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Reduced Immune Function and Malnutrition in the Elderly

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Kawakami, K., Kadota, J., Iida, K., Shirai, R., Abe, K. and Kohno, S. *Reduced Immune Function and Malnutrition in the Elderly.* Tohoku J. Exp. Med., 1999, **187**(2), 157-171 —— An important observation in elderly subjects is their susceptibility to infection associated with a decline in host immune function. Nutrition is also an important factor that influences host defense against infection. We, therefore, evaluated the relationship between nutritional status in 155 healthy subjects ranging in age from 20 to 99 years and various immunological parameters, including the phagocytic and bactericidal activities of neutrophils and monocytes, superoxide production and chemotaxis of neutrophils, lymphocyte subsets, blastoid transformation and serum immunoglobulins. Aging was associated with increased phagocytic activity of neutrophils but not bactericidal activity, superoxide production or chemotaxis of neutrophils. Aging was also associated with a significant decrease in the number of lymphocytes as well as a decline in mature T cells and helper/inducer T cells but with increased numbers of activated T cells, suppressor T cells and natural killer cells. In addition, blastoid transformation in response to phytohemagglutinin (PHA) and concanavalin A (Con A) was significantly reduced in aged subjects. A poor nutritional status was noted in individuals 60 years of age or older. The nutritional status did not influence neutrophil function but correlated significantly with the number of lymphocytes and degree of blastoid formation with PHA and Con A stimulation. Our results suggest that the cell-mediated immunity in elderly subjects is reduced as a result of malnutrition, and that improvement of the nutritional status may enhance the immune function, likely contributing to their successful aging. ———— aging; nutritional status; immune function

During the last half century, we have witnessed a tremendous increase in the mean life expectancy in several countries due to improvement in health care and environmental and nutritional conditions. However, with increased life expectancy, the elderly subject is confronted with various aging-related physiological and pathological changes. An important observation in elderly subjects is their

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susceptibility to infection (Schneider 1983), a phenomenon associated with a decline in host immune function. Several reports have examined the age-related change in immune function (Czlonkowska and Korlak 1979; Nagel et al. 1981; Goodwin et al. 1982; Hallgren et al. 1983; Hicks et al. 1983; Ligthart et al. 1986; Murasko et al. 1987; Hirokawa et al. 1992; Nakaya et al. 1992; Sansoni et al. 1993; Lesourd 1997). For example, mitogen-induced lymphocytic proliferation in the elderly is significantly less than in young subjects (Czlonkowska and Korlak 1979; Goodwin et al. 1982; Hicks et al. 1983; Murasko et al. 1987; Hirokawa et al. 1992; Nakaya et al. 1992). In addition, the percentage and absolute number of T cells, cytotoxic/suppressor cells and the ratios of naive to memory, T helper 1 subset (Th1) to Th2 in peripheral blood are low in the elderly (Nagel et al. 1981; Hallgren et al. 1983; Ligthart et al. 1986; Sansoni et al. 1993; Lesourd 1997) while the numbers of natural killer (NK) cells and activated T cells are increased (Ligthart et al. 1986; Sansoni et al. 1993). Aging also influences neutrophil function including oxidative production, phagocytosis, enzyme release and killing activity (Fulop et al. 1985; Lipschitz and Udupa 1986; McCafferty et al. 1995).

Nutrition is also an important factor that influences host defense such as neutrophil function and cellular immunity against infection (Lipschitz and Udupa 1986; Good and Lorenz 1992). Although supplementation with micronutrients enhances cell-mediated immune function, humoral immunity and NK cell activity in healthy elderly subjects (Pike and Chandra 1995; Santos et al. 1996; Meydani et al. 1997), previous studies investigating the effect of aging on the immune system simply compared differences between young and elderly subjects without examining the impact of nutritional status on the results.

In the present study, we investigated the relationship between increasing age and immune function, by examining neutrophil and monocyte function, lymphocyte subsets, blastoid transformation and the level of serum immunoglobulin, taking into consideration the effect of nutritional status on such changes.

