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Environmental and Physiological Influences on Human Natural Killer Cell Activity in Relation to Good Health Practices

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We examined the association of natural killer (NK) cell activity with a number of life style factors by a cross-sectional analysis on 2892 Japanese individuals. The following habits were found to be associated with increased NK activity: 1) alcohol drinking (males, $P<0.05$), 2) not smoking cigarettes (males and females, $P<0.001$), 3) increased intake of green vegetables (females, $P<0.001$), 4) increased intakes of meat, milk, dairy products, and soyabean products (males, $P<0.01$), 5) daily workload of less than 3 hours per day (females, $P<0.05$), 6) regular meals (females, $P<0.05$), 7) regular sleep of more than 7 hours (females, $P<0.05$), 8) proper body weight. In addition, systolic blood pressure showed a positive correlation with NK activity ($P<0.001$), while proportion of helper/inducer ($OKT4^+$) T cells and fraction of β-globulin showed negative correlations ($P<0.001$). We thus found that the living habits associated with increased NK activity were consistent with generally accepted good health practices, except alcohol drinking.

Key words: Human — Natural killer cell activity — Life style

The interindividual differences in susceptibility to cancer incidence may involve different levels of host factors, such as genetic differences in metabolic activation or detoxification of procarcinogens, suspected germline polymorphisms of oncogenes or associated genes, hormonal predisposition, and natural immune defense against development of cancer. The interaction of these host factors with the environment varies among individuals and may be responsible for different susceptibility to cancer incidence.

A prospective cohort study initiated in 1986 is aiming at an evaluation of the host-environment relationship in cancer etiology. This cohort has been used to investigate genetic susceptibility to lung cancer in terms of germ line DNA polymorphism of the human cytochrome P450IA1 gene.[1)] Besides the genetic susceptibility to chemically induced cancers, one of the main host factors investigated in this cohort study is the role of immune surveillance as a primary defense mechanism against cancer. This paper presents a cross-sectional analysis designed to clarify the influences of life style factors and physiological factors such as blood pressure, serological components and distribution of T cell subsets on the activity of natural killer (NK) cells. Among various effector cells in the cell-mediated immune response, NK cells are nonspecific and have a rapidly activatable ability to lyse various types of tumor cells. The importance of NK cells in the immune surveillance against cancer has been suggested by a number of observations, such as the high cancer incidence among beige mice[2)] and immunodeficient patients,[3)] as well as the low activity observed among familial melanoma patients[4)] and among individuals with high familial incidence of cancer.[5)]

On the other hand, considerable evidence has been accumulated on the environmental influences on NK activity. Cigarette smoking decreased the activity and cell numbers,[6, 7)] and increased cytotoxic stimulation was observed in male vegetarians.[8)] There have also been contradictory or inconsistent results on the association of NK activity with alcoholism,[9, 10)] acute physical exercise,[11, 12)] and aging.[13, 14)] All the studies so far carried out on human NK activity have suffered from limited numbers of study subjects, mostly less than a hundred, and also from specific characteristics of subjects in most of the studies, such as alcoholics, vegetarians, trained sportsmen, and subjects pretreated with various physical loads. It is therefore still necessary to investigate the influences of relevant environmental and physiological factors on NK activity among a large sample of the general population, and to conduct a follow-up study to disclose the role of NK cells in cancer etiology. This paper provides quantitative estimates of these influences on the basis of an epidemiologic and immunological survey on a total population of 2892 individuals.

MATERIALS AND METHODS

Study population We started our prospective cohort study in 1986 on the residents of mainly 40 years and older in a town in Saitama prefecture, Japan. The epidemiologic survey was carried out by use of a self-administered questionnaire on ninety life style factors

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Table I. The Age Distribution of Study Population and NK Activity

Age groups (yr)	Male		Female	
	No. of subjects	Mean NK activity±SD (%)	No. of subjects	Mean NK activity±SD (%)
<40	91	50.2±15.6	136	39.5±16.7
40–49	223	48.0±17.0	452	40.5±16.2
50–59	275	47.7±18.1	539	42.2±16.9
60–69	368	49.3±17.6	507	45.7±17.3
≧70	127	50.4±16.0	174	44.9±17.1
All age groups	1084	48.8±17.3	1808	42.8±17.0

including present and past eating habits, cigarette smoking and alcohol drinking history, conditions of work, regularity of meals, sleeping habits by duration, psychological classification of personality, assessment of mental stress, history of diseases, use of medicaments, and family history of cancer and other major diseases.

