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Immune Functions of Former Poison Gas Workers
II. Lymphocyte subsets and interleukin 2 production

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ABSTRACT

A depression has been observed in phytohemagglutinin (PHA) response of lymphocytes, one of the functions of T cells, in former workers of the Okunojima poison gas factory (Poison gas workers) having a high risk of carcinogenesis. The authors in an attempt to delineate the mechanism of this depression measured lymphocyte subsets and interleukin 2 (IL-2) production of lymphocytes in these poison gas workers and compared the results with those obtained from age matched normal controls.

1) In comparison with normal controls it was observed with regard to lymphocyte subsets that both percentage and absolute number of Leu-2a$^+$ cells in poison gas workers were significantly elevated and that both percentage and absolute number of Leu-7$^+$ cells and Leu-3a/Leu-2a ratio were significantly depressed.

2) In comparison with normal controls IL-2 production of lymphocytes in poison gas workers showed a slightly low level, but the difference was not significant.

3) A significant positive correlation was demonstrated between PHA response, IL-2 production, and Leu-3a/Leu-2a ratio in poison gas workers.

The former workers of the Okunojima poison gas factory (poison gas workers) is a high risk group of carcinogenesis, including respiratory tract neoplasms[18,23]. The authors with the aim of elucidating the relation between carcinogenesis and immune function have made determinations on various immunological parameters in these poison gas workers. As described in Part I of this report[28], an evident depression in phytohemagglutinin (PHA) response of lymphocytes considered to be one of the functions of T cells was observed in poison gas workers, but this depression was not considered to be attributable to serum factors. In this study, with the aim of further delineating the functions of T cells in poison gas workers, lymphocyte subsets and interleukin 2 (IL-2) production of lymphocytes were measured and the findings will be presented in this report.

SUBJECTS

The subjects of the present study are identical to those of Part I of this report[28]. They were all males and totalled 118 cases composed of 46 controls (68.4 ± 8.2 years of age) and 72 poison gas workers (68.3 ± 6.8 years of age). All the poison gas workers had chronic bronchitis and as shown in Table 1 were classified by type of work into group A, Group B, and Group C according to the frequency of contact with

Table 1. Type of work in the poison gas factory

Group	
A	Yperite (mustard gas) production Yperite and Lewisite production Lewisite production
B	Laboratory, Incineration, Repair Inspection, Guard
C	Akato (Sneezing gas) Tear gas and other gases Medical doctor, Clerical work

highly carcinogenic yperite gas (mustard gas)[18] and further classified into three groups by duration of work in the poison gas factory, that is, less than 2 years, 2 to 5 years, and more than 5 years.

METHODS

Preparation of peripheral blood lymphocytes

Lymphocytes were separated from heparinized peripheral blood by Ficoll-Hypaque density gradient centrifugation method[2].

Detection of lymphocyte subsets by monoclonal antibodies

As reported previously[26], detection of lymphocyte subsets was made by indirect immunofluorescence method. Used as the first antibody were monoclonal antibodies (Becton Dickinson Monoclonal Center, Inc.), that is, anti-Leu-1 as pan T cell marker, anti-Leu-2a as suppressor/cytotoxic T cell marker, anti-Leu-3a as helper/inducer T cell marker, anti-Leu-7 as natural killer (NK) cell marker, and anti-HLA-DR as marker for B cells, monocytes and activated T cells, and used as the second antibody excluding those of anti-Leu-7 were fluorescein isothiocyanate (FITC)-labelled affinity-purified goat anti-mouse IgG (Tago), and FITC-labelled IgG fraction of goat anti-mouse IgM (Cappel) as those of anti-Leu-7. Furthermore, the absolute number of Leu^+ cells was obtained by multiplying the fluorescence positive rate with the number of peripheral blood lymphocytes.

IL-2 production and determination of IL-2 activity

The supernatant obtained after 24 hr culture of 1×10^6 cells/ml of lymphocytes stimulated with 1% PHA-M (Difco) was employed as IL-2 sample. IL-2 assay was conducted in accordance with the method of Gillis et al[10]. As described previously[27], briefly, CTLL-2 cells which are IL-2-dependent mouse cytotoxic T-cell line were cultured with 8-stage serial $\log_2$ dilution of IL-2 sample and the incorporation of ^{3}H-thymidine (^{3}H-TdR) was determined. IL-2 sample obtained from one individual was used as standard IL-2 (1 unit) and employing probit analysis, IL-2 activity was expressed as unit.

