Hiroshima Journal of Medical Sciences
Vol.34, No.4, 425~430, December, 1985
HIJM 34-60

Function of Monocytes in the Retired Workers of the Okunojima Poison Gas Factory

Michio YAMAKIDO, Shigeru MATSUZAKA, Jitsuro YANAGIDA, Soichiro HOZAWA, Shinichi ISHIOKA and Yukio NISHIMOTO

The Second Department of Internal Medicine, Hiroshima University School of Medicine, 1-2-3, Kasumi, Minami-ku, Hiroshima 734, Japan

(Received September 19, 1985)

Key words: The retired workers of the Okunojima Poison Gas factory, Monocytes, Cytostatic activity, Phagocytic activity, Chemotactic activity

ABSTRACT

Monocyte function of poison gas workers was determined and the following results were obtained.

1) No difference in cytostatic activity could be observed between poison gas workers and their controls.

2) Phagocytic activity of poison gas workers was slightly depressed when compared with that of the controls, but the difference was not significant. By duration of work, it was observed that the group with duration of work exceeding five years had a significantly lower value when compared with the group with duration of work being less than two years.

3) Chemotactic activity of poison gas workers tended to be depressed when compared with that of the controls. The activity tended to be more depressed the longer the duration of work.

4) A significant positive correlation was observed between cytostatic activity and phagocytic activity.

Poison gases were manufactured by the former Japanese Army from 1927 to 1945 at Okunojima Island, a small island located in the Inland Sea of Seto[12]. The Second Department of Internal Medicine, Hiroshima University School of Medicine has been engaged for the last 33 years in a clinical observation of the former workers of the defunct Okunojima Poison Gas Factory (hereinafter referred to as poison gas workers). A high incidence of respiratory diseases including chronic bronchitis has been observed in the poison gas workers and furthermore a high frequency of malignant tumors, cancers of the respiratory tract in particular, has been reported in this population[13]. Studies made to date by the authors have demonstrated a depression in cellular immune competence among the poison gas workers such as decrease in PHA response of peripheral lymphocytes and abnormality in T lymphocyte subsets[14]. On the other hand, monocytes and macrophages are cells which have embryologically existed earlier than lymphocytes and play an important role in the defense mechanism. Alveolar macrophages play an important role as one of the defense mechanisms of the respiratory - alveolar system, but tissue macrophages originate from premonocytes and reach the tissue via the peripheral monocytes and their function as peripheral monocytes has attracted much interest as the prodromal cells of macrophages. However, no report has been published in the literature on any systematic study made on monocyte function of poison gas workers. The authors measured three types of monocyte activities and examined the monocyte function of poison gas workers.

SUBJECTS

The subjects of the present study are 73 male

poison gas workers whose age ranged from 58 to 84. In classifying these 73 workers by duration of work, there were 23 workers whose duration of work was less than two years, 31 workers whose duration was from two years to less than five years, and 19 workers whose duration of work was five or more years. For controls, 26 males not engaged in poison gas manufacture were selected with the age distribution matching the poison gas workers.

METHODS

1. Separation of monocytes

Monocytes were obtained by using a slight modification of the method of Kumagai et al[5]. Briefly, by Ficoll-Conray density gradient centrifugation method, mononuclear cell layer was separated from heparized peripheral venous blood and after washing twice with Hanks' solution it was placed in plastic dish pretreated for 24 hr at 4°C with fetal calf serum (FCS) and then incubated for one hour at 37°C. Following incubation, the plastic dish was washed four times with Hanks' solution and after removing the non-attached cells, monocytes attached to the plastic dish were obtained by detaching them with a rubber policeman.

2. Measurement of cytostatic activity

Postlabel method of ^{3}H-thymidine was employed. Target cells (MeWo were human melanoma derived subcultured cell line and SK-MES-1 were human lung cancer derived subcultured cell line) were dispensed to microtest plate (Falcon #3040) at 10^3/well. Monocytes adjusted to 1×10^5/ml (2×10^5/ml in the case of SK-MES-1) were added to the wells each at 0.1 ml (Effector: Target ratio = 10:1 or 20:1) and were incubated for 48 hr and 8 hr prior to completion of incubation, 0.4 μCi of ^{3}H-thymidine was added. Upon completion of incubation, the microtest plate was washed with Hanks' solution and ^{3}H-thymidine not incorporated by the cells was removed. After drying the microtest plate, the plate bottom was punched out and the radioactivity of ^{3}H incorporated in the target cells was determined by a liquid scintillation counter.

