Effect of micronutrient status on natural killer cell immune function in healthy free-living subjects aged ≥90 y[1–3]

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ABSTRACT

Background: Natural killer (NK) cells play a role in natural immunity against tumor and infected cells. Advanced aging is associated with functional impairment of NK cells and increased susceptibility to nutritional deficiencies.

Objective: Our objective was to test whether micronutrient status affects NK cell activity in an older population.

Design: The relations between NK cell variables (percentage of leukocytes and cytotoxicity) and blood concentrations of selected micronutrients were studied in 62 healthy, free-living northern Italian subjects (25 men, 37 women) aged 90–106 y. Anthropometric measurements were also made.

Results: All subjects were well nourished according to age-specific anthropometric norms but many of them had micronutrient deficiencies. The prevalence of micronutrient deficiency was highest for selenium (in ≈50% of both sexes), zinc (in 52% of men and 41% of women), and vitamin B-6 (in 40% of men and 59% of women), followed by vitamin A (in 16% of men and 27% of women) and vitamin E, vitamin B-12, and folate (each in <10% of both sexes). Ubiquinone-10 status was inadequate in 40% of women and 24% of men ($P = 0.02$). The percentage of NK cells was associated with serum zinc (men: $r = 0.573$, $P = 0.007$; women: $r = 0.373$, $P = 0.031$) and selenium (women: $r = 0.409$, $P = 0.018$) concentrations. In women only, NK cell cytotoxicity at different effector-target cell ratios was positively associated with plasma vitamin E and ubiquinone-10 concentrations ($P < 0.05$). No significant associations with NK cell variables were found for the other measured nutrients.

Conclusions: The results of this study strengthen the hypothesis that individual micronutrients may affect the number and function of NK cells in old age. The study also confirms the high prevalence of micronutrient deficiencies in healthy and apparently well-nourished persons aged ≥90 y. *Am J Clin Nutr* 2000;71:590–8.

KEY WORDS Natural killer cells, vitamins, trace elements, zinc, selenium, aging, nutrition, micronutrients, oldest-old age group, northern Italy

INTRODUCTION

Natural killer (NK) cells are a distinct subpopulation of lymphocytes that show spontaneous cytolytic activity against various types of tumor or infected cells (1). NK cells therefore may play an important role in early natural surveillance against cancer and infectious disease, which are among the principal causes of mortality among elderly people in the Western world (2).

There are many descriptions of changes in the leukocyte subpopulations in aging, and these are not always comparable. A progressive age-related shift in the circulating lymphocyte population from conventional T cells to NK cells is well documented (3–7), although not a universal finding (8–10), in the literature. Conflicting data were also reported with regard to the functional activity of NK cells during aging [unchanged (4, 11, 12), decreased (3, 6, 7, 13), or increased (14)]. Possible explanations for these discrepancies are differences in donor selection criteria and sample size of studies (3, 4). However, abnormalities of NK cell function with age might also be related to microenvironmental changes and primarily to endocrine and nutritional factors (15).

Although nutrition is a critical determinant of immunocompetence, and the lack of specific micronutrients may be implicated in causing depressed cell-mediated immune responses in older age (16), only a few studies specifically investigated the relation between micronutrient status and NK cell function in elderly people (17–19). Moreover, none of these studies examined this relation in the growing age segment known as the oldest old (≥90 y), even though this age group represents a particularly vulnerable population with a precarious nutritional balance that can be easily disturbed by intercurrent illness, decreased functional capacity, or increased economic hardship (20).

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We reported recently the existence of positive associations between NK cell activity and vitamin D status in a sample of healthy, free-living Italian persons ≥90 y old (15). In the present study we examined whether, in the same population, NK cell variables were associated with blood concentrations of other selected vitamins and trace elements.

SUBJECTS AND METHODS

Subjects

Details of recruitment and enrollment of participants and assessment methods were published previously (15). Briefly, 62 noninstitutionalized subjects aged 90–106 y and living in the Bologna province (Emilia Romagna region of northern Italy) were enrolled. According to the suggestions of the SENIEUR protocol admission criteria for immunogerontologic studies in humans (21), all subjects were in apparently good health, had normal hematologic and biochemical values, and were not taking nutritional supplements or medications known to affect the immune system (steroidal and nonsteroidal antiinflammatory drugs, hormones, and analgesics). Given the advanced age of these subjects, it is not surprising that, among the clinical conditions not included in the SENIEUR exclusion criteria, the most frequently recorded in this population was arteriosclerosis, manifesting itself either as hypertension (n = 10) or as a previous (>6 mo old) episode of cardiac insufficiency (n = 4); myocardial infarction (n = 3); stroke (n = 3); or arterial recanalization (n = 2). Other minor complaints were cataract (n = 3), benign prostatic hyperplasia (n = 3), osteoarthritis (n = 3), and colonic diverticula (n = 1).

