest proportion for serotypes 6B, 14, 19F, and 23F. Among those subjects with intermediate pre–dose 1 antibody concentrations, maternal antibody both interfered with response to vaccine and was insufficiently high to sustain levels $\geq$0.35 μg/mL through the first several months of life. The proportion of subjects achieving an antibody concentration $\geq$0.35 μg/mL after dose 3 did not vary by maternal antibody tertile except for serotype 4; those children with intermediate maternal antibody concentrations had the lowest proportion achieving the threshold.

Correlation between responses to PnCRM7 serotypes. The antibody response of a child to a given serotype correlated with

Table 2. Geometric mean concentrations (95% confidence interval) of antibody to each pneumococcal serotype in the intensely monitored primary efficacy subjects receiving 7-valent pneumococcal conjugate vaccine (PnCRM7) or *Neisseria meningitidis* group C protein conjugate vaccine (MnCC).

Serotype, vaccine	Before dose 1 (n = 315[a] and 295[b])	After dose 1 (n = 215[a] and 199[b])	After dose 2 (n = 202[a] and 199[b])	After dose 3 (n = 223[a] and 214[b])	Before dose 4 (n = 216[a] and 176[b])	After dose 4 (n = 154[a] and 129[b])	9–12 months after dose 4 (n = 132[a] and 129[b])
4							
PnCRM7	0.112 (0.096–0.131)	0.995 (0.838–1.181)	2.970 (2.626–3.360)	3.210 (2.867–3.594)	0.590 (0.519–0.671)	5.507 (4.668–6.498)	0.552 (0.467–0.651)
MnCC	0.124 (0.105–0.145)	0.042 (0.037–0.049)	0.025 (0.022–0.028)	0.025 (0.022–0.029)	0.030 (0.026–0.036)	0.036 (0.029–0.045)	0.059 (0.045–0.079)
6B							
PnCRM7	0.441 (0.382–0.510)	0.245 (0.211–0.286)	1.596 (1.371–1.858)	8.253 (7.124–9.562)	2.267 (1.998–2.572)	18.176 (15.7–21.041)	3.594 (3.037–4.254)
MnCC	0.518 (0.445–0.604)	0.173 (0.147–0.204)	0.097 (0.083–0.114)	0.107 (0.091–0.126)	0.165 (0.134–0.203)	0.211 (0.164–0.270)	0.428 (0.318–0.577)
9V							
PnCRM7	0.311 (0.276–0.351)	0.412 (0.356–0.476)	1.729 (1.537–1.945)	2.473 (2.237–2.735)	0.694 (0.615–0.783)	3.349 (2.935–3.821)	0.734 (0.626–0.862)
MnCC	0.334 (0.294–0.381)	0.125 (0.110–0.143)	0.083 (0.072–0.096)	0.095 (0.083–0.109)	0.150 (0.125–0.179)	0.171 (0.138–0.211)	0.28 (0.222–0.352)
14							
PnCRM7	0.437 (0.367–0.519)	0.702 (0.588–0.837)	4.576 (3.816–5.488)	6.813 (5.818–7.977)	1.782 (1.537–2.065)	8.457 (7.187–9.952)	1.286 (1.068–1.549)
MnCC	0.436 (0.365–0.520)	0.123 (0.101–0.150)	0.050 (0.042–0.059)	0.039 (0.034–0.045)	0.032 (0.026–0.039)	0.039 (0.030–0.051)	0.049 (0.038–0.064)
18C							
PnCRM7	0.228 (0.200–0.260)	0.527 (0.450–0.616)	1.591 (1.408–1.799)	2.608 (2.318–2.934)	0.491 (0.437–0.552)	4.257 (3.672–4.936)	0.528 (0.453–0.616)
MnCC	0.227 (0.199–0.260)	0.079 (0.069–0.089)	0.044 (0.039–0.049)	0.044 (0.039–0.051)	0.071 (0.059–0.087)	0.07 (0.057–0.088)	0.15 (0.112–0.200)
19F							
PnCRM7	0.568 (0.49–0.657)	0.625 (0.550–0.709)	1.682 (1.468–1.928)	2.742 (2.437–3.085)	1.038 (0.896–1.201)	4.161 (3.500–4.945)	2.201 (1.778–2.725)
MnCC	0.652 (0.561–0.758)	0.237 (0.204–0.274)	0.159 (0.136–0.186)	0.199 (0.166–0.238)	0.337 (0.271–0.418)	0.426 (0.327–0.556)	1.054 (0.814–1.360)
23F							
PnCRM7	0.276 (0.242–0.315)	0.204 (0.178–0.235)	1.023 (0.864–1.212)	2.593 (2.232–3.013)	0.685 (0.594–0.790)	6.157 (5.135–7.383)	0.892 (0.764–1.041)
MnCC	0.302 (0.263–0.345)	0.104 (0.091–0.118)	0.061 (0.054–0.069)	0.062 (0.054–0.071)	0.093 (0.077–0.111)	0.107 (0.085–0.134)	0.187 (0.148–0.237)

