Similar Antibody Concentrations in Filipino Infants at Age 9 Months, after 1 or 3 Doses of an Adjuvanted, 11-Valent Pneumococcal Diphtheria/Tetanus–Conjugated Vaccine: A Randomized Controlled Trial

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In Filipino infants, 1 dose of an adjuvanted, 11-valent pneumococcal conjugate vaccine (serotypes 1, 4, 5, 7F, 9V, 19F, and 23F conjugated to tetanus protein; and serotypes 3, 6B, 14, and 18C conjugated to diphtheria toxoid) administered alone at age 18 weeks (11PncTD1) elicited similar antibody concentrations at age 9 months as those elicited by 3 doses (11PncTD3) administered concomitantly with national program vaccines, at ages 6, 10, and 14 weeks. Geometric mean antibody concentrations ranged from 0.36 μg/mL (for serotype 18C) to 5.81 μg/mL (for serotype 4), for the 11PncTD1 vaccine, and from 0.32 μg/mL (for serotype 18C) to 5.01 μg/mL (for serotype 19F), for the 11PncTD3 vaccine. The proportion of infants with threshold antibody concentrations ≥0.35 μg/mL was also similar (ranges, 55.6%–100% for the 11PncTD1 vaccine and 42.9%–100% for the 11PncTD3 vaccine). The functional activity of antibodies expressed as opsonophagocytic activity titers was similar in the 11PncTD1 and 11PncTD3 groups. This finding is an important one for countries with financial constraints and high pneumococcal disease burden.

Vaccination with pneumococcal conjugate vaccines (PCVs), which are immunogenic in children <2 years old [1–5], now appears to be the best strategy for controlling pneumococcal disease in young children, especially in light of the increasing prevalence of pneumococcal resistance to antimicrobials worldwide [6–7]. In 2000, a 4-dose regimen of a 7-valent PCV was found to be safe and efficacious against vaccine-type pneumococcal invasive disease in children in northern California [8] and in Navajo and Apache Indian reservations [9]. It was also efficacious against vaccine-type acute otitis media in Finnish children [10]. The vaccine has been recommended for use among all infants in the United States by the Advisory Committee on Immunization Practices [11] and has been licensed in the European Union, Canada, and Australia. At present, many countries are considering the inclusion of the vaccine in their expanded program on immunization (EPI). The currently available licensed PCV is expensive ($40–$50/dose). In most countries with publicly funded EPI, the major prohibitive factor has been the high price resulting, in part, from the official recommendation of 4 doses.

After the experience with the Haemophilus influenzae type b (Hib)–conjugated vaccine, many studies on the immune response of PCV, using various protein carriers, have focused on administering 3 or 4 doses of the vaccine. These studies have shown that 3 doses of...
PCV can elicit high antibody concentrations and that the antibodies persist until the age of 9 to 15 months, when the fourth dose is administered. It is important to determine whether a reduced number of doses will be sufficient to elicit the immune response required to protect children from pneumococcal diseases. The decreased number of doses would reduce the costs of vaccination and would probably help in the inclusion of PCV into EPIs. The aim of the present study was to describe the immunogenicity of 1 and 3 doses of an adjuvanted, 11-valent PCV.

SUBJECTS, MATERIALS, AND METHODS

Vaccine recipients. From August to October 1998, healthy infants aged 6–9 weeks, who were scheduled to start their EPI vaccinations, were offered enrollment in the randomized controlled study at 6 village health centers in Tagbilaran, the capital city of Bohol, central Philippines. Written, informed consent was obtained from the parents or guardians of these infants. The study was approved by the institutional review board of the Research Institute for Tropical Medicine (Manila, Philippines).

A scratchable randomization list was used to individually randomize 180 infants enrolled in the study into 3 groups: the 11PncTD3 group, which received 3 doses of the 11PncTD vaccine; the MACV group, which received 3 doses of meningococcal AC conjugate vaccine; and the 11PncTD1 group, which received only 1 dose of the 11PncTD vaccine. Infants randomized to the 11PncTD3 and MACV groups were administered the study vaccines concomitantly with EPI vaccines, at ages 6, 10, and 14 weeks, in a double-blind manner, in contrast to infants randomized to the 11PncTD1 group, who were administered only EPI vaccines and a dose of 11PncTD, at age 18 weeks (table 1).