Detection of PHA response of lymphocytes

As described in Part I[28], with the use of 96 well microplate, lymphocytes at 1.25×10^5 cells/well were cultured for 96 hr stimulated with 1% PHA (HA-15, Wellcome) and after determination of incorporation of ^{3}H-TdR, PHA response was expressed as count per minute (cpm).

All the data were expressed as average value with standard deviation and statistical analysis of the data was made by Student's t-test.

In results, normal control is abbreviated as NC and poison gas workers as PG.

RESULTS

Lymphocyte subsets (Fig. 1 and 2)

The percentage and ratio of Leu^+ cells are shown in Fig. 1. The percentage of $Leu\text{-}1^+$ cells was 60.6 ± 10.7% in NC and 59.5 ± 10.4% in PG with no difference demonstrable between the two groups. The percentage of $Leu\text{-}2a^+$ cells was 21.2 ± 7.8% in NC and 26.3 ± 7.7% in PG with the value in PG being significantly higher than that in NC ($p<0.001$). The percentage of $Leu\text{-}3a^+$ cells was 39.9 ± 8.7% in NC and 36.7 ± 10.2% in PG with the value in PG being slightly lower than that in NC, but the difference was not significant. The percentage of $Leu\text{-}7^+$ cells was 35.8 ± 9.2% in NC and 27.5 ± 7.9% in PG with the value in PG being significantly lower than that in NC ($p<0.001$). The percentage of $HLA\text{-}DR^+$ cells was 26.4 ± 10.0% in NC and 25.1 ± 8.5% in PG with no difference being observed between the two. Leu-3a/Leu-2a ratio was 2.14 ± 0.84 in NC and 1.57 ± 0.71 in PG with the ratio in PG being significantly lower than that in NC ($p<0.001$).

The absolute number of Leu^+ cells in shown in Fig. 2. The absolute number of $Leu\text{-}1^+$ cells was 1,358 ± 602/mm^3 in NC and 1,512 ±