This survey covered 8553 individuals, with a coverage of 95% of the total residents over 40 years old. Peripheral blood samples of 2892 individuals among them were collected and subjected to biochemical and immunological assays in 1986 to 1988 at the time of yearly health screening, including about ten percent of individuals below 40 years old. The sample collection is still ongoing. In this study, only epidemiologic and immunological data on these primary groups of 2892 individuals, 1084 males and 1808 females with mean ages±SD of 56.4±12.0 and 55.1±10.9 years, were analyzed and correlated. The age distribution of the study population is shown in Table I.

Assay of NK activity The cytotoxic activity of NK cells was determined by ^{51}Cr-release assay. The effector cells were isolated from 5 ml of heparinized peripheral blood sample by density gradient centrifugation on Conray-Ficoll mixtures within 5 h after taking blood samples. All the blood samples were collected between 1 and 3 p.m. during yearly health screening periods of two weeks held in July, August and/or September. Target cells were K562, a human myeloid leukemia cell line, and labeling of target cells with ^{51}Cr was carried out by incubation with 100 μCi of $Na_2{}^{51}CrO_4$ for 1 h at 37°C. Both target cells (1×10^6/ml) and effector cells (1×10^6/ml) were suspended in a standard medium (RPMI 1640 plus 10% FBS). Effector cells in 200 μl of medium were added to target cells in 10 μl of medium in every well (effector-to-target ratio of 20) and incubated for 3.5 h. Percent specific lysis was calculated according to the standard formula.[15] We used one male control to examine variation of NK activity assay over three years: values of NK activity measured were 53 and 55% on July 7 and 21 in 1986, 54 and 51% on August 31 and September 11 in 1987, and 54% on September 4 in 1988. Three female controls gave values of 44% on July 21 in 1986 and 43% on August 31 in 1987 for control 1; 21% on July 10 in 1986, 31% on August 31, and 29% on September 10 in 1988 for control 2; 15, 14 and 20% on July 10, 15 and 24 in 1986 for control 3. The considerable increase of about 10% from 1986 to 1988 in control 2 can possibly be ascribed to her marriage and subsequent changes of life style factors in the intervening period.

Other assays Subsets of T cells were analyzed by a laser flow cytometry (FCM-1, Nihon Bunko, Tokyo) using monoclonal antibodies OKT4 and OKT8 (Ortho Diagnostic Systems, NJ) as T cell surface markers,[16] and reactivity of peripheral blood lymphocytes in the presence of phytohemagglutinin (PHA) was also examined. In addition to the immunological assays, all blood samples were also subjected to a variety of biochemical assays: total protein, albumin, globulin, serum protein fractionation (cellulose acetate electrophoresis technique), total cholesterol, triglyceride, lipoprotein fractions (agarose gel electrophoresis), glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, hemoglobin, hematocrit, chloride, potassium, sodium, serum vitamin A (high-pressure liquid chromatography), selenium (atomic absorption spectrometry) and lipid peroxides. Blood pressure was measured at the time of taking blood samples.

Statistical methods We examined the association of NK activity with life style factors among the study population by comparing the mean NK activities between the subgroups belonging to different categories in each of all ninety factors surveyed. Statistical significance of the differences between the mean activities was tested by using the standardized normal deviates. Eight factors among those examined showed significant association with NK activity (see "Results"), and confidence limits for the mean activity were also calculated assuming normal distribution.

The association between these eight factors was also examined by using the χ^2 test with contingency tables on joint distributions of every pair of factors. When a significant association of a factor with another factor was found, its influence was examined by comparing the mean NK activities of categories in a factor within each subgroup belonging to the same category of the associated factor. We also examined the mean ages of subgroups in individual categories of factors, since NK activity in females, but not in males, increased with age as shown in Table I. Among females, no significant differences in the mean ages were observed except relative weight. The influence of age on the activity was

adjusted only for relative weight among females by assuming a linear dependence of the activity on age. Otherwise no adjustment or multivariate procedure was carried out.