Cytostatic activity was obtained by the following equation: Cytostatic activity =

$$\left(1 - \frac{\text{cpm of } ^3\text{H-thymidine incorporated in target cells after incubation with monocytes}}{\text{cpm of } ^3\text{H-thymidine incorporated in target cells after incubation without monocytes}}\right) \times 100\ (\%)$$

3. Measurement of phagocytic activity

The methods and conditions employed in the measurement were reported in detail previously[8], but these will be briefly described. Yeast particles and monocytes were suspended in culture medium containing fresh serum so that the ratio of yeast particles to monocyte would be 20 : 1 and after mixing within a plastic tube (Corning #25310), it was incubated for 30 min at 37°C. Following incubation, 4°C phosphate buffer saline (PBS) was added and was mixed well so that the reaction was terminated. Thereafter, it was centrifuged at 150 G for 2 min to obtain cell sediments. One to two drops of Ziehl's carbolfuchsin solution were added to the cell sediments and after mixing well one drop was placed on a slide glass for microscopic observation at 400 power. The ability of monocytes to phagocytose yeast particles was expressed as phagocytic index (PI), the mean number of yeast particles phagocytosed per cell by counting 100 monocytes.

4. Measurement of chemotactic activity

Human AB type serum diluted solution activated by zymosan was employed as chemotactic factor. Chemotactic factor was placed in the lower chamber of blind well chamber (Laboscience, #342-0252) and was covered with 5 μm Millipore filter. After confirming the absence of air bubbles, screen-in type upper chamber was inserted, to which 0.2 ml of monocytes suspension adjusted to 1×10^6/ml was added. After allowing it to stand for 90 min at 37°C, the filter was removed and fixed in alcohol, followed by Giemsa staining. The stained filter was microscopically examined to determine the chemotactic ability. Following the method of Kataoka[4] the authors randomly counted in five visual fields at 400 power magnification the number of monocytes which migrated from the filter surface to a surface located 30 μm away and the mean thus obtained was used at chemotactic index (CI).

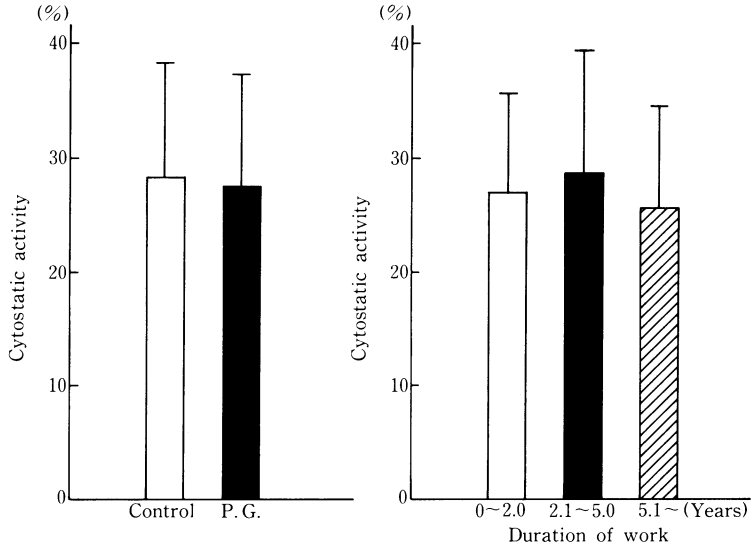

Fig. 1. Cytostatic activity of monocytes from the retired workers of the Poison Gas factory

RESULTS

1. Cytostatic activity of monocytes (Fig. 1)

The cytostatic activity of monocytes of 27.4 ± 10.0% observed in 73 poison gas workers did not demonstrate a significant difference from 28.2 ± 10.1% observed in 26 controls. Even when the subjects were divided into three groups according to duration of work, no significant difference could be observed among the three groups.

