CONCISE COMMUNICATION

Cross-Reactivity of Antibodies to Type 6B and 6A Polysaccharides of *Streptococcus pneumoniae*, Evoked by Pneumococcal Conjugate Vaccines, in Infants

Merja Väkeväinen,* Carita Eklund,* Juhani Eskola,* and Helena Käyhty

*Department of Vaccines, National Public Health Institute, Helsinki, Finland

**Streptococcus pneumoniae** serotypes 6B and 6A are important causes of infections, yet only 6B is included in current vaccines. It is, therefore, crucial to evaluate whether functional antibodies are produced against both types after vaccination. Concentration and opsonophagocytic activity (OPA) of antibodies to 6B and 6A polysaccharides were determined in serum samples from infants immunized with 3 different pneumococcal conjugate vaccines containing serotype 6B. In all vaccine groups, a significant correlation was found between the anti-6B and -6A antibody concentration and OPA. However, OPA to the vaccine serotype was detectable more commonly than OPA to the cross-reactive type. Furthermore, 5%–15% of the serum samples showed high OPA against the 6B but not the 6A strain. On average, 2–6 times more anti-6B antibodies were needed for 50% opsonophagocytic killing of the type 6A than the type 6B strain. Although pneumococcal type 6B conjugate vaccines elicit antibodies that cross-react with type 6A, not all anti-6B antibodies are functionally cross-reactive.

Pneumococcal types 6B and 6A polysaccharides (PSs) possess considerable chemical similarity: they are linear copolymers differing from each other in one bond [1, 2]. It is generally assumed that they elicit cross-reactive antibodies and that immunization with one serotype confers protection against the cross-reactive type. Therefore, and because of the better hydrolytic stability of 6B over 6A [3], the current commercial and experimental pneumococcal vaccines contain type 6B as the only representative of the serogroup 6 [2]. Both 6B and 6A are, however, frequent causes of infections. Because the data that actually show cross-protection are scarce, it is important to determine whether the conjugate vaccines truly elicit functional, cross-reactive antibodies to 6A. There is evidence from previous studies that vaccines containing 6B are not always able to induce functional antibodies to the cross-reactive serotype [4, 5]. To study the cross-reactions, we evaluated the concentration and opsonophagocytic activity (OPA) of serum IgG antibodies to pneumococcal serotypes 6B and 6A in the serum samples of infants immunized with experimental lots of 3 conjugate vaccines against *Streptococcus pneumoniae*.

Materials and Methods

PncCRM (Wyeth-Lederle), a heptavalent pneumococcal conjugate vaccine, contains PSs 4, 6B, 9V, 14, 19F, and 23F and oligosaccharide 18C conjugated to variant diphtheria toxin CRM197. The tetravalent conjugate vaccines PncD and PncT (both from Aventis Pasteur) contain PSs 6B, 14, 19F, and 23F conjugated to diphterhia (PncD) or tetanus toxoid (PncT). Pneumovax (Aventis Pasteur) and PNU-IMMUNE (Wyeth-Lederle) are commercial 23-valent pneumococcal PS (PncPS) vaccines.

At ages 2, 4, and 6 months, Finnish infants were immunized in 2 consecutive trials with one of the pneumococcal conjugate vaccines: PncCRM ($n = 53$), PncD ($n = 71$), or PncT ($n = 74$). At age 14 or 15 months, the children received a booster dose of the homologous conjugate vaccine (PncCRM [*$n = 28$] or PncD [*$n = 36$]) or of a PS vaccine (PncCRM [*$n = 25$], PncD [*$n = 35$], or PncT [*$n = 74$]). Blood samples were obtained 1 month after completion of the primary immunization series (7 months) and before (14–15 months) and 1 month after (15–16 months) booster vaccination. All prebooster serum samples are referred to as 14-month serum samples, and postbooster serum samples are referred to as 15-month serum samples.

The concentration of IgG antibodies to pneumococcal PSs was...
measured by EIA, as described elsewhere [6]. Antibody levels to 6B are given as micrograms per milliliter, as calculated on the basis of the officially assigned IgG values of the 89-SF reference serum. Because no official values were available for 6A, 10 U/mL was assigned as the anti-6A IgG concentration in 89-SF.

