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Cellular Immune Competence of Patients with Lung Cancer and Other Lung Diseases I. Analysis of peripheral blood lymphocyte subsets using monoclonal antibodies

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ABSTRACT

Lymphocyte subsets and lymphocyte responsiveness to mitogens were examined for patients with lung disease and healthy people. The results are as follows:

1) Lymphocytes derived from healthy persons showed an age dependent increase in the percentage of Leu-7$^+$ cells and a decrease in reactivity to phytohemagglutinin (PHA). The percentage of Leu-1$^+$ and Leu-3a$^+$ cells was higher in females than in males under 30 years of age, while the percentage of Leu-7$^+$ cells was higher in males at all ages.

2) Untreated lung cancer patients showed decreases in the percentage of Leu-1$^+$ cells and reactivity to PHA and concanavalin A (Con A), and an increase in the percentage of Leu-11a$^+$ cells.

3) No difference in lymphocyte subsets nor in reactivity to mitogens was observed between patients of different clinical stages. However, when the lymphocyte subsets were compared between patients at each stage and the healthy group, decreased percentages of Leu-1$^+$ and Leu-3a$^+$ in patients with stage IV cancer and Leu-2a$^+$ in those with stage I were observed, while the percentage of Leu-11a$^+$ cells was high in stage I and III patients.

4) No difference in lymphocyte subsets in relation to histological type was observed.

5) Among noncancerous respiratory disease patients, there was a reduced percentage of Leu-1$^+$ cells in sarcoidosis and pulmonary emphysema, and reduced percentages of Leu-1$^+$ and Leu-3a$^+$ cells in pulmonary fibrosis and diffuse panbronchiolitis.

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Lymphocytes, which are the major components of the immune system, consist of several subsets having different functions and surface antigens. It is known that immunological response is produced and maintained by these subsets and the interaction of these and other cells. Therefore, an analysis of lymphocyte subsets may be important not only in basic studies, but also in diagnosis of various diseases which result in immunological abnormality, and also in ascertainment of the profile and prognosis of such diseases.

The identification of human lymphocyte membrane antigens had previously been conducted by rosetting techniques and assays detecting cell surface immunoglobulins, complement receptors, and Fc receptors. However, all of these methods have problems in specificity and reproducibility.

In 1975, the hybridoma technique was developed by Kohler and Milstein[8]. Antigen-specific monoclonal antibodies produced by this hybridization technique brought a complete solution to the above problems and led to remarkable progress in investigations in this field.

In the present study, the change in lymphocyte subsets in lung cancer and other respiratory diseases and the profile of such diseases was studied by using monoclonal antibodies. Further, the *in vitro* blastogenic response of lymphocytes to mitogens which is generally regarded as a marker of cellular immune competence was made.

MATERIAL AND METHODS

Blood samples were obtained from 79 subjects with untreated lung cancer, 68 subjects with benign respiratory diseases, and six subjects with collagen diseases. All of these individuals were inpatients of either Hiroshima University Attached Hospital, Hiroshima Citizens Hospital, Hiroshima Red Cross Hospital, Hiroshima Prefectural Hospital, or Yoshijima Hospital. In addition, 128 healthy RERF staff members were included in these studies. Since the patients had to be closely matched to their normal controls for age and sex, the analysis of the lymphocyte subsets within subject groups ultimately was based on 51 lung cancer patients with a mean age of 54.0 ± 7.4 years, 51 normal controls with a mean age of 51.8 ± 9.5 years, 68 noncancerous respiratory disease patients with a mean age of 53.8 ± 15.2 years and 6 collagen disease patients. Because the tests failed for some cases, the analysis of reactivity to mitogen was based on 36 lung cancer patients, 52 normal controls, and 39 noncancerous respiratory disease patients. The investigation on control relating to reactivity to mitogen indicates that the investigation based on the control of lymphocyte subset does not always include the same person. These omitted cases were scattered throughout the age and sex categories apparently at random. As sarcoidosis patients are known to show altered frequency of lymphocyte subsets[29], they were analyzed as an independent disease and excluded from the benign disease group.