2. Phagocytic activity of monocytes (Fig. 2)

The mean value of phagocytic index of 2.16 ± 0.38 observed in poison gas workers did not show a significant difference from the mean value of 2.24 ± 0.35 observed in the control group. In examining the phagocytic index by duration of work, the phagocytic index of 2.30 ± 0.35 was seen in the group having a duration of work less than 2 years, 2.15 ± 0.38 in the group having a duration of work of 2.1 — 5 years, and 2.01 ± 0.35 in the group having a during of work exceeding 5 years, indicating a significant ($p<0.05$) depression in the phagocytic index in the group having a duration of work of more than five years compared with that of the group having a duration of work of less than two years.

3. Chemotactic activity of monocytes (Fig. 3)

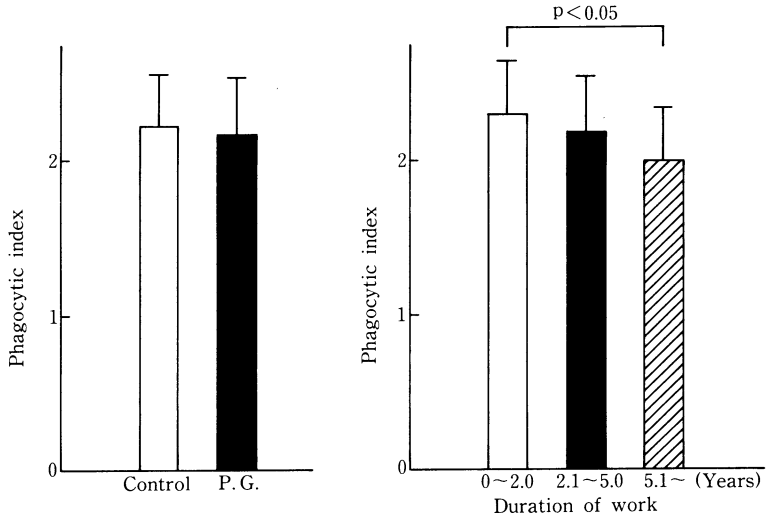

Fig. 2. Phagocytic activity of monocytes from the retired workers of the Poison Gas factory

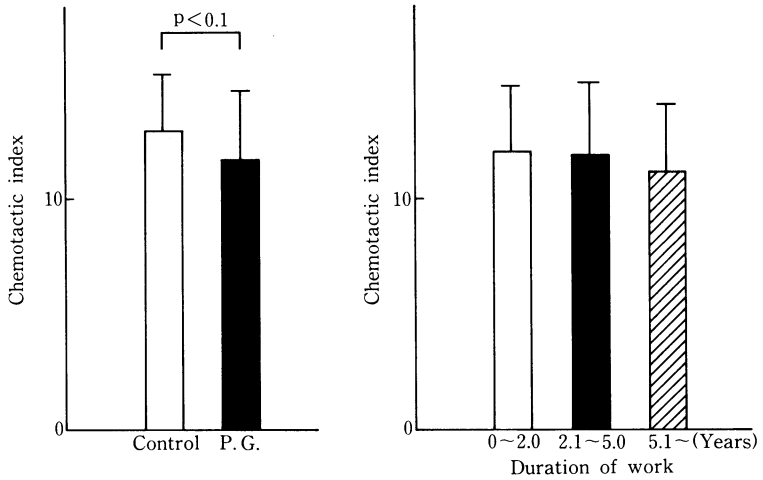

Fig. 3. Chemotactic activity of monocytes from the retired workers of the Poison Gas factory