RESULTS

Sex difference and age-dependence Our results were analyzed among males and females separately, since NK cell activity differed considerably between sexes: i.e. 48.8±17.3% (mean activity ± SD) among 1084 males and 42.8±17.0% among 1808 females. In addition, as shown in Table I, the lower activity among females was age-dependent with a positive correlation coefficient $r=0.13$ ($P<0.001$), while this was not found among males.

Alcohol drinking and cigarette smoking Alcohol drinkers clearly showed increased NK activity. Compared with the mean activity of 47.0±17.1% among 240 male non-drinkers, 248 occasional drinkers showed a nearly identical mean of 47.6±16.2% while 553 regular drinkers gave a higher mean of 50.2±17.7% with statistical significance of $P<0.05$ (43 ex-drinkers were excluded). Cigarette smoking was associated with decreased NK activity, although significant reduction was observed only among smokers with a smoking index of more than 500 (the number of cigarettes consumed per day × years). Cigarette smoking was closely associated with alcohol drinking in life style: i.e., those who smoke a lot tend to drink a lot (χ^2-test for 3×4 table, $P<0.001$). We then analyzed the activity for combinations of these two factors which could exert opposite effects. The result is shown in Fig. 1 where non-smokers/non-drinkers were never- and ex-smokers/never and ex-drinkers with abstinence duration of more than five years; the drinking index used for regular drinkers was ethanol amount (ml) consumed per day × years. In the figure, dose-response relations were observed for cigarette smoking and alcohol drinking, although the activity in the medium smoking category among regular drinkers with the index of less than 1000 was anomalously high.

The effect of smoking on the activity was significant when we restricted the subjects to non-drinkers and occasional drinkers: the mean activities of 44.9±17.5% among 188 smokers with the index of more than 500 and 45.1±17.1% among the overall 282 current smokers were lower than that of 48.8±16.5% among 167 non-smokers ($P<0.05$). Lower NK activity among smokers was also observed among females: the mean of 36.8±17.1% among 114 current smokers was lower than that of 43.3±16.9% among 1663 non-smokers ($P<0.001$). No significant effect of alcohol drinking or its association with cigarette smoking was observed among females because only 72 were regular drinkers and they consumed much less ethanol than male drinkers did.

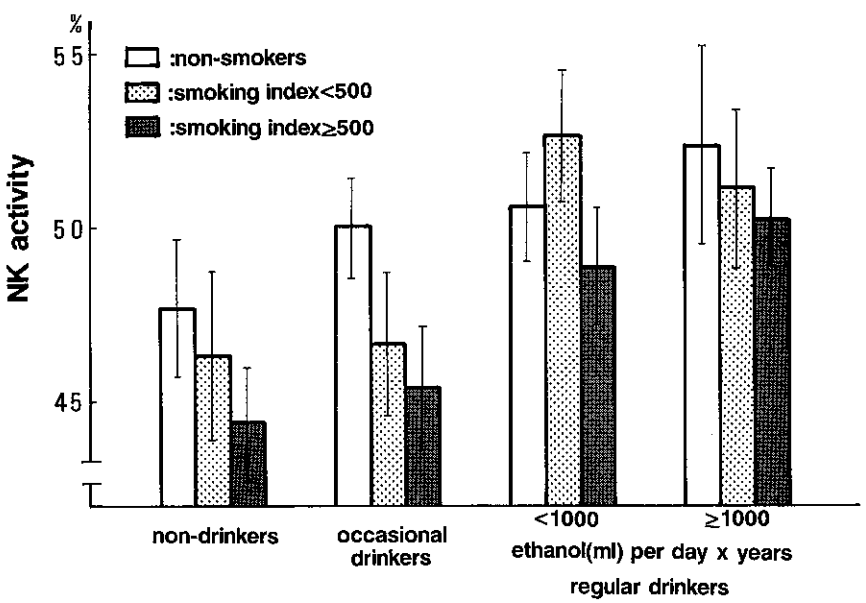

Fig. 1. NK activity in relation to smoking and drinking habits among males. Bars are 95% confidence intervals (CI). Among non-drinkers, the numbers of subjects are 82 non-smokers, 43 moderate smokers (with a smoking index of less than 500), 96 heavy smokers (with an index of more than 500); among occasional drinkers, 85, 51, 92; among moderate regular drinkers (with a drinking index of less than 1000), 87, 69, 115; among heavy regular drinkers (with an index of more than 1000), 47, 54, 131, respectively.