Study vaccine. The study vaccine was an investigational 11-valent, mixed-carrier PCV containing capsular polysaccharides of *Streptococcus pneumoniae* conjugated to either tetanus protein or diphtheria toxoid: 1 μg/dose for each of the polysaccharides for serotypes 1, 4, 5, 7F, 9V, 19F, and 23F conjugated to tetanus protein; 3 μg/dose for each of the polysaccharides for serotypes 3, 14, and 18C conjugated to diphtheria toxoid; and 10 μg/dose for the polysaccharide for serotype 6B conjugated to diphtheria toxoid (Aventis Pasteur). The vaccine was adjuvanted with aluminium hydroxide and was presented as a 0.5-mL liquid solution in a prefilled, ready-to-use glass syringe. The control vaccine was an investigational conjugate vaccine against *Neisseria meningitidis* (Aventis Pasteur) containing 4 μg each of polysaccharides for serogroups A and C and 48 μg of MACV. The vaccine was formulated in sterile, pyrogen-free, phosphate-buffered physiological saline and presented in prefilled (0.5 mL), ready-to-use glass syringes, with an appearance similar to that of the 11PncTD vaccine.

The infants received the EPI vaccines concomitantly with Hib vaccine, as described in table 1. The 11PncTD and MACV were administered by deep intramuscular route into the anterolateral aspect of the right thigh. For all infants, 5 mL of blood was obtained before administration of the first dose of the vaccines (age 6 weeks), 1 month after the third dose (age 18 weeks), and just before administration of the measles vaccine and/or the fourth dose of the 11PncTD or MACV vaccine (age 9 months).

Laboratory methods. The serum samples were stored at −20°C and transported on dry ice to the National Public Health Institute (Helsinki, Finland). A standard EIA was used to determine the antibody response to each of the 11 pneumococcal polysaccharides [1, 12]. The functional activity of antibodies was determined by use of a standardized flow cytometric opsonophagocytic assay (FACSCalibur Basic 4-color Flow Cytometer; Becton Dickinson) [13]. The assay measured the phagocytic uptake of fluorescent-labeled pneumococci by HL-60 cells (human premelocytic leukemia cells) in different serum di-

| Group          | Recommended age of vaccination at each visit of the national EPI program and schedule of vaccination for the study vaccines and blood sampling |
|----------------|--------------------------------------------------------------------------------|
|                | Group 6 weeks<sup>a</sup> | 10 weeks | 14 weeks | 18 weeks<sup>b</sup> | 9 months<sup>a</sup> |
| 11PncTD3       | 11PncTD + EPI<sup>c</sup> + Hib | 11PncTD + EPI + Hib | 11PncTD + EPI + Hib | No vaccines | Measles |
| 11PncTD1       | EPI + Hib | EPI + Hib | EPI + Hib | 11PncTD | Measles |
| MACV           | MACV + EPI + Hib | MACV + EPI + Hib | MACV + EPI + Hib | No vaccines | Measles |

**NOTE.** bacille Calmette-Guérin (BCG) was administered at 6 weeks if the infant had not received it before inclusion in the study. 11PncTD, adjuvanted, 11-valent pneumococcal conjugate vaccine (serotypes 1, 4, 5, 7F, 9V, 19F, and 23F conjugated to tetanus protein; and serotypes 3, 6B, 14, and 18C conjugated to diphtheria toxoid); EPI, expanded program on immunization; Hib, *Haemophilus influenzae* type b; MACV, meningococcal AC conjugate vaccine.

<sup>a</sup> Blood samples were obtained just before vaccines were administered.

<sup>b</sup> Not part of national EPI, only for study purposes to obtain blood sample and for the administration of 11PncTD to the 11PncTD1 group.

<sup>c</sup> BCG, diphtheria, tetanus toxoid, whole-cell pertussis, oral polo vaccine, Hib, and measles vaccines are manufactured by Aventis Pasteur. Hepatitis B vaccines are manufactured by MedFest.

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Figure 1. Profile of the randomized controlled immunogenicity study of 11-valent pneumococcal conjugate vaccine in Filipino infants. 11PncTD, adjuvanted, 11-valent pneumococcal conjugate vaccine (serotypes 1, 4, 5, 7F, 9V, 19F, 23F conjugated to tetanus protein; 3, 6B, 14, 18C to diphtheria toxoid); MACV, meningococcal AC conjugate vaccine.

The functionality of antibodies in each serum sample was expressed as the opsonophagocytic activity (OPA) titer, which is the reciprocal titer of the serum dilution determined to have 50% of the maximum uptake of the labeled bacteria. If the observed maximum uptake of pneumococci by HL-60 cells was <10%, the serum was assigned an arbitrary titer (4). If the maximum uptake was 10%–20%, the serum was assigned the lowest positive titer (8). The functionality of antibodies was measured only at age 9 months in groups receiving either the conventional 3-dose schedule of 11PncTD or 1 dose of 11PncTD at age 18 weeks. The pneumococcal serotypes chosen for the assay included the common pediatric serotypes 6B, 19F, and 23F.