Materials and Methods

Subjects

Subjects in this study consisted of 155 individuals (76 males and 79 females), aged 21 to 99 years. Ninety-five subjects were laboratory personnel (age, 20–64 years) and the remaining 60 subjects lived in an old-age home for the aged (age, 65–99 years). All were in good physical condition with free of acute infection as defined by no symptoms, signs and acute inflammatory reaction on labolatory findings, and none was on medication or treated for any immunological disease. Under informed consent, heparinised blood samples were collected from each subject. The study protocol was approved by the institutional human research review committee of Nagasaki University. The age and sex distribution of our population sample are shown in Table 1.

TABLE 1. *Study population*

Age group	Males	Females	Total
20-29	13	10	23
30-39	9	10	19
40-49	11	10	21
50-59	10	10	20
60-69	10	11	21
70-79	9	10	19
80-89	10	10	20
90-99	4	8	12
Total	76	79	155

Nutritional analysis

Nutritional assessment was performed by using a modification of the method of Buzby et al. (1980). The standard nutritional index is based on estimation of several parameters including serum albumin level (Alb: g/100 ml), triceps skin-fold thickness (TSF: mm), serum transferrin level (TFN: mg/100 ml), and results of delayed-type hypersensitivity skin tests. In the present study, however, the latter test was replaced with another closely related index of delayed-type hypersensitivity; total lymphocyte count as described by Niederman et al. (1984). The modified index was therefore calculated as follows:

$$\text{Prognostic nutritional index} = 158 - 16.6 \times \text{Alb} - 0.78 \times \text{TSF} - 0.2 \times \text{TFN} - 5.8 \times \text{LS}$$

where lymphocyte score (LS) is a scale of 0 to 2, in which 0 = total lymphocytes < 1000, 1 = 1000 to 2000 and 2 = > 2000. Using this index, good nutritional status represented a value of < 40, moderate nutritional status = 40 to 50, and poor nutritional status > 50.

Chemotaxis assay

Venous blood was collected in heparin. After mixing the blood sample with 3% dextran, neutrophils were isolated by Ficoll-Hypaque density gradient centrifugation. A suspension of 3×10^6/ml in Hank's solution (HBSS, Gibco, Grand Island, NY, USA), pH 7.2, containing 0.1% bovine serum albumin (BSA) was used for the chemotaxis assay as described by Werner et al. (1980). A 48-well microchemotaxis chamber (Neuroprobe Inc, Bethesda, MD, USA) was used to evaluate chemotaxis. To each well, we added 30 μl aliquots of the samples (FMLP: N-formyl methionyl leucyl phenylalanine 10^{-7} M, Sigma, St. Louis, MO, USA) or Hank's solution. A polycarbonate filter sheet (3 μm pores), not containing polyvinylpyrrolidone (NFB-3PVPF, Costar Corp, Cambridge, MA, USA), was placed on top of the wells in the bottom plate. The neutrophils (50 μl aliquots)

were placed in the upper wells, at a concentration of 3×10^6/ml, in Hank's solution with 0.1% BSA. Chambers were incubated for 30 minutes at 37°C in a humidified atmosphere of 95% air-5% CO_2; the filter was removed, fixed in absolute methanol, and stained with eosin-azur (Diff-Quik, Harleco; Gibbstown, NJ, USA). The cells that migrated through the filter to the other end were counted. The neutrophil chemotactic activity was measured as the mean number of cells per 9 high power fields (oil immersion fields [O.I.F], magnification $\times 1000$).

Superoxide production

Peripheral blood neutrophils were prepared as described above. The cells were suspended in Krebs Ringer phosphate solution (KRP) at a cell density of 1×10^7/ml as previously described (Kitagawa et al. 1984). The neutrophils were stimulated by 100 μg/ml concanavalin A (Con A) and 5 μg/ml of cytochalasin E. The superoxide production (SOP) was measured by the reduction of cytochrome C using a double-wavelength spectrophotometer (model 557, Hitachi, Tokyo). The results were expressed as the amount of superoxide produced (in nM) per 5×10^5 neutrophils per minute.