Dietary factors Among dietary factors, intake of green vegetables was most associated with increased NK activity, especially among females. In Fig. 2, the mean activity of 44.8±17.1% (n=644) in the category of daily intake among females differed from that of 41.9±17.0% (n=973) in the category of 2 to 4 times per week and 41.0±15.6% (n=185) in the less than once per week intake ($P<0.001$ and $P<0.01$), while males showed a similar dose-response relation but without statistical significance. The mean ages of categories showed no significant difference, ranging between 54 and 56 years old among females. Increase in the activity was not observed for yellow vegetables.

Protein sources such as meat, soyabean products and dairy products also showed enhancing effects on the activity among males. Among these food items, soyabean products are a very important source of vegetable protein in Japan, and 90% of our cohort population take the products more than 2 to 4 times per week. The observed dose-response relation for soyabean products is shown in Fig. 3, where the difference of mean activities between categories of daily intake and less than once per week is statistically significant ($P<0.01$). Meat and dairy products did not show a clear dose-response relationship but low NK activity was observed only in the category of the lowest intake frequency: 49.0±16.7% (n=217) in daily intake of meat, 49.1±17.7% (n=697) in 2 to 4 times per week and 47.6±15.9% (n=151) in less than once per week; concerning the milk and dairy products, 49.5±17.9% (n=355) in the category of daily intake, 49.7±17.0% (n=307) in 2 to 4 times per week and 47.6±16.7% (n=402) in less than once per week.

The enhancing effects of these dietary factors were not observed among females since these factors were strongly associated with each other among females (χ^2-test for 3×3 tables, $P<0.001$), but not males. Namely females compensated for the loss of protein intake from one diet with increased intake of the other diet: i.e. the observed 151 subjects in the combined category of less than once per week for the intake of both meat and dairy products was compared with the expected number of 249.7 which was calculated by assuming no interaction between the intake of these factors; the observed 65 subjects in the category of less than once per week for the intake of both meat and soyabean products was also compared with the expected number of 172.3. A similar compensation was not observed among males.

Other living habits Degree of daily work intensity was associated with NK activity among females (Fig. 4), where labor was specified as daily heavy workload such as agriculture and production work, excluding housework. It is of interest to find that the mean activity in the

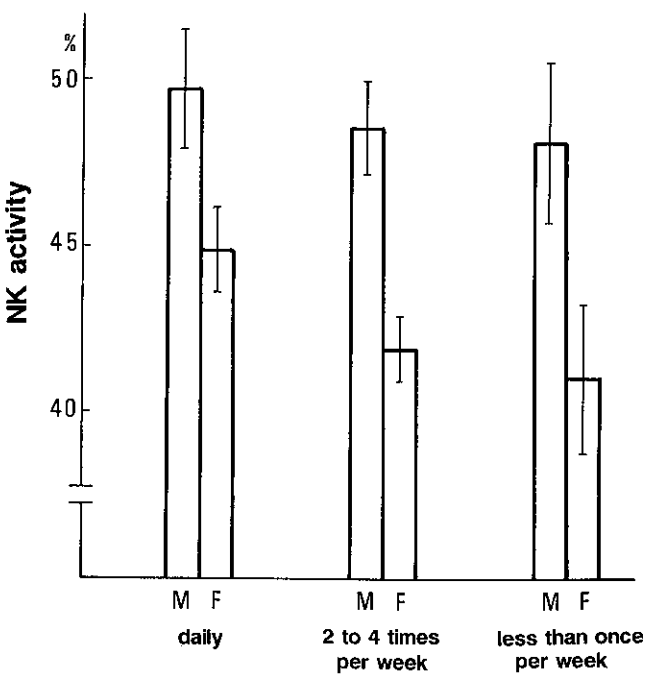

Fig. 2. NK activity (means, bars 95% CI) in relation to green vegetables intake (M: males, F: females).

