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Changes in Immunological Parameters of the Retired Workers of the Okunojima Poison Gas Factory with Administration of BCG or Nocardia Rubra Cell Wall Skeleton*)

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ABSTRACT

An immunopotentiator, BCG or Nocardia rubra cell wall skeleton, was injected to the retired workers of the Okunojima poison gas factory in order to study prophylactic approaches to the cancer and the various immunological parameters were followed.

1) γ-globulin and IgG whose pre-treatment levels were abnormally high tended to approach the normal levels.

2) α_1-AT tended to increase and α_1-AG tended to decrease in Group C. α_2-HS tended to decrease in Group A, and C_3 tended to increase in Group A.

3) Tuberculin reaction kept positive in 77.9% of the subjects studied, and it did negative in 5.2%, while 13.0% of them had a positive conversion, and 3.9% showed a negative conversion.

4) The response of lymphocytes to PHA tended to increase in all three groups, but that to PWM did not show any significant changes.

5) NK cell activity and ADCC activity showed a transient increase 2 weeks after CWS injection, and their activities returned to preinjection levels after 3 months.

INTRODUCTION

Prophylaxis of cancers in man still waits for extensive scientific and clinical works. This critical problem was discussed earlier by Hirayama et al.[13] from two aspects. primary prophylaxis, that is, the prevention of the occurence of cancer and secondary prophylaxis, that is, the prevention of their growth. The problem of primary prophylaxis has been epidemiologically approached, and secondary prophylaxis has been studied to establish practical methods of cancer prevention for the individuals or groups who may have high risks of cancer or who are in the preclinical stage of tumor development.

We have followed up the retired workers of the Okunojima poison gas factory for over 30 years, and found that the workers who had been directly engaged in poison gas (Yperite, Lewisite, etc.) production or who were directly exposed to such gases through the occupations of repair, laboratory and engineering had very high risks of bronchogenic cancer compared

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: 旧大久野島毒ガス傷害者への BCG-CWS, Nocardia-CWS の投与による免疫パラメーターの変動

to the nations average morbidity[26,27]. This is true for younger workers, and presently the occurence of cancers of the digestive tract and skin has been found in higher frequency among them[23].

Recently, the occurence of malignant tumors has been discussed in relation to suppressed immunological competence[7]. The disturbance in the immunological surveillance system seems to induce malignant changes of cell and the growth of tumor cells. On the other hand, we have demonstrated that the workers often showed impared immunity as shown by excessive reactivity in humoral immunity and suppressed cellular immunity.

On the assumption that the correction of immunity by immunological treatment would be the first approach to the prophylaxis of malignant tumor in the retired workers, we started the clinical trial to restore immunological capacities in June, 1977 using the BCG cell wall skeleton (BCG-CWS) which was replaced with Nocardia rubra cell wall skeleton (N-CWS) in January, 1978[16].

In this communication, we discuss the changes in the parameters of humoral and cellular immunity with administration of immunopotentiator.

SUBJECTS

There are about 800 workers of the Okunojima poison gas factory who were registered in our file. They were engaged in the production of Yperite, Lewisite and other gases or worked for repair, laboratory and engineering and they were high risk group of cancer. Of these, those who gave their consent to participate in this study were injected BCG-CWS or N-CWS once every 3 months. At present, a total of 206 men have been included in this study.

METHODS

Protochol of BCG-CWS or N-CWS injection

BCG-CWS was used from June to December, 1977, and it was replaced with N-CWS in January, 1978 since the latter has weaker side effects such as fever and ulcer formation but has stronger immunopotentiating action than the former. CWS were injected in a subcutaneous volume of 200 μg each time to the bilateral upper arms once every 3 months. The

volume was decreased when the subject showed abnormal reactions at the site of injection.

Assay of humoral immunity

The immunological parameters of the subjects were examined once every 3 to 6 months during the study. Humoral immunity was assayed by determinating: (1) total serum protein, (2) protein fraction by cellulose acetate membrane electrophoresis, (3) serum immunoglobulin (IgG, IgA and IgM) by the single radial immuno-diffusion method, (4) α-and β-glycoprotein [α_1-antitrypsin: α_1-AT, α_1-acid glycoprotein: α_1-AG, α_2-heat stable glycoprotein: α_2-HS and the third component of complement (C_3)] by the single radial immuno-diffusion method.