Statistical analysis. All statistical calculations were performed by use of log-transformed values. Geometric mean concentrations (GMCs), with their corresponding 95% confidence intervals, were calculated for each serotype and for each vaccine group at each of the 3 periods: 6 weeks, 18 weeks, and 9 months. Paired t tests were used to compare antibody concentrations within groups by period. The proportion of infants with antibody concentrations ≥0.35 μg/mL, the threshold of antibody concentration recently proposed by the World Health Organization [14], was calculated.

RESULTS

Trial profile. Figure 1 shows the enrollment figures and final number of infants used for data analysis. A total of 180 healthy infants who fulfilled inclusion criteria were enrolled and randomized into 3 groups, with 60 infants each in the 11PncTD3, MACV, and 11PncTD1 groups. Complete data for serologic examination were available for 166 infants. The reasons for withdrawal were dropping out of the study or insufficient amount of serum obtained for antibody analysis. There was 1 death from acute gastroenteritis that was not attributed to the vaccine. Table 1 describes the group allocation, visits to vaccination center, and the recommended age at vaccination.
Table 2. Geometric mean concentrations (GMCs) of antibodies to 11 pneumococcal serotypes at ages 6 weeks (visit 1), 18 weeks (visit 4), and 9 months (visit 5) in infants administered either 3 doses of 11PncTD, at ages 6, 10, and 14 weeks, 1 dose of 11PncTD at age 18 weeks, or no PncTD vaccine before age 9 months.

| Time of sample, | Group | GMC by serotype, μg/mL (95% CI) |
|----------------|-------|----------------------------------|
|                | doses (no. of infants) | 1    | 3    | 4    | 5    | 6B   | 7F   | 9V   | 14   | 18C  | 19F  | 23F  |
| 6 weeks        | 3     | 11PncTD (56)                  | 0.21 (0.15–0.29) | 0.27 (0.20–0.35) | 0.24 (0.17–0.33) | 0.37 (0.27–0.52) | 0.30 (0.21–0.41) | 0.54 (0.40–0.74) | 1.11 (0.80–1.54) | 0.24 (0.18–0.33) | 0.60 (0.41–0.86) | 0.31 (0.22–0.46) |
|                | 1     | 11PncTD1 (54)                 | 0.33 (0.23–0.48) | 0.32 (0.25–0.43) | 0.27 (0.19–0.38) | 0.50 (0.35–0.74) | 0.37 (0.26–0.52) | 0.49 (0.34–0.71) | 0.39 (0.29–0.54) | 0.87 (0.57–1.31) | 0.25 (0.18–0.36) | 0.72 (0.48–1.07) | 0.35 (0.25–0.50) |
|                | 0     | MACV (56)                     | 0.38 (0.26–0.56) | 0.38 (0.30–0.49) | 0.32 (0.22–0.46) | 0.58 (0.39–0.85) | 0.42 (0.29–0.59) | 0.72 (0.51–1.02) | 0.49 (0.35–0.68) | 1.07 (0.73–1.56) | 0.31 (0.21–0.45) | 0.85 (0.56–1.31) | 0.42 (0.30–0.61) |
| 18 weeks       | 3     | 11PncTD (56)                  | 11.22 (9.43–13.36) | 4.98 (4.20–5.91) | 18.40 (14.9–22.5) | 11.46 (9.41–13.95) | 1.16 (0.84–1.61) | 8.11 (6.17–10.66) | 6.61 (5.07–8.62) | 2.81 (1.95–4.04) | 3.78 (2.77–5.16) | 16.06 (11.5–22.3) | 3.15 (2.07–4.80) |
|                | 1     | 11PncTD (54)                  | 0.12 (0.10–0.15) | 0.13 (0.10–0.16) | 0.10 (0.08–0.12) | 0.17 (0.13–0.21) | 0.11 (0.09–0.14) | 0.13 (0.10–0.17) | 0.13 (0.10–0.16) | 0.28 (0.21–0.36) | 0.07 (0.06–0.09) | 0.31 (0.25–0.40) | 0.13 (0.10–0.17) |
|                | 0     | MACV (56)                     | 0.15 (0.11–0.19) | 0.13 (0.11–0.16) | 0.12 (0.09–0.15) | 0.22 (0.17–0.29) | 0.12 (0.09–0.15) | 0.16 (0.13–0.20) | 0.14 (0.11–0.18) | 0.34 (0.26–0.45) | 0.11 (0.08–0.15) | 0.36 (0.27–0.47) | 0.13 (0.10–0.17) |
| 9 months       | 3     | 11PncTD (56)                  | 2.06 (1.66–2.56) | 0.68 (0.55–0.84) | 3.92 (3.04–5.05) | 2.67 (2.13–3.35) | 0.53 (0.41–0.70) | 2.65 (2.16–3.25) | 1.30 (0.99–1.71) | 1.02 (0.74–1.40) | 0.32 (0.25–0.41) | 5.01 (3.80–6.60) | 1.00 (0.70–1.44) |
|                | 1     | 11PncTD (54)                  | 1.57 (1.29–1.90) | 1.68 (1.36–2.07) | 5.81 (4.45–7.58) | 2.29 (1.87–2.80) | 0.40 (0.30–0.55) | 3.58 (2.86–4.48) | 0.89 (0.70–1.13) | 0.91 (0.66–1.24) | 0.36 (0.27–0.47) | 4.44 (3.22–6.11) | 0.81 (0.56–1.19) |
|                | 0     | MACV (56)                     | 0.10 (0.08–0.13) | 0.23 (0.15–0.34) | 0.11 (0.08–0.15) | 0.28 (0.21–0.36) | 0.11 (0.09–0.14) | 0.16 (0.11–0.22) | 0.11 (0.08–0.14) | 0.21 (0.16–0.27) | 0.07 (0.06–0.09) | 0.25 (0.19–0.33) | 0.10 (0.07–0.13) |

