V_{H3}-Antibody Response to Immunization with Pneumococcal Polysaccharide Vaccine in Middle-aged and Elderly Persons

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Running title: V_{H3}-antibody response to pneumococcal vaccine
Keywords: pneumococcal vaccine, aging, revaccination, V_{H3} antibody

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Abstract

Background: Pneumococcal disease continues to cause substantial morbidity and mortality among the elderly. Older adults may have high levels of anticapsular antibody after vaccination, but their antibodies show decreased functional activity. In addition, the protective effect of the pneumococcal polysaccharide vaccine (PPV) seems to cease as early as 3-5 years post-vaccination. Recently it has been suggested that PPV elicit human antibodies that predominantly use \( V_{H3} \) gene segments, and induce a repertoire shift with increased \( V_{H3} \) expression in peripheral B-cells. Here we compared \( V_{H3} \)-idiotypic antibody responses in middle-aged and elderly subjects receiving PPV as initial immunization or as revaccination.

Materials: We studied pre- and post-vaccination sera from 36 (18 vaccine naïve and 18 previously immunized) middle-aged and 40 (22 vaccine-naïve and 18 previously immunized) elderly adults who received 23-valent PPV. Concentrations of IgG to four individual serotypes (6B, 14, 19F and 23F) and \( V_{H3} \)-idiotypic antibodies (detected by the monoclonal antibody D12) to the whole pneumococcal vaccine were determined by ELISA.

Results: PPV elicited significant IgG and \( V_{H3} \)-idiotypic antibody responses in middle-aged and elderly subjects, whether vaccine-naïve or undergoing re-vaccination. Age did not influence the magnitude of the antibody responses, which was evidenced by similar post-vaccination IgG and \( V_{H3} \) levels in both groups even after stratifying by prior vaccine status. Furthermore, we found similar proportions (around 50%) of elderly and middle-aged subjects experiencing two-fold increases in \( V_{H3} \) antibody titers after vaccination.
Conclusions: Age or repeated immunization does not appear to affect the $V_{H3}$-idiotypic immunogenicity of PPV among middle-aged and elderly adults.
Introduction

*Streptococcus pneumoniae* is the leading cause of bacterial pneumonia and bacterial meningitis in the United States, resulting in 175,000 hospitalizations and 7,000 to 12,000 deaths annually. Groups with the highest incidence of pneumococcal infection include the very young, the elderly, persons who are immunocompromised, smokers, and certain other demographic groups (2, 8). In persons 65 years or older, the incidence of invasive pneumococcal disease (IPD) is around 50 per 100,000 per year, and is associated with a case fatality rate of 20% whereas among those aged 85 years or older, the fatality rate increases to 40% (2, 34).

The Advisory Committee on Immunization Practices recommends vaccinating all adults aged 65 years or older with the 23-valent pneumococcal polysaccharide vaccine (PPV). One-time revaccination for this age group is also recommended if they received their first dose ≥ 5 years previously and before the age of 65 years (6). A recent meta-analysis provided evidence supporting the recommendation for PPV to prevent IPD in adults. However, it did not provide compelling evidence to support the routine use of PPV to prevent all-cause pneumonia or mortality (15). In addition, significant protection against IPD seems to be lost as early as 3-5 years after vaccination in persons older than 65 years (28, 29).

A common surrogate for antibody-mediated protection is the measurement of post-vaccination IgG antibody to capsular polysaccharides contained in PPV. Validation of this measure may be disputed given the fact that even adequate IgG concentrations in the elderly may have significant reductions in antibody functional activity to pneumococcal
polysaccharide antigens (25). Molecular characterization of the immune response to pneumococcal polysaccharides is seldom performed in clinical vaccine studies (24); however, there is a large body of literature on this subject (3, 5, 7, 22, 38). Recent studies have demonstrated that PPV stimulates an increased expression of variable region heavy chain family 3 (VH3) genes in peripheral B cells from immunocompetent subjects, yielding serum polysaccharide-specific antibodies and/or B-cells that express VH3 (1, 7, 32, 33). VH3 responses may also correlate with functional activity of anti-pneumococcal antibodies (3).