NOTE. Antibody concentrations are shown in micrograms per milliliter. n = 309, 314, 313, and 316 for dose 1 PnCRM7 serotypes 14, 18C, 19F, and 4, respectively; n = 210, 211, 212, 211, and 213 for dose 2 PnCRM7 serotypes 14, 18C, 19F, 9V, and 4, respectively; n = 200, 199, 201, and 203 for dose 3 PnCRM7 serotypes 23F, 14, 4, and 6B, respectively; n = 226, 222, 227, and 225 for post–dose 3 PnCRM7 serotypes 23F, 18C, 19F, and 4, respectively; n = 217, 217, 215, and 217 for dose 4 PnCRM7 serotypes 23F, 14, 19F, and 4, respectively; n = 153 for dose 4 PnCRM7 serotype 4; n = 299, 297, 294, 296, and 299 for dose 1 MnCC serotypes 23F, 14, 9V, 4, and 6B, respectively; n = 200, 198, 197, and 201 for dose 2 MnCC serotypes 14, 18C, 19F, and 9V, respectively; n = 191, 193, 193, and 193 for dose 3 MnCC serotypes 19F, 9V, 4, and 6B, respectively; n = 218, 216, 217, and 216 for post–dose 3 MnCC serotypes 23F, 9V, 4, and 6B, respectively; n = 177, 174, and 175 for dose 4 MnCC serotypes 14, 19F, and 6B, respectively; n = 127 and 130 for post–dose 4 MnCC serotypes 23F and 18C, respectively; and n = 128 for second post–dose 4 MnCC serotype 6B.

[a] For PnCRM7.

[b] For MnCC.