Method of Analysis

The relationship between age, sex, and various immunological parameters was studied using correlation coefficients in 126 normal controls.

For comparison, the patients and their age and sex matched normal controls were analyzed using Student's T test. The differences were considered individually and were judged to be significant when the final p-value was 0.05 or less. However to take into account the number of comparisons made, a difference should be considered statistically significant when the final p-value is p/n is 0.05 or less ($p=0.05$, $n=$the number of tests for that table).

Methods

Isolation of peripheral blood lymphocytes

From each subject, 20 ml of venous blood was collected, defibrinated by glass beads, and mixed with the same amount of phosphate buffer saline (PBS) in 50 ml centrifuge tubes (Corning Co). The specimen was underlaid with 12 ml Ficoll-Hypaque (specific gravity: 1.077) and centrifuged at 400 × *g* for 30 min at room temperature. The lymphocyte layer was collected, suspended in PBS, and centrifuged for 10 min at 510 × *g* to harvest mononuclear cells. Then 10-min centrifugation at 240 × *g* was performed twice for cell washing. The mononuclear cells were than resuspended for use.

Analysis of lymphocyte subsets using an indirect immunofluorescence antibody assay

The monoclonal antibodies employed were anti-Leu-1, anti-Leu-2a, anti-Leu-3a, anti-HLA-DR,

anti-Leu-7, and anti-Leu-11a (Becton Dickinson Co). Anti-Leu-1 is an antibody reactive to most T lymphocytes; anti-Leu-2a to cytotoxic/suppressor T lymphocytes; anti-Leu-3a to helper/inducer T lymphocytes; anti-HLA-DR to B lymphocytes and monocytes; and anti-Leu-7 and anti-Leu-11a to natural killer (NK) cells.

Lymphocytes were suspended in PBS at a concentration of 5×10^6 cells/ml and the suspension was divided into small test tubes in aliquots of 0.1 ml. Fifty microliters of monoclonal antibody solution, diluted 1/10, was added to each tube. The tubes were kept at 4°C for 20 min, centrifuged with PBS, and washed twice. After addition of 10 μl of antimouse IgG-fluorescein isothiocynate (FITC, 1:10; Tago Co), the solution was allowed to react at 4°C for 15 min, centrifuged and washed twice, and fixed with 1 ml of 1% paraformaldehyde. More than 200 lymphocytes were counted with a fluorescence microscope, and the percentage of membrane fluorescence positive cells was calculated. The counting was conducted by one observer with no knowledge of the disease, age, or sex of the subject sample.

Lymphocyte blastogenesis in culture with PHA-P, PWM, and Con A.

Lymphocytes suspended in minimum essential medium containing 10% fetal bovine serum (FBS) were dispensed into the wells of microtest plate-II (Falcon Co) so that each well would contain 0.625×10^5 cells. Mitogens PHA-P (Difco Co), concanavalin A (Con A, Sigma Co), and pokeweed mitogen (PWM, Gibco Co) were then added at a final concentration of 10 μl/ml, 20 μg/ml, and 5 μl/ml, respectively, and the cells were cultured in 5% CO_2 at 37°C for three days. Eighteen hr before completion of these cultures, 0.5 μCi of ^{3}H-thymidine (^{3}H-TdR, NEN, 5 Ci/mmol) was added. The acid insoluble fraction of the cells was harvested on glassfiber filters with a micro-cell-harvester, and radioactivity was determined with a liquid scintillation counter.

RESULTS

1) Relationship of age, reactivity to mitogens, and the percentages of lymphocyte subsets in healthy persons

The relationship between age, reactivity to mitogens, and the percentages of lymphocyte subsets was examined for healthy persons (n = 126). As indicated by the correlation coefficients in Table 1, healthy persons showed an age-dependent increase in the percentage of Leu-7$^+$ cells (NK cells, $p<0.05$) and a decrease in reactivity to PHA ($p<0.05$).