As in the SENIEUR protocol, however, none of our subjects was receiving a specific pharmacologic treatment for his or her medical condition at the time of this study. All subjects gave their informed consent to enrollment in the study, which was approved by the ethical committee of the Department of Internal Medicine, Cardioangiology, and Hepatology of the University of Bolgna.

Nutritional assessment

Anthropometric measurements

All subjects were weighed on the same scales while barefoot and in light clothing. Because shrinkage of the spine with aging can affect the validity of height measurements in the elderly, height was calculated from the knee-height measurement, according to the equations of Chumlea et al (22). Body mass index (in kg/m^2) was calculated. Arm muscle area and arm fat area were calculated from midarm circumference and triceps skinfold thickness by using standard formulas (23). These measurements were performed according to standardized procedures (24), with the patient in a recumbent position to make measurement easier and prevent falsification of results by alterations in mobility.

Blood nutrients

Peripheral blood samples were collected from 0800 to 0900, after the subjects had fasted overnight. The samples were put on ice, transported to the laboratories within 1 h, and processed immediately. Plastic tubes containing tripotassium EDTA and metal-free evacuated tubes containing no additives (Becton Dickinson, Meylan, France) were used for collecting plasma and serum, respectively. Plasma was separated by centrifugation (1500 × g, 30 min, 4°C) and analyzed immediately. Serum was separated by centrifugation (3000 × g, 30 min, 4°C) and samples were appropriately stored at −70°C until analyzed.

Plasma retinol, α-tocopherol, and ubiquinone-10 were assayed simultaneously by reversed-phase HPLC (Millennium 2010; Waters, Milford, MA) (25, 26). Because plasma lipids influence blood concentrations of lipid-soluble vitamins, plasma retinol, α-tocopherol, and ubiquinone-10 concentrations were adjusted to plasma cholesterol and triacylglycerols (27), measured according to routine procedures.

Serum selenium concentrations were measured in triplicate with a graphite furnace atomic absorption spectrometer with Zeeman background correction (Solaar 939QZ Unicam; Cambridge, United Kingdom) by using a standard addition method. Bovine serum with a low selenium concentration was used to prepare the standard curve. Human sera [standard reference material 1598; National Institute of Standards and Technology (NIST), Gaithersburg, MD] were used to validate the accuracy and precision of the method. Samples were compared with the standard curve by using the standard curve linear least-squares fit analysis. The detection limit for selenium was 0.093 μmol/L (7.4 ng/mL).

Serum zinc concentrations were measured by using a flame atomic absorption spectrometer (PU 9400, NL; Pye Unicam, Philips, Eindhoven, Netherlands) equipped with an air-acetylene flame burner. A linear calibration curve was created by using certified standard NIST solutions at 3 concentrations (0.1, 0.2, and 0.3 mg/L). Specimens were diluted 1:5 with 5% glycerol and ultrapure bidistilled and deionized water and analyzed in duplicate. Seronorm trace elements (Nycomed Pharma, Oslo, Norway) and serum trace elements control toxicology (normal range; Utak Laboratories Inc, Valencia, CA) were used as controls for validating the accuracy and precision of the method. The detection limit for zinc was 0.153 μmol/L (0.01 μg/mL).

Serum folate and vitamin B-12 concentrations were assayed by competitive immunoassay using direct, chemiluminescent technology (Chiron Diagnostics Co, East Walpole, MA). Plasma concentrations of pyridoxal-5′-phosphate (the active coenzyme form of vitamin B-6) were assayed by HPLC. Briefly, plasma proteins were precipitated by adding 1 mol perchloric acid/L (0.5 mL) to plasma (0.5 mL) and centrifuging at 13000 × g for 15 min at 4°C. Then, 0.1 mL of a 2.62% sodium bisulfate solution (pH 1.0) was added to 0.5 mL of the supernate and 50 μL of the obtained solution was injected onto a C_{18} column (Bakerbond; MetaChem, Torrance, CA) protected by a saturation column packed with 40 μm C_8 (Isolute; International Sorbent Technology, Jones Chromatography Lmt, Mid Glamorgan, United Kingdom). The mobile phase was 0.1 mmol sodium phosphate/L buffer (pH 1.5). The eluted peaks were monitored by a fluorometric detector set at an excitation of 300 nm and an emission of 400 nm. Intra- and interassay CVs were <5%. The detection limit for plasma pyridoxal-5′-phosphate was 4 nmol/L.