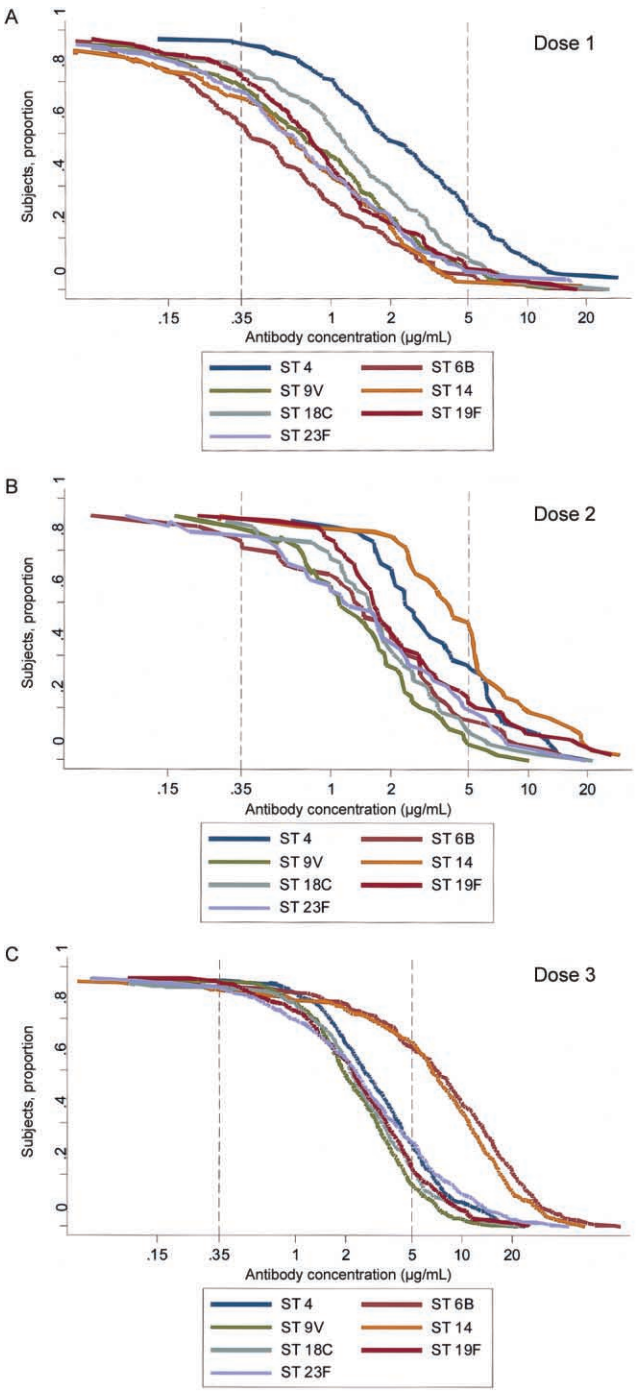

Figure 1. Reverse cumulative distribution curves of serotype (ST)–specific antibody responses to 7-valent pneumococcal conjugate vaccine (PnCRM7) in the primary efficacy (PE) group. Shown are antibody concentrations after dose 1 *(A),* dose 2 *(B),* and dose 3 *(C)* in children in the PnCRM7 PE group.

his or her response to other serotypes. We calculated the rank order correlation for all pairwise comparisons of the antibody response to all 7 serotypes after dose 3 and 4 in the PE group (Spearman correlation coefficients for dose 3 [$n = 343$], 0.40–0.72; Spearman correlation coefficients for dose 4 [$n = 207$], 0.42–0.68; $P < .05$). Of 341 PnCRM7-vaccinated subjects, 82.4% achieved or exceeded the 0.35 μg/mL threshold for all 7 serotypes after dose 3, as did 90.8% of 207 immunized subjects after dose 4.

Relationship between individual responses to various PnCRM7 doses. We evaluated the response of a given child to various doses of vaccine. For all serotypes, there was a significant positive correlation between the serotype-specific antibody response of PE subjects to 1 dose of PnCRM7 and their response to the subsequent dose (Spearman correlation coefficients, 0.24–0.58; $P < .05$).

Effect of age on the immunogenicity of PnCRM7. Because children could enroll between 2 and 23 months of age, we assessed the effect of age on the immunogenicity of PnCRM7, controlling for the number of preceding doses. With age as a categorical variable, we compared the immunogenicity of PnCRM7 among children in various age strata (table 4). Dose 1 immunogenicity was significantly greater for all serotypes except type 14 when administered at ⩾12 months of age than when administered at 2–4 months of age. There was no enhanced immunogenicity by delaying the initiation of dose 1 to 4 months of age.

The immunogenicity of dose 2 did not vary by age when administered at any time between 4 and 13 months. Dose 2 immunogenicity was, however, significantly greater when dose 1 was given at 12–23 months than at 2–4 months of age. There were no differences in the immunogenicity of dose 3 administered to infants who initiated their series at any time between 2 and 11 months of age. Delaying the initiation of the vaccine series until the second year of life resulted in enhanced immunogenicity for doses 1 and 2 at the expense of administering vaccine during the first year of life.

Comparisons of the GMCs of antibodies after the priming series show that a 3-dose priming series is significantly more immunogenic than a 2- or 1-dose priming series, even when those series are administered later during infancy or during the second year of life (tables 2–4). Similarly, the final dose in each series resulted in higher GMCs when administered after a 3-dose priming series than after a 2- or 1-dose priming series, even when the latter were administered at ages older than those for the 3-dose priming series. The final dose in each series resulted in an equivalent proportion of subjects achieving an antibody concentration ⩾0.35 μg/mL for all serotypes (data not shown).

Hib–meningococcal outer membrane protein complex (OMP) immunogenicity. After dose 1, the proportion of subjects with PRP antibody concentrations ⩾1.0 μg/mL did not differ by concomitant administration of PnCRM7, MnCC, or neither, as measured in historical control subjects (52%, 59%, and 51%, respectively) [22].

