Elderly Immune Response to a TI-2 Antigen: Heavy and Light Chain Use and Bactericidal Activity to Neisseria meningitidis Serogroup C Polysaccharide

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Previous studies of the elderly immune response to TI-2 antigens failed to correlate specific antibody levels with function and to compare responses with those of young adults. Neisseria meningitidis serogroup C capsular polysaccharide (MCPS) was used as a model TI-2 antigen. Anti–MCPS antibody levels were determined in elderly individuals and correlated with bactericidal activity. The anti–MCPS response in most persons was characterized by predominant IgG usage, with IgG2 > IgG1. No light chain or IgA subclass predominated, but some responses showed a particular chain type. Bactericidal activity correlated best with IgG2 levels. Elderly subjects had lower anti–MCPS responses than the young adults did in all chain-specific anti–MCPS levels, and levels declined more rapidly. Bactericidal activity following immunization was significantly lower in the elderly persons. These results suggest the anti–MCPS antibody repertoire in the elderly is likely maintained, and the lower level of function is related to the lower antibody levels.

Pneumonia is a major problem in the elderly, with mortality rates of 24/100,000 in those aged 60–64 years and 1032/100,000 for ages ≥85 [1]. Many studies have found that Streptococcus pneumoniae is the most common cause of community-acquired pneumonia in the elderly (reviewed in [2]). Research of age-related changes in host response to infection is unclear or contradictory [3] and is lacking at the level of antibody chain use and function. With the current expanding geriatric population, there will be a demand for this specialized knowledge and for alternative approaches to prevent infections in the elderly.

Age is an independent factor in declining immune function. Both cellular and humoral immunity are affected (reviewed in [3–5]). Total immunoglobulin production may not be affected, may decline, or, in some cases, may actually increase [5–7]. The presently available polysaccharide pneumococcal vaccine has not been shown to be efficacious in some elderly adults; despite seemingly adequate antibody levels, these persons are not protected [8–10]. The decrease in functional immunity remains largely undescribed.

The bacterial capsular polysaccharides are T cell–independent (TI-2) antigens, and antibodies to these antigens correlate with protection. To date, limited studies in humans have determined the quantitative changes in IgG, IgM, or IgA class antibodies, and no studies address antibody light chain and subclass use with functional activity in the aging response to TI-2 antigens. Most studies on the aging immune response have been performed in mice, which show loss of specificity and protective function in response to TI-2 antigens (reviewed in [3, 4, 11]).

We used the capsular polysaccharide of Neisseria meningitidis serogroup C (MCPS) as our prototype TI-2 antigen. N. meningitidis is an encapsulated gram-negative diplococcus capable of causing severe systemic disease. N. meningitidis serogroup C causes epidemic disease in developed countries [12, 13] and epidemic disease in sub-Saharan Africa [14]. The incidence of nonmeningitic N. meningitidis disease also modestly increases in the elderly [12]. Although the target population of N. meningitidis consists predominantly of the very young, there are several advantages associated with the use of this antigen in a study of the aging human immune response. First, a licensed purified polysaccharide vaccine is presently available. Second, most individuals will not receive the vaccine in their lifetime, as it is reserved for those in the military, contacts in episodic and epidemic disease, and travelers to regions of the world where meningococcal disease is endemic [15]. Third, there is a very low frequency of exposure in the community in developed countries such that most persons will not have preexisting antibodies to MCPS [16]. Finally, the specific response to MCPS can be studied without the problem of confounding antibodies, such as the nonprotective antibodies against the cell wall polysaccharide present in the sera of those vaccinated with pneumococcal polysaccharide vaccine [17]. We quantified and characterized the immune response of elderly subjects to a TI-2 antigen and compared the data with functional activity.
Materials and Methods

Study population. Twenty-two healthy young (22–31 years old, mean = 26; 14 women and 8 men) and 21 elderly (60–88 years of age, mean = 73; 11 women and 10 men) adults were recruited from the community. Each volunteer was questioned regarding medical history; those who were considered immunocompromised on the basis of medication (steroids, chemotherapy, or other immunosuppressive agents) or disease (autoimmune disease, diabetes mellitus, frequent or recurrent infections, cancer, or human immunodeficiency virus) were excluded. Volunteers with a history of exposure to meningococcal disease or vaccination or an armed services record after 1970 also did not qualify. Each volunteer received 0.5 mL of meningococcal polysaccharide vaccine (Menomune-A/C/Y/W-135; Connaught Laboratories, Swiftwater, PA) as a one-time dose given subcutaneously. Blood samples were obtained the day of vaccination and 6 weeks following vaccination and were tested for the presence of anti–MCPS antibodies. Twelve-week samples were obtained from some volunteers.