Immunologic tests

Mononuclear cell preparation and flow cytometry analysis

A complete blood profile and count was obtained for each subject. Peripheral blood mononuclear cells were separated from heparin-containing blood by conventional gradient centrifugation. The following monoclonal antibodies (MoAbs), directly conjugated with fluorescein isothiocyanate (FITC) or phycoerithrin, were used to analyze the surface antigens of peripheral blood

TABLE 1
Anthropometric data[1]

	Men (n = 25)	Women (n = 37)
Age (y)	97 ± 3[2]	98 ± 4
BMI (kg/m^2)	23.4 (20.0, 25.4)	21.9 (20.3, 24.0)
Reference range[3]	17.7, 34.4	17.8, 32.3
Arm muscle area (cm^2)	32.63 (27.42, 36.18)	23.70 (19.30, 28.10)[4]
Reference range	20.19, 52.88	15.76, 41.65
Arm fat area (cm^2)	6.10 (3.98, 7.98)	6.00 (4.8, 7.7)
Reference range	2.53, 16.54	2.14, 20.71

[1] Median (5th and 95th percentiles).
[2] $\bar{x}$ ± SD.
[3] 5th and 95th percentiles for sex-specific estimates of anthropometric variables in 98 subjects aged ≥90 y and living in the Emilia Romagna region (24).
[4] Significantly different from men, $P < 0.001$ (Mann-Whitney U test).

mononuclear cells: anti-CD3, recognizing all T cells; anti-CD16, recognizing the low-affinity receptor for FcIgG, reactive with a subset of cells with NK cell activity; anti-CD56, recognizing the nuclear cell adhesion molecule, reactive with resting and activated CD16$^+$; anti-CD2, recognizing an intercellular adhesion molecule binding the leukocyte function-associated antigen 3 (LFA-3) and expressed by both T and NK cells; anti-CD11a and anti-CD11b, recognizing different α chains of the β_2 integrin family; anti-CD18, recognizing the common β subunit of the β_2 integrin family; and anti-CD 29, recognizing the common β chain of the β_1 integrin family.

Anti-CD3, -CD16, -CD56, -CD2, and -CD29 were purchased from Becton Dickinson (Mountain View, CA). Anti-CD11a (LFA-1α, TS1/22 clone), -CD11b (L2/1 clone), and -CD18 (LFA-1β, TS1/18 clone) were prepared in our laboratory from culture supernates of hybridomas.

Incubation of 2×10^5 mononuclear cells with MoAbs was performed in V-bottom plates for 30 min at 4 °C, followed by the secondary antibody (goat anti-mouse FITC-immunoglobulin, 1:20 dilution; Becton Dickinson) when the MoAbs were not directly conjugated with fluorochrome. Incubation with 1:10 mouse normal serum (Dako, Glostrup, Denmark) was performed to reduce nonspecific binding.

Negative control cells were incubated with only immunoglobulin isotype control, FITC-conjugated goat anti-mouse immunoglobulin, or both. Mononuclear cells were then washed with phosphate-buffered saline–fetal calf serum and resuspended in 1% paraformaldehyde. The analysis was performed by using cytometry with a FACStar Plus cell sorter (Becton Dickinson) (3).

NK cell lytic activity

K562 tumor target cells (2×10^6) were incubated with 3.7 MBq radioactive sodium chromate (specific activity: 1480×10^{10}/–/4440×10^{10} /Bq/g) (NEN, Köln, Germany) for 1 h at 37 °C and shaken occasionally. Tumor cells were then washed 3 times with cold medium, centrifuged at 100 × g for 7 min at 4 °C, and resuspended at a concentration of 10^7/L. ^{51}Cr labeled target cells [10^7/L (5×10^2/50 μL)] and a varying number of effector cells [from 5×10^8 to 5×10^6/L (5×10^4 to 5×10^2/100 μL)] were incubated in triplicate in V-bottom 96-well plates [effector-target (E-T) ratios from 100:1 to 1:1]. Then, 75 mL supernate was harvested and counted in a gamma counter, and ^{51}Cr release was measured as described previously (28).

Statistical methods

Data are reported as means ± SDs, medians with 5th and 95th percentiles, or numbers of subjects with percentages, as appropriate. Because of the relatively small number of subjects and the nonnormal distribution of many nutritional variables, data were analyzed by using nonparametric statistics. Differences between groups were compared by using a Mann-Whitney U test or a chi-square test, as appropriate. The strength of the associations between NK cell immune function variables and the variables of interest was assessed by using the Spearman correlation coefficient (r_s). P values <0.05 were considered significant. Statistical calculations were performed by using SIGMASTAT 2.0 (Jandel Scientific, Erkrath, Germany).

RESULTS

Nutritional characteristics

No significant differences in age or anthropometric characteristics were found between men and women (**Table 1**), except for arm muscle area, which was higher in men than in women. Consistent with the advanced age of the study population, anthropometric measurements were on average very low, but values for all subjects fell within the normal range (5th and 95th percentiles) for the population aged ≥90 y and living in the Emilia Romagna region (24). The same was not true for blood micronutrient concentrations, which are shown in **Table 2** along with the 5th and 95th percentiles for blood nutrient concentrations obtained from a sample of 50 healthy men and women aged 20–64 y and living in the Bologna province. Subjects in the current study whose blood nutrient concentrations fell below the limits of these reference standards were defined as having deficiencies. The prevalence of micronutrient deficiency was highest for selenium (in ≈50% of both sexes), zinc (in 52% of men and 41% of women), and vitamin B-6 (in 40% of men and 59% of women), followed by vitamin A (in 16% of men and 27% of women) and vitamin E, vitamin B-12, and folate (each in <10% of both sexes). No sex-related differences in the prevalence of micronutrient deficiencies were found except for ubiquinone-10, the deficiency of which was more frequent in women (40%) than in men (24%).

