Immune Function and Vaccine Responses in Healthy Advanced Elderly Patients

Paul J. Carson, MD; Kristin L. Nichol, MD; James O’Brien; Pierre Hilo; Edward N. Janoff, MD

Background: Decline in immune function has been reported to predictably accompany advancing age. However, to our knowledge, few studies have specifically characterized the rapidly expanding advanced elderly population or controlled adequately for concurrent diseases.

Objective: To assess whether successfully reaching an advanced age in good health is associated with preserved immune function.

Methods: We prospectively compared in vivo with in vitro variables of immune function in 29 healthy, independently living elderly subjects (mean age, 80 years; age range, 75-103 years) and in 21 healthy young control subjects (mean age, 29 years; age range, 25-35 years) in a Veterans Affairs Medical Center.

Results: In vivo, among elderly and young subjects, numbers of total white blood cells, monocytes, lymphocytes, and lymphocyte subsets (CD4+ and CD8+ T lymphocytes and CD20+ B cells) were similar, as were levels of total serum IgG and IgM. Only levels of serum IgA were higher in the elderly subjects (3.0 vs 1.7 g/L; P=.001). Functionally, both groups showed vigorous responses to protein (tetanus and diphtheria toxoids) and polysaccharide (23-valent pneumococcal) vaccines. Although levels varied, the fold increases in vaccine antigen-specific IgG were not significantly different in young and elderly subjects, and the avidities of IgG to pneumococcal polysaccharides 14 and 19F were similar before and after vaccination. In vitro, proliferative responses of blood mononuclear cells to T-lymphocyte and B-cell mitogens (pokeweed mitogen, Staphylococcus aureus Cowan strain 1, and S aureus Cowan strain 1 plus interleukin 2), and lipopolysaccharide-induced production of tumor necrosis factor α, were comparable in elderly vs young subjects.

Conclusion: Successful aging, defined by reaching an advanced age with one’s overall health intact, may be associated with preserved immune function and adequate responses to vaccines.

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SUBJECTS AND METHODS

SUBJECTS

Elderly subjects older than 75 years were recruited from the Veterans Affairs Medical Center, Minneapolis, Minn, general internal medicine clinic; a community outreach influenza vaccination clinic; and a hospital volunteer pool. Subjects had to be living independently and in overall good health. Subjects were excluded for any history of major medical disease (diabetes, cancer, collagen-vascular disease, or chronic diseases of the lung, heart, liver, or kidney), taking immunosuppressive medications, smoking, alcohol abuse, hospitalization within the previous year, any lower respiratory tract infection within the preceding year, or receipt of a pneumococcal polysaccharide vaccination (by history and medical record review). Healthy control subjects aged 25 to 35 years were recruited from hospital and laboratory employees with similar exclusion criteria. Six elderly and 7 young control subjects who had received a tetanus toxoid (TT)–diphtheria toxoid (DT) booster in the previous 10 years were excluded from the tetanus-diptheria immunization arm of the study. Written informed consent was obtained from each subject in protocols approved by the Veterans Affairs Medical Center Human Subjects Committee in compliance with Department of Health and Human Services guidelines.

IMMUNIZATION

Each subject was given an intradeltoid injection of 0.5 mL of aluminum phosphate–adsorbed ultrafine TT and DT (Wyeth Laboratories Inc, Marietta, Pa) and 0.5 mL of a 23-valent pneumococcal polysaccharide (Pnu-Imune; Ledleer Laboratories, Pearl River, NY) on the same day in separate arms. Vaccines from the same lots were used for subjects and controls. Serum samples were collected from all subjects before and 4 weeks after vaccination. All serum samples were stored at −20°C until testing.

CELL POPULATIONS

Total leukocyte, monocyte, and lymphocyte counts were determined by an automated counter (model ZBI; Coulter Electronics, Inc, Hialeah, Fla). Peripheral blood mononuclear cells were separated on a gradient (Ficoll-Hypaque; Sigma-Aldrich Corp, St Louis, Mo). Peripheral blood T-lymphocyte and B-cell subsets were then measured on enrollment into the study by fluorescence-activated cell sorter analysis (FACS model 440; Becton Dickinson Immunocytometry Systems, Inc, Mountain View, Calif) with murine monoclonal antibodies to T lymphocytes (CD3, CD4, and CD8) and B cells (CD20) (Becton Dickinson Immunocytometry Systems, Inc) with appropriate isotype control antibodies.