Phagocytosis and intracellular killing

The phagocytic and bactericidal activities of neutrophils and monocytes were measured using the method described by Steinkamp et al. (1982). For this purpose, 1 ml of whole blood was placed in a flat-bottomed scintillation glass vial and incubated with 10 μl of 2′-7′ dichlorofluorescein diacetate (DCF-DA) for 10 minutes at 37°C in a water bath. After incubation, 30 μl of *Staphylococcus aureus*-labeled FITC was added to two vials, with one vial serving as a control. Both vials were incubated for 15 minutes at 37°C. Then, 100 μl of samples were added to 2 ml of FACS lysing solution at room temperature in the dark for 5 minutes, and centrifuged at 1500 rpm for 5 minutes at 4°C. The pellet was washed with PBS containing ethylenediaminetetraacetic acid (EDTA) and suspended in 0.5 ml of 1% paraformaldehyde in PBS. The phagocytic and bactericidal activities of these cells were measured by a FACScan flow cytometer (FACS Division, Becton Dickinson, Mountain View, CA, USA). A computer system (Consort 30, Becton Dickinson) was used for data acquisition and analysis.

Two-color direct immunofluorescence staining

A total of 100 μl whole blood collected by venipuncture with EDTA was placed into a 12×15 mm polystyrene tube (Falcon Plastics, Oxnard, CA, USA). The cells were stained with monoclonal antibodies against various surface markers including CD3 (T cells), CD4 (helper T cells), CD8 (suppressor T cells), CD45RA (naive T cells), CD45RO (memory T cells) and CD16/56 (natural killer, NK cells). The HLA-DR antigen was also examined as activation marker. In the next step,

5 μl of each monoclonal antibody was added and the tubes were incubated for 15 minutes at room temperature in the dark, followed by the addition of 2 ml of 1× FACS lysing solution (Becton Dickinson). The cells were mixed vigorously and incubated for 10 minutes at room temperature and then washed once in cold PBS containing 0.1% sodium azide. The cells were finally resuspended in cold PBS containing 0.5% paraformaldehyde. The lymphocyte fractions were gated and cells positive for each surface marker were analysed by FACScan flow cytometer.

Mitogen stimulation assay

Venous blood was collected in heparin. Peripheral blood mononuclear cells were separated on Ficoll-Hypaque density gradient centrifugation and washed twice in PBS. The cells were then adjusted to a concentration of 5×10^5/ml in RPMI 1640 with 10% FCS and then stimulated with 4 μg/ml of ConA, 4 μg/ml of phytohemagglutinin (PHA), or 1 μg/ml of pokeweed mitogen (PWM) (Murasko et al. 1986). After culture for 64 hours at 37°C in 5% CO_2, 0.25 μCi of ^{3}H-thymidine was added to each well, then cultured for 8 hours in 5% CO_2. The contents of the wells were harvested onto a glass filter using a cell harvester. The ^{3}H uptake was measured by a liquid scintillation counter.

Humoral immunity

We measured the levels of serum of IgG, IgM and Ig A by latex agglutination.

Statistical analysis

All data were expressed as the mean±standard error (S.E.). Significant differences were identified by non-parametric testing, using Statview 4.0 statistical package. The Kruskal-Wallis test was used to examine differences between the means of multiple unpaired groups. Probability values of <0.05 were considered significant.

Results

Neutrophil and monocyte function

The absolute number of peripheral neutrophils increased with aging (data not shown). We also analysed the chemotactic activity, superoxide production, phagocytic and bactericidal activities of neutrophils. The phagocytic activity was significantly higher in subjects older than 40 years of age than in 30-39 age group, and was significantly different between subjects aged 90-99 and 20-29 years (Table 2). However, no persistent age effect on phagocytic activity was noted. On the other hand, other indexes of neutrophil function, including bactericidal activity, superoxide production and chemotactic activities, were not influenced by aging (Table 2). We also evaluated the phagocytic and bactericidal activities of monocytes (Table 3). Both activities tended to increase with aging, although the absolute number of peripheral monocytes did not change.