Antibodies, standards, and control sera. Goat anti–human–hors eradish peroxidase conjugate antisera specific for γ, μ, or α heavy chains were obtained from Sigma (St. Louis) and used at a dilution of 1:10,000. Each anti–heavy chain class antisera was used alone to determine class-specific response. Total anti–MCPS response was determined by using a mixture of a 1:10,000 dilution of all three–anti–conjugates. Monoclonal antibodies (MAbs) to human IgG subclass and light chains were obtained (S. Johnson, CDC/WHO Collaborating Center for Research and Reagents for Human Immunoglobulin Subclasses, Centers for Disease Control and Prevention [CDC], Atlanta) and used at a dilution of 1:2500 in each chain-specific assay. The anti–human monOclonal used were as follows: anti–IgG Fc (HP6043), anti–IgG1 (HP6069), anti–IgG2 (HP6002), anti–IgG3 Fc (HP6047), anti–IgG3 hinge (HP6050), anti–IgG4 (HP6025), anti–IgA1 (HP6119), anti–IgA2 (HP6109), anti–κ (HP6156), and anti–λ (HP6054). Anti–IgG3 Fc and anti–IgG3 hinge were used together in the IgG3 assay. Goat anti–mouse IgG–horseradish peroxidase conjugate (Sigma) was used at a 1:10,000 dilution.

Several human MAb standards of known concentration, IgG subclass, and light chain composition (Institute for Cancer Research, Berne, Switzerland) were used to determine reactivities of the MAbs for assigning the IgG subclass and light chain concentrations of CDC1992 anti–N. meningitidis reference standard sera (assigned values: total = 32 μg/mL, IgG = 24.1 μg/mL, IgM = 2.0 μg/mL, IgA = 5.9 μg/mL [18]). Assigned values for CDC1992 were used to determine the anti–MCPS concentrations of all subject and control sera. Several CDC control sera with values for each immunoglobulin class determined by the standardized assay [18] were included to confirm heavy chain class values obtained by the indirect assay described below.

Sandwich-type ELISA. To determine subclass and light chain usage, a sandwich-type ELISA was used, modified from the CDC standardized assay [19]. Briefly, Maxisorp plates (Nunc, Roskilde, Denmark) were coated and blocked as described. Subjects' sera, controls, and CDC1992 were serially diluted in buffer, and 50 μL was added to plate wells. Incubations and washings were performed as described in [19]. After washing, dilutions of subclass-specific or light chain–specific MAbs in buffer were added to appropriate wells and incubated at room temperature for 2.5–3 h. After washing, subclass or light chain MAbs bound to anti–MCPS antibodies were detected using anti–mouse IgG–horseradish peroxidase conjugate (room temperature for 2 h) and o-phenylenediamine (Sigma) as substrate.

Indirect ELISA. All sera were tested at the CDC for total anti–MCPS levels only, by standardized ELISA [19]. Total anti–MCPS and heavy chain class-specific response were determined at the study site using the CDC protocol (identiﬁed as the indirect ELISA in this report). The wells were coated, blocked, and washed as described with the anti–human γ, μ, and α conjugates used alone to determine class-specific MCPS response and in a mixture to determine total anti–MCPS response.

Sandwich-type and indirect assays were performed on the same plate for each volunteer, and the CDC1992 was included as a reference standard for each assay. Pre- and postvaccination (6-week) sera were tested on the same plate. Twelve-week sera were tested separately. All sera were tested at a minimum of two dilutions and on at least two separate occasions. Antibody concentrations in control and test sera were calculated by comparing the test absorbance to absorbances obtained for the CDC1992 reference standard analyzed under identical conditions and correcting for dilutions. The standard absorbances were related to the assigned concentration in micrograms per milliliter (μg/mL) to provide a standard curve [20]. Sera that gave a nondetectable level (<0.02 μg/mL) were assigned the value of 0.01 μg/mL for statistical purposes.

