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Cellular Immunity of Patients with Lung Cancer and Other Lung Diseases II. Analysis of interleukin-2 production

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ABSTRACT

Interleukin-2 (IL-2) production by stimulated peripheral blood lymphocytes (PBL) from patients with lung cancer and noncancerous respiratory diseases was determined. The results are as follows:

1) Neither sex nor age difference was observed for IL-2 production among healthy people.

2) IL-2 production showed a positive correlation with the Leu-3a/Leu-2a ratio and a negative correlation with the percentage of HLA-DR$^+$ cells.

3) IL-2 production of patients with lung cancer and noncancerous respiratory diseases did not differ from that of healthy persons.

4) No difference in IL-2 production was found in relation to the clinical stage of lung cancer, but subjects with low IL-2 production were mostly observed in the advanced stage group (Stage IV).

IL-2 is produced by helper T cells[12] and induces responder T cells to proliferate and differentiate into effector T cells with killer or helper activity[14]. Recently, IL-2 has been employed in antitumor immunotherapy, in which killer T cells produced and expanded in *in vitro* with IL-2[2] are injected.

However, measurement of IL-2 production in various diseases has just started, and few results have yet been obtained. Thus, we measured the IL-2 production of peripheral blood lymphocytes (PBL) from lung cancer patients and evaluated and analysed the clinical relationship of the results.

MATERIALS AND METHODS

Sample

The blood samples were obtained from 55 subjects with untreated lung cancer, 53 subjects with benign respiratory diseases and six subjects

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with sarcoidosis. 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, 109 healthy RERF staff members were included in these studies.

Methods

After peripheral blood lymphocytes (PBL) were isolated, reactivity to mitogens [phytohemagglutinin (PHA), pokeweed mitogen (PWM), and concanavalin A (ConA)], percentages of lymphocyte subsets using monoclonal antibodies (anti-Leu-1, anti-Leu-2a, anti-Leu-3a, anti-HLA-DR, anti-Leu-11a, and anti-Leu-7), and IL-2 production were measured. Methods of measurement other than that of IL-2 production were described[3].

Measurement of IL-2 production

One million lymphocytes from subjects were suspended in 1 ml of test medium consisting of RPMI-1640 with 4 mM HEPES (hydroxyethylpiperazine N'-ethane sulfonic acid), 1% penicillin/streptomycin, 1% l-glutamine, 1% PHA-M (DIFCO Co.), and 2% heat-inactivated fresh human pooled AB serum. After incubation for 24 hours at 37°C in a 5% CO_2 incubator, the supernatant was harvested and stored at -80°C until assay. Culture supernatant was serially diluted twofold in Click's medium and added to wells of microplates in aliquots of 0.1 ml each. To each well, the IL-2-dependent mouse cytotoxic T cell line (CTLL-2) cells were added at the rate of 4×10^3 cells/well and cultured at 37°C in a 5% CO2 incubator for 24 hr. Four hours before harvesting, 0.4 μCi of ^{3}H-thymidine (^{3}H-TdR) was added. Cultures were harvested onto glass fiber filter strips, and ^{3}H-TdR incorporation was determined.

Units of IL-2 production were determined by probit analysis[4]. ^{3}H-TdR incorporation data were plotted against log2 dilutions of each individual IL-2 sample. The control rat IL-2 profile that intercepted the 50% activity was arbitrarily assigned a value of 1 unit, and the IL-2 production of the test sample expressed as units of activity relative to the control rat IL-2 (Fig. 1).

Method of Analysis

After the patients and their normal controls were closely matched by age and sex for comparative analysis, the analysis was made based on 34 lung cancer patients with a mean age of 55.1±6.9 years, 42 normal controls with a mean age of 52.9±6.0 years, 53 noncancerous respiratory disease patients with a mean age of 53.0±16.5 years, and six sarcoidosis patients.

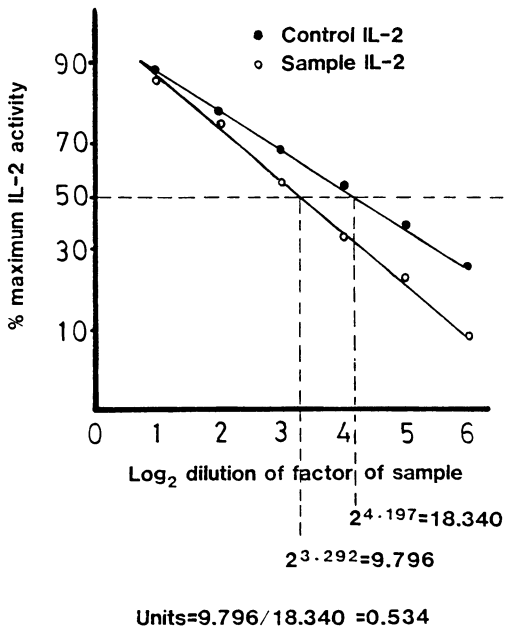

Fig. 1. Determination of units of IL-2 activity

The relationships of IL-2 production to age, sex, reactivity to mitogens, and the percentages of lymphocyte subsets were studied using correlation coefficient in 109 normal controls.

