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EVALUATION OF CELL-MEDIATED IMMUNITY IN MUSTARD GAS INJURIES

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ABSTRACT

Yamakido, et al.in 1986 have studied immunological parameters in poison gas workers, and a depression has been observed in immunologic response. Also, the frequency of cancer has reached as high as five-fold that of the general population. In the present study, cell-mediated immunity (CMI) was measured in three groups of Iranian mustard gas-injured patients.

The first group were those who had been injured three months up to one year before, and the second group were studied one to two years after injury, and the third group were studied after two years from the time of injury. The following results were obtained:

1- In comparison with normal controls (61.5±4), T lymphocytes showed a significant decrease in 50% of the three groups (50.71±15. 7; 46.95±15) of poison gas injury, and B lymphocytes were increased, but no significant difference could be seen in mitogen response to PHA.

2- In comparison with normal control (47±9), T helper cells (T4) in 52% of the first and second groups were significantly decreased (33. 14±16.59).

3- T suppressor cells (T8) in 53% of the first group, and in 22% of the second and third groups (27.29±11. 77; 21. 4±6. 89) were increased in comparison with normal controls (20±6).

4- Ratio of T4 to T8 in 71% of the first group and 60% of the second and third groups were decreased. Therefore depression of CMI in poison gas injury was observed after one, two, and three years, which will be discussed in this paper.

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INTRODUCTION

Mustard gas had been used in the First World War as a blistering agent. This was synthesized in 1882, and is divided into two kinds: nitrogen mustard, which is used for treatment of cancer, and sulphur mustard, which is used as a poison gas in the wars.[1] This poison gas is carcinogenic in animals.[2] It was first used on the western in the 1914-1918 war on the night of July 12-13, 1917 and after that date was used so extensively that areas of the country become saturated with it.

Between this date and the end of the war the total number of British cases treated for war gas poisoning is recorded as 160,970 and 80 percent of these are estimated to be due to mustard gas. 124,702 of these cases were admitted to hospitals, and 4,167 died before discharge. 1,267 of them were studied in 1930, and it was found that about all (over 80 percent) had chronic bronchitis at that date.

In subsequent years a statisically significant excess of deaths attributed to cancer of the lung and pleura has been observed among these patients (two-fold).[3] In

Okunojima city in Hiroshima, a poison gas manufacturing factory of the former Japanese army exists, which was engaged during World War II in the manufacture of such erosive and highly lethal gases as sulphur mustard and others.[4] Since 1952 and for more than 30 years, Hiroshima University School of Medicine has been engaged in a healthy survey of the former workers of this poison gas factory.[5] In this group not only has a high incidence of chronic lung diseases such as chronic bronchitis and also of respiratory tract neoplasms been observed, but the frequency has reached as high as 37-fold that of the incidence respiratory tract neoplasms in Japan.[6] Furthermore, in recent years there has been an elevated incidence of malignancy of the digestive system such as gastric cancer and hepatoma and also of skin cancer.[7] Therefore determinations on various immunological parameters were made in these workers. Depression in phytohemagglutinin (PHA) response of lymphocytes, considered to be one of the functions of T cells was observed in posion gas workers, but this depression was not considered to be attributable to serum factors.[8] Tuberculin skin reaction and number of lymphocytes was decreased in poison gas workers. Also the percentage and absolute number of suppressor cells in poison gas workers were signigicantly elevated but natural killer cells and ratio of T helper to T suppressor cells were depressed, and IL-2 production of lymphocytes also showed a slightly low level.[8,9] In the present study, cell-mediated immunity was evaluated in Iranian poison gas casualties in different times after injury and the findings are presented in this report.

PATIENTS AND METHODS

The subjects of the present study consist of 100 males, composed of 50 normal controls and 50 poison gas casualties.

They were classified into three groups according to the duration of time elapse after exposure to mustard gas.

Group A were selected for evaluation of immunology parameters, after a period of 3-12 months had passed from the time of their exposure, group B, 12-24 months, and group C, where more than 24 months had elapsed since their exposure to poison gas. They were all males from an age range between 20-30 years old. All had more than 50% surface area skin burns.