**NOTE.** 11PncTD, adjuvanted 11-valent pneumococcal conjugate vaccine (serotypes 1, 4, 5, 7F, 9V, 19F, and 23F conjugated to tetanus protein; and serotypes 3, 6B, 14, and 18C conjugated to diphtheria toxoid); CI, confidence interval; MACV, meningococcal AC conjugate vaccine.
Antibody concentrations to pneumococcal polysaccharides at age 6 weeks. GMCs ranged from 0.21 to 1.11 μg/mL, with the highest antibody concentration against serotype 14 (range, 0.87–1.11 μg/mL) (table 2). Antibody concentrations ≥0.35 μg/mL against the 11 serotypes were present in >28% of infants (range, 28.6%–85.7% of infants), with serotype 14 having the highest proportion of infants with antibody concentrations ≥0.35 μg/mL (range, 72.2%–85.7%) (figure 2).

Antibody concentrations to pneumococcal polysaccharides at age 18 weeks. In the 11PncTD3 group, GMCs of antibodies to all serotypes were significantly higher at age 18 weeks than at age 6 weeks (range, 1.1–18.4 μg/mL; P<.001), 1 month...
after the third dose of 11PncTD, with serotype 6B having the lowest GMC and serotype 4 having the highest GMC. Seventy-eight percent of infants had an antibody concentration ≥0.35 µg/mL to serotype 6B, and almost all infants in this group had an antibody concentration ≥0.35 µg/mL to all other serotypes (figure 3). In contrast, a significant decrease in GMCs to all serotypes was observed during this period in the 11PncTD1 (range, 0.07–0.31 µg/mL; P < .001) and MACV (0.11–0.36 µg/mL; P < .001) groups. The proportion of infants with an antibody concentration ≥0.35 µg/mL in these 2 groups was 3.7%–51.8% (figure 3).

Antibody concentrations to pneumococcal polysaccharides and OPA at age 9 months. In the 11PncTD3 group, the GMCs of antibodies to all serotypes were significantly lower (P < .001) at age 9 months (range, 0.32–5.01 µg/mL) than at age 18 weeks (table 2). More than 42% of infants had antibody concentrations ≥0.35 µg/mL to most serotypes (range, 42.9%–100%); the lowest proportion was against serotype 18C, and the highest proportion was against serotypes 4 and 7F (figure 4). In the 11PncTD1 group, the antibody concentrations had increased, compared with those at age 18 weeks (range, 0.36–5.81 µg/mL), and were similar to those in the 11PncTD3 group. The threshold antibody concentration of ≥0.35 µg/mL was attained by 55.6% and 69.6% of infants in response to serotype 6B; 57.4% and 42.9% of infants attained the threshold antibody concentration to serotype 18C; and 70.4% and 76.8% of infants attained the threshold antibody concentration to serotype 23F, for the 11PncTD1 and 11PncTD3 groups, respectively. For the other serotypes, the percentages were >80%. In contrast, the GMCs for all serotypes in the MACV group remained low (range, 0.07–0.28 µg/mL). The functional activity of antibodies expressed as OPA titers was similar in the 11PncTD1 and 11PncTD3 groups. The percentages of serum samples with a detectable OPA titer (≥8) were 40.0% and 32.1% for serotype 6B, 54.5% and 44.6% for serotype 19F, and 85.4% and 85.7% for serotype 23F, in the 11PncTD1 and 11PncTD3 groups, respectively.