Table 1. Correlation coefficients between lymphocyte subsets and reactivity to mitogen in healthy persons (n = 126)

Subset	Age	PHA	Con A	PWM
Age	—	−0.220*	−0.080	−0.135
Leu-1	−0.009	−0.156	−0.161	0.002
Leu-2a	−0.143	0.039	−0.040	−0.041
Leu-3a	0.074	−0.134	−0.158	−0.043
Leu-3a/Leu-2a	0.151	−0.016	0.023	0.007
HLA-DR	−0.032	0.024	0.001	0.144
Leu-7	0.249*	−0.110	−0.073	−0.200

Student's t Test; p-values are two-sided and considered for each coefficient individually. * $p< 0.05$

2) Sex differences in the lymphocyte subset percentages and reactivity to mitogens in healthy persons

Healthy persons were divided into three age-groups, under 30, 30—49, and 50 or more. Sex differences in the percentage of each lymphocyte subset and reactivity to mitogens were analyzed for each age-group.

As indicated in Fig. 1-1, in the under 30 age-group, the percentages of Leu-1$^+$ ($p<0.05$) and Leu-3a$^+$ ($p<0.05$) cells were higher in females than in males. However, the T cell subsets showed no sex differences for age-groups 30 or more except for the percentage of Leu-7$^+$ cells in the 30—49 age-group in which males showed a higher percentage than females ($p<0.01$). When all the different age-groups were pooled, males showed a higher Leu-7$^+$ percentage than females ($p<0.01$).

With regard to reactivity to mitogens, no sex differences were observed for the response to PHA and Con A, but the response to PWM in the 30—49 age-group was higher ($p<0.05$) in females than in males (Fig. 1—2).

3) Analysis of the lymphocyte subset percentages in disease

The lymphocyte subset percentages for subjects with lung cancer, noncancerous respiratory diseases, sarcoidosis, and collagen diseases were compared with those of healthy individuals.

A) Lymphocyte subsets in lung cancer patients

In the untreated lung cancer group, as shown

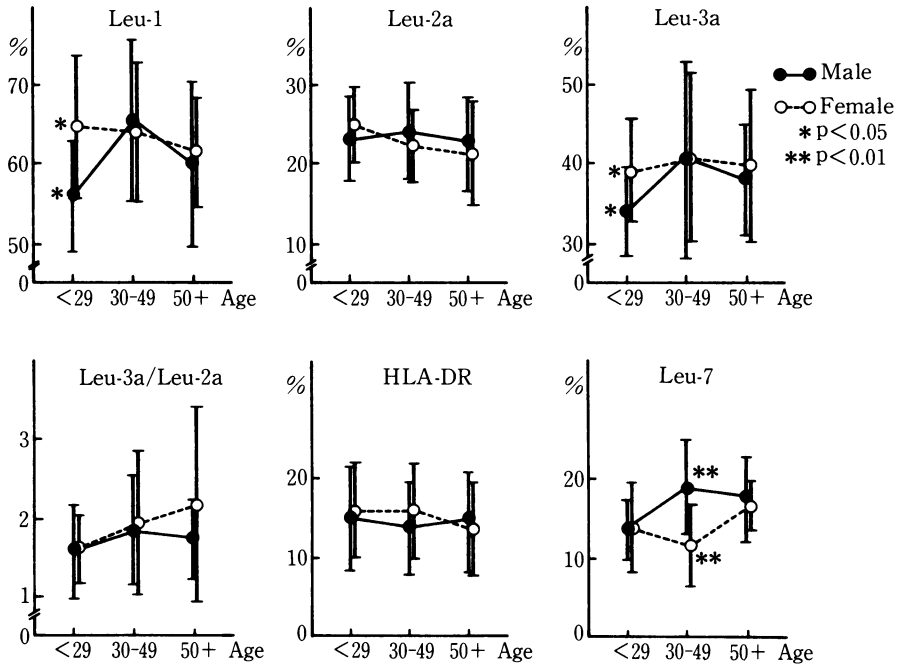

Fig. 1—1. Lymphocyte subsets determined by monoclonal antibodies in healthy individuals by age and sex

