Specific and nonspecific immune responses to fasting and refeeding differ in healthy young adult and elderly persons¹–³

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ABSTRACT

Background: Undernutrition is a main cause of immunodeficiency. Many confounding factors limit the interpretation of immune function in hospitalized elderly patients.

Objective: We compared the effects of short-term fasting and refeeding on lymphocyte subset distribution and neutrophil function in healthy subjects.

Design: Seven young adult (x ± SE age: 24 ± 2 y) and 8 elderly (71 ± 3 y) subjects were fed standardized diets (1.6 × predicted resting energy expenditure; 16% protein) for 7 d. They then fasted for 36 h and were refed for 4 h (42 kJ/kg). Lymphocyte subsets were quantified by using fluorochrome-conjugated monoclonal antibodies. Neutrophil chemotactic migration was evaluated by using a 2-compartment chamber. Neutrophil reactive oxygen species production was measured by using a luminol-amplified chemiluminescence assay and oxidation of 2′,7′-dichlorofluorescein diacetate.

Results: Baseline total and cytotoxic T lymphocyte subpopulations were lower in elderly than in adult subjects (P < 0.01). Nutritional state had a significant effect (P < 0.05) on total, helper, and cytotoxic T and B lymphocyte counts in all subjects, and the response of lymphocyte subpopulations to nutritional fluctuations was significantly affected by age. The chemotactic index was lowered by fasting in both groups (P < 0.05 compared with basal values). After refeeding, neutrophil migration was restored in adult but not elderly subjects. The superoxide anion production rate increased with fasting and reverted to prefasting values with refeeding in both groups (P < 0.05). Fasting induced a significant decrease in hydrogen peroxide production in stimulated neutrophils that was reversed by refeeding in adult but not elderly subjects.

Conclusion: The lack of response of lymphocyte subpopulation counts and neutrophil function to nutritional changes may help to explain the promness of elderly persons to infection.

KEY WORDS  Aging, lymphocyte subsets, neutrophil, fasting, refeeding, elderly, infection

INTRODUCTION

The decline in immune function with age is unanimously recognized and is supported by many epidemiologic and clinical observations (1–3). These age-related functional changes are responsible for the increased vulnerability to disease of the elderly, which contributes to a higher prevalence of infectious and neoplastic diseases. Furthermore, immune alterations delay recovery after illness. This age-related increase in morbidity will be a major public health concern in the decades to come (4, 5). The most consistent feature of the aging immune system is the heterogeneity of the changes observed. The modifications undergone by each individual component of the immune system are the subject of much controversy. For instance, T suppressor cell populations have been reported to increase (6, 7), decrease (1, 8–10), and remain unchanged (11–15) in elderly subjects. In addition, most studies generally describe a decrease in T helper subsets (1, 10, 12, 13, 16, 17), whereas some investigators detected no variations (6, 8, 9, 14, 15). Likewise, the importance of neutrophils in the immune response has been well documented during aging (18, 19), but divergent data have been reported concerning age-related changes in neutrophil number and function (20–24). The main cause of this marked heterogeneity is the presence of concomitant pathologic disorders that may themselves affect immune status. To overcome this problem, Ligthart et al (25), in the SENEREUR EURAGE protocol, set strict admission criteria for human immunogerontologic studies that include clinical information, laboratory data, and immunopharmacologic interference.

Nutritional status has long been recognized as a major factor in age-related immune impairment, especially in elderly institu-
Our institution is authorized by the French Ministry of Health to perform experiments on healthy subjects. The study was approved by the Ethics Committee of the Auvergne Region. All subjects gave informed, written consent.

**Experimental procedure**

After a 7-d stabilization period during which the subjects received a standardized diet (1.6 × predicted resting energy expenditure and containing 16% protein) to normalize energy intake, the subjects fasted for 36 h. Under these conditions, glucose is derived mainly from gluconeogenesis, which is highly dependent on amino acid availability. Immune status, which depends on biochemical pathways that are highly dependent on glucose and amino acids, is thereby altered. To avoid dehydration, water intake was carefully controlled so as not to be <1 L/24 h. After this short period of complete starvation, liquid food was reintroduced gradually (50 mL every 30 min) for 4 h (42 kJ/kg per 4-h refeeding period). The refeeding diet contained 18% protein, 32% fat, and 50% carbohydrate. This dietary intake corresponded to one meal consisting of one-third of the daily energy consumption. The diet was supplied in liquid form to optimize gastric emptying and facilitate nutrient absorption. All the subjects were kept under strict medical supervision during the experiment.