Immunologic characteristics

The immunologic characteristics of this population were extensively described elsewhere (15). Briefly, there was no significant difference between men and women in the distribution of T (CD3$^+$) and NK (CD16$^+$ and CD56$^+$) cells nor in the distribution of some adhesion molecules involved in the interaction with target cells [CD2, CD29, and the β_2 integrin family (CD11b and CD18)] both on the total mononuclear cells (**Figure 1**A) and on electronically gated CD16$^+$ cells (Figure 1B). Of note, this subject population as a whole had lower percentages of CD3$^+$ ($P < 0.001$), CD56$^+$ ($P < 0.05$), CD11a$^+$ ($P < 0.01$), CD18 ($P < 0.01$), and CD16$^+$/CD11a$^+$ cells ($P < 0.05$) than did a younger control group (10 men and 7 women aged 21–38 y; $\bar{x}$ ± SD: 27 ± 6 y). The percentage of CD16$^+$ cells in the peripheral blood of subjects ≥90 y old was directly related to the percentage of CD56$^+$ cells ($P < 0.001$) and inversely correlated with the percentage of CD3$^+$ T cells ($P = 0.002$).

TABLE 2
Blood micronutrient data

Micronutrient	Reference range[1]	Men (n = 25) Median (5th and 95th percentiles)	Men (n = 25) Percentage with deficiency[2]	Women (n = 37) Median (5th and 95th percentiles)	Women (n = 37) Percentage with deficiency
Plasma vitamin A (μmol/L)[3]	1.8, 5.4	2.36 (1.1, 4.40)	16	2.1 (0.9, 2.9)	27
Plasma vitamin B-6 (nmol/L)	11.7, 43.4	12.2 (5.5, 15.2)	40	9.0 (4.0, 10.0)	59
Serum folate (nmol/L)	5.7, 32.9	9.3 (5.0, 13.3)	8	7.7 (5.4, 12.9)	5
Serum vitamin B-12 (pmol/L)	148, 720	382 (296, 545)	0	313 (139, 495)	5
Plasma vitamin E (μmol/L)[3]	8.7, 40.3	21.2 (7.5, 25.6)	8	22.4 (16.2, 26.7)	3
Serum zinc (μmol/L)	11.70, 19.00	11.68 (9.97, 13.23)	52	12.16 (10.15, 12.98)	41
Serum selenium (μmol/L)	0.82, 1.50	0.74 (0.62, 0.90)	52	0.81 (0.67, 0.93)	51
Plasma ubiquinone-10 (mmol/L)[3]	0.42, 1.14	0.46 (0.32, 0.67)	24	0.45 (0.27, 0.56)	40[4]

[1] Based on data for 50 healthy persons aged 20–64 y.
[2] Deficiency was defined as blood nutrient concentrations below the reference values.
[3] Plasma vitamin A, vitamin E, and ubiquinone-10 concentrations were adjusted to plasma lipids.
[4] Significantly different from men, $P = 0.02$ ($\chi^2 = 5.170$).

NK cell cytolytic activity tended to be slightly but not significantly higher in women than in men (**Figure 2**). As a whole, subjects aged ≥90 y had significantly lower cytotoxic activity ($P < 0.001$ for E-T ratios from 100:1 to 12.5:1) than did younger control subjects (29 men and 10 women aged 21–38 y; $\bar{x} \pm$ SD: 29 ± 5 y). NK cell cytolytic activity of subjects aged ≥90 y was also directly correlated with their $CD16^+$ cell number for all the E-T ratios examined ($P < 0.05$).

Relation between NK cell variables and blood nutrients

Percentages of $CD16^+$ and $CD56^+$ cells of subjects aged ≥90 y were positively associated with serum zinc concentrations in both sexes (**Figure 3**). In women, percentages of total $CD16^+$ and $CD16^+$ cells expressing adhesion molecules of the β_2 integrin family (CD11b and CD18) were positively associated with serum selenium concentrations (**Figure 4**). In men, only the association of the percentage of total $CD16^+$ cells with the serum selenium concentration was nearly significant ($r_s = 0.437$, $P = 0.053$).

No significant associations were found among the selected nutrients and the percentages of $CD3^+$, $CD2^+$, $CD11a^+$, $CD11b^+$, $CD18^+$, and $CD29^+$ peripheral blood cells. No significant associations were found among the selected nutrients and the percentages of $CD16^+$ cells expressing CD2, CD11a, or CD29. Significant positive correlations of NK cell cytolytic activity with plasma lipid-adjusted vitamin E and ubiquinone-10 concentrations were found in women for all but one of the examined E-T ratios (**Figure 5**). No associations were found between NK cell cytolytic activity and blood nutrient concentrations in men.