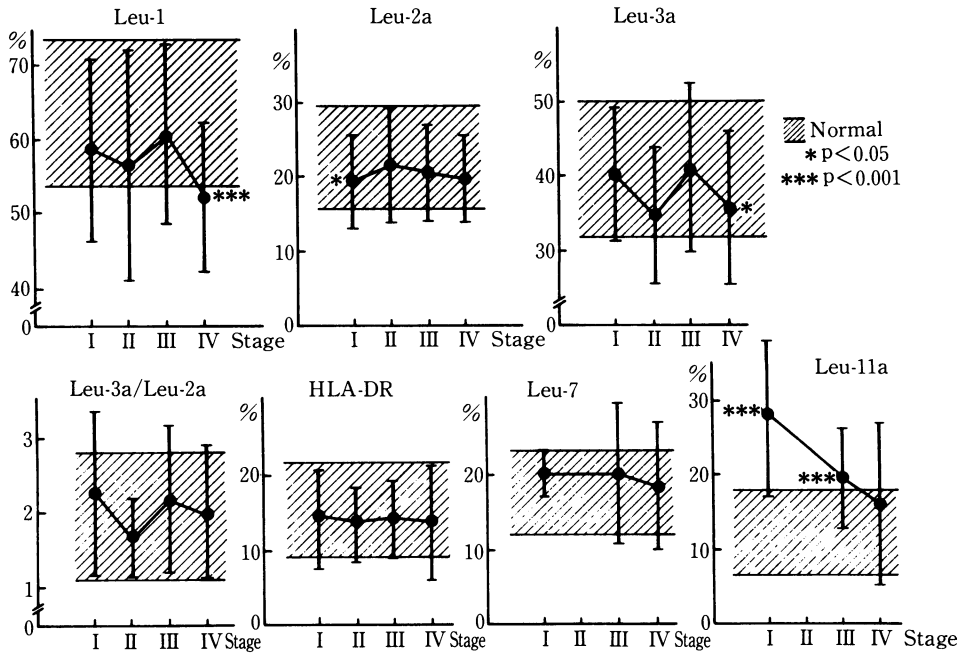

Fig. 1—2. Mitogen response by age and sex

Table 2. Lymphocyte subsets by disease

Subset	Normal (n=51)	Untreated Lung Cancer (n=51)	Noncancerous Respiratory Disease (n=68)	Sarcoidosis (n=6)	Collagen Disease (n=6)
Leu-1	63.5 ± 9.8	57.3 ± 13.5**	58.0 ± 10.5**	49.3 ± 5.9***	54.9 ± 8.9
Leu-2a	22.7 ± 6.8	20.6 ± 7.5	20.6 ± 6.7	20.1 ± 7.3	17.0 ± 10.2
Leu-3a	41.1 ± 9.0	38.2 ± 10.8	40.4 ± 11.7	33.7 ± 4.8	37.1 ± 3.6
Leu-3a/Leu-2a	1.96 ± 0.86	2.12 ± 0.89	2.35 ± 1.89	1.94 ± 0.98	2.76 ± 1.29*
HLA-DR	15.4 ± 6.2	16.2 ± 9.4	15.5 ± 5.8	14.2 ± 4.3	18.4 ± 8.7
Leu-7	17.7 ± 5.5 (n=35)	19.2 ± 9.0 (n=35)	17.0 ± 5.9 (n=35)	16.8 ± 9.2 (n=6)	15.0 ± 9.9
Leu-11a	12.3 ± 5.6 (n=23)	20.5 ± 8.6*** (n=23)	—	—	—