Assay of cellular immunity

The cellular immunity of subjects was assayed from the following parameters: (1) counts of peripheral lymphocytes, (2) tuberculin reaction, (3) reactivity of peripheral lymphocytes to various mitogens: the reactivity to PHA and PWM was expressed in terms of total count (cpm) of ^{3}H-TdR uptake at the time of DNA synthesis in the lymphocyte using the micro-volume culture technique of whole blood, (4) Natural Killer (NK) cell activity was determined by ^{51}Cr release assay[6] when K-562 derived chronic myelocytic leukemia was used the target cell or by ^{3}H-proline assay[5] when T-24 derived urinary bladder cancer was used as the target cell, and (5) the level activity of antibody dependent cell mediated cytotoxicity (ADCC) was determined by the ^{3}H-proline assay when T-24 was used as the target cell (Anti T-24 serum was diluted at 1 : 10^4).

RESULTS

Subjects were classified into three groups according to the type of work at the factory: Group A consisted of 117 subjects who directly engaged in the production of Yperite and Lewisite; Group B consisted of 40 subjects who did repair, laboratory and engineering and Group C consisted of 46 subjects who did other jobs such as the manufacture of other types of gases, clerical work, etc. Group A and B have high risks of cancer, while Group C has low risks[17]. The changes in immunity for each group were discussed below.

Changes in humoral immunity

(1) Levels of serum total protein and γ-globulin (Fig. 1):

The serum level of total protein changed within the normal range after CWS injection in all groups. The level of γ-globulin tended to gradually decrease in all groups.

(2) Level of immunoglobulin (Fig. 2):

The pre-injection level of IgG exceeded 2,000 mg/dl in all groups, however, the level gradually but significantly (p<0.05) declined in Group A and C after CWS injection. IgG tended to decrease in Group B. IgA remained slightly high in all groups during the study period. IgM did not show fluctuations beyond the normal range in all groups.

(3) Levels of α-and β-glycoprotein (Fig. 3):

α_1-AT tended to increase in Group C, but showed no significant changes in Group A and B. α_1-AG tended to gradually decrease after CWS injection in Group C, but did not show any significant changes in other groups. α_2-HS tended to decrease in Group A, but there was no significant change in Group B and C. C_3 tended to increase in Group A, but there were no changes in other groups.
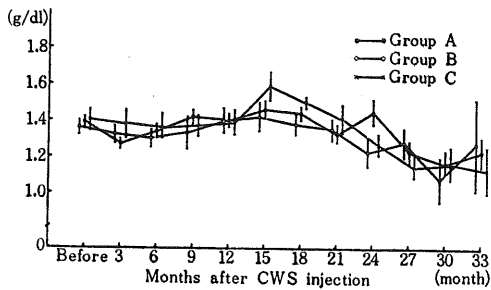

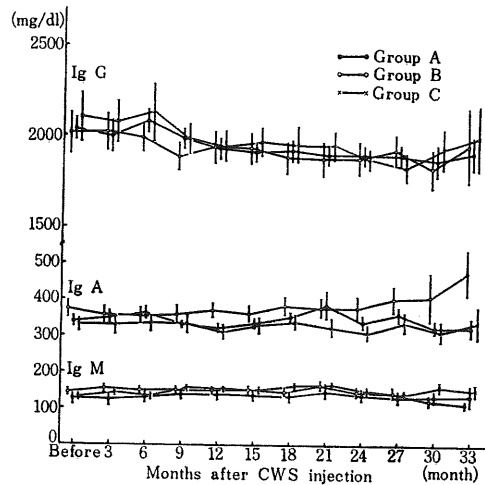**Fig. 1.** Changes in γ-globulin after CWS injection