ANTIBODY MEASUREMENTS AND AVIDITY

Levels of total serum IgG, IgM, and IgA were measured by nephelometry. Levels of pneumococcal capsular polysaccharide–specific IgG for vaccine serotypes that commonly cause invasive disease in elderly persons (types 4, 8, 12F, 14, and 19F) and TT- and DT-specific IgG in serum were measured by enzyme-linked immunosorbent assay exactly as previously described. Coefficients of variation levels of total IgA, but not IgG or IgM, in serum were increased among the elderly subjects.

IN VIVO

Humoral responses to pneumococcal polysaccharide and protein (TT and DT) antigens were measured in serum before and 4 weeks after vaccination. Compared with preimmunization values, older subjects generated a significant increase in IgG to 4 of 5 common pneumococcal capsular polysaccharides (types 4, 8, 12F, and 14) following immunization with the recommended 23-valent vaccine, as did young adults (types 4, 8, 14, and 19F) (Table 2). Neither preimmunization nor postimmunization levels of capsule-specific antibody were significantly different between the 2 age groups, nor were mean fold increases in IgG to these polysaccharides.

In addition to levels of antibody, the avidity, or functional affinity, of antibodies may contribute to their protective effects. Avidity, a quality related to the strength of antigen-antibody binding, may influence the ability of antibodies to mediate killing of encapsulated organisms. Before and following immunization, the avidity of pneumococcal capsule–specific IgG was similar among young adults and healthy advanced elderly subjects (Figure 1). Although the

### RESULTS

#### CLINICAL

Measure of absolute leukocyte, monocyte, and lymphocyte subset analysis by fluorescence-activated cell sorting showed no differences between the young and elderly subjects (Table 1). Only

<table>
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<th>Table 1</th>
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<td>Measures of absolute leukocyte, monocyte, and lymphocyte subset analysis by fluorescence-activated cell sorting showed no differences between the young and elderly subjects.</td>
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</table>

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for each assay were less than 10%. Avidities of IgG to capsular serovars 14 and 19F were assayed in duplicate by incubation of antibodies bound to the solid phase with the mild denaturing agent ammonium thiocyanate (0.1-4 mol/L) for 1 hour, as recently described. A laboratory reference serum was included on every plate to assess the coefficient of variation (<1.5%). Results are expressed as the avidity index, which is equal to the molar concentration of ammonium thiocyanate required to elute 50% of antibody bound under conditions of antigen excess.

CULTURE CONDITIONS

Peripheral blood mononuclear cells from young and elderly subjects were cultured at 1 × 10⁶ per milliliter in Roswell Park Memorial Institute 1640 medium (Biofluids, Rockville, Md) containing levoglutamidine, 2 mmol/L; penicillin, 50 IU/mL; streptomycin sulfate, 50 µg/mL; and 10% heat-inactivated fetal calf serum (GIBCO, Grand Island, NY) at 37°C in 5% carbon dioxide. Cells were cultured with or without pokeweed mitogen, 1:1000 (GIBCO); Staphylococcus aureus Cowan strain I (SAC) (CalBioChem, San Diego, Calif), 1:100,000; and SAC with or without IL-2, 20 U/mL (Peprotech, Inc, Rocking Hill, NJ), in triplicate in flat-bottom, 96-well microtiter plates (Costar, Cambridge, Mass). Lymphocyte DNA synthesis was assayed by adding 3.7 × 10⁶ Bq of tritiated thymidine (24.8 × 10⁴ Bq/mmol per liter) (New England Nuclear Co, Boston, Mass) during the final 4 hours of the 96-hour incubation at 37°C in 5% carbon dioxide. Cells were harvested on glass fiber filters for scintillation counting (Beta Plate; LKB Pharmacia, Piscataway, NJ).

Avidity of antibodies for type 14 did not change following immunization in either group, avidities for type 19F antibodies did increase significantly in both age groups. Whereas only 11% of each group showed greater than a 2-fold increase in the avidity index for type 14 after immunization, 22% of the elderly subjects and 35% of the young adults exceeded this threshold for type 19F. Such substantial changes in avidity have been shown in children immunized with capsular polysaccharides for Haemophilus influenzae type B, but they have not been described previously in adults immunized with pneumococcal polysaccharides.

When analyzed by sex, avidities for type 19F antibodies were higher among elderly men than among young men or older women before immunization (P < .02; avidity index, 0.67±0.09 vs 0.33±0.03 or 0.40±0.05, respectively). No differences in avidity were observed between groups for type 14 or 19F after immunization. Thus, levels and functional affinity of antibodies to pneumococcal polysaccharides were quite comparable among healthy young and healthy advanced elderly adults.

