Avidity and Subclasses of IgG after Immunization of Infants with an 11-Valent Pneumococcal Conjugate Vaccine with or without Aluminum Adjuvant

Tomi Wuorimaa, Ron Dagan, Merja Väkeväinen, Fabrice Bailleux, Raiili Haikala, Mansour Yaich, Juhani Eskola, and Helena Käyhty

Finnish and Israeli infants received an 11-valent mixed-carrier pneumococcal conjugate vaccine with or without aluminum adjuvant at 2, 4, 6, and 12 months of age. The relative avidity of serotype 1–, 5–, 6B–, 14–, 19F–, and 23F–specific IgG antibodies in serum obtained at 7, 12, and 13 months of age was measured by EIA, using thiocyanate as a chaotropic agent. For all serotypes, except 14, avidity increased between the ages of 7 and 12 months. After boosting at 12 months, avidity further increased for all serotypes. The adjuvant improved antibody avidity against serotype 5. The IgG antibodies produced were mainly IgG1 subclass, although some infants also produced IgG2 after boosting. In conclusion, the immunization of infants with this 11-valent pneumococcal conjugate vaccine increased avidity of IgG, suggesting successful immunologic priming.

Host defense against Streptococcus pneumoniae depends largely on type-specific capsular polysaccharide antibodies. Binding of antibodies to polysaccharide capsule leads to the activation of the classical pathway of the complement system and, hence, to rapid opsonization and phagocytosis of the bacteria. The efficiency of binding is dependent mainly on antibody affinity. The antigen-binding capacity of polyclonal antibodies with different affinities is expressed by antibody avidity. Recent evidence suggests that high antibody avidity correlates with high functional activity of antibodies and confers protection against pneumococcal challenge in mice [1–3]. In addition to the avidity of antibodies, the IgG subclass also may influence opsonic activity, since IgG1 antibodies have been suggested to display higher functional activity than IgG2 antibodies [4].

Avidity matures after antigen exposure, and high-affinity antibodies are produced after repeated exposure [5]. The increase in antibody avidity after immunization is believed to reflect successful priming and generation of immunological memory [5–7]. The avidity of IgG antibodies to capsular polysaccharides increases in infants after a primary series of immunization with pneumococcal, meningococcal, and Haemophilus influenzae type b polysaccharide–conjugate vaccines [5, 8, 9]. The avidity increases further with these conjugates after boosting [5, 9]. Studies with H. influenzae type b and pneumococcal conjugate vaccines suggest that the carrier protein used can influence the avidity of the antipolysaccharide antibodies [8, 9]. Different conjugates, therefore, may prime differently and may induce antibodies with different functional activities.

An 11-valent pneumococcal conjugate vaccine containing capsular polysaccharides from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F conjugated to diphtheria toxoid or tetanus protein carriers has been shown to be immunogenic in adults, toddlers, and infants [10–12] and to induce T cell–dependent responses in humans [13]. The efficacy of this vaccine against pneumonia in infants is being evaluated in the Philippines. The aim of our study was to assess the maturation of IgG avidity in infants to the polysaccharides of 6 serotypes (1, 5, 6B, 14, 19F, and 23F) of pneumococci after immunization with this novel conjugate vaccine, administered either with or without aluminum adjuvant. The avidity was measured after the primary series given at 2, 4, and 6 months and before and after the fourth dose at 12 months in Finnish and Israeli infants. For a descriptive analysis in a subsample of serum, we determined the IgG1/IgG2 distribution.

Material and Methods

Vaccine. The study vaccine (Aventis Pasteur) was a mixture of 11 polysaccharide-protein conjugates. Polysaccharides of pneumo-
coccal serotypes 3, 6B, 14, and 18C were conjugated to diphtheria toxoid, and polysaccharides of the types 1, 4, 5, 7F, 9V, 19F, and 23F were conjugated to tetanus toxoid. The vaccine contained 1 µg of polysaccharide per dose for types 1, 4, 5, 7F, 9V, 19F, and 23F, 3 µg of polysaccharide per dose for types 3, 14, and 18C, and 10 µg per dose of type 6B polysaccharide. The conjugate vaccine was administered with or without aluminum hydroxide adjuvant (F3 alum, adjuvanted formulation; F3, nonadjuvanted formulation).

Vaccines and immunizations. Healthy Finnish and Israeli infants (n = 251) were randomized to receive either F3 (the F3 group) or F3 alum (the F3 alum group) at 2, 4, 6, and 12 months of age. We analyzed serum specimens from the first 50 enrolled infants in each country for antibody concentration and avidity and from the first 10 in each country for the IgG1/IgG2 subclasses.