**Blood sampling**

Venous blood samples were collected at the end of the pre-fasting period (at 0700, 10 h after the last meal) for the measurement of basal values, at the end of the fasting period, and after 4 h of refeeding. Blood was collected into tubes containing EDTA for differential counting (white blood cells and total lymphocyte counts), for measurement of lymphocyte subsets, and for determination of neutrophil function.

**Biochemical characteristics**

Plasma insulin concentrations were measured by radioimmunoassay (CIS, Gif-sur-Yvette, France). Plasma albumin and C-reactive protein concentrations were measured by immunonephelometry (array protein system; Beckman, Gagny, France) and turbidimetry, respectively, with the use of human antibodies (Dako, Trappes, France).

**Assessment of blood cellularity**

The total number of leukocytes, leukocyte differential count, red blood cell number, hemoglobin, hematocrit, and platelets were measured with use of a Coulter counter (Coultronics, Margency, France).

**Indexes related to specific immune status**

Lymphocyte subpopulations were measured by flow cytometry with an Epics Profile (Beckman-Coulter, Villepinte, France) after the blood was prepared with a Q-Prep Epics immunology work station (Beckman-Coulter). Lymphocyte subsets were quantified by immunoreaction with fluorochrome-conjugated monoclonal antibodies by adding different combinations of labeled antibodies to whole blood: CD3-PC5, CD4-RD1, CD8-ECY, CD45-FITC, CD19-ECD, CD16-FITC, and CD56-PE (Beckman-Coulter), where PC is phycoerythrin-cyanin, RD and PE are phycoerythrin, ECD is phycoerythrin Texas red, and FITC is fluorescein isothiocyanate. The panel of CD designations with their appropriate cell names were as follows: CD3+ for total T lymphocytes, CD4+ for T helper-inducer lymphocytes, CD8+ for CD8+ cytotoxic T lymphocytes, CD56+ for NK cells, CD16+ for CD16+ granulocytes, CD19+ for B lymphocytes, and CD45+ for all leukocytes.
for T suppressor-cytotoxic lymphocytes, CD56+ for immature natural killer lymphocytes, and CD19+ for B lymphocytes.

Intraassay and interassay CVs were recorded by using a lyophilized preparation of human immune leukocytes that exhibited surface antigens (Cyto-Trol Control Cells; Beckman Coulter) and a suspension of fluorospheres that were uniform in size and fluorescence intensity (Flow-Count Fluorospheres; Beckman-Coulter). Intraassay and interassay CVs were <2% for all measurements made with the flow cytometer (lymphocytes subset counts and neutrophil functions). For technical reasons, we could not carry out functional tests to assess lymphocyte activity in this study.

**Indexes related to nonspecific immune status**

**Neutrophil isolation**

Six milliliters whole blood was layered onto a discontinuous Ficoll-Hypaque density gradient (Histopaque 1077 and 1119; Sigma, Saint-Quentin-Fallavier, France) and spun (700 × g, 30 min, 20°C). Neutrophils were then collected on the corresponding layer (1.077 < d < 1.119) and washed in RPMI-1640 medium (Sigma). Cells were tested for purity (>95%) and viability (>95%) by May-Grunwald-Giemsa staining and the trypan blue dye exclusion test, respectively. The final cell suspension was adjusted in RPMI-1640 medium to the cell density needed for each test by counting in a Malassez chamber (MC2, Clermont-Ferrand, France).

**Neutrophil chemotaxis**

Freshly isolated neutrophils (1 × 10⁶) were placed in a multiwell insert system containing a 3-μm membrane in a 24-well plate (Becton Dickinson, Meylan, France). Buffer with or without 1 × 10⁻⁷ mol/L formyl-Met-Leu-Phe (fMLP; Sigma) was used in the lower chamber to determine chemotaxis and spontaneous migration, respectively. Neutrophils were allowed to migrate for 90 min at 37°C in humidified air containing 5% CO₂, and were then fixed, stained, and counted under a microscope to determine their chemotaxis index. This index corresponds to the ratio of the number of neutrophils that migrated in the lower chamber in response to fMLP to the number of cells that migrated spontaneously (wells without fMLP).