Previous studies on gene expression of the B-cell repertoire of the total circulating B-cell population demonstrated a shift toward VH4 and VH1 expression in aging humans compared with predominant VH3 expression in young subjects (35). This repertoire shift has been postulated as a possible mechanism of decreased pneumococcal anti-capsular antibody function in older populations. In this regard, a preliminary report (30) found a lower expression of VH3-idiotypic antibody responses to capsular polysaccharides from serotype 4 but not serotype 14 in the elderly when compared with young individuals. A subsequent study (11) of the VH gene repertoire of human peripheral B cells specific for these two capsular polysaccharides (4 and 14) revealed that the response in both age groups was dominated by the VH3 gene family (> 90%). The VH1, VH4, and VH5 gene families were also isolated from both groups but they constituted <10% of the total heavy chain repertoire. Given the attractiveness of the study of VH3-idiotypic antibody responses to assess the immunogenicity of pneumococcal polysaccharide antigens and the need of further studies on its role in aging, we evaluated IgG and VH3-idiotypic antibody
responses after administration of PPV in sera from a subset of vaccine-naïve and revaccinated middle-aged and elderly subjects enrolled in a pneumococcal vaccine clinical trial (19).

**Material and Methods**

Studies were done with pre- and post-vaccination sera from 36 (18 vaccine-naïve and 18 previously immunized) middle-aged (50-64 years) and 40 (22 vaccine-naïve and 18 previously immunized) elderly (>=65 years) adults who received one intramuscular dose of the 23-valent pneumococcal polysaccharide vaccine (Pneumovax 23; Merck, West Point PA). This vaccine contains 25 µg of each of the following polysaccharide types per 0.5 mL: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33.

Study subjects were participants of a multisite study evaluating the safety and immunogenicity of primary vaccination and revaccination with PPV and were recruited and vaccinated at the Houston Veterans Affairs Medical Center under a protocol that was approved by the Institutional Review Board, Baylor College of Medicine, as previously described (19). Subjects were included based on the availability of sera. Re-immunized subjects had received the initial vaccine 3-5 years previously as part of their routine medical care. Blood samples were obtained before and 4 weeks after initial vaccination or revaccination. Serum was separated from whole blood samples by centrifugation and stored at -70°C until analyzed. For the current study, all samples were re-assayed as described below. Isotype expression of antibodies: The pre- and post-vaccination concentrations of IgG to pneumococcal capsular polysaccharides type 6B, 14, 19F and
23F were determined by a sandwich ELISA, as it has been described elsewhere (36). We decided to study four representative serotypes that are reported to cause a significant number of cases of IPD among middle-aged and elderly persons in the United States (37).

Serum samples were pre-absorbed with 5 µg/mL pneumococcal cell-wall polysaccharide (CWPS; Statens Seruminstitut) and 5 µg/mL type 22F CPS (ATCC) for 30 min at room temperature following the World Health Organization guidance protocol for pneumococcal antibody ELISA (36) (this methodology differs from the one described for the main study which used serotypes 25 and 72 for adsorption)(19). Dilutions of a laboratory reference serum (Pool 89-SF; Food and Drug Administration) were included on every ELISA plate. All serum samples from each individual were studied concurrently.

Idiotype expression of antibodies: The V_{H}3 expression of antibodies to the pneumococcal vaccine in pre- and post-vaccination sera was determined by ELISA, as described elsewhere (1). In brief, 96-well polystyrene plates (Costar; Corning Glass Works) were
coated with 10 µg/mL of whole PPV in PBS for 5 hours at 37°C and then kept overnight at 4°C. Before use, plates were blocked with PBS containing 1% bovine serum albumin (Fisher Scientific) for 1 hour and washed with PBS containing 0.05% Tween 20 (Fisher Scientific). ELISA plates were incubated for 2 hour at 37°C with CWPS-absorbed pre- and post-vaccine serum samples diluted 1:3, beginning with an initial dilution of 1:10 in PBS. After incubation, the plates were washed and incubated for 1 h at 37°C with the murine monoclonal antibody D12, which binds to a variable region determinant expressed by certain antibodies encoded by V_{H}3, at a concentration of 5 µg/mL. The binding of the D12-bound serum antibodies was detected with alkaline phosphatase–labeled goat anti–mouse IgG1 (Southern Biotech). The plates then were washed and developed with p-nitrophenyl phosphate, and absorbances and titers were calculated.

