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# Comparison of Pneumococcal Polysaccharide and $CRM_{197}$-Conjugated Pneumococcal Oligosaccharide Vaccines in Young and Elderly Adults

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**Conjugation of carbohydrate antigens to protein carriers significantly improves the immune response to many carbohydrates. In order to evaluate the potential for this approach to improve the performance of pneumococcal vaccine in the elderly, we evaluated pneumococcal polysaccharide-derived oligosaccharides conjugated to cross-reacting material 197 ($CRM_{197}$) (CRM-OS) in 49 older adults over 60 years of age (median age, 66 years) and compared the results to those from 50 younger adults under age 45 (median age, 27 years). Subjects were randomly assigned to receive licensed 23-valent polysaccharide vaccine (PS) which contain 25 μg of polysaccharide per serotype, or 5-valent CRM-OS, which contains 10 μg of oligosaccharide per serotype, in double-blind fashion. Both vaccines were associated with moderate local pain on administration. Antibody responses to type 14 were seen in the majority of both younger and older subjects following administration of both CRM-OS and PS, and there was no significant improvement of responses with CRM-OS in either age group. Antibody responses in young adults to the less immunogenic type 6B were seen in only 36% of subjects receiving PS and in 56% of subjects receiving CRM-OS ($P = 0.15$), and the geometric mean 6B titer 1 month after vaccination was higher in CRM-OS recipients (10.9 versus 3.7 μg/ml; $P = 0.04$). However, 6B responses were poor following the administration of either vaccine to elderly adults and there was no difference between results with CRM-OS and those with PS in this age group. Relatively few subjects developed measurable mucosal immunoglobulin A responses in nasal secretions following administration of either vaccine. Revaccination of CRM-OS recipients with PS at 2 months did not result in significant additional responses to 6B or 14. Though CRM-OS is possibly more immunogenic in young adults, the formulation of the pneumococcal glycoconjugate vaccine used in this study does not appear to offer an advantage to the elderly for types 6B or 14.**

Even with appropriate antibiotic therapy, pneumococcal infections have been estimated to result in as many as 40,000 deaths per year in the United States (11, 12). The mortality rate for bacteremic pneumococcal disease is 19%, rising to 37% in individuals over the age of 65 (23). In addition, pneumococci have gained increased resistance to penicillin and other antibiotics, complicating therapy for these serious infections. Development of an effective vaccine to prevent pneumococcal infections is therefore an object of great interest and a public health priority (9, 28).

Efforts to develop a pneumococcal vaccine have generally concentrated on generating immune responses to the pneumococcal capsular polysaccharide, since type-specific antibody to the capsule has opsonizing activity associated with protection in vitro (8). Such polysaccharide vaccines are immunogenic in healthy adults and have proven effective in controlled field trials with such individuals (4, 27, 33). Most otherwise-healthy elderly adults respond to a single dose of polysaccharide with at least a twofold rise in antibody titer (10, 14). However, some serotypes may be less immunogenic in the elderly than in younger adults (17), and elderly adults with chronic diseases, such as chronic bronchitis, may also have poor responses compared to those of healthy young controls (21). Case-control and cohort-type studies have demonstrated the protective efficacy of pneumococcal vaccine in prevention of invasive disease in elderly or high-risk individuals (31). However, the estimates of efficacy in these populations are relatively low and become progressively lower as time passes after vaccination (31). Thus, there is room for improvement in the performance of pneumococcal vaccines in the elderly.

Conjugation of carbohydrate antigens to protein carriers represents an approach to vaccine formulation which converts a T-independent antigen to a more immunogenic T-dependent antigen through addition of T-helper cell epitopes. This approach has been highly successful in the development of glycoconjugate vaccines for *Haemophilus influenzae* type b (Hib), which are immunogenic even in children less than 2 years of age and provide excellent protection against invasive Hib disease (32).

This study compared the safety and immunogenicity in young and elderly adults of pneumococcal polysaccharide in a standard formulation with a vaccine containing oligosaccharides derived from pneumococcal polysaccharides of five serotypes conjugated to diphtheria-like toxin. In addition, because of the potential advantage of generating immunologic memory with glycoconjugate vaccines by immunizing with T-cell epitopes with the saccharide, responses to subsequent exposure to pneumococcal polysaccharide were also evaluated.

