Comparison of an Opsonophagocytic Assay and IgG ELISA to Assess Responses to Pneumococcal Polysaccharide and Pneumococcal Conjugate Vaccines in Children and Young Adults with Sickle Cell Disease

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Children with sickle cell disease were immunized with either 2 doses of 7-valent pneumococcal conjugate vaccine followed by 1 dose of 23-valent pneumococcal polysaccharide vaccine or a single dose of 23-valent vaccine. Functional antibodies to 7 vaccine serotypes were measured by a flow cytometric opsonophagocytic assay (OPA) and compared with IgG capsular polysaccharide antibody concentrations measured by ELISA. Moderate correlations were found between OPA and ELISA antibody titers for all 7 serotypes (r values, 0.41–0.70; P < .001 for all serotypes). After immunization with 23-valent vaccine, geometric mean antibody titers by OPA were significantly higher in the combined schedule group for 5 of 7 vaccine serotypes but were significantly higher for only 2 of 7 serotypes as measured by ELISA. The ability of OPA to show a greater differential response to the 2 immunization schedules used in this study suggests that it may be useful in the evaluation of immunization regimens involving pneumococcal conjugate vaccines.

The development of pneumococcal conjugate vaccines appears promising for use in populations at high risk for invasive disease with Streptococcus pneumoniae. Results from a recent randomized study in healthy infants demonstrated 100% vaccine efficacy against invasive disease with a 7-valent pneumococcal conjugate vaccine [1]. Other populations at increased risk for invasive disease with S. pneumoniae may benefit from immunization with pneumococcal conjugate vaccines that elicit T cell–dependent responses and prime for increased responses to capsular polysaccharide [2, 3]. In the evaluation of pneumococcal vaccine strategies for high-risk hosts, the assessment of functional antibody responses by opsonophagocytic assays (OPAs) may be useful as a surrogate marker for protection. One recent report suggests that there may be a higher correlation between opsonophagocytic antibody titers and pneumococcal vaccine efficacy than between IgG antibody concentrations measured by ELISA and vaccine efficacy [4]. Opsonophagocytic activity can be measured by a variety of techniques and traditionally is measured with peripheral blood leukocytes as effector cells. The development of an OPA that uses differentiated HL-60 cells as effector cells has provided reproducible measurement of serotype-specific functional antibody by both manual viable and flow cytometric methods and more readily allows for assay standardization with all assay components [5, 6].

We recently evaluated the effect of a combined immunization schedule of pneumococcal conjugate vaccine followed by pneumococcal polysaccharide vaccine on IgG-binding antibody concentrations in a randomized study of older children and young adults with sickle cell disease [7]. Geometric mean IgG pneumococcal antibody concentrations measured by ELISA were higher in the study subjects who received a combined schedule of vaccines than in those who received pneumococcal polysaccharide vaccine only, although statistically significant differences between the vaccine groups were seen for only 2 serotypes (serotypes 14 and 19F). To examine whether functional antibody activity differed between the 2 vaccine groups, opsonophagocytic antibody titers measured by a flow cytometric OPA were determined in this study population.
Materials and Methods

Study subjects. Sera for the current study were obtained from subjects with sickle cell disease followed in the Children’s Hospital Hematology Clinic (Boston) who participated in a pneumococcal conjugate immunization study, as reported elsewhere [7]. In brief, 23 children and young adults with sickle cell disease were randomized to receive either a single dose of 23-valent pneumococcal polysaccharide vaccine (23v-PS group, n = 12, median age, 14 years) (Lederle Laboratories, Pearl River, NY) or 2 doses of 7-valent pneumococcal CRM197 conjugate vaccine (Wyeth-Lederle Vaccines and Pediatrics, West Henrietta, NY) 8 weeks apart, followed 8 weeks later by 1 dose of 23-valent pneumococcal polysaccharide vaccine (7v-Conj/7v-Conj/23v-PS group, n = 11, median age, 13 years). Serum was obtained from each subject at the time of each immunization and 3–6 weeks after immunization with 23-valent pneumococcal polysaccharide vaccine.

