Reactogenicity and Immunogenicity of a Protein-Conjugated Pneumococcal Oligosaccharide Vaccine in Older Adults

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Healthy adults ≥50 years old were immunized with either pentavalent *Corynebacterium diphtheriae* C7 (β197) cross-reactive material (CRM₁₉₇) protein-conjugated pneumococcal vaccine (CV) containing 10 μg each of capsular oligosaccharides from serotypes 6B, 14, 18C, 19F, and 23F or with licensed (23-valent, 25 μg/serotype) pneumococcal polysaccharide vaccine (PV). Adverse reactions, predominantly local in nature, occurred in 20 of 23 CV recipients versus 13 of 23 PV recipients ($P < .05$). Compared with mean postvaccination antibody concentrations in PV recipients, those induced by CV were not significantly different for serotypes 6B, 14, 18C, and 23F and were lower for 19F ($P < .05$). Six months later, revaccination with PV of subjects who had initially received CV elicited a slight boost in antibody concentrations to levels that were not significantly higher than those achieved after the primary vaccination or than those in persons given a single dose of PV. Pneumococcal vaccines containing protein-conjugated oligosaccharides may offer no advantage over currently licensed preparations containing unconjugated polysaccharides for immunization of healthy older adults.

Pneumococcal infection is the most common bacterial cause of lower respiratory infection in adults, accounting for ~15%–20% of all pneumonias and a substantial proportion of all cases of acute bronchitis. Both the incidence and case-fatality rate of pneumococcal infection increase with advancing age, particularly among persons with underlying disease associated with immune suppression or compromised pulmonary status. Thus, recommendations call for vaccination with at least one dose of pneumococcal vaccine of all adults ≥65 years of age and of persons ≥2 years old who have high-risk conditions associated with an increased risk of fatal bacteremic infection [1]. Revaccination is recommended at intervals of 3–6 years for certain high-risk groups [1].

The vaccine in use since 1983 contains purified capsular polysaccharides from 23 pneumococcal serotypes responsible for ~90% of bacteremic infections. Protection after immunization is thought to be dependent upon the production of circulating pneumococcal type-specific antibodies that act alone or in concert with complement proteins to opsonize bacteria and promote their phagocytosis [2]. Previous studies have documented that serologic responses to pneumococcal vaccine are reduced in elderly persons compared with those in younger adults and are attenuated in persons with chronic debilitating illnesses compared with those in healthy persons [3].

A disadvantage of pneumococcal vaccines derives from the fact that polysaccharides are T cell-independent antigens and therefore not able to recruit the participation of T helper cells, a requirement to generate B cell memory. Conjugation to protein carriers converts polysaccharides to T cell-dependent antigens, thereby enhancing their immunogenicity and enabling them to prime immunologically naive hosts for anamnestic responses [4]. This approach has been successfully applied to the development of licensed *Haemophilus influenzae* type b (Hib) conjugate vaccines [5], which are recommended for use in infants beginning at age 2 months. The availability of investigational protein-conjugate pneumococcal polysaccharide vaccines similarly offers the potential to extend protection against *Streptococcus pneumoniae* to young children, who often fail to mount antibody responses to the currently licensed vaccine containing unconjugated polysaccharides [6].

It is not known whether protein-conjugated pneumococcal polysaccharides will have any advantage over unconjugated polysaccharides for vaccination of high-risk adults. Thus, we conducted a prospective, randomized, double-blind trial to compare the safety and serologic responses in older (i.e., ≥50 years) adults immunized with a pentavalent protein-conjugated pneumococcal oligosaccharide vaccine (CV) to those elicited by the licensed 23-valent pneumococcal polysaccharide vaccine (PV). To assess whether antibody concentrations in CV-primed adults are boosted above primary postvaccination levels by reexposure to polysaccharide antigen, we also evaluated the serologic responses of CV recipients when they were revaccinated 6 months later with PV.
Materials and Methods

Vaccines

We used a pentavalent CV that contained protein-conjugated capsular oligosaccharides from pneumococcal serotypes 6B, 14, 18C, 19F, and 23F (Lederle-Praxis, Rochester, NY). Each of the oligosaccharides in the CV consisted of ~20 repeat units of the component saccharide [7]. Cross-reactive material (CRM197), a nontoxic variant of diphtheria toxin isolated from cultures of Corynebacterium diphtheriae C7 (β197), was the protein carrier used for synthesis of glycoconjugates. The pneumococcal oligosaccharides were covalently linked to CRM197 by reductive amination. Each CV dose contained 10 μg of carbohydrate per serotype. The licensed PV (Phu-Immune; Lederle, Pearl River, NY) contained 25 μg/dose of each of the 23 capsular polysaccharide components, including the five serotypes in the CV. Both vaccines were administered by intramuscular injection in a volume of 0.5 mL/dose.