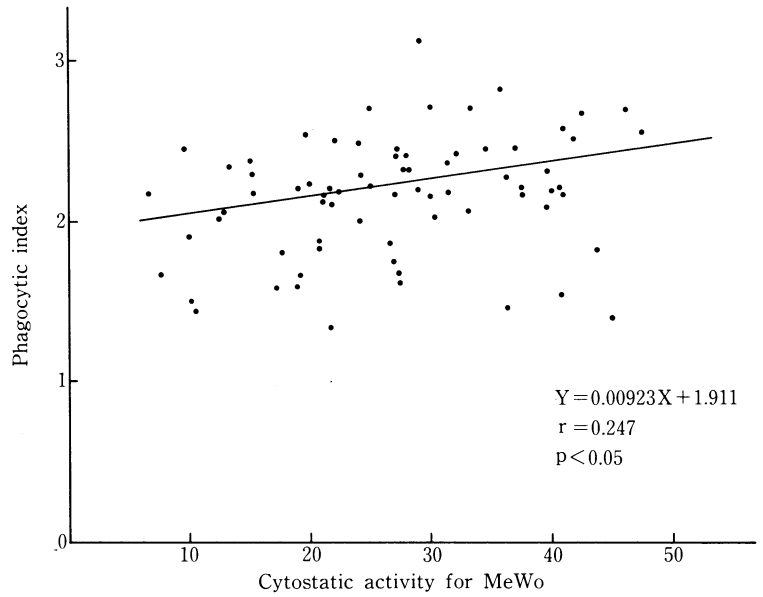

Fig. 4. Correlation between cytostatic activity and phagocytic activity of monocytes from the retired workers of the Poison Gas factory

The mean of the chemotactic activity of poison gas workers was 11.7 ± 3.0, which tends to be depressed ($p<0.1$) when compared with the mean of 12.9 ± 2.5 observed in the control group. When examined by duration of work, the mean of the group having a duration of work of less than two years was 12.0 ± 2.9, 11.8 ± 3.2 for the group with duration of work between 2 and 5 years and 11.2 ± 2.8 for the group with duration of work exceeding 5 years. The value decreased with duration of work, but the difference was not significant.

4. Correlation among monocyte activities of poison gas workers

Of the three types of monocyte activities, a positive significant correlation ($p<0.05$) was observed between cytostatic activity and phagocytic activity (Fig. 4).

DISCUSSION

Based on the studies of former poison gas workers conducted during the last 33 years, the Second Department of Internal Medicine, Hiroshima University School of Medicine has

reported that the incidence of chronic airway diseases including chronic bronchitis and lung cancer in particular is high in this population[13]. It is considered that the cause involved is long term inhalation of violent poison gases including yperite in the poison gas factory. The carcinogenic properties of yperite have been reported in a number of animal experiments[2] and also in man it has been reported that the incidence of airway cancer was high in the troops of the allied forces exposed to yperite gas during World War I[1]. With the introduction of the concept of immune surveillance system in the studies of carcinogenesis in recent years, results suggesting a close relationship between depression of immune competence and carcinogenesis have been reported. It has been reported by many workers that the frequency of malignant tumors is high among patients with transplantation and those with congenital immunodeficiency diseases[6,10].

In our study of immune competence of former poison gas workers, we have observed findings suggesting abnormal cellular immune competence such as depression of PHA reaction of peripheral lymphocytes and increase of suppressor T lymphocytes, but hardly any study has been made on the function of monocytes. In the present study, an attempt was made to determine the functions of monocytes of former poison gas workers with the use of three methods of determining monocyte activities.

No difference in cytostatic activity of monocytes could be demonstrated between poison gas workers and their controls. Even when the duration of work at the poison gas factory of these workers was divided into three groups, no difference could be observed among the three groups. Many reports have been published on the cytostatic activity of human monocytes obtained from cancer patients. Jewells et al[3] and Mashiba et al[7] have observed an elevation in activity, whereas Nakata[9] has reported a depression, but Yanagawa et al[16] has observed no difference when compared with healthy individuals, indicating no consistent tendency in the results. One of the reasons involved might be the difference in target cells employed by these workers, suggesting a need to use the same target cells among the workers in the future.

In examining the ability of monocytes to phagocytose yeast particles, no difference could be demonstrated between poison gas workers and their controls, but in classifying the workers by duration of work in the poison gas factory, the group having a duration of work exceeding five years showed a significant depression when compared with the group having a duration of work of less than two years. Furthermore, the chemotactic activity of monocytes of poison gas workers tended to be depressed when compared with that of the controls and when the group was classified by duration of work, the longer the duration of work the greater was the tendency for the chemotactic activity to be depressed. These findings suggest that inhalation of poison might damage the monocyte function to bring rise to some abnormality in the defense mechanism and to depression of resistance against infection and cancer. Yamashita[15] also focusses his interest on immune suppression due to carcinogenic factor in his studies and study of the relation between carcinogenic factor and immune competence is considered to be of importance in analyzing some types of carcinogenesis.