TABLE 2. *Immunological functions of neutrophils*

Age group	Phagocytosis (%)	Bacterial killing (%)	SOP (nM/5×10^{5}PMN /minute)	Chemotaxis (number of PMN /OIF)
20-29	93.0±2.2	91.2±2.4	3.3±0.4	95.0±6.4[b]
30-39	90.9±2.6	89.8±2.7	4.2±0.5	94.5±6.4
40-49	95.5±0.8[a]	92.2±1.2	3.5±0.4	102.8±2.8
50-59	96.8±0.5[a]	94.1±0.8	2.9±0.2[a]	99.6±2.9
60-69	96.4±0.7[a]	94.6±1.0	3.5±0.3	108.1±3.7[a,b]
70-79	95.5±1.8[a]	92.0±2.5	4.1±0.3[c]	99.3±3.6
80-89	96.2±1.1[a]	92.5±1.9	4.2±0.3[c]	100.0±2.2
90-99	98.1±0.2[a,b]	95.6±0.8	3.8±0.3	102.8±4.8

Values are expressed as mean±S.E.
OIF, oil immersion field.
[a]$p<0.05$ vs. 30-39, [b]$p<0.05$ vs. 20-29, [c]$p<0.05$ vs. 50-59.

TABLE 3. *Immunological functions of monocytes*

Age group	Phagocytosis (%)	Bacterial killing (%)
20-29	62.7±4.1	60.7±4.4
30-39	67.3±2.4	65.8±2.5
40-49	62.4±3.4	59.7±3.1
50-59	67.4±3.8	64.9±3.9
60-69	71.0±3.6	67.0±3.5
70-79	69.8±4.4	65.8±4.2
80-89	73.9±3.1[a,b]	70.3±3.1[b]
90-99	76.9±3.3[a,b]	72.3±3.4[a,b]

Values are expressed as mean±S.E.
[a]$p<0.05$ vs. 20-29, [b]$p<0.05$ vs. 40-49.

T cell subpopulations and mitogen responses of peripheral blood mononuclear cells

The percentage and absolute number of peripheral lymphocytes decreased progressively and significantly after 60 years of age (Table 4). Among these, $CD3^+$cells representing mature T cells significantly decreased with aging, while activated T cells ($CD3^+$ $HLA\text{-}DR^+$ cells) and NK cells ($CD3^-$ $CD16/56^+$ cells) increased markedly in the aged group (data not shown). $CD4^+$ cells representing helper/inducer cells decreased with aging, and there was a significant difference between the nineties (33.7±2.2%) and thirties (40.7±1.3%, $p<0.05$) and forties (44.1±1.9%, $p<0.05$) age groups. No significant differences were observed in $CD8^+$ cells representing suppressor/cytotoxic T cells among the groups. The phenotypes of $CD4^+$ and $CD8^+$ cells were further investigated using two-color

TABLE 4. *Percentage and numbers of lymphocytes*

Age group	Lymphocytes	
	(%)	(absolute number/mm^3)
20-29	36.0±4.1[b,c]	1750.4±209.5[c]
30-39	33.7±1.9[c]	1806.6±83.9[b,c]
40-49	30.6±1.7[c]	1761.1±131.9[b,c]
50-59	34.5±1.9[b,c]	1920.1±118.2[a,b,c]
60-69	31.0±1.9[c]	1618.7±125.7[c]
70-79	30.2±1.6[c]	1467.5±85.0
80-89	28.4±1.8	1413.8±101.1
90-99	23.8±1.8	1226.8±126.4

Values are expressed as mean±S.E.
[a]$p<0.05$ vs. 70-79, [b]$p<0.05$ vs. 80-89, [c]$p<0.05$ vs. 90-99.

immunofluorescence staining. The percentage of $CD4^+CD45RA^+$ cells was low while $CD4^+CD29^+$ cells tended to increase with aging. We found no age-related changes in the percentages of $CD8^+CD11b^+$ and $CD8^+CD11b^-$ cells (data not shown).

Table 5 shows the proliferative response of peripheral lymphocytes to PHA, ConA or PWM. The response to all three mitogens tended to decrease with age. Thus, the proliferative responses to PHA, ConA and PWM in subjects older than 40 years of age were significantly lower than those of subjects <39 years of age (with the exception of 40-49 vs. 20-29 and 30-39 for PHA and 40-49 and 60-69 vs. 20-29 for ConA). In addition, the proliferative response in subjects 70 years of age or older was also significantly lower than the 40-49 group for PHA and ConA (with the exception of 40-49 vs. 80-89 for ConA). The responses to PHA and ConA were the lowest in the eldest age group. Similarly, the response to ConA in the 70-79 age group was also significantly lower than in 50-59 and 60-69 age groups.