*S. pneumoniae* of serotypes 6A (reference strain from the World Health Organization collaborating Center for Reference and Research on Streptococci) and 6B (reference strain from the Centers for Disease Control and Prevention) were grown as described elsewhere [7]. In some experiments, additional strains of types 6A and 6B from American Type Culture Collection were used. OPA of serum antibodies was determined by measuring the killing of live pneumococci by fresh human polymorphonuclear leukocytes in the presence of antibody and complement [8].

Statistical comparisons were done using log-transformed data in analysis of variance, independent *t* test, paired *t* test, and Pearson’s correlation analysis. Yates’s corrected χ² test or Fisher’s exact test was used to compare the number of infants with detectable (titer ≥8) or undetectable (titer <8) OPAs.

**Results**

At 7 months, after the primary immunizations with 6B-containing pneumococcal conjugate vaccines, infants had moderate anti-6B antibody concentrations and OPAs, which decreased significantly (*P* < .05 to *P* = .001) between the ages of 7 and 14 months in all vaccine groups, except for the IgG concentration in the PncT group (figure 1). By contrast, the concentrations of anti-6A antibodies increased during the same period (*P* < .05 to *P* = .001). Anti-6A OPAs were low at 7 and 14 months. In every vaccine group, the OPA was significantly higher to 6B than to 6A strains after primary vaccinations (*P* < .001). Booster doses of PncPS or conjugate vaccines increased (*P* < .05 to *P* = .001) the anti-6B and -6A concentrations and OPAs. Still, OPAs were higher to serotype 6B than to 6A strains (*P* < .05 to *P* = .001). PncCRM was superior to the other vaccines in inducing a functional antibody response to the vaccine serotype 6B and to the cross-reactive type 6A (figure 1).

Correlations between anti-6B and -6A antibody concentrations and OPAs against 6B and 6A strains were significant at all ages in every vaccine group (figure 2). Despite the relatively good correlations, there were several serum samples that had high anti-6B antibody concentrations but low or undetectable anti-6A concentrations (figure 2, upper panels). Furthermore,
Figure 2. Correlation between anti-6B and anti-6A IgG concentration and opsonophagocytic activity (OPA) in serum samples of infants primed with PncCRM (Wyeth-Lederle [n = 53]), PncD (Aventis Pasteur [n = 71]), or PncT (Aventis Pasteur [n = 74]) conjugate vaccine and given booster doses of the homologous conjugate vaccine (PncCRM [n = 28] or PncD [n = 36]) or a polysaccharide (PS) vaccine (PncCRM [n = 25], PncD [n = 35], or PncT [n = 74]). Serum samples were obtained from 7-, 14-, and 15-month-old infants. c, Group receiving conjugate vaccine as a booster; m, month of sampling; p, group receiving PS vaccine as a booster.
the OPAs were detectable (≥8) more commonly to 6B than to 6A (figure 2, lower panels); the difference between the serotypes was significant at all ages in every vaccine group (P < .01 to P = .001), except in the PncCRM group after the conjugate booster. In each group, 5%–15% of the serum samples had high OPAs to 6B (≥100) but no OPA to 6A (<8) strain. None of the serum samples showed the opposite. To confirm this observation, part of these serum samples (n = 13) were tested with an additional pair of type 6B and 6A strains. Again, none of the serum samples had OPAs to the 6A strain, whereas all 13 serum samples had OPA titer of ≥100 to the 6B strain.

The anti-6B IgG concentration correlated strongly with the OPA against the 6B strain in all vaccine and age groups (r = .62–.93; P < .001; data not shown). The correlation between anti-6A concentration and OPA against the 6A strain was mostly significant but not as strong (r = .15–.89; P < .020 to P = .001). Likewise, there was less correlation between the anti-6B concentration and OPA against the strain type 6A (r = .16–.68; P < .20 to P = .001). No vaccine-specific differences could be found (data not shown). Of the serum samples having ≥1 μg/mL of anti-6B antibodies, a substantial proportion had no anti-6A OPA at 7 (66%–88%) and 14 (73%–93%) months. By contrast, after children were administered a booster dose of PncCRM, only 14% of their serum samples having ≥1 μg/mL of anti-6B had no OPA to 6A strain, compared with 58% for infants given booster doses of PncD (P < .01). After a booster dose of PncPS, 37%, 70%, and 67% in the PncCRM, PncD, and PncT groups, respectively, with ≥1 μg/mL of anti-6B had no OPA to 6A strain, and there were significant differences between the PncCRM group and the other 2 groups (P < .05).