Of the monocyte activities of poison gas workers, a positive correlation was demonstrated between cytostatic activity and phagocytic activity. It was regarded heretofore that the antitumor activity and phagocytic activity of monocytes and macrophages were two mutually independent activities[11], but Nakata[9] in his study of monocyte activities of lung cancer patients has reported a positive correlation between phagocytic activity and cytostatic activity. These indicate the need for further detailed study on the involvement of phagocytic activity in the mechanism of cytotoxicity.

REFERENCES

1. **Beebe, G.W.** 1960. Lung Cancer in world war veterans : Possible relation to mustard-gas injury and 1918 influenza epidemic. Natl. Cancer Inst. **25**: 1231-1252.
2. **Boyland, E. and Horning, E.S.** 1948. The induction of tumors with nitrogen mustards. Brit. J. Cancer **3**: 118-123.
3. **Jerrells, T.R., Dean, J.H., Richardson, G., Cannon, G.B. and Herberman, R.B.** 1979. Increased monocyte-mediated cytostasis of lymphoid cell lines in breast and lung cancer patients. Int. J. Cancer **23**: 768-776.
4. **Kataoka, M.** 1981. Studies on human peripheral

monocyte chemotaxis. Part I. A technical study. Okayama I.Z. **93**: 489-498.

5. **Kumagai, K., Itoh, K., Tada, M. and Hinuma, S.** 1979. Further studies of a simple method for macrophage isolation : Pretreatment of plastic petri dishes with fetal calf serum. Igaku no ayumi **110**: 611-614.
6. **Louie, S. and Schwartz, R.S.** 1978. Immunodeficiency and the pathogenesis of lymphoma and leukemia. Sem. Hematol. **15**: 117-138.
7. **Mashiba, H., Matsunaga, K., Yagawa, K., Nomura, Y., Yoshinaga, H., Furusawa, M. and Jimi, S.** 1981. Elevated cytostatic activity of monocytes from cancer patients. Gann **72**: 848-853.
8. **Matsuzaka, S., Yanagida, J., Hozawa, S., Yukutake, M., Iwamoto, K., Kodomari, N., Yamakido, M. and Nishimoto, Y.** 1984. Phagocytic activity of monocytes measured by uptake of yeast particles. J. Hiroshima Med. Ass. **37**: 949-952.
9. **Nakata, Y.** 1983. Monocyte dysfunction in patient with malignant disease. Jpn. J. Clin. Immun. **6**: 476-482.
10. **Penn, I.** 1979. Tumor incidence in human allograft recipients. Transplant. Proc. **11**: 1047-1051.
11. **Ralph, P. and Nakoinz, I.** 1975. Phagocytosis and cytolysis by a marcrophage tumour and its cloned cell line. Nature **257**: 393-394.
12. **Wada, S., Nishimoto, Y., Miyanishi, M., Katsuta, S., Nishiki, M., Yamada, A., Tokuoka, S., Umisa, H. and Nagai, M.** 1962. Review of Okunojima poison gas factory regarding occupational environment. Hiroshima J. Med. Sci. **11**: 75-80.
13. **Wada, S., Miyanishi, M., Nishimoto, Y., Kanbe, S. and Miller, R.W.** 1968. Mustard gas as a cause of respiratory neoplasia in man. Lancet **I**: 1161-1163.
14. **Yamakido, M.** 1983. Immunological competence in the high risk group of lung cancer. Lung Cancer **23**: 237.
15. **Yamashita, U.** 1983. Immunosuppression by carcinogens and carcinogenesis. Metabolism and disease **20**: 815-824.
16. **Yanagawa, E., Yasumoto, K., Manabe, H., Nagano, N., Hirota, N., Ohta, M., Hattori, T., Nomoto, K., Azuma, I. and Yamamura, Y.** 1979. Cytostatic activity of peripheral blood monocytes against bronchogenic carcinoma cells in patient with lung cancer. Gann **70**: 533-539.