Clinical Studies

Adults ≥50 years old were recruited. Volunteers were excluded from participation if they had previously received pneumococcal vaccine, had severe underlying chronic medical illness known to impair immune responsiveness (e.g., chronic renal failure or malignancy), or were receiving immunosuppressive medication. Subjects were randomly assigned to immunization with CV or PV. Six months later, subjects who initially received CV were immunized with PV, whereas those initially given PV were injected with saline placebo. All vaccinations were done in a double-blind manner. Subjects were instructed to complete a report card of adverse reactions, including local and systemic symptoms, daily for 6 days after each vaccination. Symptoms were self-graded as mild, moderate, or severe. If present, localized swelling or erythema at the injection site was estimated to be less than or greater than the area of a quarter coin (~2.5 cm in diameter). Serum specimens were obtained at the time of each vaccination and 1 month later and were processed in a blinded fashion.

Serology

IgG pneumococcal polysaccharide antibodies to capsular types 6B, 14, 18C, 19F, and 23F were measured in serum specimens by ELISA. The methods were adapted from a consensus technique developed at the October 1994 International Workshop on Immunologic Assays for Pneumococcal Vaccines (cosponsored by the World Health Organization, Centers for Disease Control and Prevention, National Institute of Allergy and Infectious Diseases, and Food and Drug Administration [FDA]).

Antigen coating. ELISA conditions were optimized individually for each capsular antigen. Commercial lots of all five antigens were obtained from the American Type Culture Collection (Rockville, MD). The coating concentrations were 10 μg/mL for 6B, 19F, and 23F and 1 μg/mL for 14 and 18C. Individual antigens were diluted in 0.01 M PBS prepared with type 1 pyrogen-free water (Baxter Scientific, McGaw Park, IL), then added to 96-well plates (Nunc-Bacti; Fisher Scientific, St. Louis) in a volume of 100 μL/well and incubated at 37°C for 5 h. Just before sample addition, the plates were rinsed three times with wash buffer consisting of PBS with 0.05% Tween 20 (PBS-T).

Sample preparation. The assays were standardized using reference serum 89SF (provided by C. Frasch, FDA). Two additional serum samples known to contain high and low levels of antibodies were used as controls to ensure assay reproducibility. Each plate contained the reference and control sera in addition to pre- and postvaccination test sera from study volunteers. All 4 serum specimens from each volunteer were included on the same plate. The reference and control sera were initially diluted 1:200 and the test sera 1:100 in PBS-T containing 10 μg/mL C-polysaccharide (CPs; Statens Seruminstitut, Copenhagen) and incubated at 25°C for 30 min. Subsequently, eight 2-fold dilutions of each serum sample were prepared in PBS-T, and 100 μL of each serum dilution was transferred to individual wells of the washed antigen-coated plates. The plates were incubated at 25°C for 2 h and maintained overnight (16–24 h) at 4°C.

Addition of anti-IgG conjugate and substrate. Horseradish peroxidase–conjugated mouse monoclonal anti-human IgG Fe (HP-6043; Hybridoma Reagent Laboratory, Baltimore) was used at a 1:4000 dilution in PBS-T for all assays. After three washes with PBS-T, 100 μL of conjugated antibody was added to each well, and the plates were incubated at 25°C for 2 h. Substrate solution was prepared by dissolving o-phenylenediamine dihydrochloride (Sigma) in buffer containing phosphate-citrate with urea hydrogen peroxide (Sigma) ≤30 μL/mL before use. Plates were washed three times immediately before addition of 100 μL/well substrate solution and then incubated for 60 min at 25°C before the reaction was stopped with 50 μL/well 2.5 N sulfuric acid. Colorimetric measurements were made using a microtiter plate spectrophotometer at 492 nm with a dual beam at 550 nm.

Calculations. Optical density readings for each well were plotted as a function of the log10 reciprocal dilution of the corresponding serum specimen. Capsular polysaccharide-specific IgG antibody concentrations (micrograms/milliliter) in the test sera were calculated by comparing the resulting curves to those of the reference serum using the parallel line bioassay method described for the Bordetella pertussis ELISA [8]. Antibody concentrations were calculated only when the curves for test samples were parallel with the reference serum (slope ≥0.9). If the antibody concentration of a serum sample was too high to calculate over the range of dilutions that were tested, the assay was repeated using higher serum dilutions. If the antibody concentration was too low to generate a linear curve parallel to that of the reference serum, the sample was declared to have a concentration below the minimum level of detection (MLD) for the assay and was arbitrarily assigned a value 50% of the minimum. The MLDs (in micrograms of total IgG antibody/milliliter) were 0.20 for 6B, 0.25 for 14, 0.10 for 18C, 0.15 for 19F, and 0.10 for 23F. For concentrations above the MLD of the assays, the coefficients of variation were 9.4% for 6B, 7.9% for 14, 12.5% for 18C, 11.8% for 19F, and 10.5% for 23F.