Similar to results with pneumococcal polysaccharide vaccine, elderly subjects generated significant humoral responses to recall protein antigens TT and DT (Figure 2). However, when compared with the younger control group, elderly subjects had signifi-

**CYTOKINE PRODUCTION**

To detect the ability of mononuclear cells to produce the proinflammatory cytokine tumor necrosis factor α (TNF-α), peripheral blood mononuclear cells were stimulated in triplicate wells with increasing concentrations of lipopolysaccharide from Escherichia coli serovar O127:B8 (Sigma-Aldrich Corp), and culture supernatants were harvested after 4 hours of incubation in 3% carbon dioxide at 37°C and frozen at −20°C until tested. Tumor necrosis factor α levels in culture supernatants were measured by immunassay using a murine anti-TNF-α monoclonal antibody (Miles, Inc, West Haven, Conn) as the capture antibody in phosphate-buffered saline on plates (Maxisorp; Nunc, Inc, Naperville, Ill). Tumor necrosis factor α in samples, standards, and controls was detected with rabbit anti-human TNF-α antibody (Genzyme Corp, Cambridge) and a horseradish peroxidase–conjugated goat anti-rabbit antibody (Southern Biotechnology Associates, Inc, Birmingham, Ala). Tetramethylbenzidine (Miles Scientific, Naperville) and hydrogen peroxide substrate stopped by 4 N sulfuric acid was used for color development. The assay, which did not detect IL-1, IL-2, IL-4, IL-6, IL-10, interferon γ, or transforming growth factor β, had a sensitivity of 20 pg/mL.

**STATISTICS**

Values were compared using a statistical program (StatView; Abacus Concepts, Berkeley, Calif) to compare means and variance by an unpaired t test between clinical groups for all numeric data. Mean fold increases for antigen-specific IgG were calculated by dividing 1-month values by preimmunization baseline values for each patient. Data are given as mean ± SEM.

**Table 1. Clinical and Immunological Characteristics of the Study Participants**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Advanced Elderly</th>
<th>Young</th>
</tr>
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<tbody>
<tr>
<td>Clinical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. studied</td>
<td>29</td>
<td>21</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>80.0 ± 6.0</td>
<td>29.0 ± 3.3</td>
</tr>
<tr>
<td>Range</td>
<td>75-103</td>
<td>25-35</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Female</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Cell count, × 10⁹/L*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total leukocytes</td>
<td>6.10 ± 0.30</td>
<td>5.90 ± 0.40</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.67 ± 0.12</td>
<td>1.76 ± 0.07</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.52 ± 0.028</td>
<td>0.52 ± 0.040</td>
</tr>
<tr>
<td>Lymphocyte subset, cells/µL*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4⁺ T lymphocytes</td>
<td>668 ± 53</td>
<td>728 ± 66</td>
</tr>
<tr>
<td>CD8⁺ T lymphocytes</td>
<td>353 ± 39</td>
<td>401 ± 44</td>
</tr>
<tr>
<td>CD20⁺ B cells</td>
<td>115 ± 13</td>
<td>132 ± 13</td>
</tr>
<tr>
<td>Immunoglobulin level, g/L*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>11.66 ± 0.55</td>
<td>10.87 ± 0.57</td>
</tr>
<tr>
<td>M</td>
<td>1.34 ± 0.15</td>
<td>1.57 ± 0.14</td>
</tr>
<tr>
<td>A</td>
<td>3.02 ± 0.32</td>
<td>1.69 ± 0.15</td>
</tr>
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*Data are given as mean ± SEM.
†P < .003 compared with the elderly participants.
significantly lower prevaccine levels of TT-specific antibodies ($P<.01$), and convalescent levels for TT- and DT-specific antibodies tended to be lower in the elderly subjects ($P=.06$). Despite these lower absolute levels in the elderly subjects, the ability to generate a response on antigenic challenge (eg, mean fold increase in TT- and DT-specific antibodies) was comparable or greater in the elderly subjects ($P=.02$ for TT-specific IgG). Moreover, the absolute increase in antibody to TT ($50.3\pm11.1$ vs $65.5\pm14.4$ µg/mL) and DT ($6.2\pm2.2$ vs $11.8\pm3.0$ µg/mL) was comparable in the elderly subjects and in young adults, respectively. Thus, static measures of immune competence (cell numbers and lymphocyte subset distributions) and the functional immune reserve (responsiveness to vaccines) were relatively intact in the healthy advanced elderly subjects compared with those in younger adults.