Assignment of subclass and light chain antibody concentrations to CDC1992. Values in μg/mL of light chain– and subclass-specific responses to MCPS in the CDC1992 meningococcal standard have not been determined. To determine these light chain and IgG subclass–specific antibody levels of the CDC1992 standard, one-third of a Maxisorp plate (Nunc) was coated with 100 μL of doubling dilutions of each appropriate human monoclonal standard, that is, IgG1α and IgG1κ were serially diluted for determining the anti–IgG1 mAb reactivity. The other two-thirds of the plate were coated with MCPS-methylated human albumin and serial dilutions of CDC1992 and CDC control serum 900306 (CDC306) were tested by the sandwich ELISA as described above.

Human monoclonal standards were reacted with the same solution of anti–Fc, subclass, or light chain monoclonal, and all other conditions were kept constant. All standards and controls were done in duplicate. The reactivity of each monoclonal was shown to be linear and comparable at each dilution of standard at the tested dilution mAb (1:2500). Because the reactivity of each mAb was equivalent at the dilutions used in the subclass assays, the absorbance obtained for each dilution of CDC1992 in individual assays was matched to an absorbance obtained for a dilution of CDC306 IgG2. Analysis of CDC306 showed this serum to contain only an IgG2 subclass anti–MCPS response. This control was used as a subclass standard with the anti–MCPS IgG2 level assigned 74.4 μg/mL, equal to the IgG level measured by the standardized assay (anti–MCPS values for CDC306 are IgG = 74.4 μg/mL, IgM = 0.4 μg/mL, and IgA = 0.7 μg/mL [18]).

The concentration of anti–MCPS-specific antibody of each subclass for CDC1992 was calculated from the concentration of CDC306, then corrected for the dilution of the standard. The total of IgG subclasses 1–4 was compared with the total IgG antibody obtained using the γFc-specific mAb. The κ and λ light chain con-
centrations were calculated from absorbance obtained for CDC306 compared with the absorbance obtained with anti-γFc mAb (total, 80.0 μg/mL, CDC value by standardized assay [18]). The $\kappa + \lambda$ sum was compared with total anti–MCPS levels.

No human monoclonal standards were available for testing the IgA subclass monoclonal reagents. IgA subclass values were assigned to CDC1992 by assuming linearity of the monoclonal as seen with the IgG subclass–specific reagents and assigning the sum of the absorbance of the separate assays equal to 100% the total IgA-specific concentration. Each IgA subclass was assigned a proportional amount of the total. To confirm the appropriateness of these assigned values, the IgA1 + IgA2 sum was compared with IgA anti–MCPS levels in control sera (previously determined by the CDC [18]).

Bactericidal assay. Serum bactericidal activity was assayed at the CDC, by the standardized procedure [21]. This assay detects serogroup-specific antibody-mediated complement-dependent lysis of the target strain of bacteria. Briefly, N. meningitidis C11 was grown on serum-containing agar for 4 h, harvested, and suspended in glucose buffer. Dilutions of heat-inactivated sera were mixed with bacterial cells and 25% baby rabbit serum in a microtiter plate and incubated for 60 min. After the incubation, melted soft agar was added to each well of the microtiter plate and incubated overnight. Bactericidal assays were performed on two separate occasions: pre- and postvaccination samples of donors done together, with several sera from the first batch included in the second. Values from the first batch were lower overall than those from the second batch and may have been the result of different reactivities of batches of rabbit sera.

The serum bactericidal titer was defined as the reciprocal of the highest dilution yielding $\geq 50\%$ killing. Bactericidal levels of 4 (next 2-fold lower dilution) were used for statistical analysis when the reported bactericidal titer was $< 8$ (undetectable). A bactericidal titer of 65,536 (next 2-fold higher dilution) was used when the levels were reported as $> 32,768$. To allow comparison of postvaccination cidal activity, the fold increase in bactericidal activity was calculated as log$_2$ of an individual’s postvaccine/prevaccine bactericidal titer [21]. The amount of antibody required for 50% killing was calculated as total anti–MCPS (μg/mL) × (increase in bactericidal activity)$^{-1}$.