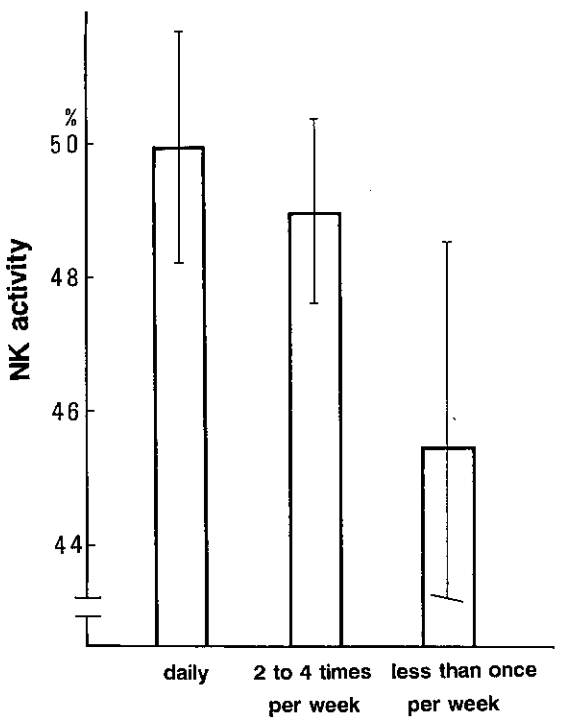

Fig. 3. NK activity (means, bars 95% CI) in relation to soyabean products intake among males.

category of less than 3 h of working time per day was the highest at 44.4±17.5% (n=497) and that the activity decreased with increased hours of gainful work. The mean activity in the category of less than 3 h was higher than the mean of 42.2±16.7% (n=760) with no heavy workload ($P<0.05$) or 41.5±17.1% (n=264) with working time of more than 6 h ($P<0.05$). Among males, the same trend was observed in never-smokers: 50.2± 16.0% (n=65) with no heavy workload, 53.6±19.5% (n=38) with working time of less than 3 h, 48.3±16.2% (n=28) with 3 to 6 h and 46.4±15.5% (n=41) with more than 6 h. When we included smokers, this trend disappeared because of a strong association between laboring status and smoking habit observed only among males.

Regular sleep was also closely associated with increased NK activity. The mean activity of 43.3±17.0% (n=1297) in the category of regular sleep of more than 7 h among females was higher than that of 40.6±16.9% (n=231) with irregular sleep ($P<0.05$), and females with regular sleep of less than 7 h showed an intermediate mean of 42.3±16.6% (n=260). Regularity of meals was also influential: i.e., the mean activity of 42.9±17.0% (n=1749) in the category of regular meals was higher than that of 38.2±16.6% (n=55) with irregular meals ($P<0.05$). Here regularity means not only in mealtime but also in eating three meals a day and not eating between meals. Among males, these two factors were associated with smoking and drinking habits, and the number of subjects with irregular sleep or meals among non-smokers or non-drinkers was too small for analysis. It should be noted that no significant differences in mean ages between categories of workload, regularity of sleep or meals were found among females and that the observed associations with NK activity were not confounded by age.

Diseases High NK activity was observed among 141 males and 255 females with hypertension to be 52.6± 16.5% and 46.3±17.0% and also among those with history of hypertension to be 50.7±18.0% (n=68) and 44.8±15.9% (n=99), compared with the mean activity of 48.1±17.3% (n=875) and 42.0±17.0% (n=1454) among those without the disease. The increase in the mean activity among those with the disease showed statistical significances of $P<0.005$ for males and $P<0.001$ for females.

Among four male patients with gout, a very high mean activity of 63.3±9.3% was also observed ($P<0.005$).

Relative weight Relative weight used here means the ratio of observed individual weight to the standard one calculated for individual height, sex and age in the Japanese population.[17] It should be noted that the mean ages in relative weight categories of ≥1.20, 1.19–1.10 and 1.09–0.90 among females were within a narrow range of 52.3 to 53.4 years old, although the higher mean ages of 57.4 and 63.6 years were observed in the categories of relative weight 0.80–0.89 and ≤0.79. The mean activities in these two categories were adjusted in Fig. 5 to the mean age of 53.4 years old in the central category of 0.90–1.09, assuming the age-dependence of NK activity observed in females. The relative weight of 0.90–1.09