Numbers are mean percentage ± SD except Leu-3a/Leu-2a
Student's t Test; comparison made with normal group; p-values are two-sided and considered for each comparison individually.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$

in Table 2, the percentage of Leu-1$^+$ (total T-cells) was significantly lower ($p<0.01$) than that in healthy people while that of Leu-11a$^+$ cells was higher ($p<0.001$). No differences were observed for other markers. Next, a more detailed analysis was made of the lung cancer group. First, the lymphocyte subsets were analyzed by clinical stage. As indicated in Fig. 2, when the lymphocyte subset percentages and the ratio of Leu-3a/Leu-2a were compared by stage, no differences were observed at the respective stages. However, when the lymphocyte subset percentages of lung cancer patients of each stage were compared with those of healthy persons, the percentages of Leu-1$^+$ cells and Leu-3a$^+$ cells of patients with stage IV cancer showed a significant decrease ($p<0.001$, $p<0.05$). The decreased percentage of Leu-2a$^+$ cells was observed only in stage I lung cancer patients ($p<0.05$) while the Leu-11$^+$ cell percentage was significantly higher ($p<0.001$) in stage I and III patients.

Results of lymphocyte subsets according to histological type (squamous cell carcinoma, small cell carcinoma, adenocarcinoma) are given in Table 3. No differences were observed in the percentage of lymphocytes subsets or the ratio of Leu-3a/Leu-2a.

B) Noncancerous respiratory diseases

Respiratory disease patients as a group demonstrated lower percentages of Leu-1$^+$ cells than that of healthy persons ($p<0.01$, Table 2). Further analyses for each disease (Table 4) revealed a decrease of Leu-1$^+$ cell percentage in pulmonary emphysema ($p<0.01$); a decrease of Leu-1$^+$ cell and Leu-3a$^+$ cell percentages in pul-

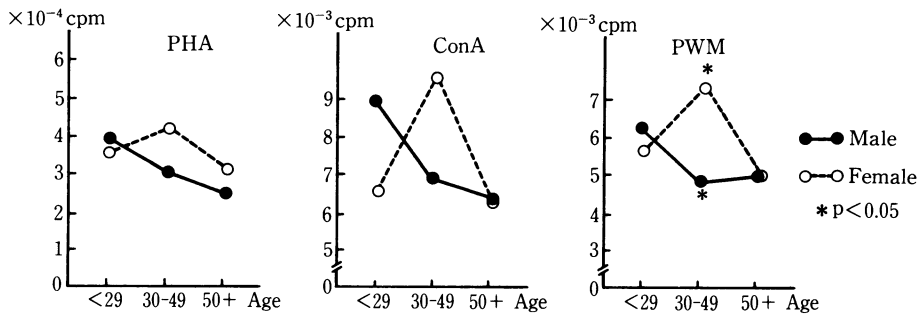

Fig. 2. Lymphocyte subsets in untreated lung cancer patients by clinical stage
Student's t-test; comparison made with normal group; p-values are two-sided and considered for each comparison individually

Table 3. Lymphocyte subsets in lung cancer patients by histological group

Subset	Normal (n=51)	Lung Cancer Patient		
		Squamous cell (n=23)	Small cell (n=6)	Adenocarcinoma (n=41)
Leu-1	63.5 ± 9.8	58.5 ± 14.0	60.5 ± 8.5	57.2 ± 12.0
Leu-2a	22.7 ± 6.8	20.4 ± 6.3	17.0 ± 7.1	20.5 ± 6.2
Leu-3a	41.1 ± 9.0	39.0 ± 9.9	45.8 ± 12.9	38.0 ± 10.3
Leu-3a/Leu-2a	1.96 ± 0.86	2.07 ± 0.75	2.68 ± 1.05	2.04 ± 0.87
HLA-DR	15.4 ± 6.2	12.4 ± 4.9	13.5 ± 3.3	14.8 ± 5.8
Leu-7	17.7 ± 5.5 (n=35)	22.4 ± 11.1 (n=9)	19.7 ± 9.2 (n=5)	19.8 ± 6.5 (n=21)