DISCUSSION

This study showed that NK cell immune function of healthy, free-living subjects aged ≥90 y was associated with blood concentrations of certain individual micronutrients of which deficiencies are very common in advanced age. Our results agree with the data of Chandra (19), which showed an improvement in NK cell variables of healthy subjects aged >65 y after 12 mo of supplementation with physiologic amounts of micronutrients. In contrast, Dowd et al (17) reported no significant correlations between NK cell function and individual blood nutrients, except for vitamin C. Their study population, however, included hospitalized patients, and very old subjects were scarcely represented.

Our results also differed from those of Payette et al (18), who found no correlations between NK cell function and micronutrient status in a group of elderly Canadian subjects. Comparisons with this study, however, are difficult because the Canadian study included subjects taking micronutrient supplements (29), and more than half of the study population had unusually low concentrations of interleukin 2.

On the basis of age- and region-specific anthropometric norms, no one in the population of the current study had clinically overt protein-energy undernutrition. On the basis of blood nutrient reference values for young subjects living in the same area and with similar dietary habits, however, about half of the study subjects had zinc and selenium deficiencies. Dietary intake was not estimated, but several conditions common in advanced age (reduction in total energy intake, lack of variety and characteristic self-selection of food items, poor socioeconomic conditions, malabsorption, and drug-nutrient interactions) were likely responsible for the reduced concentrations of these trace elements in this and other studies (20). Our data also agree with previous reports of possible micronutrient deficiencies even in independently living and apparently healthy elderly populations (30).

A remarkable finding in the current study was the strong association between the relative number of peripheral lymphocytes expressing markers of NK cell activity and zinc and selenium concentrations. Several studies in animals and humans showed decreased NK cell activity during zinc-deficient states (31). Zinc influences the activity of multiple enzymes involved in activation, replication, and programmed death of lymphocytes and also acts as an antioxidant (32). However, exposure of NK cells to high concentrations of zinc in vitro appears to inhibit NK cell cytotoxicity, perhaps inducing a down-regulation of CD16 (33). Selenium is also involved in NK cell–mediated immunity because it appears to up-regulate the interleukin 2 receptor on the surface of NK cells, resulting in enhanced proliferation and clonal expansion of cytotoxic precursor cells (34).

In a previous study (3) we found that 35 SENIEUR study subjects aged 71–95 y had a progressively higher number of NK cells than did young control subjects, whereas in the current study we had a consistent population of SENIEUR study subjects aged ≥90 y who did not show such a difference. In our previous study, how-