Statistics. Paired Student’s $t$ test was used to compare the sum of individual IgG or IgA subclass anti–MCPS antibody levels by sandwich assay to the IgG- or IgA-specific antibody level by indirect ELISA (respectively) and the $\kappa + \lambda$ light chain levels to the total anti–MCPS level for each serum tested. Paired Student’s $t$ test was also used to compare anti–MCPS antibody levels obtained by the ELISA procedure described above and by the CDC standardized ELISA. Correlation coefficients and paired $t$ tests were determined for comparison of totals of subclass assays to total class assays. Geometric means, medians, and 95% confidence intervals (based on geometric SDs) were calculated for each group, and the Mann–Whitney $U$ test was used to compare the young with the elderly response in each assay. Arithmetic means, confidence intervals, and $t$ tests were used to compare ratios such as class-specific/total anti–MCPS. Correlation coefficients were determined for total and each individual light and heavy chain level (log) to the log, of fold increase in bactericidal activity. $P < .05$ was considered as significant for all analyses.

Results

Comparisons of the assays. The CDC1992 N. meningitidis reference standard was assigned subclass and light chain concentrations as follows: $\kappa = 16.4$ μg/mL, $\lambda = 14.6$ μg/mL, IgG1 = 8.9 μg/mL, IgG2 = 14.0 μg/mL, IgG3 = 0.6 μg/mL, IgG4 = 0.02 μg/mL, IgA1 = 3.2 μg/mL, and IgA2 = 2.7 μg/mL. Values for the test and control sera were calculated based on these assigned values for CDC1992.

The anti–MCPS values obtained by the indirect ELISA were compared with total anti–MCPS levels determined by the standardized ELISA performed at the CDC, and no significant difference was found by the paired Student’s $t$ test ($P < .05$, 2-tailed). Correlation coefficients were $\geq .95$. The calculated levels of $\kappa + \lambda$ and IgG1 + IgG2 + IgA were compared with the total anti–MCPS antibodies and were not found to be significantly different ($r > .95$, $P > .05$). Measured IgG levels correlated to the sum of the IgG subclasses ($r > .95$, $P > .05$). Individual sera vary in their chain composition of anti–MCPS from the geometric means such that the overall profile of one serum cannot be used to predict the profile of another.

Pre-versus postvaccination antibody levels. Most persons (91%) had low preexisting levels of anti–MCPS-specific antibodies ($< 2.00$ μg/mL, the level that has been associated with protection [15]). Four persons had preexisting antibodies $> 2.00$ μg/mL (2 elderly and 2 young persons). The low or undetectable levels of anti–MCPS in the heavy or light chain assays prevented meaningful statistical analysis between data for the young and elderly subjects. Vaccinated individuals showed a significant increase (paired Student’s $t$ test, $P < .05$) in anti–MCPS antibody levels following vaccination compared with prevaccination levels. This was seen in total, IgG-, light chain-, and subclass-specific anti–MCPS antibodies except IgG4 (see tables 1–3). Most individuals (42/43) had anti–MCPS levels following vaccination of $> 2.00$ μg/mL (individual $< 2.00$ μg/mL was elderly) and would be considered protected [15].

The overall relationship of class-specific response in all the study subjects was 73% IgG, 13% IgM, and 19% IgA. This type of class-specific response was seen in all the young and most of the elderly persons. One elderly individual had only an IgA response (no IgG or IgM), and 3 other elderly subjects had only an IgG response. The IgG subclasses were distributed overall as 27% IgG1, 66% IgG2, 5% IgG3, and 2% IgG4 of total IgG anti–MCPS. IgG2 anti–MCPS antibodies were present in most postvaccination sera, with 39 of 41 IgG class–responding individuals having IgG2 $> $ IgG1. IgG3 anti–MCPS response was not detectable in most postvaccination sera, but a few persons showed some IgG3 response (postvaccination range: $\leq 0.02$–18.0 μg/mL, geometric mean = 0.20 μg/mL; pre- vs. postvaccination levels, paired Student’s $t$ test, $P = .01$). Levels of IgG4 anti–MCPS-specific antibodies remained largely undetectable before and after vaccination (postvaccination range: $\leq 0.02$–5.6 μg/mL, geometric mean = 0.02 μg/mL; pre- vs. postvaccination levels, paired Student’s $t$ test, $P = .01$).
TABLE 1. Anti-MCPS heavy chain use in elderly and young adults at 6 weeks after vaccination.