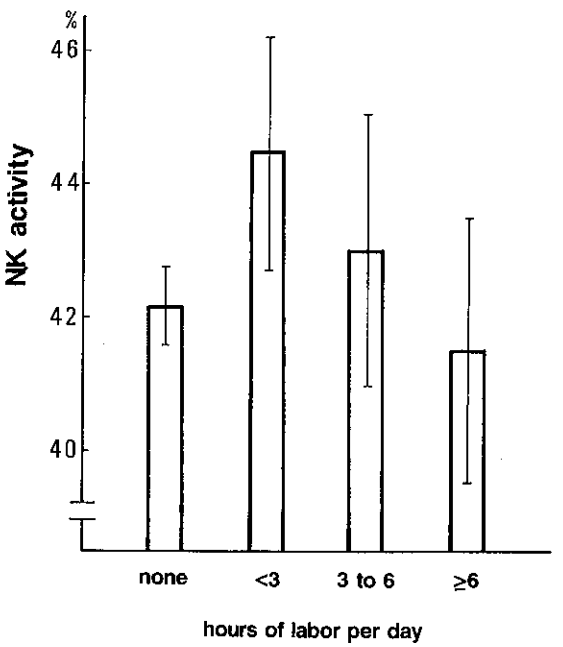

Fig. 4. NK activity (means, bars 95% CI) in relation to degree of daily work among females.

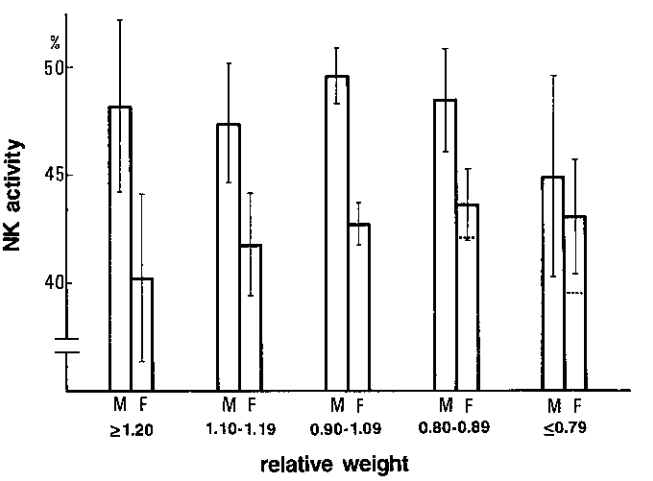

Fig. 5. NK activity (means, bars 95% CI) in relation to relative weight. Dotted lines are the mean activities adjusted for age assuming a linear age-dependence observed among females.

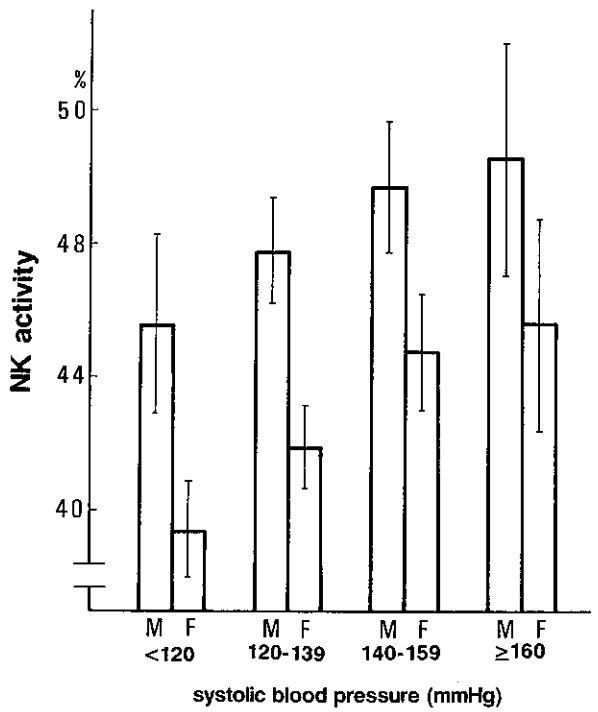

Fig. 6. NK activity (means, bars 95% CI) in relation to systolic blood pressure (M: males, F: females). Users of hypotensive drugs were excluded. Correlation coefficients were 0.11 and 0.12 among males and females, respectively.