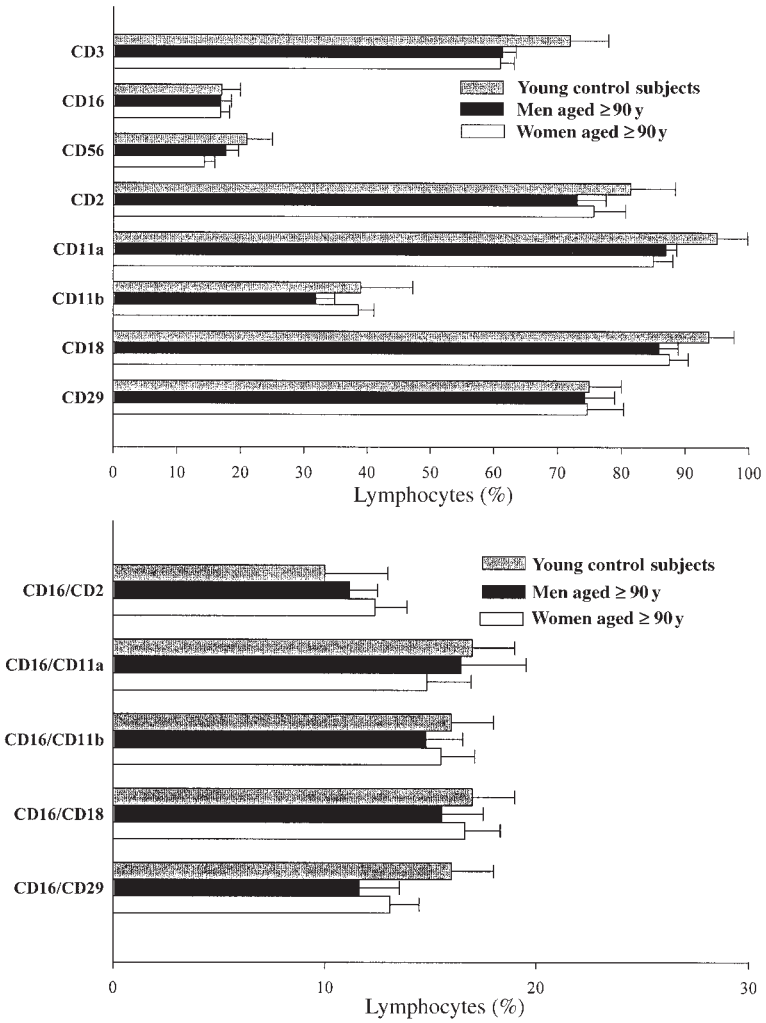

FIGURE 1. Surface marker expression of fresh peripheral blood lymphocytes and electronically gated $CD16^+$ lymphocytes from men (n = 25; lymphocyte count: 1.5 ± 0.6 × 10^9/L) and women (n = 37; lymphocyte count: 1.8 ± 0.5 × 10^9/L) aged ≥90 y. The young control subjects (n = 17; lymphocyte count: 1.9 ± 0.7 × 10^9/L) were aged 21–38 y. Results are expressed as mean (±SD) percentages of positive cells.

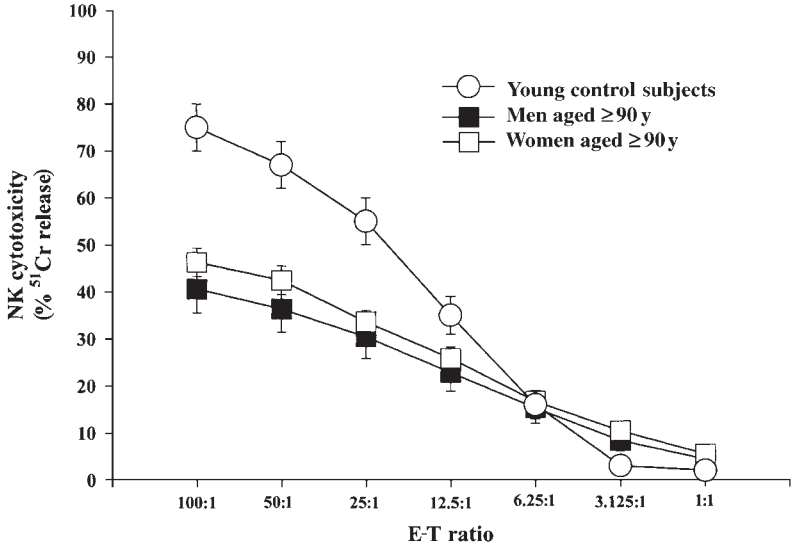

FIGURE 2. Natural killer (NK) cell cytotoxicity for different effector-target (E-T) ratios in healthy, free-living men (n = 25) and women (n = 37) aged ≥90 y. The young control subjects (n = 39) were aged 21–38 y. Results are expressed as mean (±SD) percentages of ^{51}Cr release.

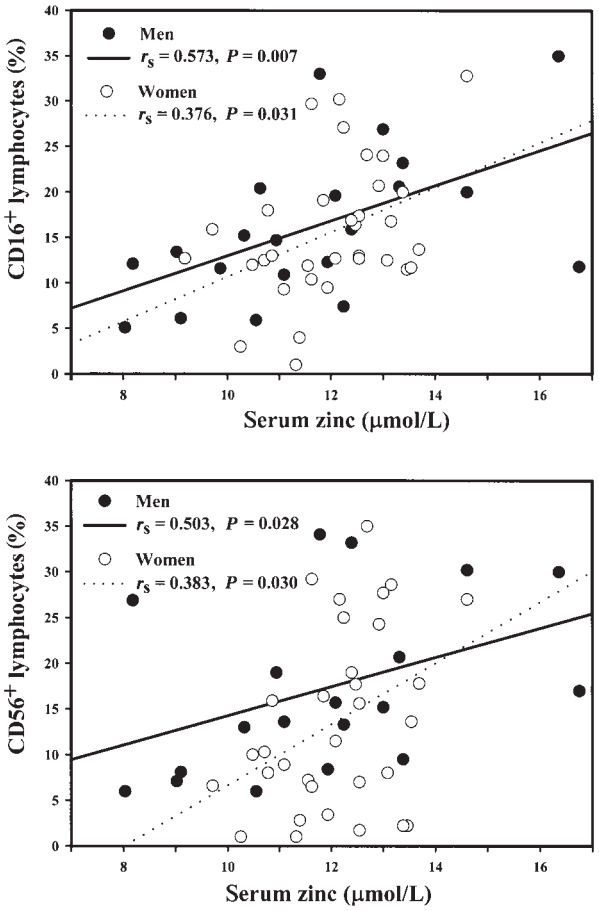

FIGURE 3. Relations among percentages of $CD16^+$ and $CD56^+$ lymphocytes and serum zinc concentrations in healthy, free-living men and women aged ≥90 y.

ever, as well as in many other studies of leukocyte subsets in very old people (4–11), zinc and selenium were not taken into account. Indeed, the evaluation of zinc and selenium is not required by the SENIEUR protocol and marginal deficiencies of trace elements are hardly ever recognized during clinical examination. On the basis of the strong association between the number of NK cells and blood concentrations of both zinc and selenium found in the current study, it is possible that differences in trace element status might explain the controversial results in the literature about the effect of aging on the number of NK cells.