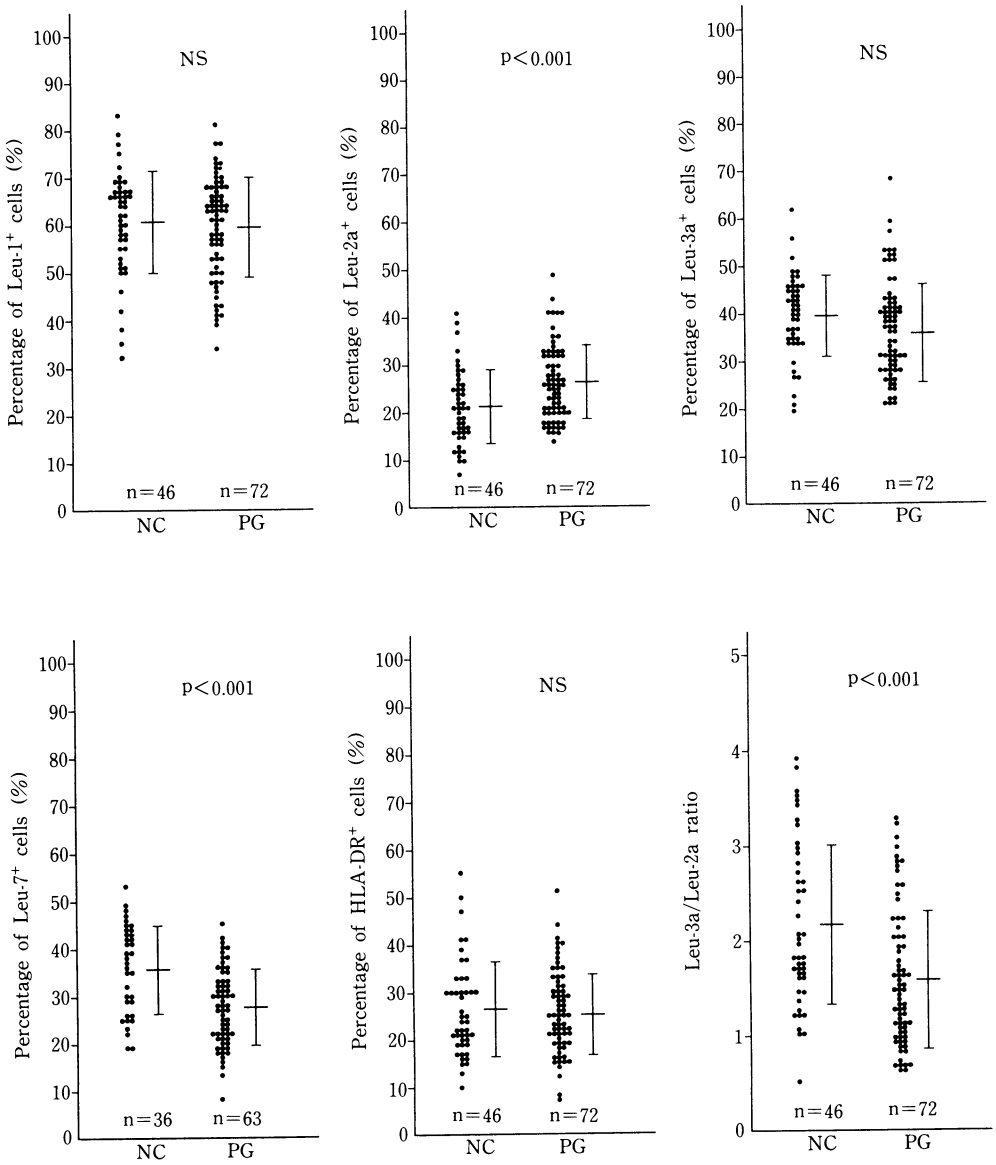

Fig. 1. Percentage and ratio of lymphocyte subsets in normal controls (NC) and poison gas workers (PG). Results are mean value ± SD.

618/mm^3 in PG with no significant difference being observed between the two groups. The absolute number of Leu-2a$^+$ cells was 482 ± 275/mm^3 in NC and 677 ± 364/mm^3 in PG with the value in PG being significantly higher than that in NC ($p<0.01$). The absolute number of Leu-3a$^+$ cells was 889 ± 398/mm^3 in NC and 922 ± 391/mm^3 in PG with no difference being observed between the two groups. The absolute number of Leu-7$^+$ cells was 885 ± 408/mm^3 in NC and 699 ± 342/mm^3 in PG with the value in PG being significantly lower than that in NC ($p<0.01$). The absolute number of HLA-DR$^+$ cells was 562 ± 235/mm^3 in NC and 643 ± 338/mm^3 in PG with no significant difference being observed between the two groups.