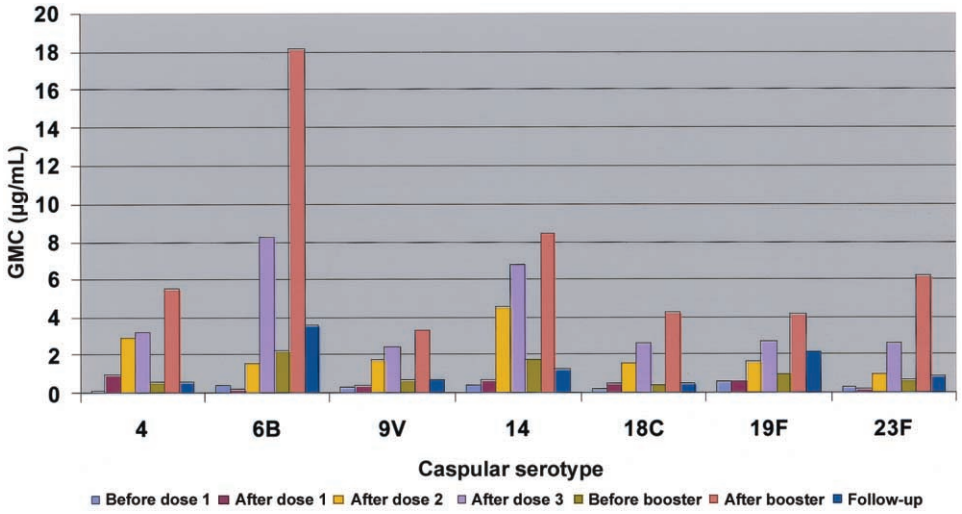

Figure 2. Serotype-specific antibody responses to 7-valent pneumococcal conjugate vaccine (PnCRM7), by dose and serotype. Shown are antibody responses over time in primary efficacy subjects receiving PnCRM7. GMC, geometric mean concentration.

DISCUSSION

This is 1 of only 2 reports evaluating the immunogenicity of PnCRM7 after each of the 4 doses used in the US infant immunization schedule [10] and exploring the comparative immunogenicity of various infant and toddler vaccine schedules in a single population [9]. The GMCs of antibodies after PnCRM7 vaccination in the present American Indian children are equivalent to or greater than those observed in children in the general populations of the United States [1, 2], the United Kingdom [9], Finland [10], The Gambia [6], or South Africa [8]. Thus, there is no evidence that high rates of invasive pneumococcal disease among American Indian populations are attributable to an impaired immune response to pneumococcal polysaccharide antigens, at least as they are presented in the form of PCVs.

Maternal antibody interference with the immune response to viral vaccines, such as measles, and bacterial polysaccharide vaccines, such as Hib, is well recognized [23, 24]. We have shown that increasing maternal pneumococcal antibody concentration is associated with a reduced response to PCV when administered during the first 6 months of life. Maternal antibodies to the carrier protein (i.e., diphtheria), which could be associated with the response to PnCRM7 in infants, were not assessed [25, 26]. There was no evidence of maternal pneumococcal antibody interference of the memory response as assessed by the post–dose 4 antibody response. The distribution of maternal pneumococcal antibody concentrations in this population may differ from that of other populations in which early PCV administration is considered (table 2). The proportions of subjects with antibody concentrations $\geq$0.35 μg/mL before dose 1 among PnCRM7-vaccinated children were 20%, 54%, 44%, 51%, 34%, 59%, and 39% for serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, respectively.

Other variables explored, such as sex, breast-feeding, age at dose 1 (between 2–6 months), and dosing interval, did not predict a response to PCV over the range that they were observed. For any dose of PnCRM7, a subject's response to 1 serotype correlated positively with his or her response to other serotypes in the vaccine. Also, a subject's response to a given dose of vaccine correlated positively with his or her response to a subsequent dose of vaccine.

The catch-up immunization schedules for children initiating vaccine at 7–23 months of age were highly immunogenic; both a single dose and a second dose administered during the second year of life were significantly more immunogenic than those doses administered early during infancy. However, there was no enhanced immunogenicity by delaying the immunization schedule within the first year of life. Higher antibody GMCs were achieved when the final dose was administered after a 3-dose primary series than after a 2- or 1-dose primary series, even though the latter were administered at older ages. The proportion of subjects who achieved an antibody concentration $\geq$0.35 μg/mL after the final dose in the series did not differ according to the series administered, but the series did affect the proportion achieving an antibody concentration $\geq$5.0 μg/mL.