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## MATERIALS AND METHODS

**Subjects.** Two groups of subjects were enrolled in this study. Younger adults were healthy individuals between the ages of 18 and 45. Older adults were over 60 years of age and were in stable health. Subjects with known immunocompromising illness or those taking medication (including systemic steroids) were excluded, as were those with a history of previous pneumococcal vaccination or those known to have had a tetanus-diphtheria vaccination within three years of enrollment.

**Vaccines.** The pneumococcal conjugate vaccine (cross-reacting material–oligosaccharide [CRM-OS]) was obtained from Wyeth-Lederle Vaccines and Pediatrics, Henrietta, N.Y., and consisted of oligosaccharides derived from pneumococcal polysaccharides of types 6B, 14, 19C, 19F, and 23F conjugated to the mutant diphtheria toxin $CRM_{197}$ by reductive amination (3). Each vaccine dose contained 10 μg of oligosaccharide for each pneumococcal serotype conjugated at a ratio of between 0.5:1 and 1:1 $CRM_{197}$ to oligosaccharide by weight, resulting in a total protein content of ≤50 μg of $CRM_{197}$ per dose. The protein-oligosaccharide combination was adsorbed to aluminum phosphate and given in a volume of 0.5 ml intramuscularly.

The polysaccharide vaccine (PS) was licensed 23-valent pneumococcal polysaccharide vaccine (PNU-IMUNE 23; Wyeth-Lederle Vaccines and Pediatrics). Each 0.5-ml dose contained 25 μg of polysaccharide for each of the 23 included serotypes. A 0.5-ml sterile saline solution served as a placebo. All vaccines were administered intramuscularly.

**Study design.** Within each age group, subjects were randomly assigned to receive 23-valent PS or 5-valent CRM-OS. Vaccine was administered by a study nurse who was aware of the type of vaccine administered but who was not otherwise involved in the evaluation of the subject. Two months after the initial vaccination, subjects who received CRM-OS were given PS while subjects who initially received PS were given a saline placebo. Sera were obtained before and 1 month after each vaccination and 3 months after the second vaccination. Nasal secretions were obtained by nasal wash prior to the first vaccination and 1 month afterward and stored at −70°C until tested.

Subjects kept a diary card for 7 days on which they noted symptoms of pain at the injection site, myalgias, arthralgias, and headache on a scale of 0 (not present) to 3+ (severe) and on which they noted use of analgesic medications. Subjects measured the maximum diameter of any redness at the vaccination site in millimeters and monitored their own oral temperatures. Oral temperatures of >38°C were considered fever. Subjects returned on days 3 and 7 after vaccination for review of the diary card.

**Serologic assays.** Type-specific total immunoglobulin G (IgG) to serotypes 14 and 6B was measured by enzyme-linked immunosorbent assay (ELISA). We chose to analyze responses to these two serotypes because they represent two contrasting patterns of expected responses; the response to serotype 14 is usually vigorous, while the response to type 6B polysaccharide is usually much weaker (20, 29).

Type-specific *Streptococcus pneumoniae* capsular polysaccharides from serotypes 6B and 14 (American Type Culture Collection, Rockville, Md.) were bound to 96-well polystyrene microtiter plates (model 25801; Corning, Corning, N.Y.) at 0.5 and 0.125 μg per 100 μl per well, respectively, by overnight incubation. To reduce nonspecific signal, each well was then postcoated with 1% bovine serum albumin (BSA) in phosphate-buffered saline, pH 7.4, for 2 h. Serum samples were applied to plates at an initial dilution of 1:50 or 1:100 in 1% BSA with 0.1% (vol/vol) Tween 20 in phosphate-buffered saline, pH 7.4 (dilution buffer), to which was added cell wall polysaccharide (Statens Seruminstitut, Copenhagen, Denmark) at a final concentration of 10 μg/ml to absorb non-type-specific antibody. After incubation for 2 h, the plates were probed with alkaline phosphatase-labeled goat anti-human IgG (TAGO Immunologicals, Camarillo, Calif.) in dilution buffer for 2 h. The substrate paranitrophenol phosphate (Sigma Chemicals, St. Louis, Mo.) in diethanolamine buffer, pH 9.0, was added, and the reaction was developed for 30 min and quenched with 3 N NaOH. All incubations took place at room temperature (approximately 21°C). The absorbance was read in a dual-wavelength ELISA reader at 405 and 605 nm.