This reflects the higher immune response against type 6B in the PncCRM groups after boosting (figure 1). The respective percentages of serum samples with ≥1 μg/mL of anti-6B but no OPA to 6B strain were significantly lower (P < .05 to P = .001): 2%–6% at 7 months, 16%–35% at 14 months, 0%–4% after booster of conjugate vaccine, and 0%–11% after booster of PncPS.

On average, 2–6 times higher concentrations of anti-6B antibodies were required for 50% opsonophagocytic killing of type 6A (0.045–0.252 μg) than type 6B (0.016–0.068 μg) bacteria, depending on the vaccine. The difference was significant in all groups (P < .05 to P = .001), with the exception of children primed with and given booster doses of PncD (P = .065).

Discussion

The three 6B-containing experimental conjugate vaccines used in this study elicited functional, cross-reactive antibodies to pneumococcal serotypes 6B and 6A, but the quality of antibodies against the 2 serotypes differed. Vaccination generally induced lower functional activity of antibodies to 6A than to 6B. Several serum samples that showed good functional activity against 6B strains had no activity against 6A strains, and no serum sample showed the opposite. These results are in agreement with studies reporting some, though not perfect, in vitro cross-reactions [1, 4, 5] and in vivo cross-protection [9–12] between the 2 types of serogroup 6.

Differences in the kinetics of anti-6B and -6A antibody concentration and OPA suggest that immunization with 6B-containing vaccines induces a response more efficiently to type 6B than to type 6A. The anti-6B antibody concentration and OPAs were moderate in most children after primary immunizations, followed by a significant decrease between ages 7 and 14 months, the period without vaccination. Meanwhile, no decrease in anti-6A OPA and an increase in anti-6A concentration were seen, which indicates that the kinetics of anti-6A formation were less dependent on vaccination than were the kinetics of anti-6B formation.

The chemical composition of group 6 pneumococcal PSs is identical, except for the rhamnopyranosyl-ribitol linkage: 6A has 1→3 linkage, whereas 6B has 1→4 linkage [1, 2]. This linkage has not been considered to be an immunodominant part of the tetrasaccharide repeating unit of group 6 PSs. According to this study, part of the antibodies seem to be specific to this nonidentical epitope, as suggested by the presence of serum samples lacking activity against 6A but having good activity against 6B and by the fact that anti-6B antibody concentrations needed for 50% killing of types 6B and 6A bacteria were substantially different.

In agreement with previous studies [4, 5, 8], concentration and OPA of antibodies to the 6B strain correlated strongly with each other. The relationship between anti-6B concentration and OPA against the 6A strain was interesting; a considerable number of serum samples with an anti-6B concentration ≥1 μg/mL had no OPA to the 6A strain, whereas most of those serum samples had OPA to the 6B strain. We could not find any vaccine-specific differences in the correlations between anti-6B antibody concentration and OPA against the 6A strain. If in vitro OPA of antibodies to pneumococcal capsular polysaccharides is considered to be a correlate of their in vivo functional activity, anti-6B antibody concentrations might be useful at the individual level in predicting protection against 6B but not always against 6A strains. However, our data clearly show that the mean functional activity against the 6A strain was highest in the PncCRM group that also had the highest mean concentrations of anti-6B antibody. Thus we can conclude that, at the population level, the higher the anti-6B antibody response, the higher the protection also against 6A strains.

Our laboratory findings agree with the results of a Finnish efficacy trial of PncCRM vaccine, which was found to be 84% efficacious against acute otitis media caused by 6B strains, whereas its efficacy to acute otitis media caused by 6A strains was 57% [11]. The same vaccine has prevented invasive infections caused by 6A pneumococci [10]. However, in a South African study, 9-valent PncCRM reduced carriage of 6B but not of 6A pneumococci, which suggests that the cross-reactive
protection may be different, depending on the nature of the infection [13]. These findings and the results of in vitro studies [4, 5] should be kept in mind when reevaluating whether to include type 6A PS into the future generations of multivalent conjugate vaccines. The accumulating data of finished and ongoing efficacy and immunogenicity trials hopefully can be used to find the serologic correlates or surrogates of protection not only to the vaccine serotypes but also to the cross-reactive types.

Acknowledgments

We thank Maijastiina Voutilainen, Hannele Lehtonen, Arja Vuorela, and Sirkka-Liisa Wahlman for technical assistance and Heidi Åhman for anti-6B concentration results. We also thank personnel of the study centers for their help in the clinical part of the study.

References