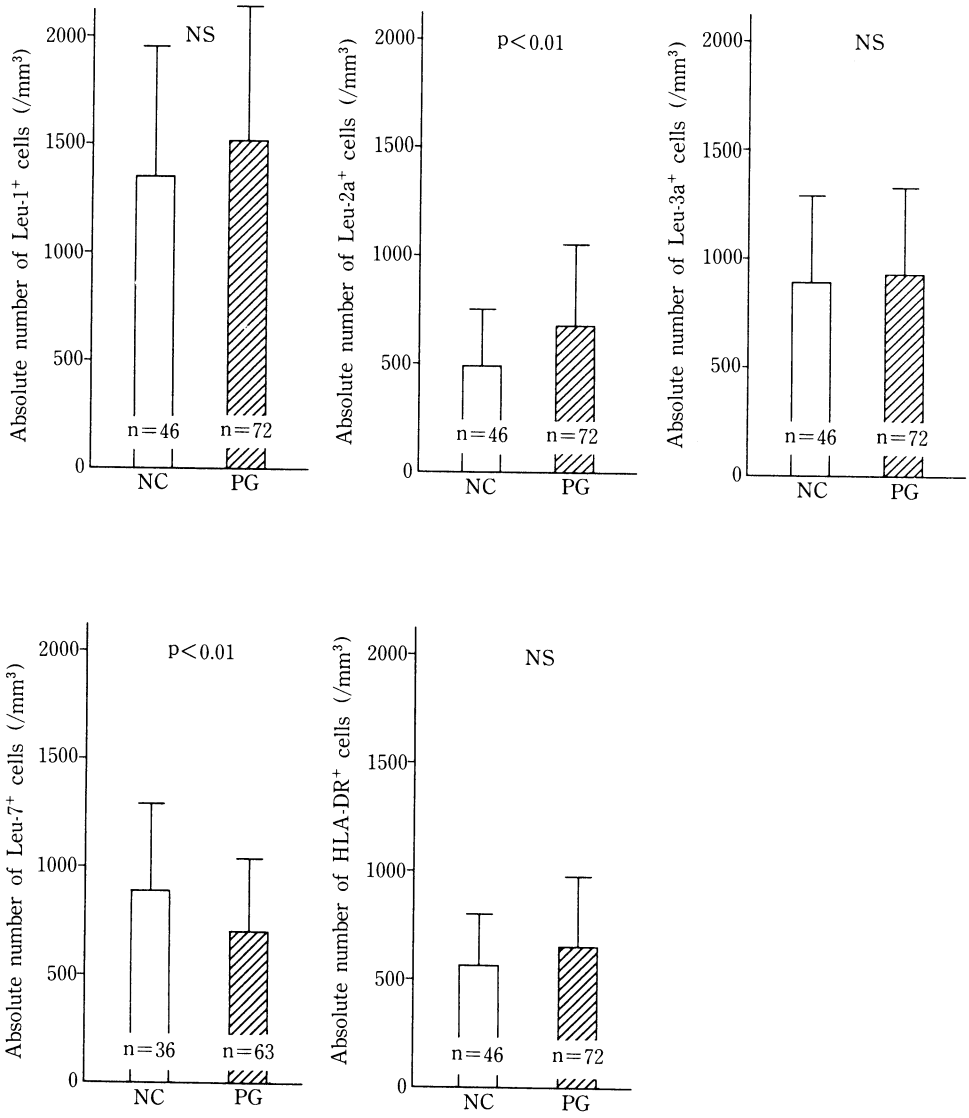

Fig. 2. Absolute number of lymphocyte subsets in normal controls (NC) and poison gas workers (PG). Results are mean value ± SD.

IL-2 production of lymphocytes (Fig. 3)

IL-2 production in shown in Fig. 3. It was 2.76 ± 1.38 units in NC and 2.58 ± 1.05 units in PG with the value in PG being slightly lower than that in NC. The difference was not significant.

Influence of type and duration of work in the poison gas factory

No effect of type and duration of work in the poison gas factory could be observed in lymphocyte subsets and IL-2 production (data not shown).

Correlation between PHA response, IL-2 production and lymphocyte subsets (Table 2)

The following correlations could be observed between these parameters in poison gas workers. A significant positive correlation could be observed between PHA response and IL-2 production ($p<0.001$). PHA response showed a significant negative correlation with Leu-2a+