Humoral immunity

We also measured the level of serum immunoglobulins, including IgG, IgA and IgM. The level of IgG and IgA tended to be higher in elderly subjects albeit insignificantly, but age had no effect on IgM (Fig. 1).

Nutritional status and immunity

A gradual decrease in serum albumin and serum transferrin concentrations was observed with aging, but no acute phase reactions were seen in all individuals (data not shown). The mean prognostic nutritional index started to increase significantly with age at 60-69 years (Fig. 2), and was significantly high in 80-89

TABLE 5. *Lymphocyte proliferation* (mean ± S.E., cpm)

Age group	PHA	ConA	PWM
20–29	43027.7 ± 2372.5	37442.7 ± 1821.6	20471.7 ± 1606.3
30–39	42889.3 ± 2979.4	39908.4 ± 2390.7	19922.1 ± 1254.5
40–49	41013.7 ± 1886.8	33040.6 ± 1223.0	13285.4 ± 2159.7
50–59	36324.1 ± 1327.3	31467.5 ± 2970.5	10766.2 ± 1420.6
60–69	35424.6 ± 1983.2	32423.9 ± 1820.0	13786.0 ± 1843.7
70–79	30861.0 ± 2018.4	24346.7 ± 2018.9	12286.3 ± 1463.4
80–89	33082.5 ± 1487.1	28228.7 ± 2002.6	11539.9 ± 1063.9
90–99	23018.8 ± 1983.3	17564.3 ± 2549.3	9974.0 ± 1365.5

$p<0.05$	PHA	ConA	PWM
	20–29 vs. 50–59, 60–69, 70–79, 80–89, 90–99	20–29 vs. 50–59, 70–79, 80–89, 90–99	20–29 vs. 40–49, 50–59, 60–69, 70–79, 80–89, 90–99
	30–39 vs. 50–59, 60–69, 70–79, 80–89, 90–99	30–39 vs. 40–49, 50–59, 60–69, 70–79, 80–89, 90–99	30–39 vs. 40–49, 50–59, 60–69, 70–79, 80–89, 90–99
	40–49 vs. 70–79, 80–89, 90–99	40–49 vs. 70–79, 90–99	
	50–59 vs. 90–99	50–59 vs. 70–79, 90–99	
	60–69 vs. 90–99	60–69 vs. 70–79, 90–99	
	70–79 vs. 90–99	70–79 vs. 90–99	
	80–89 vs. 90–99	80–89 vs. 90–99	

and 90–99 age groups, representing a poor nutritional status in these age groups. The relationships between nutritional status and several immunological parameters are summarised in Table 6. The majority of moderate to poor nutritional status group was 80–99 age. Neutrophil function was not influenced by the nutritional status, while monocyte function, such as phagocytosis and bactericidal activity, increased significantly in the presence of poor nutritional status (>50) compared with <40. The absolute number of peripheral lymphocytes (<40 vs. >50, $p<0.05$) and the percentages of $CD3^+$ (<40 vs. >50, $p<0.05$) and $CD4^+$ cells (<40 vs. >50, $p<0.05$) were significantly low, while $CD8^+$ cells were significantly high in the presence of a poor nutritional status (<40 vs. >50, $p<0.05$). On the other hand, there was no relationship between T cell activation markers, such as the DR antigen, and nutritional status. The percentage of $CD3^-$ $CD16/56^+$ cells significantly increased with a decline in nutritional status (<40 vs. >50, $p<0.05$). Furthermore, the response to PHA and ConA diminished significantly with the decline in nutritional status (Table 6). Finally, there was no relationship between nutritional status and serum immunoglobulins, including