A standard human serum for comparison was generated by combining five postvaccination human sera. This pool was standardized by multiple comparisons with the international standard serum 89-SF (a gift of Carl Frasch, Food and Drug Administration, Bethesda, Md.), for which the concentrations of type-specific IgG antibody have been established (26). The range of dilutions of the pool corresponding to the standard serum was determined, and these dilutions of the pooled serum were included as a standard curve on each plate.

Serum samples were initially tested at a 1:100 dilution in duplicate; if the difference between wells was over 10%, the sample was run again. All samples from a subject were run in parallel on the same plate. Samples which exceeded the linear portion of the standard curve were repeated with twofold dilutions until they were within the range of the standard curve. To determine the relative quantities of IgG equivalents, the log of the absorbance was compared to a least-squares line fit of the log of the absorbance of the serum control against the log dilution and the antibody levels were calculated as micrograms per milliliter of IgG equivalents.

The format for antibody subclass was similar to that for the analysis of total serotype-specific IgG, with the following exceptions. For the subclass assay, polysaccharides were adsorbed to plates at 2 μg/well. Serum dilutions for initial testing were at 1:50. A postvaccine serum with high activity was used on each plate as a control to standardize the results with the test sera, but no standard reference serum of known serotype-specific IgG subclass activity was available. Plates were probed with mouse monoclonal anti-human subclass-specific antibody (Southern Biotechnology Associates, Birmingham, Ala.).

We also adapted the protocol for measurement of serum IgG to the measurement of antigen-specific IgA in nasal wash specimens. For the IgA assay, wells were coated with 1 μg of polysaccharide per well (e.g., 100 μl of 10 μg/ml polysaccharide). Wells were postcoated with 1% BSA, and unconcentrated nasal wash specimens were diluted in buffer containing 10 μg of cell wall polysaccharide per ml at an initial dilution of 1:2. In preliminary studies, we established a standard nasal wash pool of seven nasal washes which exhibited high levels of serotype-specific activities to both type 6 and type 14; these washes were pooled as a standard to be run with each plate of samples. The activity of this pool was arbitrarily defined as 100 U of activity and used as a reference standard for calculation of specific IgA. The total IgA content of the nasal wash was also determined by ELISA with a standard curve of purified placental IgA (DAKO, Carpenteria, Calif.) for comparison. Values are reported as units of pneumococcal polysaccharide-specific IgA activity after adjustment for total IgA content.

**Statistics.** Antibody levels and fold rises in titers were log transformed to approximate a normal distribution for purposes of graphical presentation and comparisons. Because the fold rises in titers were not normally distributed even after log transformation, they were compared by the nonparametric Wilcoxon rank sum statistic test. Response rates and side effects were compared with the chi-square or Fisher exact test as appropriate. To adjust for multiple comparisons, a modified Bonferonni method was used (15).

This study was approved by the Human Investigations Committee of the University of Rochester. All subjects signed appropriate informed consent documents prior to study entry.

## RESULTS

A total of 99 subjects were enrolled in the study, including 50 younger adults and 49 older adults. The median age of the younger adults was 27 years, ranging from 18 to 45. The older adults ranged in age from 60 to 78, with a median age of 66 years.

Younger adult subjects were healthy and did not have acute or chronic medical conditions. Older adults were also generally healthy. Four older recipients of PS and four older recipients of CRM-OS had a history of angina and/or had had coronary bypass surgery; two subjects in the PS group and one subject in the CRM-OS group had a history of asthma or had had lung surgery, and none of the PS recipients and six subjects in the CRM-OS group had diabetes mellitus. All medical conditions were stable and under good control at the time of enrollment.