Simultaneously, 50 normal controls were also under medical observation. Mustard gas in high doses is lethal, but the casualties mentioned had been in contact with low doses of mustard gas and low defective lung disease.

Methods:

A: Percentage of T and B lymphocyte by rossette method:

Lymphocytes were separated from heparinized peripheral blood by ficol-hypaque density gradient centrifugation method (Boyum).[10] T and B lymphocytes were separated into sheep erythrocytes rossette forming cell (T lymphocytes) and human cells coated with complement- anitibody rossette(B lymphocytes), according to the method of Jondal, with some modifications.[11] Briefly, equal volumes of lymphocytes (5×10^6/ml) and SRBC (2%) were mixed and inoculated at 4° C. After 16-20 hours, the percentage of lymphocytes binding two or more SRBC were determined as T cells.

For B cells equal volumes of lymphocytes $(2.5\times10)^6$ and human cells (sensitized with hemagglutinin and complement) were mixed, then after centrifugation, the percentage of B lymphocytes was determined.

Mitogen stimulation

For the in-vitro proliferative response, the lymphocytes were suspended in RPMI enriched with 20 percent AB serum and antibiotic at a concentration of 0.2×10^6/ml. The cultures were incubated with 0.1 ml of PHA (Difco, Detroit, Mich.) for 72 hours, at 37° C, and in 5% CO_2 atmosphere. All experiments were performed in triplicate. One uCi of tritiated thymidine were added to cultures 16 hrs prior to harvest. Count, per minutes, were determined in liquid scintillation counter (Packard). The results were reported as transformation index which is expressed as:

$$TI=\frac{\text{C.P.M. of stimulated cells}}{\text{C.P.M. of unstimulated cells}}$$

Detection of B and T lymphocytes and also T helper and T suppressor cells was by indirect immunofluorescent method.[12] Monoclonal antibodies were used as the first antibody, that is, anti Leu-1 as T cell markers, anti-Leu-2a as suppressor T cell markers, anti-Leu-3a as helper T cell marker, and anti -HLA-Dr as a marker for B cells, and fluorescein isothiocyanate (FITC) was used as the second antibody, labelled affinity-purified goat anti-mouse Ig G. Furthermore, the absolute number of Leu + cells was obtained by multiplying the fluorescence- positive rate with the number of peripheral blood lymphocytes. All the data were expressed as average value with standard deviation and statistical analysis of the data was made by students's T-test.

RESULTS

Percentage of T and B lymphocytes with the two

Table I. T and B lymphocytes in mustard gas injuries and controls

Lymphocytes		control	(a) group one	(b) group two
T lymphocytes	rossette method	(c) 61.5±4	50.71±15.7 ↓ 50% (d)	46.95±15.75 ↓ 50%
	monoclonal method	66±9	64.52.±14 24% ↓	54.56±18 30% ↓
	P value	-	p<0.005	p<0.005
B lymphocytes	rossette method	19±3	24±4	28.5±6.89
	P value	-	P<0.005	P<0.005
number		50	22	28

a=Patientswhohadbeeninjured threemonths upto one year before.
b= group who had been injured one to two years before.
c = Mean±SD
d= percentage of injured group whose T lymphocytes were lower than normal.

Table II. T helper and T suppressor cells in mustard gas injuries

lymphocytes		control	(a) group one	(b) group two
T helper	Mean+SD	(c) 47±9	33.14±16.59 52% (d)	34.57±14.52 50%
	P value	20±6	p<0.005	p<0.005
T suppressor	fnean+SD	-	27.29±11.77	21.14±6.89 22%
	P value	-	p<0.005	p<0.01
T helper / T suppressor	Mean+SD	2.1±1.3	1.44±0.98 71.4%	1.53±0.9 60.26%
number		17	22	28

a= group who had been injured three months up to are year before
b = group who had been injured one to two years before.
c= Mean±SD
d = percentage of injured group whose lymphocytes were lower than normal.