DISCUSSION

In the present study, we have confirmed that the 11PncTD vaccine is immunogenic when administered in 3 doses simultaneously with national EPI and Hib vaccines, at ages 6, 10, and 14 weeks. However, the finding that a single dose of the vaccine at age 18 weeks and the conventional 3 doses, at ages 6, 10, and 14 weeks, elicited a similar concentration of similarly functionally active antibodies at age 9 months was unexpected.

The most plausible reason for the immunogenicity and persistence of antibodies, despite the lower number of doses, is carrier priming due to diphtheria toxoid and tetanus proteins. These infants had received 3 doses of diphtheria-tetanus-whole-cell pertussis (DTwCp) vaccine in their EPI schedule before administration of the 11PncTD vaccine. Studies have shown that immunity to carrier proteins enhances the immune response to polysaccharides in subsequent immunizations with conjugate vaccines [15–16]. Similarly, another study showed that a single dose of a Hib tetanus vaccine was found to elicit good antibody response when administered to infants who had earlier
received tetanus vaccine [17]. The comparable GMCs of antibodies and functional activity at age 9 months in the 11PncTD1 and 11PncTD3 groups, for all serotypes, irrespective of the carrier, suggests that similar priming may also occur for diphtheria toxoid–conjugated vaccines. Since the infants in the 11PncTD1 group received DTwcP at least 4 weeks before they received 11PncTD, in contrast to the 11PncTD3 group, which had received DTwcP simultaneously with 11PncTD, the 11PncTD3 group was presented with a large load of carrier protein from DTwcP and from 11PncTD. In some studies, this has been found to cause a reduction in subsequent antibody response [18]. However, the clinical relevance of this finding is not known.

Another factor that could have facilitated the persistence of antibodies until age 9 months is the early nasopharyngeal carriage acquisition of Staphylococcus pneumoniae, which is very common in developing countries [19]. In a previous study conducted in the Philippines, the overall pneumococcal carriage rates in infants not receiving pneumococcal vaccine were 24% and 44% at ages 6 and 18 weeks, respectively [20]. The contacts to pneumococci may have acted like a natural booster between the last dose of vaccine, at age 14 weeks (11PncTD3) or 18 weeks (11PncTD1), and the serum sample collection, at age 9 months.

The GMCs of antibodies after 3 doses of 11PncTD are comparable or similar to those of the currently licensed 7-valent PCV in other parts of the world [8, 21]. Since the present study was not designed to compare 1 dose with 3 doses of 11PncTD, the results can be considered as preliminary. In future studies, a single dose of PCV administered during the EPI visits at 6, 10, or 14 weeks could be compared with 2 doses administered at initial EPI visits and at age 9 months (EPI visit for the measles vaccine) or with a conventional 3 or 3-plus-1 dose schedule. These new studies should also consider the long-term effect of the vaccination by analyzing the persistence and functional capacity of antibodies, as well as the formation of immunologic memory. In the present study, the OPA titers for serotypes 19F, 23F, and 6B did not differ between the 11PncTD1 and 11PncTD3 groups. As we have demonstrated elsewhere [22], the low antibody concentration against serotype 23F results in good functional activity, whereas the opposite is true for serotype 19F. More than one-half of the serum samples did not show functional activity against serotype 6B, but this is probably explained by the low sensitivity of the opsonophagocytic assay to detect activity if the antibody concentration is <1.0 μg/mL.

The less-expensive alternative regimens, such as a 1- or 2-dose schedule of PCV, could greatly benefit children in developing countries where the mortality and morbidity from pneumococcal diseases, particularly pneumonia, remain high. It might also benefit other countries currently considering whether the economic burden of pneumococcal diseases outweighs the cost of vaccination.

Acknowledgments

We thank P. Helena Mäkelä, for her significant contribution to the design of the study, and Rolando Giancarlo Inciong and Rhea Naval-Parreño, for additional statistical analysis and SAS programming.

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