Signs and symptoms within the first seven days following vaccination are shown in Table 1. Both PS and the conjugate vaccine were associated with moderate pain at the injection site in a substantial proportion of young volunteers. Muscle aches and joint aches were noted on the diary card in smaller numbers of subjects following vaccination with both the conjugate vaccine and PS. There was a greater frequency of pain ($P = 0.04$) and of headache ($P = 0.02$) following administration of CRM-OS than following administration of PS in younger adults. Severe pain was noted in one recipient of PS and one recipient of CRM-OS. When symptoms occurred, they lasted a maximum of 4 days following vaccination. Although judged as moderate or severe on the diary card, symptoms were not disabling and there were no instances of missed days of work or school because of symptoms. Older adults experienced local pain less frequently than did younger adults, and there were no significant differences in the frequency of pain or other symptoms between subjects who received CRM-OS and those who received PS in this age group.

Revaccination of younger adults with PS 2 months after vaccination with the conjugate vaccine was associated with increased pain ($P = 0.052$), redness ($P = 0.08$), and swelling ($P = 0.004$) compared to results of the initial PS vaccination. The difference in frequency of pain among subjects after the initial vaccination and after revaccination with PS was also statistically significant in older adults ($P = 0.02$). Symptoms

TABLE 1. Signs and symptoms within 7 days of administration of CRM-OS or PS to younger and older adults and after revaccination with PS or a saline placebo

| Group | Vaccine administered | No. of subjects | No. of subjects with: | | | No. of subjects with moderate or severe symptoms of: | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Temp ≥38°C | Redness >20 mm | Swelling >20 mm | Pain | Muscle aches | Joint aches | Headache |
| Younger adults | CRM-OS | 25 | 1 | 5[a] | 3 | 19[b] | 6 | 2 | 11[c] |
| | PS | 25 | 0 | 1[a,d] | 0[e] | 12[b,f] | 8 | 2 | 4[c] |
| | PS after CRM-OS | 24 | 0 | 5[d] | 7[e] | 18[f] | 11 | 5 | 6 |
| Older adults | CRM-OS | 25 | 0 | 2 | 1 | 1 | 1 | 1 | 1 |
| | PS | 24 | 0 | 0 | 0 | 1[g] | 0 | 0 | 1 |
| | PS after CRM-OS | 23 | 1 | 3 | 3 | 7[g] | 0 | 0 | 0 |

[a] $P = 0.08$, Fisher exact test.
[b] $P = 0.04$, chi-square test.
[c] $P = 0.02$, Fisher exact test.
[d] $P = 0.08$, Fisher exact test.
[e] $P = 0.004$, Fisher exact test.
[f] $P = 0.052$, chi-square test.
[g] $P = 0.02$, Fisher exact test.

following revaccination resolved within 5 days, were judged to be severe in two younger adults and no older adults, and did not limit activity. Administration of a saline placebo to individuals who had previously received PS did not result in local signs or symptoms (data not shown).

The levels of type-specific anti-capsular polysaccharide IgG antibody in sera before and after vaccination are shown in Fig. 1. As expected, antibody responses to type 14 PS were seen in most young and elderly recipients of PS and the two age groups responded similarly to vaccination. A total of 16 of 25 (64%) younger adults and 16 of 24 (67%) older adults manifested fourfold or greater increases in antibody levels to type 14 following PS vaccination, and the geometric mean antibody levels in the two age groups were similar 1 month after vaccination (12.6 versus 18.2 μg/ml in young and old adults, respectively; the *P* value was not significant). Conjugation of the type 14 oligosaccharide to CRM did not significantly improve responses to this serotype in young adults. Fourfold responses to type 14 were seen in 17 of 25 (68%) young adults following CRM-OS vaccination, and the geometric mean titer 1 month following vaccination with CRM-OS (17.3 μg/ml) was only slightly higher than after vaccination with PS (the *P* value was not significant). Type 14 responses of older adults to CRM-OS were slightly decreased compared to those to PS. Fourfold responses to type 14 were seen in 8 of 25 (32%) older adults following CRM-OS vaccination. This rate was significantly lower than the rate following PS vaccination in older adults ($P = 0.015$) and lower than the rate of response to CRM-OS in younger adults ($P = 0.01$). The level of antibody in older adults following CRM-OS vaccine was also lower (8.8 μg/ml). However, the difference between geometric mean titers to type 14 of older adults administered CRM-OS and those administered PS was not statistically significant.