Statistics: Geometric mean concentrations (GMC) of IgG to individual capsular polysaccharides and geometric mean V_{H}3 titers to the whole vaccine and their corresponding 95% confidence intervals were calculated. All antibody concentrations and
titers were log_{10}-converted before any statistical comparison was made. The pre- and post-vaccination levels were compared between groups (middle-aged and elderly) by unpaired Student’s t test. The pre- and post-vaccination levels within each group were compared by paired Student’s t test. Stratification by vaccination status was also studied and comparison of multiple subgroups was done with analysis of variance. Pearson’s correlation coefficient was calculated to measure the level of association between IgG and V_{H3}-idiotype antibody responses (defined as post minus pre-vaccination log-converted antibody levels). Multiple comparisons were accounted for by using Bonferroni correction. Statistical analyses were implemented using STATA 9.2 (College Station, Texas) and p-values < 0.05 were considered significant.

Results

IgG antibody response: GMC of IgG to individual pneumococcal capsular polysaccharides (serotypes) according to age group and vaccination status are shown in Table 1. Pre- and post-vaccination IgG levels were similar between middle-aged and elderly groups. When stratified by vaccine status, pre-vaccination IgG level was higher in previously immunized subjects than in vaccine-naive in both groups and for all serotypes; however this difference was statistically significant only for serotype 14 in both middle-aged and elderly groups (P < 0.03 for both comparisons). Despite these differences in pre-vaccine levels, post-vaccination IgG levels were similar in all subgroups. Middle-aged and elderly groups showed significant increases (within-group comparisons) in their IgG levels to all four serotypes (P < 0.01 for all comparisons). Stratified analysis also
showed significant IgG increases in all subgroups to all serotypes (P < 0.05 for all comparisons).

**VH3-idiotype antibody response:** Pre- and post-vaccination VH3 levels were similar between middle-aged and elderly groups even after stratification by vaccine status. Both groups showed significant increases (within-group comparisons) in their VH3 antibody titers after vaccination with PPV (P < 0.01 for both comparisons) (Table 2). Stratified analysis also showed significant increases in VH3 levels in all subgroups (P < 0.03 for all comparisons) (Figure 1). Comparable proportions of middle-aged and elderly patients experience two-fold increases in VH3 antibody titers (54.8% and 45.7%, respectively, P = 0.46). Although not a statistically significant difference, a higher proportion of vaccine-naïve subject had 2-fold increases in VH3 antibody titers when compared to revaccinated groups (75% and 55% vs. 33. 3% and 33.3% for middle-aged and elderly patients, respectively) (P = 0.06).

**Correlations:** VH3-idiotypic antibody responses were correlated with IgG responses to serotypes 14 (r= 0.36, P = 0.03) and 23F (r = 0.45, P < 0.01). No significant correlations were observed for the other two serotypes.

**Discussion**

In our study, age did not influence the magnitude or the quality of the antibody responses elicited by the pneumococcal polysaccharide vaccine among either vaccine-naïve or previously immunized subjects. This was evidenced by similar post-vaccination IgG and VH3 levels in middle-aged and elderly groups even after stratifying by prior vaccine
status. Furthermore, our study showed similar proportions of healthy elderly and middle-aged subjects experiencing two-fold increases in $V_{H3}$ antibody titers after pneumococcal polysaccharide vaccine.

Several previous studies had shown a similar IgG antibody response to pneumococcal polysaccharides in elderly individuals compared with their younger counterparts (4, 17, 26, 27); however, these levels do not always accurately correlate with the functional antibody activity (25) nor with protection against pneumococcal disease (20). In addition, other studies have suggested that humoral responses among elderly subjects correlate better with their functional status than with their chronological age (4, 23). Our patients were all ambulatory subjects without concomitant debilitating acute or chronic illness, who were expected to survive for 5 years after study enrollment (19). Therefore our results on $V_{H3}$ response among ambulatory elderly subjects may not be extrapolated to elderly frail patients.