It has also been speculated that the increase in the number of NK cells with age could be a compensatory mechanism for coping with the reduced cytolytic activity of single NK cells (3, 4). If so, zinc and selenium might be of central importance in maintaining an effective natural immunity in the oldest-old age group. However, there is no definite evidence that differences in the number of NK cells among elderly subjects actually influence the ability of the elderly to fight infections and cancer. Ligthart et al (11) reported that NK cell function was directly proportional to the number of $CD16^+$ cells in the peripheral blood. Ogata et al (7), however, reported that the activity, but not the number of, NK cells was related to the subsequent development of severe infections in elderly Japanese subjects. Our study also showed positive associations between NK cell cytotoxicity and plasma vitamin E and ubiquinone-10 concentrations in women. The lack of similar associations in men must be considered with caution because of the relatively small number of subjects in this subgroup and their better ubiquinone-10 status.

The low frequency of vitamin E deficiency in our subjects agrees with previous reports that aging per se has little effect on plasma vitamin E concentrations (30, 35), although other authors found low age-related vitamin E platelet values (36). Ubiquinone-10, a vitamin-like substance that is found in small amounts in a wide variety of foods but that can also be synthesized in all human tissues, is well known as a redox component in the mitochondrial respiratory chain and is an effective membrane antioxidant (37). The basal plasma concentrations of ubiquinone-10, which reflect metabolic demand (37), have been described as both increasing (38) and decreasing (39) with aging. Chronic malnutrition is presumed to affect ubiquinone-10 status both directly (by reducing ubiquinone-10 dietary intake) and indirectly (by reducing the dietary intake of other vitamins, such as vitamin B-6, folic acid, and vitamin B-12, that are involved in its biosynthesis) (37).

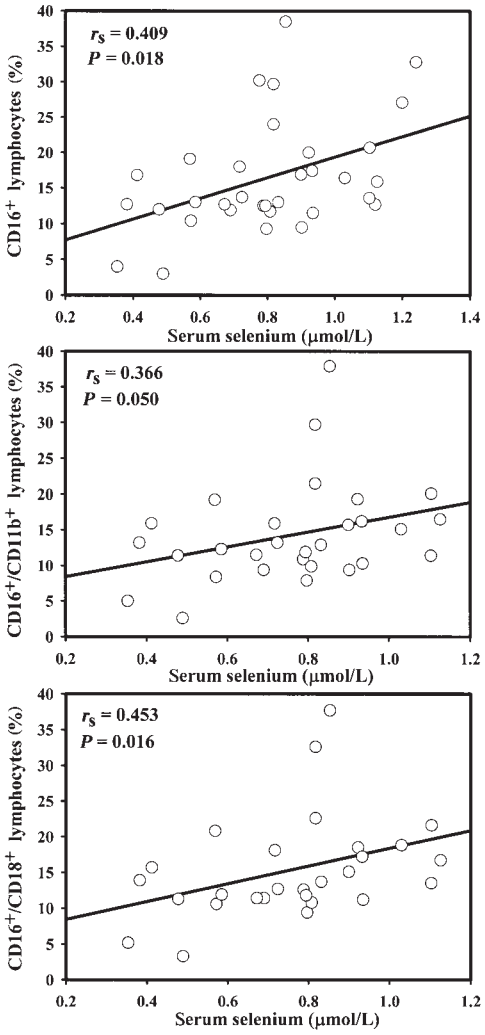

FIGURE 4. Relations among percentages of $CD16^+$, $CD16^+/11b^+$, and $CD16^+/18^+$ lymphocytes and serum selenium concentrations in healthy, free-living women aged ≥90 y.