Compared with the GMC after dose 2 of PnCRM7 during infancy, dose 3 resulted in a significantly greater GMC for all serotypes except type 4. The impact of dose 3 observed here was similar to that seen in infants in the general US population, in which only serotypes 4 and 19F remained unchanged [1]. By contrast, there were no significant GMC increases for any serotype among infants in the United Kingdom who received

Table 3. Geometric mean concentrations (95% confidence interval) of antibody to 7-valent pneumococcal conjugate vaccine (PnCRM7) in children initiating vaccination between 7–11 (infant catch-up [ICU] group) or 12–23 (toddler catch-up [TCU] group) months of age.

	ICU group				TCU group			
	Before dose 2		Before dose 3		Before dose 1		Before dose 2	
Serotype	PnCRM7 (n = 48)	MnCC (n = 43)	PnCRM7 (n = 27)	MnCC (n = 30)	PnCRM7 (n = 155)	MnCC (n = 152)	PnCRM7 (n = 139)	MnCC (n = 135)
4	3.296 (2.345–4.632)	0.029 (0.021–0.041)	4.523 (3.321–6.160)	0.044 (0.025–0.076)	2.959 (2.446–3.579)	0.042 (0.034–0.053)	3.692 (3.169–4.301)	0.056 (0.044–0.071)
6B	1.588 (1.035–2.436)	0.125 (0.083–0.188)	9.577 (6.352–14.438)	0.191 (0.118–0.307)	0.621 (0.506–0.762)	0.180 (0.143–0.226)	5.009 (4.043–6.207)	0.269 (0.206–0.352)
9V	1.367 (0.983–1.902)	0.107 (0.070–0.164)	2.892 (2.053–4.062)	0.166 (0.096–0.285)	1.003 (0.831–1.210)	0.131 (0.104–0.164)	1.909 (1.609–2.264)	0.241 (0.182–0.321)
14	4.278 (2.897–6.317)	0.022 (0.016–0.031)	8.202 (5.883–11.435)	0.026 (0.019–0.036)	0.818 (0.661–1.010)	0.029 (0.025–0.034)	6.899 (6.026–7.897)	0.038 (0.030–0.048)
18C	1.805 (1.287–2.533)	0.055 (0.039–0.077)	3.874 (2.693–5.571)	0.076 (0.051–0.115)	1.415 (1.171–1.710)	0.097 (0.079–0.119)	2.375 (2.039–2.766)	0.134 (0.106–0.170)
19F	2.436 (1.653–3.560)	0.237 (0.137–0.409)	3.881 (2.703–5.571)	0.483 (0.285–0.818)	1.034 (0.862–1.241)	0.303 (0.236–0.388)	3.547 (3.010–4.182)	0.462 (0.353–0.604)
23F	1.590 (1.051–2.405)	0.074 (0.052–0.105)	4.317 (2.699–6.906)	0.089 (0.062–0.128)	0.975 (0.773–1.229)	0.113 (0.093–0.139)	3.050 (2.543–3.657)	0.154 (0.121–0.196)

NOTE. Antibody concentrations are shown in micrograms per milliliter. $n = 47$ for ICU group post–dose 2 PnCRM7 for serotypes 18C and 4, respectively; $n = 26$ for ICU group post–dose 3 PnCRM7 for serotype 18C; $n = 42$, 42, 44, and 44 for ICU group post–dose 2 MnCC for serotypes 23F, 14, 18C, and 9V, respectively; $n = 29$ for ICU group post–dose 3 MnCC for serotypes 23F, 9V, and 4, respectively; $n =$ 152, 151, 152, and 153 for the TCU group post–dose 1 PnCRM7 for serotypes 23F, 14, 18C, and 9V, respectively; $n = 138$, 137, and 138 for the TCU group post–dose 2 PnCRM7 for serotypes 18C, 9V, and 4, respectively; $n = 132$, 136, and 133 for the TCU group post–dose 2 MnCC for serotypes 23F, 18C, and 4, respectively; and $n = 153$, 154, 156, and 155 for the TCU group post–dose 1 MnCC for serotypes 23F, 18C, 4, and 6B, respectively.