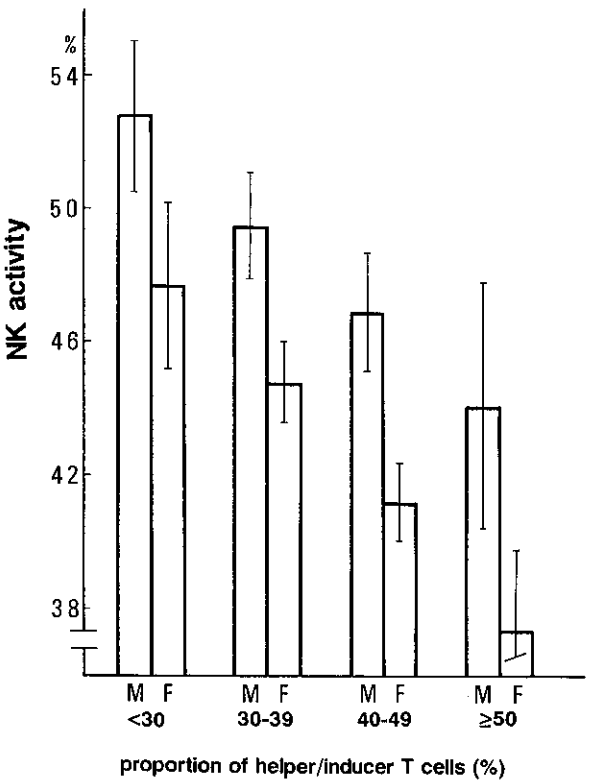

Fig. 7. NK activity (means, bars 95% CI) in relation to proportion of helper/inducer T lymphocytes.

(near to the standard weights) thus showed the highest mean activity among both males and females ($P=0.08$ for difference of the mean values in males, and no significance test was applied for the age-adjusted means of females), and the relation had a concave-downwards shape.

Blood pressure The observed association of NK activity with hypertension suggested that blood pressure may be an influencing factor, although the influence of hypotensive drugs used was uncertain. A clear correlation can be seen in Fig. 6 between the activity and systolic blood pressure, where users of hypotensive drugs were excluded from the subjects. Correlation coefficients were 0.11 and 0.12 among males and females, respectively, with a significance level of $P<0.001$ by the t test. Since systolic blood pressure significantly increased with age, we examined the correlation with NK activity in each of the age groups among females. A similar relationship was observed with correlation coefficients ranging between 0.09 and 0.22. A similar correlation was also observed among users of hypotensive drugs. On the other hand, less or no correlation was observed between the activity and diastolic blood pressure: i.e., $r=0.02$ and 0.07 among males and females.

Subsets of T cells Human T cells can be classified into two major subpopulations using the monoclonal antibodies OKT4 (equivalent to CD4) and OKT8 (CD8) specific for surface antigens on T cell membranes: helper/inducer ($OKT\,4^+$) and cytotoxic/suppressor ($OKT8^+$) T lymphocyte subsets. The proportion of $OKT4^+$ cells showed a negative correlation with NK activity (Fig. 7), with $r=-0.14$ and -0.18 among males and females ($P<0.0001$). On the other hand, the proportion of $OKT8^+$ cells did not show any significant correlation: $r=0.05$ and -0.02 among males and females. Accordingly, the ratio of $OKT4^+/OKT8^+$ also correlated with the activity: $r=-0.12$ and -0.09 among males and females ($P<0.001$). It should be noted that the proportion of $OKT4^+$ cells also showed a correlation with systolic blood pressure: $r=-0.14$ and -0.15 among males and females ($P<0.001$).

Serum proteins Serum proteins were separated by electrophoresis and classified as α_1, α_2, β and γ fractions of globulin. Among them, only β-globulin was associated with NK activity, and the correlation coefficients between proportion of β-globulin in total globulin and NK activity were calculated to be $r=-0.14$ and -0.10 among males and females ($P<0.001$). Proportion of

β-globulin had no association with proportion of $OKT4^+$ cells and a little association with systolic blood pressure (only females showed a correlation of $r=0.07$ with $P<0.01$).