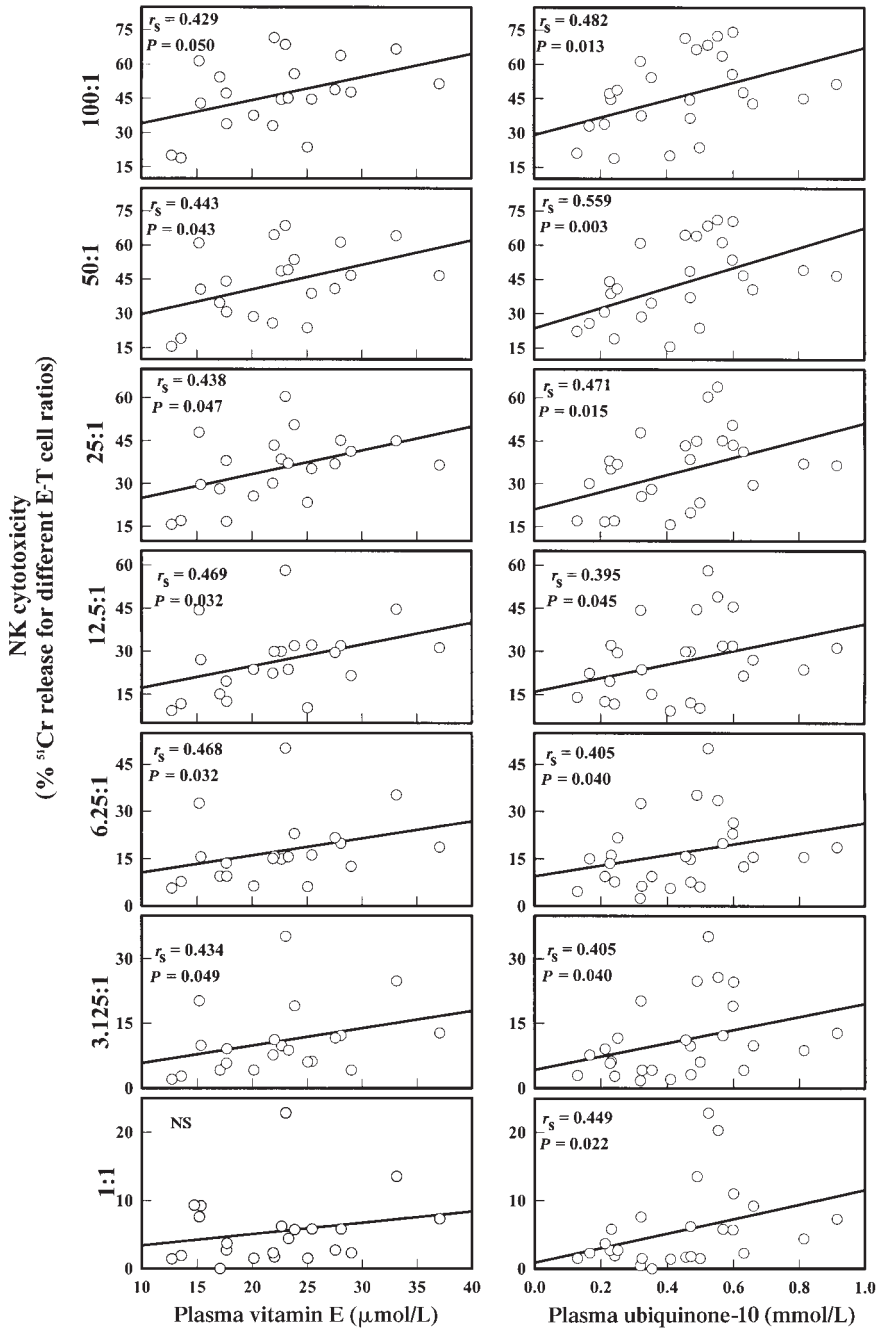

FIGURE 5. Relations among natural killer (NK) cell cytotoxicity (^{51}Cr release) and plasma vitamin E and ubiquinone-10 in healthy, free-living women aged ≥90 y.

Several animal and human studies showed that adequate vitamin E intake is essential for immune function and that vitamin E supplementation can successfully improve some aspects of the age-related decline in immunity (40). Although the mechanism behind the immunostimulatory effect of vitamin E is still unknown, there is compelling evidence that vitamin E may exert its immunoenhancing effect by regulating prostaglandin synthesis, decreasing free radical formation, or both (41).

By contrast, little is known about the role of ubiquinone-10 as an immunomodulating agent. Folkers et al (42) reported an increase in IgG and T4 lymphocyte blood concentrations after ubiquinone-10 administration in human subjects, but no data are available about the possible influence of ubiquinone-10 on NK cell activity. In our study, plasma ubiquinone-10 and vitamin E concentrations were associated with NK cell activity. A possible explanation for our findings could be the sparing effect of ubiquinone-10 on vitamin E as a result of its effective antioxidant action and, alternatively (or additionally), its ability to recycle oxidized vitamin E (43).

In contrast with a few reports suggesting that vitamin A (44), vitamin B-6 (45), folate (46), and vitamin B-12 (47) may affect

NK cell activity in overtly deficient subjects with various diseases, we did not find associations between NK cell variables and blood concentrations of these vitamins in our study population. Differences in age and health status of subjects in these studies may have contributed to this inconsistency in findings. Alternatively, many of these vitamins might indirectly affect NK cell function through their involvement in the multistage process of endogenous ubiquinone-10 biosynthesis.

In conclusion, the results of this study strengthen the hypothesis that individual micronutrients are related to NK cell function in old age. They also confirm the high prevalence of micronutrient deficiencies in apparently healthy and well-nourished subjects aged ≥90 y. The cross-sectional design of the study, however, does not permit one to infer that individual micronutrient deficiencies actually caused impairment of NK cell function. Further intervention studies are required to determine whether the provision of nutritional supplements effectively enhances NK cell immune function in elderly subjects. Meanwhile, a thorough nutritional assessment, paying particular attention to the dietary intake of essential nutrients, is strongly recommended in geriatric practice.

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