Numbers are mean percentage ± SD
No significant differences are present

Table 4. Lymphocyte subsets in patients with noncancerous respiratory disease, sarcoidosis, and collagen disease

Diagnosis	n	Subset Mean Percentage ± SD					
		Leu-1	eu-2a	Leu-3a	Leu-3a/Leu-2a	HLA-DR	Leu-7
Normal	51	63.5± 9.8	22.7± 6.8	41.1± 9.0	1.96±0.86	15.4±6.7	17.7± 5.5 (n=35)
Bacterial pneumonia	4	63.4±10.8	23.3± 7.4	41.3±10.2	1.90±0.69	15.8±2.6	– –
Allergic pneumonia	3	57.0± 6.6	21.6± 4.8	33.2± 6.2	1.60±0.58	13.9±6.9	– –
Chronic bronchitis	9	64.6± 8.0	22.0± 7.0	46.7±14.0	1.93±0.80	13.5±5.7	18.4± 1.9
Emphysema	5	50.6± 8.1**	19.2± 7.6	37.2± 7.1	2.10±0.71	19.1±6.2	15.2± 3.1
Bronchial asthma	14	58.2±11.3	21.5± 4.6	41.5±12.9	2.06±0.99	15.1±6.4	18.1± 7.9
Bronchiectasis	4	59.9± 4.5	26.7± 7.4	41.6±13.6	1.73±1.00	19.6±5.0	20.7± 2.1
Pneumoconiosis	5	55.4±13.7	19.0± 3.4	35.8±13.3	1.91±0.67	15.6±4.6	19.0±10.4
Pulmonary fibrosis	4	43.8± 8.6***	20.3±16.2	26.4± 6.3**	1.81±1.42	14.5±7.9	19.5± 3.5
Pulmonary tuberculosis	8	61.4±10.2	20.0± 8.6	41.7±12.4	2.43±1.13	13.1±7.0	16.7± 3.9
Diffuse panbronchitis	6	49.7± 9.6**	18.4± 8.0	30.7± 8.3*	1.57±0.42	15.1±3.7	12.8± 5.0
Sarcoidosis	6	49.3± 5.9**	20.1± 7.3	33.7± 4.8	1.94±0.98	14.2±4.3	16.8± 9.2
Collagen disease	6	54.7± 8.9	17.0±10.3	37.1± 3.6	2.76±1.29	18.4±8.7	15.0± 9.9

Student's t Test; see Table 2

Table 5. Mitogen response by disease

	Normal (n=52)	Untreated Lung Cancer (n=36)	Noncancerous Respiratory Disease (n=39)
PHA (cpm)	40,250 ± 25,650	30,105 ± 14,533*	37,931 ± 16,662
Con A (cpm)	10,020 ± 8,360	6,406 ± 8,028*	9,324 ± 7,662
PWM (cpm)	4,730 ± 2,940	4,714 ± 5,381	4,680 ± 3,920

Student's t Test; see Table 2

Table 6. Mitogen response in lung cancer patients by clinical stage

	Normal (n=52)	Clinical Stage of Lung Cancer Patient			
		I (n=13)	II (n=4)	III (n=8)	IV (n=9)
PHA (cpm)	40,250 ± 25,650	36,580 ± 19,530	26,510 ± 7,060	25,925 ± 4,051	24,288 ± 9,031
Con A (cpm)	10,020 ± 8,360	7,510 ± 1,037	3,560 ± 2,790	3,533 ± 2,578*	4,712 ± 2,833
PWM (cpm)	4,730 ± 2,940	7,600 ± 8,050	3,490 ± 2,380	4,771 ± 2,372	2,650 ± 2,148

Student's t Test; see Table 2

monary fibrosis ($p<0.001$, $p<0.01$) and in diffuse panbronchiolitis ($p<0.01$, $p<0.05$).