DISCUSSION

Etiological associations of biologic markers with human cancer incidence have been investigated mainly by means of case-control comparison. However, the comparison of phenotypic expressions such as blood components, metabolic activities and immunological responses involves an inevitable and serious problem in interpretation of the results: i.e., the observation on patients may involve the effect of cancer, and even hospital controls with benign diseases used in some studies have the same problem. Accordingly, prospective cohort studies will be required, although repeated observation of biologic markers in the surviving cohort population may be necessary. Our cohort study was designed for the purpose of examining the etiological association of several biologic markers with cancer.

An investigation as to whether biologic markers are possibly involved in cancer etiology or not first requires examination of the association of expressed levels of markers with life style factors known to enhance or reduce risk of cancers. We examined this association of NK activity with many life style factors in terms of a cross-sectional analysis. Factors associated with increased NK activity were found to be: 1) alcohol drinking, 2) not smoking cigarettes, 3) increased intake of green vegetables, 4) increased intake of meat, soyabean, milk, and dairy products, 5) daily and moderate work, 6) regular meals, 7) regular sleep, 8) maintenance of proper body weight (avoiding of over- or under-weight). However, items 3, 5, 6, and 7 showed statistical significance only among females, and an increase of cohort population will be required to examine this further.

It is of much interest that these factors are very similar to the seven well-known good health practices, except alcohol drinking (never smoking cigarettes, regular physical activity, moderate or no use of alcohol, 7–8 h sleep regularly, maintaining proper weight, eating breakfast, and not eating between meals), which were shown to reduce the mortality from major causes of deaths including cancer.[18] Out of seven practices, three (never smoking cigarettes, engaging in regular physical activity, and getting proper sleep) were recently demonstrated to reduce the mortality from all cancers down to about a half of the standard mortality among U.S. whites.[19] Moreover, physical activity and physical fitness were separately shown to reduce risk of cancers.[20, 21] Since these practices were not associated with exposures to carcinogenic factors except cigarette smoking, it has been speculated that they may be associated with some immunologic defense mechanisms against cancer progression, although no evidence has been available. Our cross-sectional study has provided conclusive evidence that good health practices are associated with enhanced NK activity. Having provided this, the evaluation of cancer incidence is a central theme of our cohort study. In addition, the effect of green vegetables, but not yellow vegetables, on NK activity suggests that the observed reduction of cancer risk by green & yellow vegetables may involve immunologic mechanisms besides the effect of β-carotene as an antioxidant, and cigarette smoking not only supplies procarcinogens in the body but also impairs NK activity.

NK activity may be one of main factors involved in primary defense against cancer, and we have to take into account other host factors in different stages of carcinogenesis so as to obtain a generalized view of cancer etiology. For example, NK activity is not a factor that can explain sex-difference or age-dependence of cancer incidence. In this regard, other factors such as accumulated damage to DNA with age, age-dependent DNA repair and physiological differences between the sexes must be considered as well as different exposure levels to carcinogens between sexes.

We also showed the significant association of NK activity with systolic blood pressure, proportion of helper/inducer T cells and β-globulin fraction among physiological factors examined. Among them, only helper/inducer T cells are documented to participate in control of NK activity, since proliferation of NK cells is known to be under T cell control.[22] The observed negative correlation between NK activity and proportion of helper/inducer T cells may be consistent with the increased NK activity and decreased proportion of $OKT4^+$ cells observed as an immediate effect of short-term exercise.[11] Additionally, in our cohort population, the proportion of helper/inducer T cells was increased by cigarette smoking ($P<0.001$) and decreased by frequent intake of green vegetables ($P<0.05$), moderate workload ($P<0.01$) and regular meals ($P<0.01$) (data not shown). These factors influenced the distribution of T cell subsets and enhanced NK activity, suggesting that the mechanism of NK activity control involves in part changes in T cell subsets. Involvement of serum factors, which are known to enhance (interleukin 2 and interferon) or inhibit (catecholamines, corticosteroids) NK activity, was not examined in this study. As for the systolic blood pressure and β-globulin fraction, whether they are controlling factors or confounding ones is unknown, and a further investigation on immunologic or biologic aspects may be required.

The present study has not only established baseline characteristics of our cohort population as regards NK

activity, but also revealed for the first time various exogenous and endogenous factors influencing NK activity among a large sample of the general population, providing clear evidence that some life style factors or behaviors reducing cancer risk are associated with enhanced NK activity. Given the potential implications of our findings, a follow-up study is warranted to assess the association of NK activity with cancer incidence.

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