Statistical Analyses

Serum antibody concentrations were logarithmically transformed for statistical analysis and determination of geometric mean levels of the study groups. A significant antibody response to the
primary vaccination was defined as a rise in concentration ≥2-fold between the baseline specimen and either the 1-month or the 6-month postvaccination specimen. Differences between vaccine groups were analyzed by Mann-Whitney U test to compare mean log2 antibody levels and Fisher’s exact test to compare proportions of subjects with antibody responses or adverse reactions. Booster responses to PV in the group that had received a primary dose of CV were analyzed using the Wilcoxon signed ranks test to compare antibody levels achieved 1 month after each immunization.

Results

Subject characteristics. Forty-six volunteers (30 women) aged 50–85 years (mean, 67 ± 8 SD) were enrolled and randomly assigned to receive primary vaccination with CV or PV (n = 23 in each group). The mean age and sex ratios of subjects were comparable in the 2 vaccine groups. All subjects completed the study.

Reactogenicity. During the 6 days after the first vaccination, one or more adverse reactions were reported by 20 (87%) of the CV recipients compared with 13 (57%) of the PV recipients (P < .05). Adverse reactions to vaccination were predominantly local in nature, mild in severity, and 1–2 days in duration. Localized pain, arm stiffness, and induration at the injection site occurred more frequently in the CV than in the PV group, although these differences were not statistically significant. Furthermore, 3 of the 18 CV recipients who experienced localized pain or arm stiffness graded the intensity of their symptoms as moderate or severe, whereas all 12 of the PV recipients who reported these symptoms graded them as mild. Localized erythema, induration, or both, when present, were usually less than the area of a quarter coin in size, except for 2 subjects in each vaccine group.

Six months after primary vaccination, subjects who initially received CV were given a booster dose of PV, whereas those initially vaccinated with PV were injected with saline placebo. At the time of the booster immunization, all subjects remained blinded regarding vaccine assignment. Local reactions were reported significantly more often by PV than by placebo recipients, whereas systemic reactions were comparable in the 2 groups. Local reactions to a booster dose of PV occurred with about the same frequency as they did following primary CV immunization. Four of the 16 subjects who experienced pain at the injection site after booster immunization with PV graded their symptom as moderate in severity.

Immunogenicity. Serologic responses measured as total IgG antibody to each of the five capsular serotypes within the CV are summarized in figure 1. Baseline geometric mean antibody concentrations ranged from 1.25 to 4.17 μg/mL and were comparable in the 2 vaccine groups. Increases in geometric mean antibody levels after immunization varied among serotypes and ranged from 2-fold for 19F to 10-fold for 18C. Mean titers of all antibodies declined slightly between 1 and 6 months after vaccination.

Among the 5 serotypes analyzed, there were no consistent differences between the two vaccines in the magnitude or frequency of serologic responses. Against 6B, 14, and 18C, CV and PV elicited postvaccination geometric mean antibody levels that were about equivalent. On the other hand, CV recipients tended to mount lower levels of antibody to 19F, but higher levels to 23F, compared with those achieved by PV recipients. Due to the variance of the data, these differences were not statistically significant with the exception of the lower 6-month postvaccination geometric mean concentration of 19F antibody in persons who received CV compared with PV (P < .05). The proportions of subjects who responded to vaccination with a ≥2-fold increase in antibody level exceeded 60% (except that only 39% of CV recipients responded to 19F) and were not significantly different between vaccine groups (table 1). There were no consistent or statistically significant age-related differences in the magnitude or frequency of antibody responses in subjects 50–64 years old and in those ≥65 (data not shown).

Volunteers whose primary vaccination was with CV had modest responses after booster immunization with PV, ranging from 1.1- to 2.4-fold rises in geometric mean antibody concentrations from levels at the 6-month time point (figure 1). For serotypes 6B, 18C, and 23F, the antibody concentrations at 1 month after the booster were no greater than peak levels 1 month after the primary vaccination. Levels of antibody to 14 and 19F were somewhat higher after booster vaccination than after primary vaccination, although this difference was statistically significant only for serotype 19F (P < .02). Furthermore, the final geometric mean concentrations of antibodies to 14 and 19F were not significantly different in subjects after booster immunization with PV compared with subjects who received a single dose of PV.

Discussion

A major limitation to the use of purified polysaccharides from encapsulated bacteria as vaccines is their inconsistent immunogenicity in children ≤18 months old [6, 9] and in persons of any age with severely compromised immune function [10]. Protein conjugation has been a successful strategy for overcoming the developmental delay in immune responsiveness to polysaccharide antigens. Licensed Hib conjugate vaccines can induce protective antibody levels even in 2-month-old infants [3] and have led to a dramatic reduction in the incidence of invasive Hib disease in children.