The lymphocyte subset percentages did not differ from those of healthy persons for bacterial and allergic pneumonia, chronic bronchitis, bronchial asthma, bronchiectasis, and pulmonary tuberculosis.

For sarcoidosis, the percentage of Leu-1$^+$ cells was decreased ($p<0.01$), but no difference was observed in other T cell subsets. No differences were observed for the subset percentage in collagen disease patients (three cases each of systemic lupus erythematosus and systemic sclerosis).

4) Reactivity of patient lymphocytes to mitogens

Untreated lung cancer patients showed decreased reactivities to PHA and Con A compared with healthy persons ($p<0.05$, $p<0.05$). Noncancerous respiratory diseases patients did not show altered reactivities to the mitogens (Table 5).

When responses to mitogen were analyzed according to the clinical stage of lung cancer (Table 6), the responses to all mitogens showed a tendency to decrease with advancing clinical stage, but only reactivity to Con A showed a decrease in stage III patients ($p<0.05$).

DISCUSSION

Effect of age in healthy people

It is well known that aging is one of the factors involved in the alteration of immune response. Therefore, healthy persons were first examined for the effects of aging on reactivity to mitogens and the percentages of lymphocyte subsets.

As for changes in reactivity to mitogens due to aging, many reports state that no fixed trend is observed in reactivity to PWM, but that reactivity to PHA and Con A decreased[6,30]. A decrease of reactivity to PHA with aging was observed in this study. The possible causes for the decrease of reactivity to mitogens with aging are: a decrease of reactive cells; disorders of receptors and metabolism in cells; disorders in intercellular interaction; and a decrease of thymic hormone, but the exact mechanism is unknown.

As for changes of lymphocyte subsets due to aging, the percentage of Leu-7$^+$ cells increased with age has been reported by Abo et al[1]. Leu-7$^+$ cells are granulocytic lymphocytes which have the morphological characteristics of NK cells. Some reports indicate that NK cell activity decreases with aging[25] or that there is no definitive trend[19,26]. Therefore, the present finding that the percentage of Leu-7$^+$ cells increases with age suggests an increase in Leu-7$^+$ cells with decreased NK cell activity may occur with aging.

Although Nagel et al[16,17] reported an age-dependent decrease in the percentages of Leu-4$^+$, OKT-3$^+$, Leu-2a$^+$, and OKT-8$^+$ cells, Mascart-Lemone et al[10] found that the percentage of OKT-8$^+$ cells increased with aging. Hallgren et al[7] reported that OKT-3$^+$ cells (similar to Leu-1$^+$) and OKT-8$^+$ cells (similar to Leu-2a$^+$) are not affected by aging, and the present study confirmed their results.

Also, there are conflicting reports on the relationship between B cells and aging. Matsumoto et al[11] observed an increasing trend of OKla-1$^+$ cells (B cells and monocytes) with aging, but Yamakido et al[32] and the present study did not find differences between age groups. The disagreements among investigators may be attributable to the paucity of study subjects and differences in the methods of detection.

Effect of sex in healthy people

As for differences in lymphocyte subsets by sex, there are many reports that females show a higher rate of OKT-4$^+$ cells (similar to Leu-3a$^+$) and a lower rate of OKT-8$^+$ cells[10,11,17]. In the present study, the percentages of Leu-3a$^+$ cells were higher only in females aged under 30. This finding may be associated with the higher frequency of development of certain types of autoimmune diseases, such as systemic lupus erythematosus in young females.

As with Abo et al[1], a higher percentage of Leu-7$^+$ cells was observed in males. It has been reported that estrogen inhibits the maturation of NK cells[23], which may explain this sex difference. Also, a significantly higher percentage of NK cells in females aged 50 or more as compared with that of those under 50 may be explained by the possible activity of sex hormones on NK cell activity *in vivo*.