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>IgM</th>
<th>IgA</th>
<th>Total</th>
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<tbody>
<tr>
<td>Elderly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM/median (CI) µg/mL&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.2&lt;sup&gt;b&lt;/sup&gt; (5.2–28)</td>
<td>0.88&lt;sup&gt;b&lt;/sup&gt; (0.34–2.3)</td>
<td>1.7&lt;sup&gt;b&lt;/sup&gt; (0.63–4.8)</td>
<td>24&lt;sup&gt;b&lt;/sup&gt; (15–39)</td>
</tr>
<tr>
<td>% of total anti-MCPS (CI)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71 (55–87)</td>
<td>14 (6–22)</td>
<td>19 (11–27)</td>
<td>98&lt;sup&gt;b&lt;/sup&gt; (92–103)</td>
</tr>
<tr>
<td>Correlation with cidal&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.54</td>
<td>0.08</td>
<td>0.09</td>
<td>0.64</td>
</tr>
<tr>
<td>Young</td>
<td></td>
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</tr>
<tr>
<td>GM/median (CI) µg/mL&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34&lt;sup&gt;b&lt;/sup&gt; (22–52)</td>
<td>3.9&lt;sup&gt;b&lt;/sup&gt; (2.9–5.1)</td>
<td>7.2&lt;sup&gt;b&lt;/sup&gt; (4.7–11)</td>
<td>46&lt;sup&gt;b&lt;/sup&gt; (31–69)</td>
</tr>
<tr>
<td>% of total anti-MCPS (CI)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76 (68–84)</td>
<td>12 (7–17)</td>
<td>18 (14–23)</td>
<td>97&lt;sup&gt;b&lt;/sup&gt; (91–103)</td>
</tr>
<tr>
<td>Correlation with cidal&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.61</td>
<td>0.42</td>
<td>0.35</td>
<td>0.59</td>
</tr>
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</table>

NOTE: MCPS, N. meningitidis serogroup C polysaccharide.
<sup>a</sup> Geometric mean (GM)/median (95% confidence interval [CI] using geometric SD).
<sup>b</sup> Significantly lower than corresponding result for young adults (P < .05).
<sup>c</sup> Mean (95% CI), where x = % (subclass/total anti-MCPS).
<sup>d</sup> Correlation coefficient, where x = bactericidal log, fold increase and y = log<sub>10</sub>, subclass-specific anti-MCPS.

test, P = .55). The IgA subclasses were 51% IgA1 and 49% IgA2 of total IgA in those who had an IgA anti–MCPS response. Light chains were 63% κ and 37% λ. The κ/λ ratio of all postvaccination test and control sera was 1.7, with a range of 0.54–9.14. This is not significantly different from the normal ratio in human light chain usage of 2.0 [22].

Correlation with bactericidal activity. Postvaccination bactericidal titers were significantly increased (>4-fold) in all but 3 individuals (2 elderly and 1 young). The log<sub>2</sub> of the fold increase in bactericidal activity did not correlate with IgM class-specific (r = .25) or with IgA class- or IgA subclass-specific responses (r = .25). There was correlation with the postvaccination IgG anti–MCPS levels (r = .59). Increased bactericidal activity correlated best with IgG2 levels (r = .53). IgG1 levels correlated at .48. Correlation with bactericidal activity was not assessed on IgG3 or IgG4 levels, as levels were lower than the lower limit of detectability. The correlation between light chain usage and fold increase in bactericidal activity was r = .52 for κ and r = .47 for λ light chains.