methods in three groups of Iranian mustard gas casualties and controls are shown in Table I. The percentage of T lymphocytes showed a significant decrease in 50% of the three groups of poison gas casualties in comparison with normals, as B lymphocytes were increased in the three groups. The percentage and ratio of T helper (T_4) and T suppressor cells (T_8) in mustard gas casualties and controls are shown in Table II.In comparison with normal, T helper cells (T_4) in 52% of first and second group, and in 22% of the second and third group were increased, so ratio of T helper to T suppressor in 71% of the first group and 60% of the second and third groups were decreased.

Lymphocyte *in vitro* reactivity to PHA is shown in Table III. No significant difference could be seen in poison gas casualties vs. controls.

DISCUSSION

Since 1970 when Burnet first reported the concept of immunological surveillance, interest has been focused on the relation of decreased immunity to carcinogenesis and extensive analyses have been conducted.[13] These studies have shown that various immunological abnormalities are present in tumor-bearing hosts. With regard to peripheral blood T cells of cancer patients, Braun, et al[14] have reported that in solid tumors such as lung cancer and breast cancer, both the percentage and absolute number of peripheral blood T cells decreased with progression of cancer. On the other hand with regard to cancer patients, McClurkey[15] has reported that in breast cancer the percentage of T suppressor increased and the T helper and ratio of T helper to T suppressor were depressed. Nishimote, et al[16] have reported that poison gas workers are a high risk group of cancer, and lung neoplasms have increased in those exposed to poisonous gas during the First World War, so carcinogenesis of mustard gas is proved. A group of Japanese authors have studied immunological parameters in poison gas workers, and found that cell-mediated immunity was depressed. T suppressor cells are elevated, and ratio of T helper to T suppressor is decreased. Lymphocyte response to PHA is also depressed. Some Iranian authors have studied C.M.I in poison gas injuries within one month of their exposure, but we have studied the immune system in three groups of Iranian mustard gas injuries where a long period of time had elapsed since their exposure to evaluate first the immune system, second the risk of cancer in these groups and, thirdly, evaluation of phagocytosis and humoral immunity in these groups which will be discussed in other papers. In contrast to this report, in poison gas injuries, the percentage of T cells showed significant decrease in comparison with normal controls- the relation between this abnormality and the high frequency of cancer is

Table III. lymphocytes transformation responses in mustard gas injuries and control

	Control	(a) group one	(b) group two
CPM cells per minute	6152±7705 [c]	9947.6±4708	8818±5474
SI	18.56±12.39	17.98±13.36	29.85±1.5

a=group who had been injured three months up to one year before
b=group who had been injured one to two years before
c= Mean±SD

thought-provoking. That B lymphocytes are increased in comparison with normal controls maybe due to infection in these patients, since Ab is also elevated (which will be discussed in other papers) but this elevation maybe due to a decrease in the percentage of T lymphocytes and an increase of B lymphocytes.

Lymphocyte *in vitro* reactivity to PHA was normal in most of the cases whereas in the skin test with PPD, most cases of mustard gas injuries showed a marked depression of responsiveness to specific antigen, so a specific depression is present *in vivo*.

Depression of T helper lymphocytes in 52% of the first and second groups of mustard gas injuries is due to immune deficiency in different times after injury, and the same resultis seen by other authors. We observed elevation of T suppressor lymphocytes (53% ofthe first and 22% of the second groups of mustard gasinjuries)as the result of depression of ratio of T_h/T_s so C.M.I. deficiency is due to T suppressor cells and these abnormalities are similar to those of cancer patients.

In conclusion as we have seen, after exposure to mustard gas, the immune sytem is defective. Fortunately after several years, some of them will recover, but others will not and T suppressors are high after many years. It is necessary in the future not only to pursue clinically the possible relationship of these abnormalities to carcinogenesis, but also to evaluate the immune system in these patients.

We suggest immunotherapy as a treatment for mustard gas casualties in the evaluation of their immune system.

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