Clinical trials are underway with experimental conjugate vaccines containing saccharides from other encapsulated bacteria including pneumococcus, meningococcus, and group B streptococcus. Pneumococcal conjugate formulations in particular are immunogenic in young children (unpublished data) [11]. In contrast to the encouraging findings from pediatric studies, the present results suggest that pneumococcal vaccines containing protein-conjugated oligosaccharides have little if
any advantage over licensed preparations containing unconjugated polysaccharides for the immunization of healthy older adults.

Because the ≥50-year-old population has the most persons at high risk of serious pneumococcal infection, we selected this group for the present study. However, since volunteers with significant underlying medical problems were excluded, our conclusions may not apply to persons with severe immunodeficiency. It is conceivable that the relative advantage of protein-conjugated over unconjugated saccharides for eliciting antibody responses in adults is inversely related to the immunocompetency of the host. Further studies are warranted to examine the potential usefulness of protein-conjugated pneumococcal vaccines in specific subsets of immunocompromised adults, including persons with hematologic malignancy, chronic renal failure, and human immunodeficiency virus infection.

Since the CV contained less than half the antigenic content of each capsular serotype in the licensed PV, the similarity of immune responses to the two vaccines may be construed as evidence that the conjugated formulation was more potent on a dose-adjusted basis. On the other hand, the CV was more reactogenic than PV, and it seems likely that this relative disadvantage would offset any enhancement of immunogenicity that...

**Table 1.** Serum IgG capsular-specific antibody response rates in older adults after immunization with protein-conjugated pneumococcal oligosaccharide vaccine (CV) or licensed pneumococcal polysaccharide vaccine (PV).

<table>
<thead>
<tr>
<th>Vaccine group</th>
<th>No. (%) of subjects with ≥2-fold rise in antibody to serotype</th>
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<tr>
<td></td>
<td>6B</td>
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<tr>
<td>CV</td>
<td>16 (70)</td>
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<td>PV</td>
<td>14 (61)</td>
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**Figure 1.** Serum IgG antibody responses to capsular types 6B, 14, 18C, 19F, and 23F in older adults after immunization with protein-conjugated pneumococcal oligosaccharide vaccine (CV) or licensed pneumococcal polysaccharide vaccine (PV). Arrows indicate times of first and second vaccine injections. • = subjects immunized with CV at month 0 and PV at month 6; ○ = subjects immunized with PV at month 0 and placebo at month 6. * Significant difference in geometric mean antibody concentrations between vaccine groups (P < .05).
might result from the incorporation of larger quantities of conjugated oligosaccharides. An unacceptably high incidence or severity of adverse reactions might also result from the inclusion of additional serotypes that were not present in the pentavalent CV used in this study and is another potential drawback of a multivalent pneumococcal conjugate vaccine intended for use in adults.

It is possible that only a limited number of pneumococcal serotypes are rendered more immunogenic for the majority of adults by protein-conjugation. If so, the optimal pneumococcal vaccine for this age group may contain a mixture of conjugated and unconjugated saccharides. It is also possible that glycoconjugates made with other protein carriers are superior to CRM197 conjugates for adult immunization. In a previous report, adults responded better to a diphtheria toxoid conjugate of pneumococcal serotype 12F than to the unconjugated polysaccharide [12]. Likewise, we recently observed that diphtheria toxoid-conjugated Hib polysaccharide stimulated significantly higher antibody levels in elderly volunteers than did unconjugated Hib polysaccharide [13]. Future studies of pneumococcal conjugate vaccines should investigate whether the immune response in adults is influenced by the nature of the protein carrier, the method of coupling, or the molecular weight of the saccharide(s) used to synthesize the glycoprotein conjugates.

Even though serologic responses to CV and PV were quantitatively similar in terms of total IgG antibody measured by ELISA, the antibodies elicited by the two vaccines may be qualitatively different with respect to IgG subclass distribution or functional activity. The in vitro bactericidal and in vivo protective activities of capsular polysaccharide antibodies induced by alternative forms of Hib conjugate vaccine are profoundly influenced by vaccine-related differences in antibody avidity that are independent of the absolute antibody concentration [14]. There is also evidence that antibodies to Hib capsular polysaccharide exhibit distinct patterns of immunoglobulin variable region expression that vary according to host age and vaccine form and may be an important determinant of the aforementioned functional characteristics [15]. Studies are underway to examine whether qualitative differences similarly exist between antibodies induced in children and adults by vaccines containing unconjugated and alternative forms of protein-conjugated pneumococcal polysaccharides.

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References