Young versus elderly persons. The results of class-, IgG and IgA subclass-, and κ and λ light chain–specific anti–MCPS responses for the young and elderly groups are shown in tables 1, 2, and 3, respectively. Following vaccination, the elderly adults had a significantly reduced response to MCPS (P < .05) compared with the young adults in total anti–MCPS, IgG, IgG1, IgG2, IgM, IgA, and IgA subclasses, and light chain assays. Anti–MCPS IgG1 levels in the elderly subjects were markedly decreased compared with those in the young (P < .001). The difference in IgG1 levels between elderly and young adults was demonstrated further by the elderly’s significantly lower percentage IgG1 of IgG and the elderly adults’ IgG1/IgG2 ratio (table 2), whereas all other chain-specific relationships were maintained. The κ/λ ratio was not different between the 2 groups. The fold increase in bactericidal levels was significantly lower in the elderly than in the young adults (tables 1–3). The amount of total anti–MCPS required to achieve 50% killing was higher in the elderly than the young adults, but the difference was not significant (P = .10).

To determine whether the decreased antibody levels in the elderly adults were related to a higher rate of decline in antibody levels, we obtained blood samples from some of the elderly (n = 9) and young (n = 11) persons 12 weeks after immunization and compared the total anti–MCPS antibody levels with their levels 6 weeks after immunization (table 4). At 6 weeks, these elderly persons had lower total anti–MCPS than the young persons, but the difference between these 2 groups was not significant. All of the young persons’ antibody levels were maintained or continued to increase at 12 weeks. The
elderly persons’ total anti–MCPS at 12 weeks and fold increase was significantly lower than for the young adults; only 2 of 9 elderly persons had levels increasing over this time period.

Discussion

Characterization of the human response to MCPS. We performed a vaccine study to determine the relationship between quantitative and functional immune response in the elderly to TI-2 antigens using the capsular polysaccharide of N. meningitidis serogroup C. Most individuals (42/43) responded to the vaccine with total anti–MCPS levels of ≥2.00 μg/mL and a >4-fold increase in bactericidal activity and would be considered protected [15, 21, 23].

Overall, the persons in our vaccine study show the expected class and subclass distribution for an antipolysaccharide response and IgG2 subclass antibodies correlated best with bactericidal function. With regard to class distribution, our results are similar to the predominant IgG response seen in the S. pneumoniae 6B polysaccharide response [24], antipneumococcal response to 14 other serotypes [25], and response to Haemophilus influenzae type b (Hib) polysaccharide [26]. Previous studies with N. meningitidis serogroup A polysaccharide (MAPS) and MCPS responses show equal IgG and IgM class distribution of anti–MAPS [27] or predominant IgG anti–MCPS in some individuals and IgM in others [28–30]. The differences between these anti–MCPS studies and ours are not critical and may be explained on the basis of previous exposure, timing of sample collections, and the age distribution of the sample groups.

A light chain preference has been demonstrated in antibodies to some polysaccharide antigens. The predominant and protective response to Hib is composed of antibodies with κ light chains [22]. The κ/λ ratio of ~4.5 seen in anti–MAPS antibodies is higher than the normal serum ratio, which suggests a κ chain preference [27]. The response to S. pneumoniae 6B polysaccharide shows a preference for λ light chains, with the κ/λ ratio at 0.8 [24]. In the response to MCPS, Ambrosino et al. [22] found significantly increased κ/λ ratios relative to total immunoglobulin. Our data indicate that overall there is no predominant light chain usage in the anti–MCPS response, yet some individuals do show a predominant light chain, possibly as the result of differential expansion of responding clones among individuals. We observed no light chain preference in several subjects with only an IgG2 anti–MCPS response. These data together suggest that the heavy chain variable regions may be more likely involved in antigen specificity and binding than the light chain variable regions.

The IgG anti–MCPS response had both IgG1 and IgG2 subclass antibodies present, with IgG2 subclass predominant. Our data are similar to the IgG2 > IgG1 response seen in adults to other polysaccharide antigens such as Hib [31, 32], pneumococcus [17, 33, 34], and group A streptococcus [35] but differ from other studies in which the response to MAPS [27] and Hib [36] showed predominant IgG1 subclass antibodies in some individuals with IgG2 predominating in others.