**Neutrophil superoxide anion production**

Superoxide anion (O₂⁻) production by neutrophils was measured with a luminol-amplified chemiluminescence assay. Neutrophil suspensions (5 × 10⁶) containing luminol (1 mmol/L; Sigma) were placed in disposable polystyrene vials and the vials were placed in the light-proof chamber of a luminometer (model 1250; LKB Pharmacia, Saint Quentin-en-Yvelines, France) at 37°C. Neutrophils were then stimulated with phorbol 12-myristate 13-acetate (PMA, 1 μmol/L; Sigma) and the resulting light output was continuously recorded on a chart recorder and simultaneously printed out. All results are expressed as mV by using the chemiluminescence emission peak.

**Neutrophil hydrogen peroxide production**

Hydrogen peroxide production by stimulated neutrophils was measured as previously described (31). Briefly, neutrophils (1 × 10⁶) were preincubated for 15 min with 5 μmol 2⁻³⁻dichlorofluorescein diacetate/L (DCFH-DA; Fluka, Saint-Quentin-Fallavier, France) in a water bath with permanent shaking and temperature control (37°C). Neutrophils were then stimulated with PMA (1 μmol/L). This results in a leukocyte oxidative burst, during which nonfluorescent intracellular DCFH-DA is oxidized to highly fluorescent dichlorofluorescein (DCF) by hydrogen peroxide. DCF fluorescence was recorded by flow cytometry (Beckman-Coulter). Results are expressed as the ratio of hydrogen peroxide produced by PMA-stimulated neutrophils to that produced by unstimulated cells.

**Statistical analysis**

Data are presented as means ± SEMs and statistical analysis was performed with PCSM software (Deltasoft, Grenoble, France). The experimental design comprised 2 crossed fixed factors with the factor aging as 2 classes (adults and elderly) and the factor nutritional treatment as 3 classes (control, fasting, and refeeding). When the nutritional treatment effect was significant, we used the letters C (control), F (fasting), and R (refeeding) and the symbols >, <, and = to specify the main effects of these treatments. This design allowed statistical analysis by two-way, repeated-measures analysis of variance (ANOVA). This two-way repeated-measures ANOVA was performed to discriminate between the effects of aging, nutritional treatment, and their interaction. The level of significance was set at P < 0.05 for this test. When the ANOVA indicated significant interactions, the Neuman-Keuls test was used to identify differences between individual means. When no significant interaction was found, the Neuman-Keuls test was used to calculate and compare the marginal means. Because the Neuman-Keuls procedure does not control for type I error, we used a Bonferroni correction, for which the significance level was set at P < 0.02.

**RESULTS**

**White blood cell count**

The total leukocyte population, which includes lymphocytes, monocytes, and neutrophils, was increased by both fasting and refeeding in both groups (C < F = R), with no significant effect of age (Table 2). This variation was partly due to an increase in neutrophil number during the fasting and the refeeding periods (C < F = R).

**Indexes related to specific immune status**

Nutritional manipulations were associated with a reduction in the lymphocyte proportion (C > F = R; Table 2). Additionally, the total lymphocyte count was significantly affected by age and treatment (C > F = R).

The percentage of T lymphocytes was significantly altered by the dietary treatment (C > F < R; Table 3). In addition, the proportion of T lymphocytes was significantly lower in the elderly subjects than in adults. The absolute number of T lymphocytes was affected by nutritional treatment (C > F = R), by age, and by an age × nutritional treatment interaction. Specifically, T subsets decreased significantly in adult subjects after the fasting period, and this alteration persisted after refeeding. In addition, these lymphocyte subpopulations were significantly lower during the prefasting, fasting, and refeeding periods in the elderly subjects than in the adults.

An increase in the percentage of B lymphocytes was observed in both groups after refeeding (C = F < R; Table 3). Moreover, as shown by the comparison of marginal means with the
affected by age (Table 3).