Lymphocyte subsets in lung cancer patients

It has been reported that the OKT 4/OKT 8

ratio is decreased in cancer patients[5]. However, no significant difference in the Leu-3a/Leu-2a ratio was observed in the present study. When the lymphocyte subsets at each stage were compared with those of healthy persons, the percentages of Leu-1$^+$ cells and Leu-3a$^+$ cells were decreased in patients with stage IV cancer. Anti-Leu-3a antibody recognizes helper and inducer T cells. Helper T cells are considered to play a facilitative role in inhibiting tumor cell growth *in vivo*. Therefore, the decrease of the percentage of Leu-3a$^+$ cells may be related to immunologic dysfunction in advanced cancer.

It is reported that although the NK cell activity of cancer patients is barely decreased with localized tumors[22], it tends to decrease with cancer progression[3,27]. However, there is a report[18] that no difference was observed in the percentage of Leu-7$^+$ cells between healthy persons and lung cancer patients as observed in the present study. Steinhauer et al[24] postulated that reduced NK cell activity in terminal stage cancer patients is not due to a decrease of the absolute number of NK cells, but to the paucity of cells demonstrating cytotoxicity.

Leu-11a antibody[21] has recently been developed and it is reported to react with cells with stronger NK cell activity than the cells identified with Leu-7 antibody. That is, Leu-11a$^-$/Leu-7$^+$ cells show weak NK cell activity while Leu-11a$^+$/Leu-7$^-$ cells show strong NK cell activity[9]. In agreement with the results of Nishikawa et al[18], the present study showed a higher percentage of Leu-11a$^+$ cells in lung cancer patients than in healthy persons. Although no cancer stage differences were observed in the percentage, it was slightly higher in patients of stages I and III. This may indicate an increase of Leu-11a$^+$ cells *in vivo* as a reactive defense mechanism before the lung cancer cells metastasizes.

Lymphocyte subset in noncancerous disease

There are few reports on the analysis of lymphocyte subsets in pulmonary emphysema, pulmonary fibrosis, and diffuse panbronchiolitis. A decrease in the percent of T cells was observed in all three diseases. The cause for the decrease could be a temporary immune deficiency in as much as these subjects were all inpatients and generally showed exacerbated symptoms.

It is said that sarcoidosis cases demonstrable systemic deterioration of cellular immunity, such as a decrease of total T cells, negative tuberculin response, and a decrease of reactivity of lymphocytes against mitogens[2]. We observed a decrease of the percentage of total T cells but no difference in T cell subset percentages, although others[12] have reported a decrease of OKT-8$^+$ cells. This discrepancy might be due to the degree of activity of sarcoidosis in our patients because active sarcoidosis shows changes of lymphocyte subsets, while inactive sarcoidosis shows no change[4].

Lymphocytes of patients with collagen diseases are generally known to show a decrease of OKT-8$^+$ cells and an associated increase of the OKT 4/OKT 8 ratio[15,28]. No changes of the percentage of Leu-2a$^+$ cells and the Leu-3a/Leu-2a ratio were observed in this study. This disagreement may be also attributable to the degree of activity of disease or differences in the method of detection.

Reactivity to mitogens in lung cancer patients

Mitogens react with corresponding receptors on the membrane surface of lymphocytes and induce blastogenesis. The process is quite similar to that of the division and proliferation of lymphocytes after they recognize antigens. Therefore, reactivity to mitogens is often measured as an index of the functional activity of lymphocytes. It is known that PHA reacts with helper T cells, and Con A with helper T cells and suppressor T cells[14]. PWM is believed to stimulate both T and B cells[31]. Many reports indicate a decreased reactivity of lymphocytes to PHA in cancer patients[13,20] which was also observed here.

As yet there is no one specific parameter that reflects all aspects of immune competence. Thus, studies using many parameters should be performed for the elucidation and development of a comprehensive clinically meaningful immune profile.

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