**IN VITRO**

Results of in vitro tests of immune function complemented those in vivo. Peripheral blood mononuclear cells from the elderly subjects showed a robust proliferative response to T-lymphocyte- and B-cell mitogenes (pokeweed mitogen and SAC, respectively) that paralleled that of young subjects (Figure 3, top). Moreover, although the elderly subjects have been described to show impaired responses to the T-lymphocyte-derived lymphotrophic cytokine IL-2,40,41 this factor was equally effective in augmenting proliferative responses to SAC in both age groups at the concentration tested (20 U/mL). Immunoglobulin production also increased significantly and comparably in response to each mitogen in the 2 age groups (IgG and IgM not shown). Spontaneous and mitogen-stimulated production of IgA, levels of which were increased in serum samples from older subjects, was similar in the 2 age groups (Figure 3, bottom).

Production of the proinflammatory cytokine TNF-α by peripheral blood mononuclear cells with and without stimulation with increasing concentrations of lipopolysaccharide was evaluable in 24 of the elderly subjects and in 16 of the young controls (Figure 4). Despite a trend for the older subjects to produce higher levels of TNF-α after stimulation with the midrange concentra-
We have shown that a range of immune functions were relatively intact in a healthy group of advanced elderly subjects compared with those in young adults 50 years their junior. In almost all areas of in vitro, in vivo, and clinical examination, including responses to vaccine, we found few statistically significant differences between the 2 groups. Consistent with previous observations, aging did not appear to affect absolute numbers of most cell lines,3,5,10,41 including the number of monocytes, lymphocytes, and their subsets (B cells and CD4+ and CD8+ T lymphocytes). Only levels of serum IgA were increased, as variably reported in the literature.3,5,10,42 Such polyclonal elevations may result from cumulative antigen exposure over time, increased mucosal exposure, or perturbations of B-cell activation. Deficits in cell-mediated immunity, as evidenced by poor lymphocyte responses to mitogens, have been one of the more consistent abnormalities described in elderly persons, particularly with T-lymphocyte mitogens.3,5,10,41,44,45 This defect has been hypothesized to be due, in part, to decreased production of and response to IL-2, a crucial growth factor for lymphocyte proliferation to mitogen or antigen, and dysregulation of activation requirements.14,15,46-47 However, in the healthy advanced elderly persons described herein, proliferative responses to T-lymphocyte (pokeweed) and B-cell (SAC) mitogens were intact. Furthermore, elderly and young subjects showed comparable proliferative responses with IL-2 supplementation. In each case, optimal doses of mitogen or IL-2 were used; dose responses may have revealed more subtle defects.

Aside from the IL-2-related responses noted, to our knowledge, few studies have examined other cytokine responses in elderly humans. Interleukin 1 production has been normal or decreased in elderly persons, whereas TNF-α levels were elevated in the serum samples from elderly subjects in one study46 but production of TNF-α in vitro was normal when compared with young subjects in another.44,48-50 We showed that in vitro production of TNF-α, a key cytokine in the immediate inflammatory response to infection, by peripheral blood mononuclear cells with and without stimulation with lipopolysaccharide was not appreciably affected by age. These data are consistent with limited previously published data51 from this group of elderly and young subjects, among whom plasma levels of TNF-α, IL-6, IL-10, and transforming growth factor β were all comparable.

Perhaps the greatest relevance of these data to the care of advanced elderly adults is their responses to these recommended vaccines. That prevaccine levels of TT-specific antibodies were lower in the elderly subjects compared with the younger adults likely reflects the longer intervals since the former’s last immunization. In some cases, subjects had not been immunized since their military service in World War II, and others were uncertain if they had ever even received a primary immunization series. Despite these limitations, advanced elderly subjects showed vigorous responses to immunization with TT and overall higher mean fold increases in TT-specific antibodies compared with those in younger adults. This pattern of response was similar to that seen with DT. Earlier studies10,22 reporting lower responses to immu-
Complement-dependent killing of the organisms may well reflect a failure to immunize these adults, rather than a failure of elderly adults to respond to immunization.

Nevertheless, tetanus and diphtheria remain uncommon diseases in elderly persons; pneumococcal disease has a much greater clinical impact. Our elderly subjects had comparable or greater preimmunization levels of antibodies against pneumococcal capsular polysaccharides, reflecting, perhaps, their greater cumulative lifetime exposure to Streptococcus pneumoniae compared with younger adults. Indeed, Mushor et al have reported that asymptomatic colonization elicits capsule-specific antibodies in serum. Moreover, elderly subjects generated appreciable antibody responses following immunization. That these results conflict with some previous reports of an overall lower response to pneumococcal vaccine in elderly persons may be related to differences in methods (23- vs 14-valent vaccine and enzyme-linked immunosorbent assay vs radioimmunoassay for antibody measurement) and to our selection of healthy subjects.