As a vaccine composed of polysaccharide antigens, PPV induces a T-cell-independent response and thus, an anamnestic response (i.e., booster) is unlikely (31). Immunologic tolerance or hyporesponsiveness induced by prior polysaccharide antigen exposure constitutes a potential concern when considering revaccination with PPV (9, 21). Although pre-vaccination IgG levels to some serotypes were higher in previously immunized subjects, post-vaccination levels actually reached similar levels in all subgroups. However, as a result of higher pre-vaccination titers, a lower proportion of revaccinated subjects achieve a 2-fold response. Our results are concurrent with those of the main study (19). In addition, in a different substudy, Manoff and collaborators
showed that revaccination of adults aged ≥65 years was comparable to primary vaccination for inducing elevated and persistent functional (measured by opsonophagocytic assay) specific anticapsular antibodies; in this study the results of the functional assays correlated with the IgG antibody responses (14). These results agree with those of earlier studies that revaccination elicits a significant response but not of greater intensity than that to the initial vaccination (10, 14, 16, 34); and most importantly, it indicates that any possible difference initially observed in the antibody response of revaccinated and vaccine-naïve subjects do not persist over time. Among HIV infected patients on HAART, the V<sub>H</sub>3 response to polysaccharide antigens after revaccination was similar to that of initial vaccination (32). Similarly, our data indicate that adults aged 50-65 years or >65 do mount a significant serotype- and V<sub>H</sub>3-specific antibody response to revaccination with PPV. However, we have not evaluated the functional activity of the V<sub>H</sub>3 specific antibodies, and correlation of V<sub>H</sub>3 specific antibodies with function, although supported by one small study (3), continues to be speculative.

We found a linear correlation between V<sub>H</sub>3 antibodies to the whole vaccine and IgG responses to two serotypes (14 and 23F). In a previous study (1) utilizing the same assay, antibodies to capsular polysaccharides of serotypes 3, 6B and 14 did not yield V<sub>H</sub>3 determinants recognized by the D12 monoclonal antibody. The prior study evaluated the antibody response among a younger group of subjects. It is possible that there is a differential expression of V<sub>H</sub>3 epitopes among different serotypes that is influenced by age [11,29].
We measured $V_{H3}$ response with the use of the D12 monoclonal antibody and a whole vaccine ELISA because we had previously demonstrated that the determination of whole vaccine D12-positive antibodies is a reliable method of demonstrating pre- to post-vaccine increases in D12-positive antibodies (32). With this assay, 23 (35%) of 66 patients had no detectable $V_{H3}$ response. The D12 monoclonal antibody does not appear to recognize all $V_{H3}$ family antibodies, and it is possible that by the use of a panel consisting of several monoclonal antibodies that recognize different $V_{H3}$ epitopes, we would have been able to detect greater $V_{H3}$ responses (1). Hence, we are limited on our ability to detect additional differences in age related responses, responses based on prior immunization status, or correlation with IgG responses. In addition, we did not study any other $V_{H}$ gene families; which limits our ability to detect any subtle shifts in $V_{H}$ family use. More complex PCR-based methodology would have increased our ability to better characterize the entire $V_{H}$ repertoire (12, 39).

A decreased production of $V_{H3}$ antibodies and a shift to a $V_{H5}$ gene family after PPV have been postulated as contributing factors to the increased susceptibility to *S. pneumoniae* infections among HIV infected patients (1, 7, 32). Thus, it appears reasonable to hypothesize that the study of $V_{H3}$ antibody responses may offer an additional approach to test the antibody response after administration of the pneumococcal polysaccharide vaccine. We demonstrated that vaccination with pneumococcal polysaccharide vaccine elicits significant IgG and $V_{H3}$-idiotypic antibody responses in middle-aged and elderly subjects, whether vaccine-naïve or undergoing re-vaccination 3 -5 years after a primary immunization. $V_{H3}$ antibody response was not
impaired among elderly adults. Vaccination and revaccination with pneumococcal polysaccharide vaccine after 3-5 years appears to be an effective strategy to elicit isotypic and $V_{H}3$-idiotypic immunogenicity in middle-aged and elderly adults (10, 13, 16, 18, 27, 28, 34).

**Bibliography**

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Table 1: Geometric mean concentrations of IgG antibodies to individual pneumococcal capsular polysaccharides in pre- and post vaccination sera from middle-aged and elderly subjects according to vaccine status