Neuman-Keuls test, a significant age effect was noted. A nutritional effect on the absolute number of B lymphocytes was also observed: the number of B lymphocytes was significantly lowered by the fasting period and corrected by the refeeding regimen ($C > F < R$). Natural killer cell counts were also significantly affected by age (Table 3).

The percentage of CD4+ cells was reduced after fasting and corrected after refeeding ($C > F < R; Table 4$) in adult and elderly subjects. By contrast, the absolute number of CD4+ cells was reduced after fasting and this alteration persisted during refeeding ($C = F = R$). The absolute number of CD4+ cells was also significantly affected by age: the T helper lymphocyte subpopulation was significantly lower in the elderly subjects than in the adults. In addition, modifications of T helper subset counts during nutritional manipulations were correlated with variations in albuminemia after fasting and refeeding in the adult subjects ($r^2 = 0.24, P < 0.05$) but not in the elderly ones (data not shown).

In both the adult and elderly subjects, the percentage of CD8+ cells was significantly affected by refeeding ($C = F > R; Table 4$). The absolute number of cytotoxic T lymphocytes was significantly affected by dietary treatment ($C < F < R$), age, and their interaction: the absolute number of CD8+ cells was lower during fasting and refeeding than during the control period in adult subjects and was lower in the elderly subjects than in the adults during all treatments. These changes in the cytotoxic T lymphocytes were correlated with variations in albuminemia induced by the dietary treatments (data not shown) in the elderly group ($r^2 = 0.77, P < 0.01$). Because both the CD4+ and the CD8+ populations were affected by nutritional treatment, the ratio of CD4+ to CD8+ cells was lower in fasted subjects ($C > F < R; Table 4$).

Indexes related to nonspecific immune function

**Neutrophil chemotaxis**

During fasting, the neutrophil chemotaxis index was lower than basal control values in adult and elderly subjects (Figure 1). Refeeding induced a significant enhancement of neutrophil migration responsiveness in the adults. In addition, a correlation between the chemotaxis index and variations in glycemia induced by nutritional manipulations (data not shown) was found only in

| TABLE 2 | Blood cell indexes in adult and elderly subjects during the control condition (C) and after the fasting (F) and refeeding (R) periods |
| --- | --- | --- | --- | --- | --- | --- |
| | Adults | | Elderly | | ANOVA |
| | Control | Fasting | Refeeding | Control | Fasting | Refeeding |
| Leukocytes ($\times 10^9$/L) | 6.48 ± 0.51 | 7.14 ± 0.45 | 5.75 ± 0.52 | 5.39 ± 0.55 | 6.91 ± 0.92 | 6.85 ± 0.78 |
| Lymphocytes (%) | 30.7 ± 1.8 | 24.5 ± 3.2 | 20.3 ± 1.9 | 25.8 ± 2.5 | 20.2 ± 3.5 | 19.7 ± 2.8 |
| Monocytes (%) | 1.98 ± 0.19 | 1.70 ± 0.21 | 1.53 ± 0.18 | 1.32 ± 0.09 | 1.19 ± 0.11 | 1.21 ± 0.09 |
| Neutrophils (%) | 8.36 ± 0.71 | 7.33 ± 1.01 | 8.49 ± 0.93 | 8.85 ± 0.87 | 7.96 ± 0.48 | 8.95 ± 0.73 |
| Neutrophils ($\times 10^9$/L) | 57.2 ± 1.7 | 66.0 ± 3.9 | 69.5 ± 2.6 | 60.3 ± 3.9 | 68.9 ± 4.1 | 68.9 ± 3.5 |

1 ± SEM; $n = 7$ or 8 subjects per group. Indexes are expressed both as percentages of the total leukocyte number and as absolute values. Two-way repeated-measures ANOVA was performed to discriminate among the effects of age ($A; P < 0.05$), nutritional treatment ($C, F,$ and $R$), age, and their interaction ($I; P < 0.05$). When no significant interaction was noted, a comparison of marginal means was valid and was carried out with the Bonferroni-corrected Neuman-Keuls test, which indicated a significant effect of age on T and B lymphocyte subpopulations as a percentage of total leukocytes, $P < 0.02$.