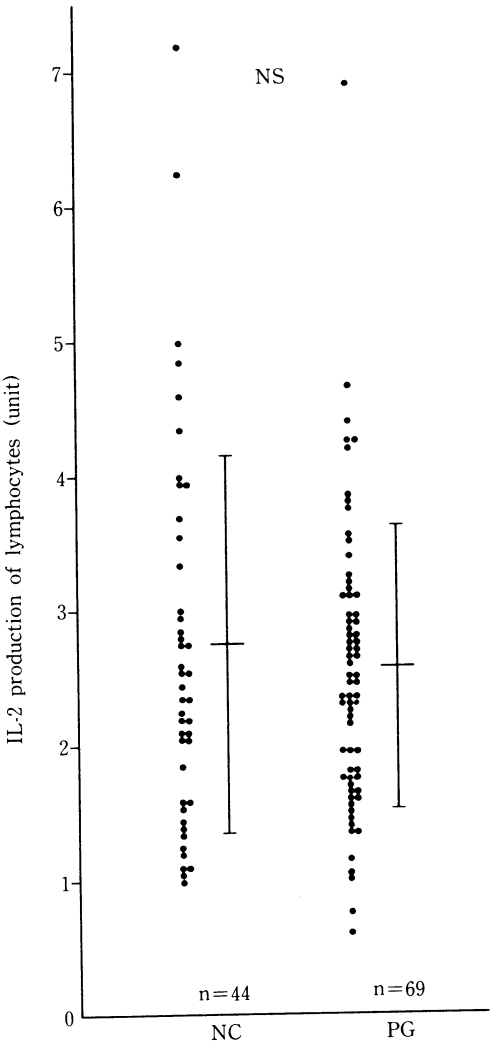

Fig. 3. IL-2 production of lymphocytes in normal controls (NC) and poison gas worker (PG). Results are mean value ± SD.

cells ($p<0.01$) and a significant positive correlation with Leu-3a$^+$ cells ($p<0.01$) and with Leu-3a/Leu-2a ratio ($p<0.001$). IL-2 production showed a significant positive correlation with Leu-3a$^+$ cells ($p<0.01$) and with Leu-3a/Leu-2a ratio ($p<0.01$).

DISCUSSION

It has become apparent from many reports that the proliferation and differentiation of T cells through the stimulation of antigen or mitogen are the results of the organic action of functionally differentiated T cell subsets and macrophages through the cytokines which they produce[6,7,9,15]. Farrar et al[5] have summarized these complex relationships into a concept called "lymphokine cascade", which can be outlined as follows. Briefly, T cells stimulated by antigen or mitogen produce colony stimulating factor (CSF). Macrophages stimulated by antigen or CSF produce IL-1. Helper T cells stimulated by antigen or mitogen bring about the appearance of IL-1 receptors on the surface of cell membrane. When this receptors bind with IL-1, helper T cells produce IL-2. The various effector cells or precursors of effector cells which are stimulated by antigen or mitogen bring about the appearance of IL-2 receptors on the surface of cell membrane, and by the binding of IL-2 to these receptors, these cells proliferate and differentiate. This proliferation is evaluated as mitogenic response of lymphocytes or mixed lymphocyte reaction. Furthermore, of these effector cells, cytotoxic T cells or NK cells when stimulated by IL-2 not only proliferate and differentiate but also produce interferon γ (IFNγ). By the stimulation of this IFNγ, cytotoxicity of these cells is increased.

Table 2. Correlation between PHA response, IL-2 production and lymphocyte subsets in poison gas workers

	PHA response (cpm)	Leu-1 (%)	Leu-2a (%)	Leu-3a (%)	Leu-7 (%)	HLA-DR (%)	Leu-3a/ Leu-2a
PHA response (cpm)		n=69 r=0.204 NS	n=69 r=−0.449 p<0.001	n=69 r=0.364 p<0.01	n=62 r=0.055 NS	n=69 r=−0.236 NS	n=69 r=0.519 p<0.001
IL-2 production (unit)	n=69 r=0.392 p<0.001	n=69 r=0.048 NS	n=69 r=−0.225 NS	n=69 r=0.336 p<0.001	n=62 r=0.112 NS	n=69 r=−0.038 NS	n=69 r=0.347 p<0.01

In view of the foregoing, in exploring the causes of depression of PHA response of lymphocytes observed in poison gas workers, it was considered meaningful to measure lymphocyte subsets and IL-2 production of lymphocytes. It is well known that PHA response of lymphocytes is depressed in malignancy. A review of the literature was made to examine what changes develop in lymphocyte subsets and IL-2 production of lymphocytes in malignancy and a comparison was made with the data of poison gas workers.