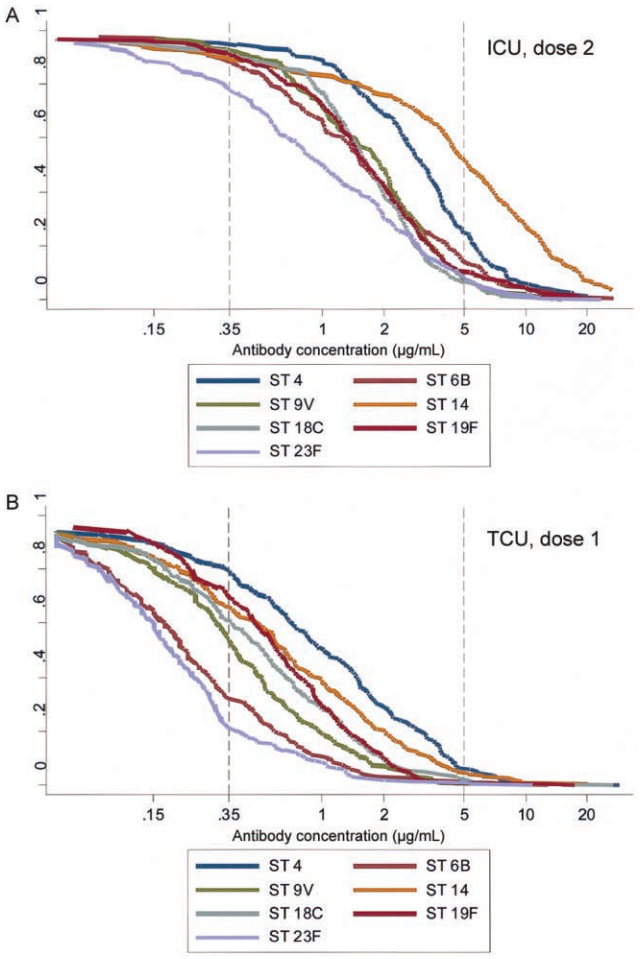

Figure 3. Reverse cumulative distribution curves of serotype (ST)–specific antibody responses to 7-valent pneumococcal conjugate vaccine (PnCRM7) after dose 2 in subjects in the infant catch-up (ICU) group *(A)* and after dose 1 in subjects in the toddler catch-up (TCU) group *(B)*.

a 2-dose versus a 3-dose priming series [9]. In that study, the 3-dose priming series was given at 2, 3, and 4 months of age; the shorter interval between doses, compared with that given in the United States (i.e., 2, 4, and 6 months of age), may reduce the immune response and explain why no differences in the 2-dose and 3-dose priming series GMCs were observed. This hypothesis is supported by the observation that the post–dose 3 GMCs in American Indian children were greater than those in the UK infants for all serotypes except types 14 and 19F, whereas there were almost no differences in the post–dose 2 GMCs between the 2 populations. Other factors, such as population variability or natural exposure to pneumococcal antigens through nasopharyngeal carriage, may also contribute to observed differences in immunogenicity; controversy exists about the impact of differences in ELISAs (i.e., absorption with 22F) [20, 27].

Although the GMCs were greater after dose 3 than after dose 2 for most serotypes, the proportion of subjects who achieved an antibody concentration ⩾0.35 μg/mL increased for types 6B and 23F only, which is consistent with findings from several other studies [1, 28, 29]. There was a significant increase in the proportion of infants achieving an antibody concentration ⩾5.0 μg/mL—a proposed threshold for protection against nasopharyngeal colonization [21]—for all but 1 serotype. Thus, the need for a third priming dose is not entirely clear. Significant efficacy against invasive disease has been documented in post-licensure US studies for 2-dose priming schedules, although there appears to be added efficacy conferred by a third priming dose [30, 31]. Our immunogenicity data suggest that the third dose in infancy may play a significant role in the indirect effects of vaccine by increasing the proportion of subjects with antibody concentrations required for protection against nasopharyngeal colonization. The relative longevity of the immune response after a 2-dose or 3-dose priming schedule is not known. As exposure and natural boosting to vaccine-serotype strains is reduced in the community through PCV use, the longevity of the primary immune response may become very important. The impact of various reduced-dose schedules in infancy on direct effects, carriage, and indirect effects should be evaluated further.