Serotype 6B responses were decreased in comparison to type 14 responses in both younger and older adults. Fourfold responses to 6B were seen in 9 of 25 (36%) younger adults and 9 of 24 (38%) older adults following PS vaccination, and postvaccination antibody levels to type 6B were lower than those to type 14 (Fig. 1). Conjugation of the 6B oligosaccharide to $CRM_{197}$ improved the 6B response in young adults, with 14 of 25 (56%) subjects achieving a fourfold increase in antibody level ($P = 0.15$ compared to the rate with PS) and a significantly higher geometric mean antibody level after vaccination with CRM-OS than after vaccination with PS (10.9 versus 3.7 μg/ml; $P = 0.04$). However, no improvement by oligosaccharide conjugation was seen in elderly adults, who had both similar rates of fourfold responses (11 of 25 [44%] versus 9 of 24 [38%]) and similar mean antibody levels to 6B (3.2 versus 4.4 μg/ml) after CRM-OS and after PS vaccination, respectively.

There was relatively little response to revaccination with PS in subjects who initially received CRM-OS. No subject had more than a twofold rise in titer to serotype 6B (mean fold rise, 1.12; 95% confidence interval [CI], 1.05 to 1.20). Only two subjects (one older and one younger) had over a twofold (but not a fourfold) increase in antibody titer to type 14 (mean fold rise, 1.18; 95% CI, 1.06 to 1.31). This was also true of the subjects who did not respond to the initial CRM-OS injection. Older subjects exhibited a slightly greater increase in antibody titer following revaccination, with a median fold rise of 1.2 (95% CI, 1.08 to 1.32) against type 14 and a fold rise of 1.2 (95% CI, 1.12 to 1.34) against type 6B.

Figure 2 depicts the magnitude of the IgG1 and IgG2 subclass responses against the two polysaccharides, expressed as the geometric mean fold rises, or ratios, between prevaccination and first postvaccination samples. Because there was little measurable IgG3 and IgG4 activity, these subclasses were not analyzed further.

The results of the subclass analysis generally paralleled those for total IgG responses. The fold increase in type-14-specific IgG1 and IgG2 was greater than the fold increase in type-6B-specific IgG1 and IgG2 following both PS and CRM-OS vaccinations in young and elderly adults. Polysaccharide vaccine generally resulted in a relatively greater increase in IgG2 than in IgG1, although the differences in mean fold rises between subclasses were not statistically significant between any group. The fold increase in IgG2 was greater than the fold increase in IgG1 against type 14 in 34 of 49 and against type 6B in 33 of 49 individual recipients of PS. Conjugated vaccine also resulted in relatively greater increases in IgG2, although there was a trend towards a slight increase in IgG1 responses in young adults, particularly to type 6B, since 12 of 25 young recipients of CRM-OS had an IgG1 response greater than their IgG2 response. Overall, however, the differences in fold increases and