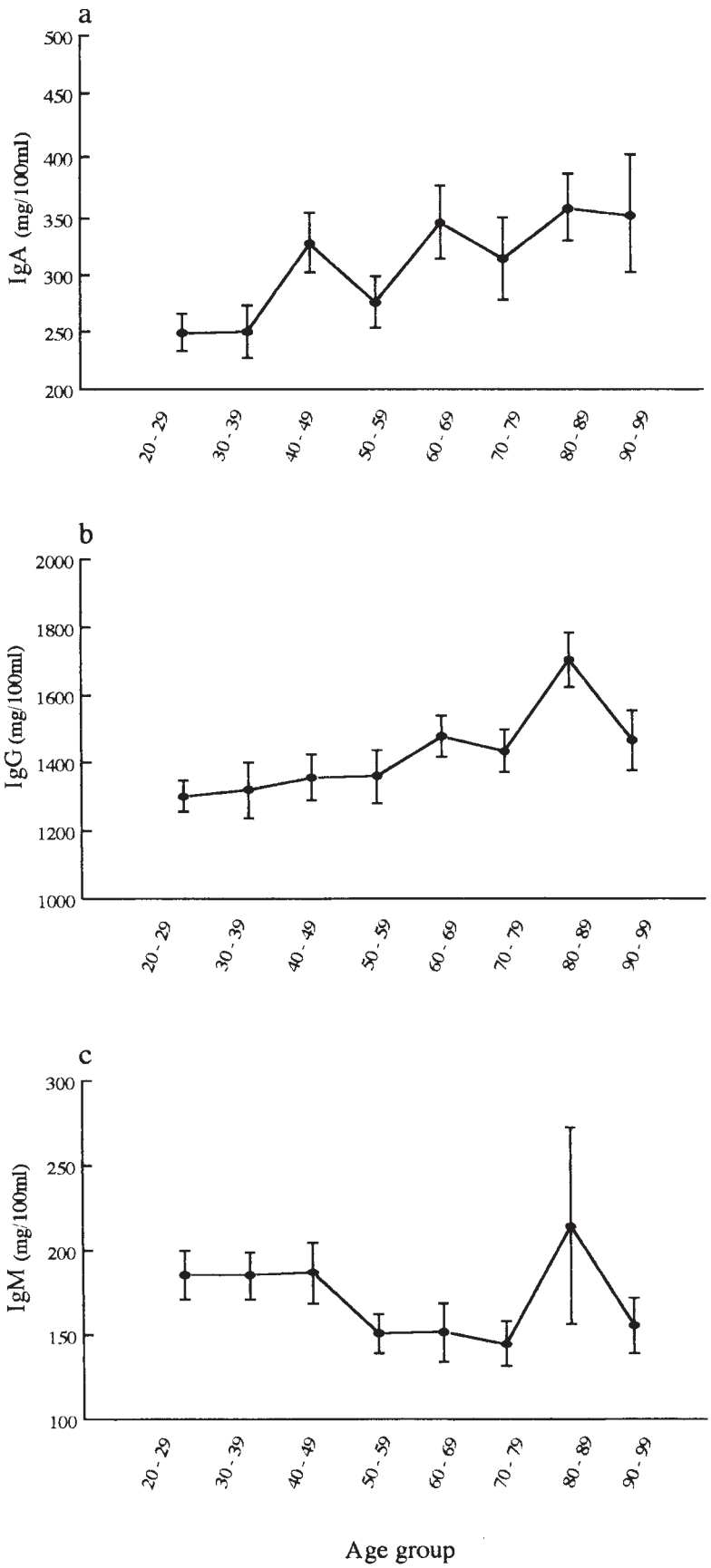

Fig. 1. Serum immunoglobulin concentrations in each age group. Values are the mean ± S.E. a, IgA; b, IgG; c, IgM.