The present study also assessed the pneumococcal antibody concentrations beyond the immediate post–dose 4 period. The GMCs and the proportion of subjects with concentrations ⩾5.0 or ⩾0.35 μg/mL fell significantly for most vaccine serotypes during the 12 months after the administration of the fourth dose, similar to observations in Finnish infants [10]. Despite the decline in antibody concentrations, the proportion with concentrations ⩾0.35 μg/mL remained significantly higher in PnCRM7-vaccinated than in MnCC-vaccinated subjects. The proportion of PnCRM7-vaccinated subjects with an antibody concentration ⩾5.0 μg/mL was <10% for most serotypes at 12 months after dose 4. If passive transudation of serum anticapsular antibody is the primary means of protection against vaccine-serotype nasopharyngeal acquisition, it is not clear how the reduction in vaccine-serotype nasopharyngeal colonization is maintained over the long term. A clearer understanding of the local and systemic immune mechanisms of nasopharyngeal protection are needed to fully interpret and predict the impact of pneumococcal vaccine in communities.

We observed no interference of PnCRM7 with the Hib-OMP vaccine antibody response. This is critical for epidemiologic settings in which invasive Hib disease occurs very early during life, necessitating a conjugate Hib vaccine that is highly immunogenic after dose 1. The present study is the only one to have evaluated concomitant Hib-OMP and PCV administration.

The limitations of this study include the lack of racial diversity among the children studied and the limited sample size of the older age cohorts, compared with that of the younger cohorts.

Table 4. Serotype-specific antibody responses (geometric mean concentration [GMC]) to various doses of 7-valent pneumococcal conjugate vaccine administered at various ages.

Dose	Age at dose, months	Age at previous doses, months	4		6B		9V		14		18C		19F		23F	
			Subjects, no.	GMC	Subjects, no.	GMC	Subjects, no.	GMC	Subjects, no.	GMC	Subjects, no.	GMC	Subjects, no.	GMC	Subjects, no.	GMC
1	2	NA	175	1.014	177	0.268	173	0.411	173	0.789	173	0.525	174	0.639	176	0.203
	4	NA	16	1.579	16	0.140[a]	15	0.568	16	0.747	16	0.500	16	0.639	16	0.287
	12–24	NA	139	3.170[a]	139	0.659[a]	137	0.980[a]	136	0.929	137	1.455[a]	139	1.143[a]	136	0.966[a]
2	4	2	156	2.659	156	1.474	155	1.469	154	3.964	156	1.493	156	1.584	154	0.903
	6	4	13	2.409	13	1.342	13	1.729	13	3.643	13	1.354	13	1.781	13	1.362
	9–13	7–11	19	1.928	20	1.353	20	0.978	20	2.636	19	1.071	20	1.737	20	1.106
	14–26	12–24	115	3.844[a]	116	4.954[a]	115	1.904[a]	116	6.778[a]	116	2.376[a]	116	3.675[a]	116	3.112[a]
3	6	2, 4	150	2.995	150	6.747	151	2.176	149	5.911	149	2.349	153	2.310	152	2.432
	8	4, 6	16	3.764	16	9.279	16	2.755	16	8.159	16	2.678	16	3.181	16	3.464
	12–15	7, 9	27	3.991	27	9.568	27	2.688	27	7.796	26	3.292	27	3.401[a]	26	3.712

NOTE. Antibody concentrations are shown in micrograms per milliliter. NA, not applicable.

[a] $P < .05$, compared with the 2-, 4-, 6-, and 12–15-month schedule.

In conclusion, the present study has demonstrated the relative merits of various PCV schedules through the first 2 years of life. It has also shown that high maternal antibody concentrations are correlated with a reduced immune response to PCV during infancy but do not interfere with the induction of the immune memory response to PnCRM7. As we learn more about the correlates of protection against nasopharyngeal acquisition and the long-term community effect of the vaccine, our understanding of immunogenicity by dose and age will be critical for optimizing vaccine schedules and vaccine impact.

Acknowledgments

We acknowledge the research program assistants and nurses of the Center for American Indian Health, the children and their families who participated in this study, the institutional review boards that oversaw this trial, the data-monitoring committee for this trial, and the invaluable support of the Indian Health Service. We also thank Kate Williams for assistance in the preparation of the manuscript.

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