Fig. 2. Changes in immunoglobulin levels after CWS injection
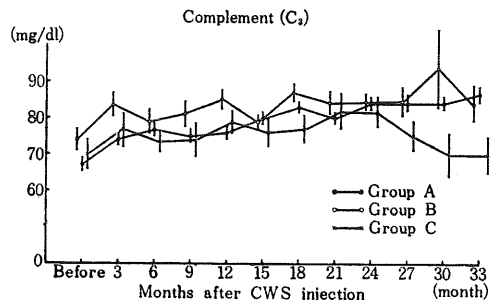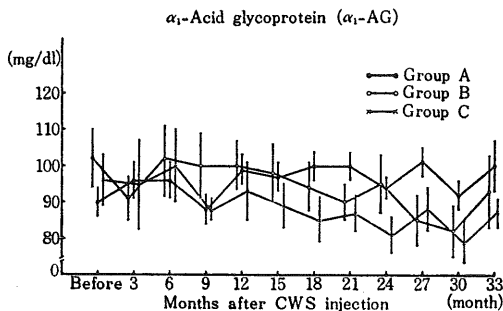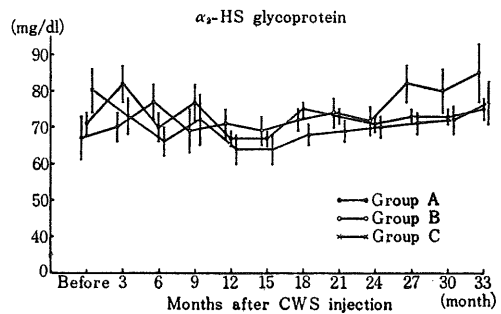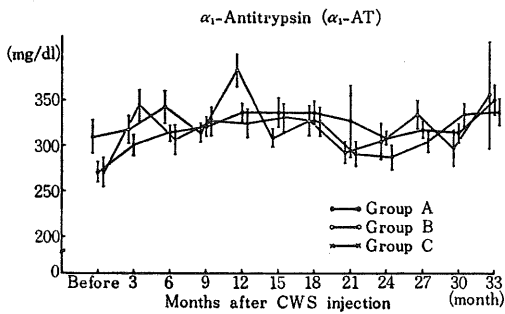

Fig. 3. Changes in α-and β-glycoprotein levels after CWS injection

Table 1. Changes in PPD skin test after CWS injection

| Changes of PPD skin test	Type of work (%)			Total
	Group A	Group B	Group C	
pos. → pos.	70(81.5)	27(77.1)	23(69.6)	120(77.9)
neg. → pos.	7(8.1)	7(20.0)	6(18.2)	20(13.0)
neg. → neg.	6(6.9)	0(0)	2(6.1)	8(5.2)
pos. → neg.	3(3.5)	1(2.9)	2(6.1)	6(3.9)
Total	86(100)	35(100)	33(100)	154(100)

Changes in cellular immunity

(1)　Lymphocyte count in peripheral blood:

The count fluctuated within the range of 2,000-3,000/mm³, and there were no definite trends of the changes.

(2)　Tuberculin reaction (Table 1):

Among 154 subjects whose tuberculin reaction could be tested, 120 (77.9%) kept a positive reaction and 8 (5.2%) had a negative reaction. The other 20 (13.0%) showed a positive conversion, but the remaining 6 (3.9%) had a negative conversion.

(3)　Mitogen responses of peripheral lymphocytes (Fig. 4) :
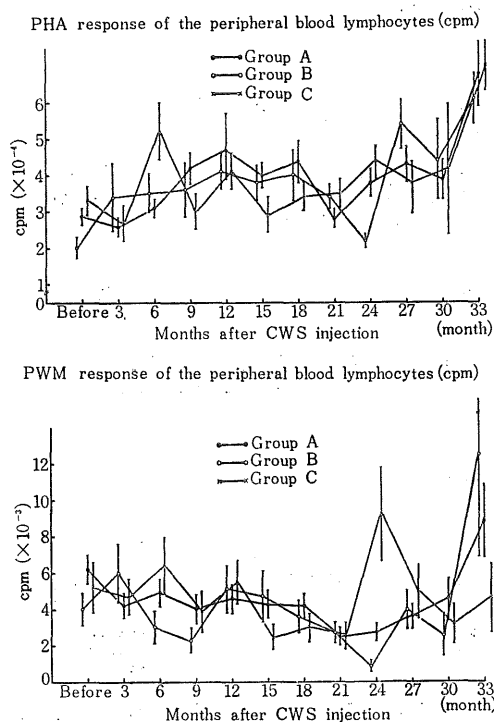
Fig. 4. Changes in mitogen response of the peripheral blood lymphocytes after CWS injection