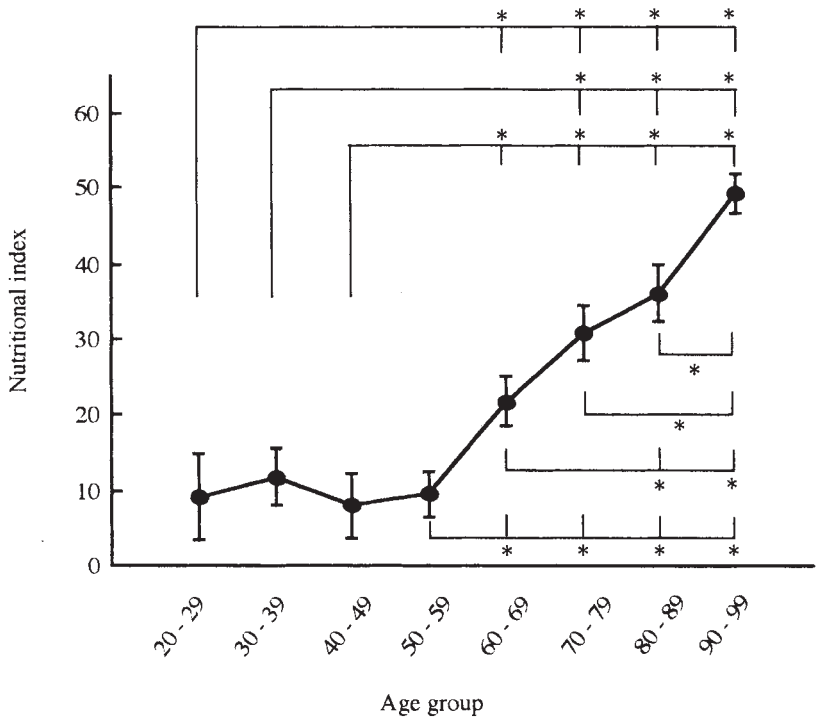

Fig. 2. Changes in nutritional status with aging. Values are the mean±S.E. $^{*}p<0.05$.

IgG, IgA, and IgM (data not shown).

Discussion

The process of aging is associated with increased susceptibility to infections (Terpenning and Bradley 1991). Some of this has been ascribed to a declining capacity in cell-mediated and humoral immunity, alteration of nutritional factors and chronic underlying diseases. Most studies of immune function in the aged host have focused on the cell-mediated immune system (Czlonkowska and Korlak 1979; Nagel et al. 1981; Goodwin et al. 1982; Hallgren et al. 1983; Hicks et al. 1983; Murasko et al. 1987; Hirokawa et al. 1992; Nakaya et al. 1992), while a few reports examined humoral immunity, NK cell and neutrophil function (Fulop et al. 1985; Ligthart et al. 1986; Lipschitz and Udupa 1986; Sansoni et al. 1993; McCafferty et al. 1995; Lesourd 1997). Nutrition is also an important factor in host defense against infection.

The major findings of the present study were a lack of effect of aging on neutrophil function, except for enhanced phagocytosis, while advanced age was associated with enhanced monocyte function such as phagocytosis and bacterial killing. These results suggest that aging is not associated with abnormal phagocytosis. Moreover, these functions were independent of the nutritional status. McCafferty et al. (1995) have recently reported that age was associated with a decrease in the luminol enhanced chemiluminescence (CL) response to zymosan and in unstimulated CL, while Niwa et al. (1989) found no significant differences in either phagocytic level, superoxide or hydrogen peroxide production

TABLE 6. *Immunological parameters with nutritional status*

	Nutritional index		
	<40 ($n=88$)	40–50 ($n=14$)	>50 ($n=13$)
Neutrophil			
Phagocytosis (%)	95.5±0.6	97.8±0.6	96.6±1.4
Bacterial killing (%)	92.4±0.9	95.6±0.9	93.6±1.2
Superoxide production (nM/5×10^{5}PMN/minute)	3.6±0.2	3.8±0.4	4.1±0.3
Chemotaxis (number of PMN/OIF)	104.2±1.7	100.9±3.7	97.5±4.9
Monocyte			
Phagocytosis (%)	65.9±1.9	73.5±3.5	78.0±3.7[a]
Bacterial killing (%)	63.1±1.8	69.0±3.4	74.6±3.7[a]
Number of lymphocytes (/mm^3)	1743.6±55.3	1321.8±75.7[a]	1039.8±106.8[a]
Surface marker			
CD3(%)	62.7±1.2	63.0±3.3	50.4±3.1[a,b]
CD4(%)	41.4±1.0	36.1±2.1[a]	32.0±2.1[a]
CD8(%)	29.0±0.9	34.8±2.4[a]	34.3±2.4[a]
CD16/56(%)	18.8±1.0	22.8±2.5	33.9±3.7[a,b]
Blastoid transformation (cpm)			
Response to PHA	37170.4±1067.5	31987.3±1968.5	25037.54±2190.89[a]
ConA	32072.0±1175.1	25360.2±2963.4[a]	20182.62±1672.44[a]
PWM	13896.2±858.2	12032.7±1478.8	9825.23±1279.78

Values are expressed as mean±S.E.
[a]$p<0.05$ vs. <40. [b]$p<0.05$ vs. 40–50. OIF, oil immersion field.