Student's T test were used for comparative analysis on the patients and their normal controls. The differences were considered individually and were judged to be significant when the 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 p-value is p/n or less ($p=0.05$, n = the number of tests for that table), and this was done.

RESULTS

1) Relationship of IL-2 production to age, reactivity to mitogens, and the percentages of lymphocyte subsets in healthy persons

As indicated in Table 1, IL-2 production showed a positive correlation with the Leu-3a-to-Leu-2a ratio ($p<0.05$) and a negative corre-

Table 1. Correlation coefficients between IL-2 production and several immunological parameters in healthy persons (n=109)

	Age dependent	Mitogen response			Lymphocyte subsets					
		PHA	Con A	PWM	Leu-1	Leu-2a	Leu-3a	Leu-3a/ Leu-2a	HLA-DR	Leu-7
IL-2 (units)	0.146	0.097	0.054	−0.037	−0.055	−0.142	0.080	0.213*	−0.193*	0.006

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

Table 2. Interleukin-2 production in healthy men and women

	Sex	Age			
		Total	<29	30 – 49	50+
IL-2 (units)	Men	1.00 ± 0.65 (n=63)	0.79 ± 0.53 (n=7)	1.00 ± 0.61 (n=26)	1.04 ± 0.71 (n=30)
	Women	0.91 ± 0.51 (n=46)	0.90 ± 0.45 (n=19)	0.87 ± 0.47 (n=16)	1.00 ± 0.69 (n=11)

Numbers are mean ± standard deviation units.
Student's t-test; comparison made between men and women; p-value are two-sided and considered for each coefficient individually. No significant differences were found.

Table 3. IL-2 production by disease

	n	IL-2 (unit)	
Normal	42	1.11 ± 0.71	
Lung cancer	34	1.31 ± 0.95	NS
Noncancerous respiratory disease	53	1.31 ± 0.84	NS
Sarcoidosis	6	0.74 ± 0.38	NS

Student t-test; comparison made between normal and disease; NS: no significant difference.

lation with the percentage of HLA-DR$^+$ cells ($p<0.05$). It showed no correlation with other parameters.

2) Sex differences in IL-2 production

Healthy persons were divided into three age-groups, under 30, 30—49, and 50 or over. No sex difference was observed for IL-2 production (Table 2).

3) IL-2 production in disease

Table 4. IL-2 production in lung cancer patients by clinical stage

	Normal	Clinical stage of lung cancer patients			
		I	II	III	IV
IL-2 (units)	1.11 ± 0.71 (n=42)	1.18 ± 0.86 (n=20) NS	1.38 ± 0.70 (n=6) NS	1.30 ± 0.90 (n=15) NS	0.97 ± 0.95 (n=14) NS

Numbers are mean ± S.D. units
Student t-test; see Table 3

Table 5. Cases with low IL-2 production (those with IL-2 levels less than 1SD below the mean of the normal cases)

Case	Clinical stage	TNM classification	Histology	IL-2 production (units)
1	IV	$T_3N_2M_1$	Small cell	0.022
2	IV	$T_3N_2M_2$	Squamous cell	0.145
3	IV	$T_3N_1M_1$	Adenocarcinoma	0.192
4	IV	$T_2N_1M_1$	Unknown	0.263
5	III	$T_2N_2M_0$	Adenocarcinoma	0.294
6	III	$T_3N_1M_0$	Squamous	0.369

Table 6. Cases with high IL-2 production (those with IL-2 levels greater than 1SD above the mean of the normal cases)