The reactivity of lymphocytes to PHA tended to increase in all groups, and the increase was particularly significant ($p < 0.001$) in Group A. On the other hand, the reactivity to PWM did not show any changes in all three groups.

(4)　Changes in NK cell activity (Fig. 5):

A total of 16 subjects could be observed for the changes in NK cell activity. A significant
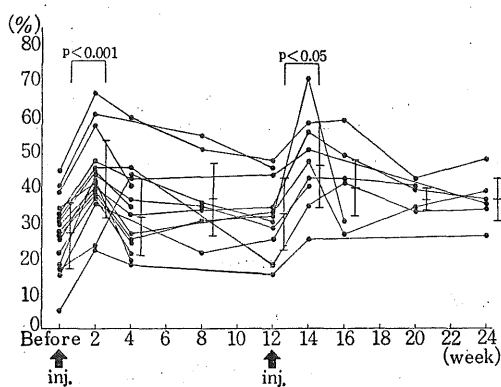
Fig. 5. Changes in NK cell activity against K-562 after N-CWS injection
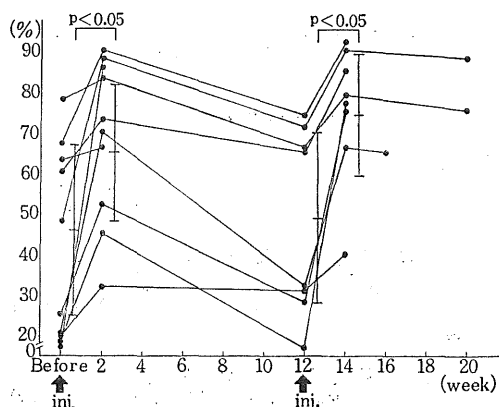
Fig. 6. Changes in ADCC after N-CWS injection

(p<0.01) increase in NK cell activity against K-562 and T-24 was observed 2 weeks after N-CWS injection, and then the levels tended to return to the pre-injection level. The significant increase was again noted 2 weeks after N-CWS injection at 12 weeks.

(5) Changes in ADCC activity (Fig. 6):

The activity of ADCC was determined simultaneously with the NK cell activity determination. The activity significantly increased at 2 weeks after N-CWS injection, but returned to the pre-injection level later. The second injection caused similar increases.

DISCUSSION

The retired workers of the poison gas factory whom we have been observing are quite unique clinical groups. There is still a high incidence of cancer among them[17], although more 30 years have passed since their exposure to poison gas. They provide us excellent oppotunities to study prophylactic approaches of cancer.

In current cancer research, the concept of immunological survellance system was introduced[7], and many clinical reports[20,25] have been published which indicate the relationship between depressed immunity and cancer development.

The workers who were affected by the poison gas, as reported earlier[16], frequently showed abnormal responses in humoral and cellular immunity. Therefore, we thought that the potentiation of immunity in such groups might possibly prevent the occurrence of malignant tumors, and so attempted to use immunopotentiators for that purpose in these individuals. In the actual treatment, we used BCG-CWS and N-CWS, which were developed by Yamamura and Azuma[2,3], once every 3 months and followed up the changes in various immunological parameters and clinical corses of the subjects. In this paper, we studied the effect of CWS with various immunological parameters for 33 months.

The subjects in this study were diveded into Group A, B and C according to the type of work at the factory, and the immunological parameters were monitored.