Serologic assays. Serum samples were obtained 1 month after the completion of the primary series and before and 1 month after the fourth dose. IgG antibody concentrations to capsular polysaccharides of types 1, 5, 19F, and 23F (tetanus conjugates) and 6B and 14 (diphtheria conjugates) were measured by an EIA [11]. We neutralized unspecified cell-wall polysaccharide antibodies to increase specificity of the assays. The coefficient of variance (CV) for interassay variation for 2 control serum specimens remained <15%. IgG1 and IgG2 subclasses of antibodies to capsular polysaccharides of types 6B, 14, and 23F were measured by use of mouse monoclonal antibodies to either human IgG1 or human IgG2 (Zymed Laboratories) and alkaline phosphatase–conjugated antimouse IgG (Jackson ImmunoResearch) [14]. The CV for interassay variation for 2 control serum specimens remained <20%.

Relative avidity of IgG antibodies to pneumococcal capsular polysaccharides of types 1, 5, 6B, 14, 19F, and 23F was determined by a modified EIA method [9]. The antibody avidity method has been described elsewhere for types 6B, 14, 19F, and 23F [9]. Before study serum specimens were measured, we evaluated the reproducibility of the assay for the new serotypes 1 and 5 by analyzing both intra- and interplate variance (i.e., within and between, respectively, plates prepared on separate days). The CV for both intra- and interplate variance remained <10%. Sodium thiocyanate (NaSCN) served as a chaotropic agent to dissociate low-avidity antibodies. Due to variability of antibody avidity among serotypes [9], an optimal NaSCN concentration was titrated for each serotype: 0.5 M for types 1, 5, 6B, and 23F and 0.65 M and 0.80 M for types 19F and 14, respectively. Each serum sample dilution series was assayed with and without NaSCN treatment. Results are given as avidity index, indicating the percentage of antibodies that remained bound to antigens after the NaSCN treatment [9]. Individuals having an OD reading of ≤0.3 in the lowest dilution (1:50) of any of the 3 samples (at 7, 12, or 13 months) were excluded from the analysis. The CV for interassay variation for 2 control serum specimens remained <10%.

Statistical methods. Antibody concentrations are given as geometric mean concentrations (GMCs), and relative avidity results are given as mean of avidity indices (MAIs) with 95% confidence interval. Antibody and avidity response over time was analyzed by analysis of variance (ANOVA). The linear contrast test was used to investigate the linear increase in the avidity after immunizations. One-way ANOVA and the least significance difference test were used to determine the statistical significance of the differences in the antibody concentration and avidity between the vaccine groups. Statistical tests were considered to be significant at the level of P < .005. Because of a small sample size for subclass assays, the IgG2 and IgG1 data were analyzed descriptively.

Results

IgG antibody concentrations. For the 100 infants included in the avidity assays, the GMCs of IgG antibodies at all time points for types 1, 5, 6B, 14, 19F, and 23F were similar to those for the whole study cohort (n = 251; figure 1) [12]. Antibody concentrations were higher in Israeli than in Finnish infants. The differences were significant (P < .005) for types 6B and 14 at 7 months and for types 1, 6B, 19F, and 23F at 13 months. Both vaccine formulations elicited increases in pneumococcal polysaccharide–specific antibody concentrations after the primary series in both countries. Antibody concentrations for all serotypes declined between ages 7 and 12 months but increased for all types after boosting (figure 1). Although no significant differences were found, the IgG antibody concentrations were almost always higher in the F3 alum than in the F3 groups.

IgG1 and IgG2 subclasses. At 7 months, antipolysaccharide IgG antibodies against types 6B, 14, and 23F were primarily of subclass IgG1. The concentration of IgG2 also remained low after boosting. At 13 months, the concentration of IgG1 to type 14 was higher than that of IgG2 in all infants. Similarly, the concentration of IgG1 for types 6B and 23F was higher than that of IgG2 in 80% and 85% of all infants, respectively.

IgG avidity. The numbers of serum samples with sufficient antibody concentrations for the avidity assays were 44, 45, 34, 32, 47, and 43 in the F3 group and 50, 50, 46, 43, 50, and 50 in the F3 alum group for types 1, 5, 6B, 14, 19F, and 23F, respectively. Avidity data from Finnish and Israeli infants are shown combined (figure 2), because the comparisons between the 2 populations revealed no significant differences in MAIs.