Flow cytometric OPA. Functional antibody activity to the 7 pneumococcal serotypes contained in both vaccines were measured by a standardized flow cytometric OPA [5]. In brief, sera were heat inactivated at 56°C for 30 min. Eight 2-fold dilutions starting with 10 μL of each subject’s serum were incubated with 20 μL of suspension containing 4 × 10^5 nonviable carboxyfluorescein-labeled S. pneumoniae for 30 min at 37°C. Then, 10 μL of sterile 3–4-week-old baby rabbit serum (Pelfreeze, Brown Deer, WI) was added as a source of complement and incubated for 15 min at 37°C. Forty microliters of a suspension containing 2 × 10^6 differentiated HL-60 cells (granulocytes) was then added to each dilution well and incubated for 15 min at 37°C before being resuspended in 80 μL of buffer and transferred to titer tubes for flow cytometric analysis. Control wells contained only HL-60 granulocytes and bacteria. Analysis was performed as previously described, with 1 marker region (M1) set with use of the HL-60 cell control fluorescence peak to include 98% of this population, and a second marker region (M2) to determine the percentage of differentiated HL-60 granulocytes with phagocytized S. pneumoniae (i.e., fluorescence greater than the M1 region for each serum dilution). Titers were reported as the reciprocal of the highest serum dilution yielding ≥50% of the maximum phagocytic uptake. Samples with a maximum phagocytic uptake <20% were considered negative and reported as half the lowest dilution tested (titer of 4 for all serotypes measured except for serotype 4, which was reported as a titer of 8). This assay has good reproducibility: 87% of the assays performed on the same serum sample yield results within 1 dilution of the median titer for all 7 vaccine serotypes [5].

IgG ELISA. IgG antipneumococcal antibody to serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F were measured by ELISA as described elsewhere [8]. All sera were preabsorbed with C-polysaccharide (lot B-2; National Institute of Allergy and Infectious Diseases Reference Laboratory, University of Rochester, Rochester, NY). The assay was standardized by use of reference serum 89-SF (Food and Drug Administration, Bethesda, MD) with assigned IgG values for each serotype [9].

Statistics. Antibody titers were log transformed for statistical calculations. Linear correlations were made by least-squares analysis. Comparisons of geometric mean antibody concentrations and titers measured by ELISA and OPA, respectively, between the immunization groups were performed by unpaired 2-tailed t test for parametric analyses and Mann-Whitney rank sum test for non-parametric analyses.

Results

The correlation of antibody measured by OPA and ELISA was examined for each pneumococcal serotype (figure 1). There was a moderate correlation between the assays with r values from 0.41 for serotype 18C to 0.70 for serotype 4 (P < .001 for all serotypes). Geometric mean antibody titers measured by OPA were compared between the 2 immunization groups (figure 2, left panels). There were no significant differences in opsonophagocytic antibody titers between the 7v-Conj/7v-Conj/23v-PS group and the 23v-PS only group before immunization for each of the 7 serotypes measured. After immunization with 23v-PS vaccine, geometric mean opsonophagocytic antibody titers for the 7 serotypes measured were higher in the 7v-Conj/7v-Conj/23v-PS group than in the 23v-PS group, reaching statistical significance for serotypes 6B (962 vs 42.7, P = .02), 14 (1810 vs. 215, P = .05), 18C (512 vs. 12.7, P = .004), 19F (290 vs. 30.2, P = .009), and 23F (659 vs. 6.00, P = .0001). Fold increases in geometric mean opsonophagocytic antibody titers from before immunization to after immunization with 23-valent vaccine ranged from 64 to 226 (median, 128) in the 7v-Conj/7v-Conj/23v-PS group, compared with fold increases of 1.5–38.1 (median, 4.8) in the 23v-PS only group. IgG geometric mean pneumococcal antibody concentrations measured by ELISA were also higher in the 7v-Conj/7v-Conj/23v-PS group after immunization with 23v-PS vaccine compared with those of the 23v-PS only group, but the differences were less marked (figure 2, right panels). Statistically significant differences were noted only for serotypes 14 (21.8 μg/mL vs 5.16 μg/mL, P = .04) and 19F (16.7 μg/mL vs 4.10 μg/mL, P = .02). Fold increases in IgG geometric mean antibody concentrations before and after immunization with 23v-PS vaccine were 4.1–22.3 (median, 7.8) in the 7v-Conj/7v-Conj/23v-PS group for the 7 serotypes measured and 2.2–5.4 (median, 4.1) in the 23v-PS only group.