**TABLE 3**

Lymphocyte distribution in adult and elderly subjects during the control condition (C) and after the fasting (F) and refeeding (R) periods

<table>
<thead>
<tr>
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<th>Adults</th>
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<th>Elderly</th>
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<th>ANOVA</th>
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<tr>
<td></td>
<td>Control</td>
<td>Fasting</td>
<td>Refeeding</td>
<td>Control</td>
<td>Fasting</td>
</tr>
<tr>
<td>T lymphocytes (%)</td>
<td>78.1 ± 1.8</td>
<td>74.5 ± 1.8</td>
<td>75.7 ± 2.1</td>
<td>70.8 ± 3.9</td>
<td>67.8 ± 3.0</td>
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<tr>
<td>(×10^9/L)</td>
<td>1.54 ± 0.15</td>
<td>1.28 ± 0.18</td>
<td>1.24 ± 0.18</td>
<td>0.94 ± 0.08</td>
<td>0.82 ± 0.10</td>
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<tr>
<td>B lymphocytes (%)</td>
<td>12.1 ± 0.8</td>
<td>12.3 ± 1.5</td>
<td>14.3 ± 1.6</td>
<td>16.1 ± 2.6</td>
<td>16.2 ± 2.6</td>
</tr>
<tr>
<td>(×10^9/L)</td>
<td>0.24 ± 0.03</td>
<td>0.19 ± 0.02</td>
<td>0.21 ± 0.03</td>
<td>0.21 ± 0.03</td>
<td>0.20 ± 0.04</td>
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<tr>
<td>NK lymphocytes (%)</td>
<td>5.8 ± 1.3</td>
<td>6.4 ± 1.6</td>
<td>3.1 ± 1.1</td>
<td>1.4 ± 0.4</td>
<td>4.0 ± 1.8</td>
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<tr>
<td>(×10^9/L)</td>
<td>0.11 ± 0.03</td>
<td>0.11 ± 0.03</td>
<td>0.05 ± 0.02</td>
<td>0.02 ± 0.00</td>
<td>0.04 ± 0.01</td>
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1 ± SEM; $n = 7$ or 8 subjects per group. Lymphocytes are expressed both as percentages of the total lymphocyte number and as absolute values. Two-way repeated-measures ANOVA was performed to discriminate among the effects of age ($A; P < 0.05$), nutritional treatment ($C, F,$ and $R$), age, and their interaction ($I; P < 0.05$). When no significant interaction was noted, a comparison of marginal means was valid and was carried out with the Bonferroni-corrected Neuman-Keuls test, which indicated a significant effect of age on T and B lymphocyte subpopulations as a percentage of total leukocytes, $P < 0.02$. Because a significant interaction was found for the absolute number of T lymphocytes, comparison of individual means was valid and was carried out with the Bonferroni-corrected Neuman-Keuls test. NK, natural killer.

2 Significantly different from the control adult group, $P < 0.02$.

3 Significantly different from adults, $P < 0.02$. 

Immune Responses to Fasting and Refeeding in the Elderly

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CD8+ cells with the Bonferroni-corrected Neuman-Keuls test.

Neutrophil superoxide anion production had no beneficial effect on neutrophil chemotaxis in the elderly subjects.

Because we selected only healthy subjects unimpaired by any illness and taking no medication liable to exert immunomodulating effects, we can postulate various underlying mechanisms, and that nutritional state might be a major factor in a limited immune response even before hospitalization. To test this hypothesis, we chose a population free of pathologic disorders according to the SENIEUR protocol. This stringent selection is crucial because many confounding factors such as inflammation, sepsis, cancer, and ischemic heart disease modify immune status. We found marked differences between adult and elderly healthy individuals in both the number of some lymphocyte subsets and neutrophil functions. The results of our study show that age by itself affected not only basal immune function but also the response to various nutritional states, implying that nutritional state is an important consideration in the clinical management of elderly patients. As previously described (1), our results indicate that basal counts of total T lymphocytes and the cytotoxic T lymphocyte subset were lower in the elderly than in the adult subjects. Because we selected only healthy subjects unimpaired by any illness and taking no medication liable to exert immunomodulating effects, we can postulate various underlying mechanisms.