One month after priming at 7 months of age and before boosting at 12 months, MAIs were similar between the F3 and F3 alum groups for all serotypes. Between 7 and 12 months, MAIs increased for all serotypes with both vaccines, except for type 5 in the F3 group. The rise in MAIs was most evident for type 5 in the F3 group. The rise in MAIs was most evident for type 14 (figure 2) and was significant (P < .005) for type 14 in the F3 group and for types 5 and 14 in the F3 alum group. The avidity increase during follow-up (month 7 vs. 13) was statistically significant (P < .0001, 2 degrees polynomial linear
Figure 1. Geometric mean concentrations (GMCs [μg/mL]) of type 1, 5, 6B, 14, 19F, and 23F antibodies in Finnish (Fin) and Israeli (Isr) infants who were immunized with an 11-valent pneumococcal conjugate vaccine with (F3al) or without (F3) aluminum adjuvant at 2, 4, and 6 months of age and who received a homologous booster vaccine at 12 months of age. All the booster responses were statistically significant ($P < .05$).

Discussion

In this study, we show that the immunization of infants with an 11-valent pneumococcal conjugate vaccine induces an increase in IgG antibody concentration and avidity to capsular polysaccharides of types 1, 5, 6B, 14, 19F, and 23F. As expected, the IgG antibody produced was mainly IgG1. Aluminum adjuvant did not seem to provide a significant benefit in immunogenicity, although an overall tendency for higher antibody concentration and relative avidity appeared with the adjuvanted contrast test) in the F3 and F3 alum groups for types 5, 6B, 19F, and 23F.
Figure 2. Mean avidity indices and 95% confidence intervals (error bars) of type 1, 5, 6B, 14, 19F, and 23F IgG antibodies in infants who were immunized with an 11-valent pneumococcal conjugate vaccine with (F3al) or without (F3) aluminum adjuvant at 2, 4, and 6 months of age and who received a homologous booster vaccine at 12 months of age.

formulation in most serotypes. The data also suggest that differences in the sensitivity to the adjuvant might exist between polysaccharide-protein conjugates; of the 6 types studied, type 5 conjugate benefited from the adjuvant.

Previous studies and the present data indicate that antibody concentrations decline during the months after priming and that boosting evokes a significant antibody response. At the same time, antibody avidity continues to improve during the months after priming, and boosting can further increase avidity [5, 9]. This difference in kinetics of antibody and avidity de-
velopment after antigen exposure might be the result of a diminishing production of antibody-forming cells and increased production of memory B cells during maturation of immune response [15].

In our study, the avidity maturation differed between the serotypes. Antibody avidity to serotype 1 showed only a small increase after the primary immunization series and after the fourth dose at 12 months. The avidity maturation to serotype 14 similarly showed no increase in the period between 7 and 12 months but increased significantly after the fourth dose. Conversely, antibody avidity to types 5, 6B, 19F, and 23F was already higher in the period between 7 and 12 months, and a further increase was observed after boosting. The kinetics of IgG avidity for types 6B, 14, and 19F resembled those described elsewhere in infants [9]. The variation in avidity maturation suggests that conjugates of different serotype specificity have different capacities to generate memory. However, the overall kinetics of avidity maturation observed in this study indicates that the 6 conjugates were adequate for induction of B cell maturation.

Antibody avidity has been claimed to be an important parameter for documenting the immunological properties of a polysaccharide-protein conjugate vaccine [5, 7, 8]. First, in healthy infants, antibody avidity matures after immunization with a pneumococcal conjugate vaccine and correlates with bactericidal and opsonophagocytic activity [1, 2, 4]. Second, an increase in avidity after immunization with a conjugate vaccine, predicting induction of memory, can be a useful surrogate of protection [5, 7].

In conclusion, we have shown that the 11-valent mixed-carrier pneumococcal conjugate vaccine primes for development of immunological memory in infancy. The character of the antibody response after the boosting and of the avidity maturation evoked by vaccination suggests adequate priming.

Acknowledgments

We thank Orly Zamir, Pirjo-Riitta Saranpää, Anna-Stina Leinonen, and Minna Koivuniemi for their professional contribution in the clinical arrangements; Laurence Pollissard for clinical monitoring; and Beatrice Chabot, Jukka Jokinen, and Esa Ruokokoski for data management. We appreciate the work done by clinical study physicians and nurses in health care centers, and we thank the parents and children who participated in this study in Finland and Israel.

References