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Serotype 6B</th>
<th>Serotype 14</th>
<th>Serotype 19F</th>
<th>Serotype 23F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre Post</td>
<td>Pre Post</td>
<td>Pre Post</td>
<td>Pre Post</td>
</tr>
<tr>
<td>Middle-aged</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=36)</td>
<td>1.97</td>
<td>3.03</td>
<td>3.26</td>
<td>9.33</td>
</tr>
<tr>
<td></td>
<td>(1.46 – 2.65)</td>
<td>(2.35 – 3.92)</td>
<td>(2.38 – 4.47)</td>
<td>(6.33 – 13.74)</td>
</tr>
<tr>
<td>Vaccine-naïve</td>
<td>1.68</td>
<td>2.73</td>
<td>2.06</td>
<td>11.20</td>
</tr>
<tr>
<td>(n=18)</td>
<td>(1.17 – 2.40)</td>
<td>(2.01 – 3.71)</td>
<td>(1.59 – 2.67)</td>
<td>(5.51 – 22.06)</td>
</tr>
<tr>
<td>Previously</td>
<td>2.30</td>
<td>3.36</td>
<td>5.17</td>
<td>7.89</td>
</tr>
<tr>
<td>vaccinated</td>
<td>(1.39 – 3.80)</td>
<td>(2.17 – 5.22)</td>
<td>(3.10 – 8.63)</td>
<td>(5.20 – 11.97)</td>
</tr>
<tr>
<td>(n=18)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elderly</td>
<td>2.21</td>
<td>3.18</td>
<td>3.89</td>
<td>7.45</td>
</tr>
<tr>
<td>(n=40)</td>
<td>(1.67 – 2.93)</td>
<td>(2.26 – 4.49)</td>
<td>(2.81 – 5.41)</td>
<td>(5.19 – 10.68)</td>
</tr>
<tr>
<td>Vaccine-naïve</td>
<td>1.99</td>
<td>3.32</td>
<td>2.67</td>
<td>6.65</td>
</tr>
<tr>
<td>(n=22)</td>
<td>(1.34 – 2.97)</td>
<td>(1.91 – 5.77)</td>
<td>(1.78 – 4.00)</td>
<td>(3.97 – 11.14)</td>
</tr>
<tr>
<td>Previously</td>
<td>2.17</td>
<td>3.50</td>
<td>6.20</td>
<td>8.55</td>
</tr>
<tr>
<td>vaccinated</td>
<td>(1.63 – 3.85)</td>
<td>(1.98 – 4.61)</td>
<td>(3.80 – 10.11)</td>
<td>(4.95 – 14.75)</td>
</tr>
<tr>
<td>(n=18)</td>
<td></td>
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</tbody>
</table>

Significant difference (P < 0.03) in log-converted pre-vaccination concentrations (µg/mL) between previously vaccinated and vaccine-naïve subjects

*Significant difference (P < 0.05) in IgG concentrations (within-group comparisons) for middle-age and elderly groups

95% confidence intervals are shown in parentheses.
Table 2. Geometric mean V<sub>H</sub>3 titers to pneumococcal polysaccharide vaccine in pre- and post-vaccination sera from middle-aged and elderly subjects according to vaccine status

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Pre-vaccination</th>
<th>Post-vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle-aged (N=31)</td>
<td>6.48 (5.10 – 8.24)</td>
<td>16.12† (10.92 – 23.78)</td>
</tr>
<tr>
<td>Vaccine-naïve (n=16)</td>
<td>5.65 (4.73 – 6.74)</td>
<td>21.60† (13.45 – 34.70)</td>
</tr>
<tr>
<td>Previously vaccinated (n=15)</td>
<td>7.51 (4.66 – 12.13)</td>
<td>11.80† (6.18 – 22.49)</td>
</tr>
<tr>
<td>Elderly (n=35)</td>
<td>7.38 (5.87 – 9.27)</td>
<td>20.86† (13.27 – 32.78)</td>
</tr>
<tr>
<td>Vaccine-naïve (n=20)</td>
<td>6.06 (4.81 – 7.63)</td>
<td>21.05† (11.26 – 39.35)</td>
</tr>
<tr>
<td>Previously vaccinated (n=15)</td>
<td>9.59 (6.19 – 14.84)</td>
<td>20.60† (9.84 – 43.16)</td>
</tr>
</tbody>
</table>

No significant differences in log-converted pre- or post-vaccination titers (units) between middle-aged and elderly subjects (95% confidence intervals are shown in parentheses)

†Significant increase (P < 0.03) in log-converted V<sub>H</sub>3 titers (within-group comparisons) for middle-age and elderly groups
Figure 1: Log10-converted \(V_{\mathrm{H}3}\)-idiotypic antibody titers to pneumococcal polysaccharide vaccine in pre- and post-vaccination sera from middle-aged and elderly subjects according to vaccination status (\(^*\) denotes a P-value < 0.05)