Discussion

The optimal evaluation of a new vaccine is to determine the efficacy of the vaccine to protect a target population from disease. There are situations, however, in which a vaccine efficacy trial may not be ethical or logistically feasible. In these cases, a serologic correlate of protection that could serve as a surrogate marker may be useful. The protective levels of serotype-specific pneumococcal antibody have not been clearly defined, although data from the recent vaccine efficacy trial in infants may provide some guidelines. In addition, a focus of discussion among vaccine experts has been whether functional antibody as measured by the uptake of opsonized bacteria in cultured
For this report, we measured functional antibody titers by a standardized flow cytometric OPA in older children and young adults with sickle cell disease who received 2 different regimens of pneumococcal vaccines. We examined the correlation of functional antibody titers with IgG binding antibody concentrations measured by ELISA. For the 7 pneumococcal serotypes measured that are in both the conjugate and polysaccharide vaccines, geometric mean opsonophagocytic antibody titers correlated moderately with those measured by ELISA. Previous studies have shown similar correlations of pneumococcal antibody measured by ELISA with those measured by manual viable OPAs [6, 10]. Of note, many subjects in this study had pre- or postimmunization sera with detectable IgG antibody concentrations measured by ELISA but with negative opsonophagocytic antibody activity. This discrepancy may represent the presence of IgG antibody that binds to antigen-specific capsular polysaccharide fixed to a solid plate that lacks functional activity. Although not investigated in this report, the lack of functional activity may be due to differences in antibody avidity, IgG subclasses, or complement fixation activity of the antibodies measured by these assays [11–14]. Results of a recent study among elderly persons immunized with 23-valent polysaccharide vaccine suggest that low avidity antibody may result in serum assays that show a discrepancy of moderate-to-high IgG ELISA antibody concentrations and low opsonophagocytic activity [11]. Another explanation for the disparity between ELISA and OPA may relate to measurement by ELISA of nonspecific antibodies that bind to contaminants (other than C-polysaccharide) present in the pneumococcal polysaccharide antigen preparations used to coat the ELISA plates [15]. The clinical relevance of these distinctions between OPA and ELISA measurements in the evaluation of pneumococcal conjugate vaccines for use in different high-risk populations remains to be determined.

Comparison of opsonophagocytic antibody responses of the combined schedule group (7-valent conjugate plus 23-valent
Figure 2. Geometric mean pneumococcal opsonophagocytic titers (left panels) and geometric mean pneumococcal IgG concentrations measured by ELISA (right panels) for each of 7 serotypes in both 7-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine. Dashed line with ○, titers of 7v-Conj/7v-Conj/23v-PS vaccine group before and after each immunization; solid line with ●, titers of 23v-PS-only group before and after immunization with 23-valent vaccine.

polysaccharide vaccine) with those of the 23-valent polysaccharide vaccine group showed greater differences than did comparison of the vaccine groups of IgG pneumococcal antibody concentrations measured by ELISA. We found statistically significant differences between the vaccine groups in opsonophagocytic antibody titers for 5 of the 7 serotypes after immunization with 23-valent polysaccharide vaccine, whereas we found statistically significant differences in IgG antibody measured by ELISA between the groups after immunization with 23-valent vaccine only for 2 serotypes. The opsonophagocytic assay also demonstrated that functional antibody titers of some serotypes tended to increase in the combined schedule group after the second dose of conjugate vaccine and/or after immunization with 23-valent polysaccharide vaccine. In contrast, there were minimal increases in IgG pneumococcal antibody concentrations measured by ELISA after the second dose of conjugate vaccine or subsequent immunization with 23-valent polysaccharide vaccine. If functional activity is a better predictor of protection, then the merits of a combined vaccine schedule would be underestimated by IgG ELISA assessment. These findings suggest that functional antibody activity as measured by OPA is useful in the evaluation of pneumococcal conjugate vaccines.

References