The level of γ-globulin tended to decrease after CWS injection, and the chief immunoglobulin contained in γ-globulin, IgG, was found to be decreased in all three groups with a paticularly significant decrease in Group A and C. In regard to the significant high levels of IgG in the retired workers having high risks of cancer, Nishimoto et al.[18] thought that the high level might have been caused by frequent airway infection, but basically such a group appeared to have a high response to external stimulation. On the other hand, Furue et al.[10] observed that IgG levels were high in cancer patients and that the combination of chemotherapy with immunotherapy controlled the increase of IgG well, but the immunotherapy alone was not effective at all. Therefore, we are uncertain of the immunological significance of IgG reduction; however, the injection of CWS to the workers enhanced their resistance to influenza virus compared to the non-injecting group and so this reduction was presumed to be the initial step of immunological normalization.

α-and β-glycoprotein are closely related to immunological reaction in vivo and, therefore, there have been many reports[4,9] about their serum levels and cancer development since the Higgins' report[12]. In particular, α-glycoprotein is considered to be involved in the suppression of immunological reactions and immunological tolerance in cancer patients[8,28]. We also reported earlier that the increase of glycoprotein had a good correlation with the suppression of immunological reactions in lung cancer patients[28]. In this study, we have determined the level of 4 proteins, α_1-AT, α_1-AG, α_2-HS and C_3, and have found in group A, the subjects having the highest risks of cancer, that α_2-HS tended to decrease and C_3 tended to increase despite the fact that α_1-AT and α_1-AG remained no remarkable change. However, there is a report[1] that α_2-HS decreases with the increase in the complement value in lung cancer patients, and so further careful monitoring of these parameters is necessary. On the other hand, Group B did not show any significant changes in all serum proteins. In Group C, α_1-AT tended to increase, while α_1-AG tended to decrease. These serum proteins are known to increase as acute phase reactants at the occurrence of acute inflammation[28]. However, α_1-AT and α_1-AG are also reported[24] to increase in patients with malignant tumors, and variant type of α_1-AT in such patients increased[21]. Now we think that the

type of α_1-AT should be further studied.

Among the cellular immunological parameters determined, the count of lymphocytes did not show any significant changes after CWS injection in all groups. When the subpopulation of lymphocytes was determined using monoclonal antibody, the number of helper T cells was found to increase in Group A compared to non CWS injected group, however, changes after CWS injection have not been studied yet and, therefore, require further examination.

Among the 154 subjects in whom tuberculin reaction was studied, 6 (3.9%) who showed a negative conversion from the positive state during the study period seemed to be an important matter of discussion. Tuberculin reaction is a skin test using a recall antigen, PPD. The negative convertion seemed to reflect secondary damage of the skin response mechanism in the body immunized with PPD antigen[15]. Clinical examination has been continued in these individuals who showed negative conversion. On the other hand, the positive conversion was obtained in 13.0% of the 154 individuals during the study.

The PHA responses of peripheral blood lymphocytes tended to increase in all 3 groups. The response of the lymphocytes to mitogen might be the first step of foreign substance identification by the host following antigen invasion into the body. The response of lymphocytes closely relates to the function of the lymphocyte, and, therefore, the level of the mitogen responses provides a reliable means to determine the level of cellular immunity[19]. PHA chiefly stimulates T cell; therefore, CWS injection might have enhanced the activity of T cell in the workers. Hirao et al.[14] reported the increase in the reactivity to PHA after combinational use of CWS in lung cancer models of rabbits induced by methylcholanthlene. It is thought that NK cell which exhibit anti-tumor activity in the host unimmunized with tumor antigens react with tumor cells in a short time, and it is considered to play an important role in suppressing cancer development or growth in the early stage[11]. In 16 subjects NK cell activity showed a significant increase with the peak at the second weeks after CWS injection. A similar response was obtained when CWS was re-injected at 3 months. Saijo et al.[22] performed an experiment about

the changes of the activity of NK cell after CWS injection into mouse, and found that NK cell was activated with treatment of CWS. The NK cell activity returned to the pre-injection level at 3 months of the study, and, therefore, our CWS injection schedule of once 3 months may be appropriate. The ADCC activity by Killer (K) cell that has properties similar to NK cell was increased transiently. Although the physiological role of ADCC has not been clarified yet, ADCC has been presumed to have an effect in some types of malignant tumors.

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