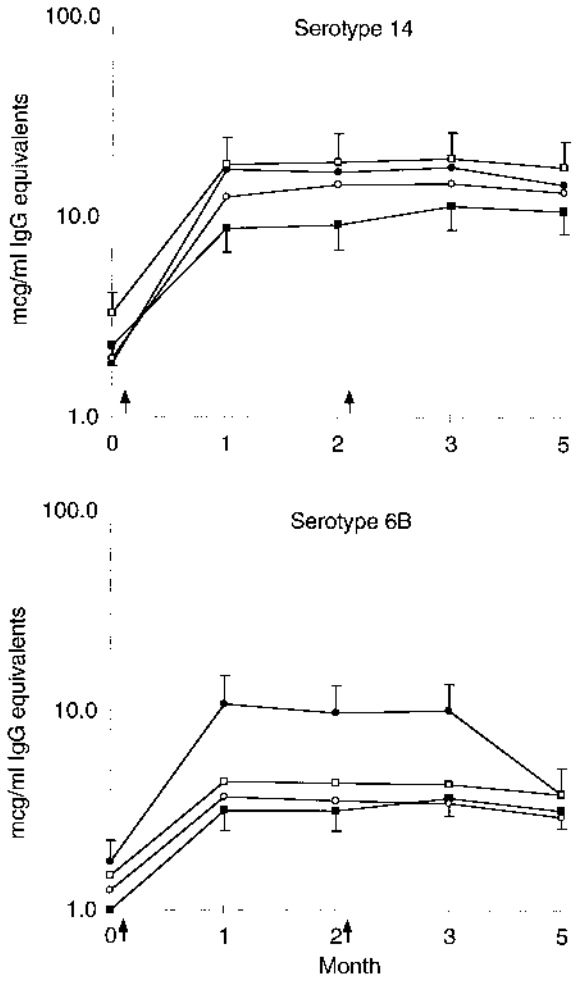


FIG. 1. Antibody levels against type 14 (top) and type 6B (bottom) by month of study. Vaccines were given at months 0 and 2, as indicated by arrows. Circles represent younger adults, and squares represent older adults. Subjects represented by filled symbols received CRM-OS and then PS at month 2, while subjects represented by open symbols received PS and then a saline placebo at month 2. T bars represent standard errors. Statistical comparisons are given in the text.

response rates between subclasses were small and not statistically significant.

We also evaluated mucosal antibody responses to capsular polysaccharide by analyzing serotype-specific IgA in nasal secretions by ELISA before and after the first vaccination, as shown in Table 2. Twofold or greater increases in serotype-specific mucosal IgA were detected relatively infrequently following immunization, and there were no fourfold increases in IgA. There was a statistically significant difference between nasal IgA response rates in young adults vaccinated with CRM-OS and rates in those vaccinated with PS. However, overall, there was no difference between pre- and postimmunization antigen-specific mucosal IgA titers for either serotype with either vaccine.

## DISCUSSION

Administration of either licensed PS or conjugated oligosaccharide vaccine to young adults was associated with moderate local pain in the majority of recipients; these symptoms occurred following the administration of conjugated vaccine more frequently than following the administration of PS. No subject refused a second immunization because of pain. As expected, those receiving the conjugated vaccine who were reimmunized with PS had increased frequency of transient, moderate pain compared to those receiving PS initially; these results are similar to but less severe than local reactions described when polysaccharide vaccines were readministered within 1 year (6, 7). It has been suggested that the risk of such local reactions on revaccination may be related to the level of existing antibody to the pneumococcal PS (14, 24). For reasons that are not clear, older individuals were significantly less likely to complain of pain following administration of either conjugated vaccine or PS than their younger counterparts; similar observations have been made with influenza vaccine (34).

Conjugation of the 6B oligosaccharide to $CRM_{197}$ significantly enhanced the immunogenicity of this antigen in young adults, compared to that of PS alone to 6B. In contrast, despite enhanced immunogenicity in the younger adults, older adults exhibited no increased immunogenicity of $CRM_{197}$-conjugated 6B oligosaccharide compared to that of PS. For the more immunogenic type 14, conjugation of oligosaccharide to $CRM_{197}$ appeared to slightly decrease the immunogenicity of the vaccine in the elderly, compared to results with PS. This may reflect alteration of the carbohydrate during derivation of the oligosaccharides or be reflective of the lower total 6B carbohydrate dose in CRM-OS. This may be similar to the findings for patients with Hodgkin's disease, in whom reduction of the vaccine dose to 1 μg of PS/serotype resulted in a decrease in titer to one-half that seen with the standard PS vaccine (20).