In vitro bactericidal and opsonophagocytic assays are used to measure the functional activity of a specific response, and increased levels of such activities may correlate with protection [21, 37–40]. Previous studies on the human response to bacterial polysaccharides show that, of all the class and subclass Ig chains, IgG2-specific antibodies correlate best with serum bactericidal activity and protection [31, 34], whereas IgG1 antibodies function better as opsonins and may be superior to IgG2 in protection against infection [41]. IgM antibodies have been shown to be bactericidal but function poorly as opsonins [40]. IgA1 antibodies have also demonstrated the ability to fix complement and initiated strain-specific lysis with N. meningitidis serogroup C [42], but their protective efficacy remains to be proven. In this study, IgM and IgA anti–MCPS antibodies were present at significant levels in most postvaccine sera, but correlation to cidal activity was lower than IgG1 and IgG2 subclass anti–MCPS antibodies, which correlated similarly.

Response to MCPS in the elderly. Many studies have attempted to define the age-related decline in response to TI-2 antigens, in particular, the response to S. pneumoniae capsular

### Table 3. Anti-MCPS light chain use in elderly and young adults at 6 weeks after vaccination.

<table>
<thead>
<tr>
<th></th>
<th>κ (95% CI)</th>
<th>λ (95% CI)</th>
<th>κ/λ (95% CI)</th>
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<tr>
<td>Elderly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM/median (CI) μg/mL</td>
<td>11/12 (5.1–24)</td>
<td>4.9/11 (1.8–13)</td>
<td>2.3/1.8 (1.2–4.4)</td>
</tr>
<tr>
<td>% of total anti-MCPS (CI)</td>
<td>57 (47–65)</td>
<td>35 (24–45)</td>
<td>NA</td>
</tr>
<tr>
<td>Correlation with cidal</td>
<td>0.43</td>
<td>0.43</td>
<td>NA</td>
</tr>
<tr>
<td>Young</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM/median (CI) μg/mL</td>
<td>27/26 (18–40)</td>
<td>16/16 (10–24)</td>
<td>1.5/1.4 (0.97–2.2)</td>
</tr>
<tr>
<td>% of total anti-MCPS (CI)</td>
<td>60 (55–65)</td>
<td>37 (31–42)</td>
<td>NA</td>
</tr>
<tr>
<td>Correlation with cidal</td>
<td>0.63</td>
<td>0.46</td>
<td>NA</td>
</tr>
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</table>

**NOTE.** MCPS, N. meningitidis serogroup C polysaccharide. NA, not applicable.

* a Geometric mean (GM)/median (95% confidence interval [CI] using geometric SD).

* b Significantly lower than corresponding result for young adults (P < 0.05).

* c Mean (95% CI), where x = % (light chain-specific/total anti-MCPS).

* d Correlation coefficient, where x = bactericidal log₂ fold increase and y = log₂ chain-specific anti-MCPS.
poly saccharide vaccine. Studies using the pneumococcal polysaccharide vaccine report a satisfactory antibody response in 61%–87% of elderly persons 35 days after vaccination [43], and antipneumococcal antibody levels in the aged are not significantly lower than young adults [25, 44, 45]. Others have observed that elderly patients had higher levels of antibodies to some pneumococcal polysaccharide serotypes, but their sera failed to opsonize pneumococci [46, 47]. Decreased protection following vaccination was seen in some individuals [8–10, 44, 48], and vaccine efficacy studies show only 75% of immunocompetent individuals >65 years of age are protected [49] compared with 100% of younger adults [50]. The loss of protection and opsonization despite high anti–S. pneumoniae antibody levels suggests a loss of affinity/avidity and, consequently, function with age. These observations are supported by studies with aged mice immunized with the phosphorylcholine antigen of S. pneumoniae that show that the increase in antiphosphorylcholine antibodies is due to high levels of low-affinity antibodies and a loss of the protective high-affinity antibodies [51–54]. None of the S. pneumoniae studies have correlated class- and subclass-specific responses with functional activity nor compared such data to young adults.

On the basis of the studies in mice and the lower efficacy of the pneumococcal vaccine in humans, we expected to see anti–MCPS antibody levels maintained in the elderly with a loss of bactericidal activity, suggesting a loss of specificity. Instead, we found that most of the elderly had a postvaccination pattern of heavy and light usage similar to that in young adults. Lower antibody levels following immunization explain the lower fold increase in bactericidal activity in the elderly compared with the young adults, rather than a loss of specificity of the antibodies. The increase in antibodies required by the elderly adults to achieve 50% killing in the bactericidal assay, while not significantly increased, suggests that some of the loss of bactericidal activity may be due to the markedly lower IgG1 antibody levels. Bactericidal activity of affinity-isolated IgG1 and IgG2 anti–MCPS is required to determine definitively if any loss of bactericidal function is due to a repertoire shift.