in elderly adults compared with young adults. On the other hand, other studies have shown that aging has little or no influence on monocyte and macrophage function (Gardner et al. 1981; Horan and Fox 1984). Considered together, the present results as well as those of earlier studies indicate that there is a heterogeneity in age-associated phagocyte dysfunction probably reflecting the use of different methodologies in these studies. Our results using whole blood samples were supported by the findings that opsonisation and complement levels are, in general, unaffected by aging (Phair et al. 1978) and that the ability of neutrophil in killing *S. aureus* is independent of age (Phair et al. 1978; Nagel et al. 1982). In this context, our results showed that the levels of IgG, IgA and IgM in elderly subjects were not different from those in younger subjects.

We also analysed cell-mediated immunity. While results of previous studies showed a lack of effect of age on the number of lymphocytes (Ligthart et al. 1986; Terpenning and Bradley 1991), the present findings indicated that the total number of lymphocytes was significantly low in the 90–99 age group relative to that in the 20–59 groups. Furthermore, we also examined different subpopula-

tions of mononuclear cells and found that aging was associated with a reduced percentage of mature T cells ($CD3^+$ cells), increased percentage of activated T cells ($CD3^+$ $HLA\text{-}DR^+$ cells) and natural killer cells ($CD3^-$ $CD16/56^+$ cells). These results are in agreement with those reported previously by other investigators (Facchini et al. 1987; Sansoni et al. 1993; Licastro et al. 1995). The high proportion of NK cells is likely to be compensatory in nature in response to a decrease in mature T cells and NK cell cytotoxicity in the elderly (Facchini et al. 1987). Our results that aging was associated with a reduction in $CD4^+$ but not $CD8^+$ cells are different from those of other investigators (Nagel et al. 1981; Hallgren et al. 1983; Ligthart et al. 1986; Hirokawa et al. 1992). This discrepancy may be explained by genetic and racial differences since the population sample in the present study consisted of Japanese subjects. However, memory T cells increased with advanced age in agreement with a previous report (Hirokawa et al. 1992; Lesourd 1997), indicating that most T cells in an elderly person have already had prior antigenic exposure.

The blastoid transformation in response to PHA, ConA and PWM showed a progressive and significant reduction with aging, which is in agreement with previous reports (Goodwin et al. 1982; Hicks et al. 1983; Murasko et al. 1987), although conflicting results have been reported in previous studies examining cellular immunity in the elderly. These results also indicate that the decline in T cell function occurs in the 40's for PWM or 50's for PHA and ConA, supporting previous findings that in the majority of the elderly population, a decline in cell-mediated immune response to PHA, ConA and PWM occurs prior to the age of 70 (Murasko et al. 1986). In contrast, active elderly subjects demonstrated a significantly greater proliferative response to PHA and to PWM (Venjatraman and Fernandes 1997), suggesting that exercise should help our elderly subjects in an old-age home to reverse the adverse effects of aging upon cell-mediated immune response.

Protein-energy malnutrition is associated with a significant impairment of cell-mediated immunity, phagocyte function, complement system, humoral immunity and cytokine production, and micronutrients deficiency such as zinc, selenium, iron, copper and vitamins mimics a decrease in immune responses with aging (Beisel 1982; Chandra 1997; Lesourd 1997). Zinc administration in humans corrects some of the age-related decline in immunological responses (Duchateau et al. 1981), and supplementation with vitamins increases cell-mediated immune function and the number of NK cells in elderly subjects (Pike and Chandra 1995; Meydani et al. 1997). Beta-carotene supplementation also enhances NK cell activity in elderly men (Santos et al. 1996). Furthermore, our results showed that the nutritional status in persons 60 years or older decreased significantly, and that malnutrition assessed by the prognostic nutritional index negatively influenced cell-mediated immunity. Since the majority of moderate to poor nutritional status group was 80–99 age, these findings suggest that the

immune response in elderly subjects is reduced as a result of malnutrition.

In conclusion, the cell-mediated immunity is thought to be more important than other host mechanisms in the development and combating infections in elderly subjects. Our results together with other reports suggest that improvement of the nutritional status in elderly subjects may lead to the enhanced immune function and protection against infection, likely contributing to their successful aging.

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