Although mean fold increases to pneumococcal polysaccharides tended to be lower among elderly subjects, the peak levels at 1 month were not significantly lower. This peak value, and the persistence of the antibodies, is an important determinant of vaccine-derived protection against pneumococcal disease. We did not address the duration of antibody responses, but we did characterize the quality of the antibodies produced, or antibody avidity. The avidity, or functional affinity, of capsule-specific antibodies correlates with their ability to mediate complement-dependent killing of the organisms by phagocytes. In this context, healthy advanced elderly adults were also able to generate antibodies with functional affinity comparable with those of healthy young adults.

Overall, these results may have implications for clinical practice as it pertains to adult immunization. Despite the universal recommendation for pneumococcal vaccination in persons older than 65 years, vaccination rates in the United States for this group have tended to be poor, at 25% to 50%. These low rates are believed to be due, in part, to the widespread perception that pneumococcal vaccine is poorly immunogenic in elderly persons. Although our study does not purport to suggest clinical efficacy, it does suggest that advanced age per se may not be a risk factor for lowered immune responses. Limitations in our data include that, as noted, we did not assess the duration of these antibody responses. Indeed, some investigators have found that responses to various vaccines may be less long lasting among elderly persons. However, consistent with our results, a recent study of responses to pneumococcal vaccine in elderly persons found comparable avidity and durability of capsule-specific IgG antibodies in most elderly subjects and in younger control subjects. In addition, we did not measure the ability of these serum samples to mediate killing of S pneumoniae by phagocytes in vitro, and groups of debilitated advanced older adults with underlying illness showed impaired phagocytic activity in vitro.20 These data in healthy advanced elderly adults would lend support to the several retrospective case-control studies that do suggest vaccine efficacy in this patient population against serious pneumonia and invasive S pneumoniae infections, although controlled data of efficacy against all pneumococcal pneumonia in older adults are more limited.

Our findings challenge the conventional notions of inherent immunosenescence. These contrasting results are likely explained in large part by differences in subject selection. Prior studies have varied considerably in the definition of “elderly” and “healthy” subjects. Many recruited healthy elderly subjects from clinics and nursing homes and did not specify strict case definitions and rarely included any discussion of other factors (underlying illnesses, medications, vitamins, smoking, or alcohol use) that may affect immune function.24 We used rigorous screening for potential immune-altering medications and comorbidities. Our data are somewhat limited by examination of a small, highly selected sample of healthy older adults and may not be readily generalizable to a large segment of the elderly population. Similarly, we cannot extrapolate these results to predict responses to other vaccines commonly used in elderly persons, such as the influenza virus vaccine or, more recently considered, the varicella vaccine. However, our results emphasize the need to more systematically characterize the impact of comorbidity and other factors that may contribute to the aging process as causes of immunosenescence.

Comorbidity has been a neglected area of investigation in studies of immune function in elderly persons. Although nutritional assessment was not available in our group of healthy advanced elderly adults, several variables of immune function were reported to be worse among elderly subjects with regular clinic visits for health problems than among healthy elderly subjects. Indeed, factors such as diet, exercise, personal habits, and psychosocial stress are often overlooked when studying “normal” physiologic losses associated with aging.27 Nutrition, including protein undernutrition and deficiencies of vitamins and trace elements, may underlie many immune deficits attributed to age. The association of vitamin B12 deficiency with impaired responses to pneumococcal polysaccharide vaccination in elderly persons highlights that cofactors may contribute to immune integrity or dysregulation more than putative preprogrammed immunosenescence.

In summary, we have shown that in a group of healthy advanced elderly individuals, several measures of immune function remain essentially intact when compared with those in healthy adults 50 years younger. A prominent component of this immune competence is their ability to generate robust antibody responses to the panel of vaccine antigens tested. Our subjects represent a distinct subgroup of people who successfully reach an advanced age with their overall health intact. Our study did not measure all possible components of immune function and was not designed to establish primacy between functional immunity and attaining “healthy advanced elderly” status. Indeed, these data raise the question as to
whether immunocompetence leads to or derives from overall good health and longevity, and what role genetics and environment play on these outcomes. Answers to these questions require long-term prospective longitudinal studies in which immune function and clinical outcome in a large cohort are monitored over time. Such investigations should characterize the roles of illness (co-morbidity), nutrition, and stress on the development of immunosenescence or on the immunologic vitality of advanced elderly persons.

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