TABLE 4

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<th>Adults</th>
<th>Elderly</th>
<th>ANOVA</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Fasting</td>
<td>Refeeding</td>
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<tr>
<td>CD4+ cells (%)</td>
<td>50.7 ± 3.1</td>
<td>48.0 ± 2.7</td>
<td>50.9 ± 3.0</td>
</tr>
<tr>
<td>(×10^9/L)</td>
<td>1.00 ± 0.10</td>
<td>0.83 ± 0.12</td>
<td>0.79 ± 0.11</td>
</tr>
<tr>
<td>CD8+ cells (%)</td>
<td>27.8 ± 1.8</td>
<td>28.4 ± 1.9</td>
<td>25.6 ± 1.9</td>
</tr>
<tr>
<td>(×10^9/L)</td>
<td>0.56 ± 0.07</td>
<td>0.48 ± 0.07^a</td>
<td>0.39 ± 0.05^a</td>
</tr>
<tr>
<td>CD4+CD8+</td>
<td>1.91 ± 0.23</td>
<td>1.76 ± 0.20</td>
<td>2.09 ± 0.25</td>
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^a Significantly different from the control adult group, P < 0.02.
^b Significantly different from the fasting adult group, P < 0.02.
^c Significantly different from adults, P < 0.02.

Adults (r^2 = 0.38, P < 0.01). By contrast, refeeding had no beneficial effect on neutrophil chemotaxis in the elderly subjects.

Neutrophil superoxide anion production

Fasting resulted in a significant increase in O_2^- production in both the adult and elderly subjects (C < F > R; Figure 2). Refeeding led to a recovery of basal O_2^- generation by stimulated neutrophils whatever the subject’s age.

Neutrophil hydrogen peroxide production

Nutritional modifications were characterized by alterations in hydrogen peroxide generation by PMA-activated neutrophils in adult and elderly subjects (C > F = R; Figure 3).

DISCUSSION

We hypothesized that subtle or obvious alterations in immune function might precede the development of disease in elderly subjects, and that nutritional state might be a major factor in a limited immune response even before hospitalization. To test this hypothesis, we chose a population free of pathologic disorders according to the SENIEUR protocol. This stringent selection is crucial because many confounding factors such as inflammation, sepsis, cancer, and ischemic heart disease modify immune status. We found marked differences between adult and elderly healthy individuals in both the number of some lymphocyte subsets and neutrophil functions. The results of our study show that age by itself affected not only basal immune function but also the response to various nutritional states, implying that nutritional state is an important consideration in the clinical management of elderly patients.

As previously described (1), our results indicate that basal counts of total T lymphocytes and the cytotoxic T lymphocyte subset were lower in the elderly than in the adult subjects. Because we selected only healthy subjects unimpaired by any illness and taking no medication liable to exert immunomodulating effects, we can postulate various underlying mechanisms.

FIGURE 1. Mean (±SEM) neutrophil chemotaxis index (ratio of the number of neutrophils that migrated in response to formyl-Met-Leu-Phe to the number that migrated spontaneously) in healthy adult (n = 7) and elderly (n = 8) subjects. The subjects were fed a standardized diet for 7 d (control; □), fasted for 36 h (■), and were then refed with a liquid diet introduced gradually over 4 h (42 kJ/kg; ■). There was a significant age × nutritional treatment interaction, P < 0.05 (two-way repeated-measures ANOVA). Individual means were compared by using the Bonferroni-corrected Neuman-Keuls test: *Significantly different from the respective control treatment, P < 0.02; †Significantly different from fasting in adults, P < 0.02; ‡Significantly different from refeeding in adults, P < 0.02.
especially for the antigen expression of lymphocytes, which appears to be affected by aging itself. First, age-related changes in the thymus tissue may explain the decline in T cell–dependent immunity (32). Nevertheless, because the thymus undergoes involution before the onset of age-related changes in immune function, the thymus may be only partly responsible for the changes in T lymphocytes with age. Another explanation may be antigen expression on T lymphocyte subsets, which is subject to qualitative and quantitative modifications with advancing age (13, 33, 34). The CD25 antigen, which is identified as the \( \alpha \) chain of the high-affinity interleukin 2 receptor, decreased with age in both helper and cytotoxic lymphocytes (13), making interleukin 2 less effective for lymphocyte proliferation (8, 12). Conversely, a marked CD3\(^{+}\)HLA-DR\(^{+}\) up-regulation was described with increasing age, suggesting an increase in antigen presentation by T lymphocytes (6). Such qualitative and quantitative age-related changes in antigen expression on T lymphocytes may have a major influence on cell activation and functions during adaptive challenges (infectious diseases, inflammation, trauma, and protein-energy malnutrition). In addition, T helper lymphocytes have a specific role in helping B lymphocytes to synthesize antibodies, so that a reduction in the number of these cells favors a decrease in humoral response capacity (12, 35).