Because they influence the interactions between T-helper cells and B cells, glycoconjugate vaccines may alter the subclass of antibody generated. Most studies have shown that IgG2 is the most abundant subclass of antibody produced following pneumococcal PS vaccination (1, 30). However, increases in opsonizing antibody have been primarily correlated with increases in serum IgG1 and IgG4 antibody, while the presence of complement phagocytosis is more strongly correlated with the IgG1 and IgG2 subclasses (5, 16). Glycoconjugate vaccines could have an advantage if they are associated with shifts in the subclass of antibody to more functional forms. Consistent with previous studies, we also found that responses to PS tended to favor the IgG2 subclass. Conjugation of oligosaccharide to CRM did not significantly affect the ratio of IgG1 to IgG2 responses to vaccination, although there were trends in this direction in young adults.

Polysaccharide vaccines do not induce memory, and revaccination generally does not result in levels of antibody which exceed those seen on initial vaccination. Because glycoconjugate vaccines probably induce T-cell memory, boosted responses might be seen when individuals were revaccinated or were subjected to natural exposure to pneumococci. This phenomenon of T-cell memory may improve the protective efficacies of such vaccines if such boosted responses occur in response to nasopharyngeal colonization. In order to approximate this effect, CRM-OS recipients were revaccinated with PS 2 months after the initial vaccination. While revaccination was well tolerated, significant antibody responses to this maneuver were not detected in either younger or older adults. However, preliminary studies of animals (22) suggest that a greater interval between vaccinations and/or use of a subimmunogenic challenge dose of PS may be more effective in demonstrating such an effect, if it is present.

Because *S. pneumoniae* is a mucosal pathogen, we evaluated by ELISA mucosal antibody responses in nasal secretions collected by nasal wash. It has been suggested that mucosal re-

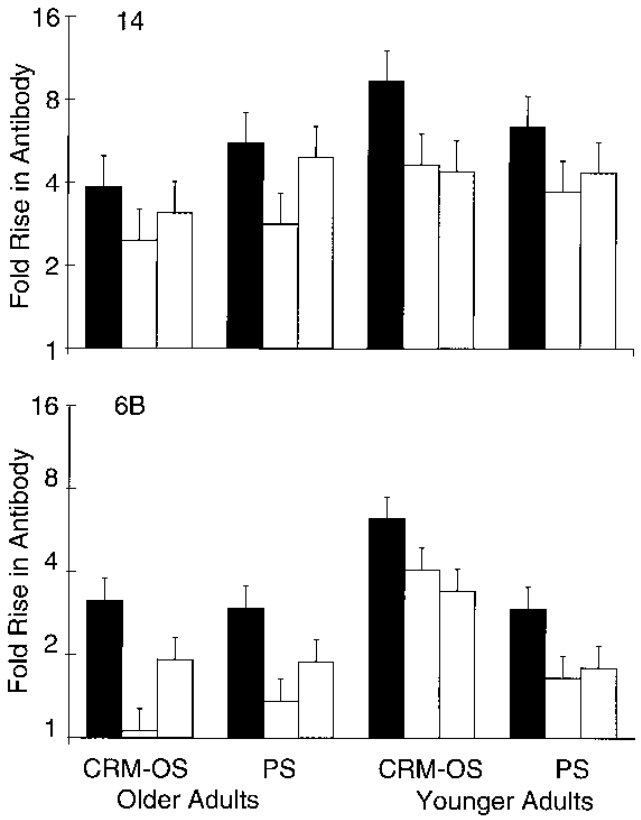


FIG. 2. Geometric mean fold increases in antibody levels at the time of prevaccination (month 0) and 1 month after the first vaccination (month 1) against type 14 (top) and 6B (bottom) pneumococci; data are categorized by vaccine and age group with standard errors noted with T bars. Filled bars indicate the fold increases in total IgG, open bars indicate the fold increases in IgG1, and shaded bars indicate the fold increases in IgG2. Statistical comparisons are given in the text.

sponses to parenterally administered vaccines may occur in individuals who have had previous mucosal exposure to antigen (19), as is expected for *S. pneumoniae*. However, we did not detect an overall significant increase in the level of type-specific IgA antibody to either serotype tested in nasal secretions following administration of either the conjugated vaccine or PS, although some individuals did manifest twofold or greater increases in titers.