With regard to peripheral blood T cells of cancer patients, Braun et al[3] have reported that in solid tumors such as lung cancer and mammary cancer both the percentage and absolute number of peripheral blood T cells decrease with progression of cancer. In contrast to this report, in poison gas workers both the percentage and absolute number of Leu-1$^+$ cells did not show a significant difference from those of normal controls. In comparison with normal controls, in poison gas workers the percentage and absolute number of Leu-2a$^+$ cell showed a significant increase, the percentage of Leu-3a$^+$ cells slightly decreased, and Leu-3a/Leu-2a ratio was significantly depressed. On the other hand, with regard to cancer patients, McCluskey et al[13] have reported that in mammary cancer the percentage of OKT8 (homologous to Leu-2a) increased and the percentage of OKT4 (homologous to Leu-3a) and T4/T8 ratio were depressed. Ginns et al[11] have also reported that T4/T8 ratio was depressed in metastatic lung cancer patients. In Japan, many similar reports[16,19,29] have also been made on solid tumors and such abnormalities have been reported to become remarkable with progression of disease stage. The findings made in these reports are extremely similar to the changes observed in poison gas workers. The involvement of suppressor T cells in the development and progression of cancer has been suggested[8], and it is considered that the abnormalities of T cell subsets in poison gas workers are primarily increase of suppressor T cells. The relation between this abnormality and high frequency of cancer is provoking. In comparison with normal controls, both the percentage and absolute number of Leu-7$^+$ cells are significantly depressed in poison gas workers. Balch et al[1] in studying 247 solid tumor patients have reported that the percentage of Leu-7$^+$ cells was significantly depressed in colon cancer, lung cancer, and mammary cancer. Also in Japan, similar findings have been reported in hepatoma[17] and pharyngeal cancer[14]. In cancer patients it is known that NK cell activity of peripheral blood lymphocytes becomes depressed with progression of disease stage[22] and the relation has been suggested between this fact and the decrease in the percentage of Leu-7$^+$ cells. On the other hand, the authors[25] have reported previously that in poison gas workers there are many cases whose NK cell activity is abnormally depressed in comparison with normal controls. The relation between this fact and the decrease in Leu-7$^+$ cells provokes much interest. In comparison with normal controls, no difference in the percentage and absolute number of HLA-DR$^+$ cells could be observed in poison gas workers. There is no report in the literature on the study of HLA-DR$^+$ cells in cancer patients.

Many reports have been published on the changes in IL-2 production of lymphocytes in tumor bearing hosts. According to Burger et al[4], IL-2 production is depressed in tumor-bearing mouse, in which is involved suppressor T cells. In man, it has been reported that IL-2 production is depressed in solid tumors[21], malignant lymphomas[24], and metastatic tumors[20]. Among these reports, some have observed depression of response to IL-2[4,21] and have given as cause the depressed appearance of IL-2 receptors[4]. In contrast to these reports, IL-2 production of lymphocytes was slightly depressed in poison gas workers when compared with normal controls, but the difference was not significant.

No effect of type and duration of work at the poison gas factory on lymphocyte subsets and IL-2 production of lymphocytes could be demonstrated.

In lymphocytes of poison gas workers, a positive correlation was observed between PHA response and IL-2 production, and a negative correlation between these tow and percentage of Leu-2a$^+$ rate and a positive correlation between these two and percentage of Leu-3a$^+$ rate and Leu-3a/Leu-2a ratio were observed. PHA response of lymphocytes implies proliferative function of lymphocytes in response to IL-2

and these two are in themselves closely related to each other. However, in poison gas workers, the depression of IL-2 production was not as evident as depression of PHA response and it is possible that the response of lymphocytes to IL-2 is impaired. With regard to the correlation between these two and lymphocytes subsets, Leu-3a$^+$ cells contain helper T cells which are IL-2 producing cells[5], while Leu-2a$^+$ cells contain suppressor T cells which are assumed to inhibit IL-2 production[12], therefore, the ratio of the two subsets has been suggested to have a close relationship to PHA response and IL-2 production. According to this, a close relation has been suggested between depression of PHA response and abnormality of lymphocyte subsets in poison gas workers.

As described in the foregoing, a depression in PHA response and also abnormality of lymphocyte subsets have been observed in lymphocytes of poison gas workers and these abnormalities are similar to those of cancer patients. Furthermore, the presence of a close relation between IL-2 production and these abnormalities has been suggested. It is considered necessary in future not only to pursue clinically the possible relation of these abnormalities to carcinogenesis but also to study the response of lymphocytes to IL-2 and IL-1 and the productivity of IL-1 and prostaglandin E_2 by macrophages.

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