For natural killer lymphocytes, we showed a significant decline in the CD3\(^{+}\)CD16\(^{+}\)CD56\(^{+}\) phenotype (immature cells) with age. Such an immunosenescent change in the immature natural killer cell subset is a potential immunologic disadvantage for elderly subjects because these lymphocytes have high cytotoxic potential (36, 37). Nevertheless, Krishnaraj et al (38, 39) reported that the decrease in immature natural killer cells was accompanied by an up-regulation of CD3\(^{+}\)CD16\(^{+}\)CD57\(^{+}\) mature natural killer cells in elderly persons.

Because baseline changes in immune status occur in elderly subjects, it is of major interest to determine the influence of
Fasting clearly induced an increase in \( \text{O}_2^{\cdot -} \) generation. The oxidase burst depends on the activity of NADPH,H\(^+\) oxidase, a transmembrane electron transport chain that reduces oxygen to \( \text{O}_2^{\cdot -} \) (52). When neutrophils are activated by a stimulant, PMA, the multicomponent system of NADPH,H\(^+\) oxidase, is rapidly assembled and activated (21). PMA activates protein kinase C (18), which is involved in the activation of NADPH,H\(^+\) oxidase (18). Some authors have reported that fasting is accompanied by a marked increase in protein kinase C activity, resulting in stimulation of NADPH,H\(^+\) oxidase activity (53, 54). This last observation may then explain the increase in \( \text{O}_2^{\cdot -} \) production we noted during fasting. In addition, \( \text{O}_2^{\cdot -} \) dismutation leads to the formation of hydrogen peroxide, which participates in the microbicidal action of neutrophils (18). Some differences in hydrogen peroxide generation between the adult and elderly persons in our study were observed during starvation. As previously described (29, 55, 56), elderly individuals under nutritional stress exhibit metabolic pathway imbalances in neutrophil hydrogen peroxide scavenging enzymes, resulting in a decrease in total cellular myeloperoxidase, catalase, and glutathione peroxidase. The dysregulation of the reactive oxygen species generation pathway during aging may explain the differences in hydrogen peroxide generation noted in this study after starvation. After the refeeding period, \( \text{O}_2^{\cdot -} \) production by neutrophils from the adult and elderly subjects decreased and returned to normal values. These last results imply that NADPH,H\(^+\) oxidase displays a high sensitivity to nutritional variations. We also showed that elderly persons do not adapt to nutritional repletion, presenting a high rate of hydrogen peroxide production despite a decrease in \( \text{O}_2^{\cdot -} \) generation during refeeding.

In conclusion, despite the limited number of subjects in this study, we showed that short-term fasting and refeeding affect several immunologic indexes in elderly persons, in whom aging already favors intrinsic changes in immunity. The mechanisms of the impairment in the ability of lymphocytes and neutrophils to adapt to nutritional manipulations are still a matter of speculation and may involve either an intrinsic defect in the immune cells that exists before the cells are released into the bloodstream or an acquired defect related to environmental components depending on both the aging process and nutritional status. These findings highlight the specific effects of age and nutritional state on immune function and suggest that age is a risk factor for reduced immunity, even in a healthy population. A superimposed infection or inflammation will presumably amplify these alterations. Finally, besides age and pathologic disorders, undernutrition has an adverse effect on immune function. This finding needs to be taken into account in the care of vulnerable elderly hospitalized patients. In this population, protein-energy supply or even specific immunomodulating nutritional agents must be carefully administered to aid the recovery of immune function and limit the consequences of infectious challenge.

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