Studies in the variable gene repertoire utilized by humans in response to S. pneumoniae and N. meningitidis lag far behind the studies in response to H. influenzae. A recent study by Lucas and Reason [55] showed that elderly and young adults had an identical antibody repertoire in response to Hib conjugate vaccines. Furthermore, antibody function was maintained in the elderly response. Our observations and those of Lucas and Reason are in contrast to the repertoire shift and loss of specificity in antibodies to some capsular serotypes of S. pneumoniae seen in aged mice and suggested by human vaccination trials. Our results suggest that the anti–MCPS antibody variable gene repertoire in the elderly is likely maintained, and the lower level of function is related to the lower antibody levels. A decrease in all classes and subclasses in the specific response to a TI-2 antigen in the elderly has not been shown previously, although decreases in particular classes, such as IgM in pneumococcal vaccination [25] and anticommensal bacteria antibodies [56] in the aged have been described.

There is an increasing awareness of the critical role of T cells and accessory cells in the response to T cell–dependent antigens. Much of the decline in the response to these antigens may, in fact, relate to the decreased function of T cells (reviewed in Miller [57]), macrophages [58], splenic NK cells [59, 60], and dendritic cells [3] with aging. Ambrosino et al. [61] suggest that the production of IgG2 antibodies by human B cells is relatively T cell–independent based on their experiments with highly purified cell populations in severe combined immunodeficient mice, whereas production of IgG1 antibodies relies upon cytokine signals from T cells and accessory cells [62]. The lower levels of anti–MCPS in the elderly subjects at 6 weeks and the more rapid decline in levels by 12 weeks may be due to innate B cell defects with age or a decline in cytokine support for clonal expansion. The markedly decreased IgG1 levels in the elderly adults, as seen in the significantly decreased percentage of IgG1 and IgG1/IgG2 ratio, supports the loss of accessory cell support for B cell clonal expansion and, consequently, isotype switching. A more rapid decline in antibody levels with age has been demonstrated with the response to S. pneumoniae vaccination, after which the antibody response declines to pre-vaccination levels as early as 3–4 years later [9, 63].

Many of the studies on immune cell aging suggest a mosaic model for immunosenescence, in which the proportion of cells with a particular function declines more dramatically than the response of an individual cell (reviewed in [57]). Our data on the response to MCPS suggest that each TI-2 antigen response needs to be evaluated separately and that data for one TI-2 antigen cannot be used to predict the response to another. This conclusion is supported by the recent data on the H. influenzae

Table 4. Total anti-MCPS in elderly and young adults at 6 and 12 weeks after vaccination.

<table>
<thead>
<tr>
<th></th>
<th>6 weeks</th>
<th>12 weeks</th>
<th>Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elderly, GM/median (CI)</td>
<td>32/23 (16–65)</td>
<td>26/14 (13–51)</td>
<td>0.88±0.70 (0.59–1.2)</td>
</tr>
<tr>
<td>Young, GM/median (CI)</td>
<td>51/72 (26–99)</td>
<td>76/102 (34–173)</td>
<td>1.7/1.4 (1.2–2.2)</td>
</tr>
</tbody>
</table>

NOTE: MCPS, N. meningitidis serogroup C polysaccharide; CI, confidence interval.

a Geometric mean (GM)/median (95% confidence interval [CI] using geometric SD).

b Mean (95% CI).

c Significantly lower than corresponding result for young adults (P<.05).
response [55]. The type of analysis we have performed on class, subclass, and light chain data is important for the study of alternative vaccine strategies for TI-2 antigens such as polysaccharide/protein conjugate and peptide-mimic vaccines. Such alternative vaccine strategies are important for the very young and the very old, groups at highest risk for infection with encapsulated organisms. This type of characterization of the normal responses in young and elderly adults is needed to compare future studies with T-dependent formulations of clinically relevant vaccines for the elderly, such as an S. pneumoniae vaccine.

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References