Case	Clinical stage	TNM Classification	Histology	IL-2 production (units)
1	III	$T_2N_2M_0$	Squamous cell	3.53
2	IV	$T_2N_2M_1$	Adenocarcinoma	3.42
3	Ia	$T_1N_0M_0$	Squamous cell	3.38
4	Ia	$T_4N_0M_0$	Adenocarcinoma	2.73
5	Ia	$T_1N_0M_0$	Adenocarcinoma	2.60
6	IV	$T_2N_1M_1$	Adenocarcinoma	2.49
7	III	$T_1N_2M_0$	Small cell	2.28
8	III	$T_3N_2M_0$	Squamous cell	2.22
9	II	$T_2N_1M_0$	Adenocarcinoma	2.14
10	II	$T_2N_1M_0$	Unknown	2.02
11	III	$T_1N_2M_0$	Adenocarcinoma	1.94
12	Ia	$T_1N_0M_0$	Small cell	1.92
13	II	$T_1N_2M_0$	Adenocarcinoma	1.86

Table 7. Comparison of lymphocyte subsets between the low IL-2 production group and the high IL-2 production group

	Lymphocyte subsets				
	Leu-1	Leu-2a	Leu-3a	Leu-3a/Leu-2a	HLA - DR
Normal (n=51)	63.5 ± 9.8	22.7 ± 6.8	41.4 ± 9.0	1.96 ± 0.86	15.4 ± 6.2
Lung cancer (Untreated)					
Total (n=51)	57.3 ± 13.5	20.6 ± 7.5	38.2 ± 10.8	2.12 ± 0.89	16.2 ± 9.4
Low IL-2 group (n=6)	57.3 ± 9.3	23.2 ± 5.9	34.2 ± 8.6*	1.57 ± 0.66*	20.1 ± 8.1*
High IL-2 group (n=12)	60.3 ± 13.9	19.4 ± 5.2	45.1 ± 8.1*	2.49 ± 0.84*	12.4 ± 3.9*

Numbers are mean ± SD percentage
Student t-test; comparison made between the low IL-2 production group and the high IL-2 production group;
* = $p<0.05$

IL-2 production of normal persons and patients with lung cancer, noncancerous respiratory diseases, and sarcoidosis was determined (Table 3).

No significant difference was observed in IL-2 production between the normal group and each disease group.

Neither did detailed analysis on the stage of lung cancer show any significant differences in IL-2 production (Table 4). However, the four out of six lung cancer subjects with IL-2 production less than 1 standard deviation lower than the mean of the normal group were in stage IV and the remaining two, in stage III (Table 5). On the other hand, four of the 13 lung cancer subjects with IL-2 production more than 1 standard deviation above the mean of the normal group were in stage I, three in stage II, four in stage III, and two in stage IV. No difference in the distribution was observed by stage (Table 6). Furthermore, the lymphocyte subset percentages were reviewed for six subjects with low IL-2 production and 12 subjects with high IL-2 production. As indicated in Table 7, the low IL-2 production group showed, compared with the high production group, a significantly lower percentage of Leu-3a$^+$ cells ($p<0.05$) and Leu-3a-to-Leu-2a ratio ($p<0.05$) and a higher percentage of HLA-DR$^+$ cells ($p<0.05$).

DISCUSSION

A number of reports have been published on the effect of aging on IL-2 production. Gillis et al[5] and Yamakido et al[13] reported that IL-2 production is impaired in aged people. However, we observed no relationship between age and IL-2 production. This disagreement is due to the age distribution. Gillis et al[5] examined a group of person 67—81 years of age and Yamakido et al[13], a group with a mean age of 68.9 ± 8.0 years, while we examined those under 61 years of age. Sex difference was not observed in IL-2 production, a finding which agrees with the result of Yamakido et al[13].

Autoimmune diseases such as systemic lupus erythematosus (SLE)[1,6] and a certain T cell leukemia[10] are well known to show abnormal IL-2 production. Paganelli et al[9] reported reduced IL-2 production in children with serious immunodeficiency. With regard to IL-2 activity of cancer patients, Nakayama et al[8] reported that IL-2 activity was much lower in most patients with cancer who had metastasis than in those without metastasis. Similarly, Rey et al[11] observed impaired IL-2 production in cancer patients. We did not observe any significant difference in IL-2 production between normal persons and lung cancer patients with or without metastasis. However, subjects with lower IL-2 production were predominantly observed in stage IV. These findings may indicate that the decrease of IL-2 production is related to the tumor progress in cancer patients.

Further, we observed that the percentage of Leu-3a$^+$ cells was lower in the lower IL-2 production group than in the higher production group. Reduced IL-2 presumably is due to the paucity of IL-2 producing subsets.

No difference was observed in IL-2 production by peripheral blood lymphocytes between sarcoidosis patients and normal persons in the present study. Hunninghake et al[7] have reported the presence of large amount of IL-2 production by lung T cells from patients with sarcoidosis and high intensity alveolitis, while detectable amounts of IL-2 were not found in supernatant of blood T cells.

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