There are relatively few previous reports of measurements of mucosal IgA responses to pneumococcal vaccines in humans. In one study, Lue et al. reported approximately twofold increases in specific IgA in tears on days 7 and 14 following administration of licensed PS to healthy adults (18); pneumococcus-specific IgA levels by day 28 after immunization were not different than such levels before immunization. The results of the two studies are difficult to compare because of significant differences in methodology, including the use of tears rather than nasal wash, evaluation of mucosal responses at earlier time points, and the use of a poly-L-lysine-conjugated 23-valent pneumococcal vaccine as the ELISA coating antigen in the previous report (18). Although there is no clear-cut relationship between mucosal antibody and pneumococcal vaccine efficacy, further standardization of measurements of pneumococcal mucosal antibody may be useful in future pneumococcal vaccine studies.

Several factors which may influence the immunogenicities of glycoconjugate vaccines have been identified. These include the physical nature of the carbohydrate antigen as an oligosaccharide or polysaccharide, the ratio of carbohydrate to protein present in the material, the presence of free polysaccharide, the use of adjuvants, and the specific carrier protein used (2). Thus, a greater effect of conjugation on immune responses might have been seen in our study if a more immunogenic form of glycoconjugate had been available. However, it should be noted that the form of conjugation used in this study did result in increased responses to the poorly immunogenic serotype 6B in young adults, in contrast to the lack of enhancement in the elderly.

Finally, the presence of previous priming to the carrier protein could significantly influence the response to glycoconjugate vaccines, particularly for diphtheria toxin-based conjugates (13). We did not measure antibody to diphtheria toxin in these subjects and specifically excluded subjects with recent tetanus-diphtheria vaccination in order to reduce the risk of arthus-like reactions to CRM-OS. Thus, it is possible that the subjects in this trial, particularly the older subjects, may have had a remote history of diphtheria vaccination and may have been poorly primed for initial responses to CRM-OS. Under these circumstances, a multiple-dose regimen of CRM-OS vaccination may have improved responses, as seen with $CRM_{197}$-conjugated Hib vaccines in infants (13).

The results of this study are similar to those of a recent study evaluating pneumococcal $CRM_{197}$-OS in adults over the age of 50 (25) and extend those results by evaluation of IgG subclass and mucosal IgA responses and by concurrent comparison with responses to the same lot of vaccine in young adults. Both studies suggest that, while reasonably well tolerated, the CRM-OS formulation of pneumococcal glycoconjugate vaccine used does not appear to offer an advantage over polysaccharide pneumococcal vaccines in older adults. However, the potential for enhanced immunogenicity in the elderly by more potent formulations or more effective administration schedules of conjugated pneumococcal vaccines exists, and such vaccines

TABLE 2. Type-specific IgA responses in nasal secretions of younger and older adults following administration of PS or CRM-OS

| Group | Vaccine | Response to 6B | | | Response to type 14 | | |
|---|---|---|---|---|---|---|---|
| | | Mean $\log_2$ units of IgA ± SE | | No. of subjects/no. of subjects tested with twofold rise (%) | Mean $\log_2$ units of IgA ± SE | | No. of subjects/no. of subjects tested with twofold rise (%) |
| | | Prevaccination | Postvaccination | | Prevaccination | Postvaccination | |
| Younger adults | PS | 3.50 ± 0.14 | 3.10 ± 0.17 | 0/25 (0) | 3.37 ± 0.16 | 3.11 ± 0.19 | 0/25 (0) |
| | CRM-OS | 3.73 ± 0.22 | 3.79 ± 0.23 | 5/25 (20)[a] | 3.55 ± 0.23 | 3.70 ± 0.23 | 5/24 (21)[a] |
| Older adults | PS | 3.10 ± 0.19 | 3.11 ± 0.18 | 3/24 (8) | 3.04 ± 0.17 | 3.14 ± 0.20 | 1/24 (4) |
| | CRM-OS | 3.03 ± 0.20 | 2.90 ± 0.21 | 3/24 (8) | 3.05 ± 0.20 | 3.16 ± 0.20 | 3/23 (13) |

[a] $P = 0.02$ when results from recipients of CRM-OS and PS are compared by the Fisher exact test.

remain an option for enhancing pneumococcal vaccine performance in this age group.


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