

Changes of Human Immunological Parameters by PSK Administration^{*}

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ABSTRACT

PSK was administered to the retired workers of the Poison Gas factory who showed high incidence of malignancy and immunological abnormalities. Furthermore, changes of various immunological parameters were studied. In the group in which Leu 1 positive rate showed a low value, Leu 1 positive rate showed significant increase after PSK administration. NK cell activity as well as Leu 7 positive rate showed significant elevation after PSK administration. It seems that PSK potentiates the immunity of non-tumor bearing individuals with depressed immunity.

INTRODUCTION

With the dramatic advances made in tumor immunology in recent years, immunotherapy of tumors has been made with the use of various non-specific immunotherapeutic drugs and a good number of basic and clinical reviews have been reported. The action mechanism of these immunotherapeutic drugs is chiefly of the host mediated type and their property as biological response modifier (BRM) has been emphasized.

PSK [Krestin (R)], a protein-bound polysaccharide derived from basidiomycetes, is one of those immunotherapeutic drugs clinically applied in Japan. The authors have administered PSK to the retired workers of the Poison Gas factory established by the former Japanese army who constitute a high risk group of malignant neoplasms such as lung cancer^{23,24)} and who show such immunological abnormalities as decrease in cell mediated immunity¹³⁾ and have attempted to elucidate the action mechanism of PSK as BRM by measuring various immunological parameters.

MATERIALS AND METHODS

Subjects

Group A: Eight volunteers among the retired workers of the Poison Gas factory were employed as subjects. They were all males whose average age was 60.0 ± 2.7 years. Group B: Four volunteers among the retired workers of the Poison Gas factory whose peripheral blood lymphocyte subsets had been examined prior to PSK administration and whose Leu 1 positive rate showed a low value were used as subjects. They were all males whose average age was 75.0 ± 5.8 years.

Method and period of PSK administration

Oral administration of PSK at the usual dose of 3.0 g/day was given three times a day after meals. The period of administration was 8 weeks for Group A and 4 weeks for Group B. *The employed immunological parameters and their methods*

Number of peripheral blood lymphocytes and PPD skin reaction. Number of peripheral blood lymphocytes was obtained by multiplying the absolute number of peripheral blood leu-

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kocytes by lymphocyte ratio. Furthermore, PPD (Japan BCG) for general diagnostic use was employed. The average longitudinal diameter and transverse diameter of erythema were expressed in mm.

PHA and ConA response of peripheral blood lymphocytes. PHA and ConA response of peripheral blood lymphocytes separated by Ficoll-Hypaque gradient centrifugation was expressed in c. p. m. with incorporation of $^3\text{H-TdR}$ ²⁾.

Peripheral blood lymphocyte subsets. By indirect immunofluorescence technique using Leu series monoclonal antibodies, anti-Leu 1⁵⁾, anti-Leu 2a⁶⁾, anti-Leu 3a⁶⁾, and anti-Leu 7¹⁾, (Becton-Dickinson), peripheral blood lymphocyte subsets were identified and then expressed in %. Furthermore, the ratio of Leu 3a to Leu 2a was obtained.

NK cell activity. Peripheral blood lymphocytes, as effector cells, were cultured for 3 hr with K-562 derived from chronic myelogenous leukemia as target cells at E:T ratio of 25:1 and NK cell activity was obtained by ^{51}Cr release assay method⁴⁾ by the following equation:

$$\text{Cytotoxic activity} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100(\%)$$

Moreover, maximum release was obtained by adding detergent (1% Nonidet-P40).

Macrophage mediated cytostatic activity. Peripheral blood mononuclear cells obtained by Ficoll-Hypaque gradient centrifugation were added to plastic dishes coated with fetal calf serum (FCS), and after removing non-adherent cells, monocytes were obtained by stripping away by rubber policeman and then used as effector cells. Using MeWo derived from human melanoma as target cells, they were cultured for 48 hrs with E:T ratio of 10:1, and using the post-label method^{8,18)} of $^3\text{H-TdR}$, cytostatic activity was obtained using the following equation.

$$\text{Cytostatic activity} = \left\{ 1 - \frac{\text{cpm of } ^3\text{H-TdR incorporated in viable target cells with effector cells}}{\text{cpm of } ^3\text{H-TdR incorporated in viable target cells without effector cells}} \right\} \times 100(\%)$$

Serum interferon activity. The inhibitory effect of serum on the cytopathic effect (CPE)

of Sindbis virus against FL cells was determined by the dye uptake method¹⁰⁾ using 0.02% neutral red as unit from 50% CPE value.

Various serum proteins. Such glycoproteins as α_1 -Antitrypsin (α_1 -AT), α_1 -Acid glycoprotein (α_1 -AG), α_2 -Heat stable glycoprotein (α_2 -HS) and C₃ component (C₃) and such immunoglobulins as IgG, IgA, and IgM were determined by single radial immunodiffusion method.

In Group A, the changes of parameters are presented prior to administration, 4 weeks after administration, and 8 weeks after administration, and in Group B the changes of parameters are shown prior to administration and 4 weeks after administration.

Statistical analysis was done by means of Student's t-test. All data were expressed in average and standard deviation.

RESULTS

Changes of immunological parameters in Group A

Number of peripheral blood lymphocytes and PHA and ConA response of peripheral blood lymphocytes (Fig. 1)

No significant change in number of blood lymphocytes and PHA and ConA response of peripheral blood lymphocytes could be observed by PSK administration.

Peripheral blood lymphocyte subsets (Fig. 2)

Leu 1, Leu 2a and Leu 3a positive rate was studied, but no consistent trend could be observed. No significant change could be observed in these parameters including Leu 3a/Leu 2a ratio.

NK cell activity (Fig. 3)

NK cell activity was $23.3 \pm 4.3\%$ prior to PSK administration, but it elevated to $25.1 \pm 5.0\%$ 4 weeks after PSK administration and to $37.7 \pm 8.3\%$ 8 weeks after PSK administration. A significant elevation ($p < 0.01$) was observed between that prior to PSK administration and that 8 weeks after PSK administration.

Macrophage mediated cytostatic activity (Fig. 4)

Large variations in activity was observed by case and no significant change could be demonstrated. Moreover, no remarkable change could be seen in individual cases.

Others (Table 1)

No significant change could be seen in serum interferon activity by PSK administration. Fur-

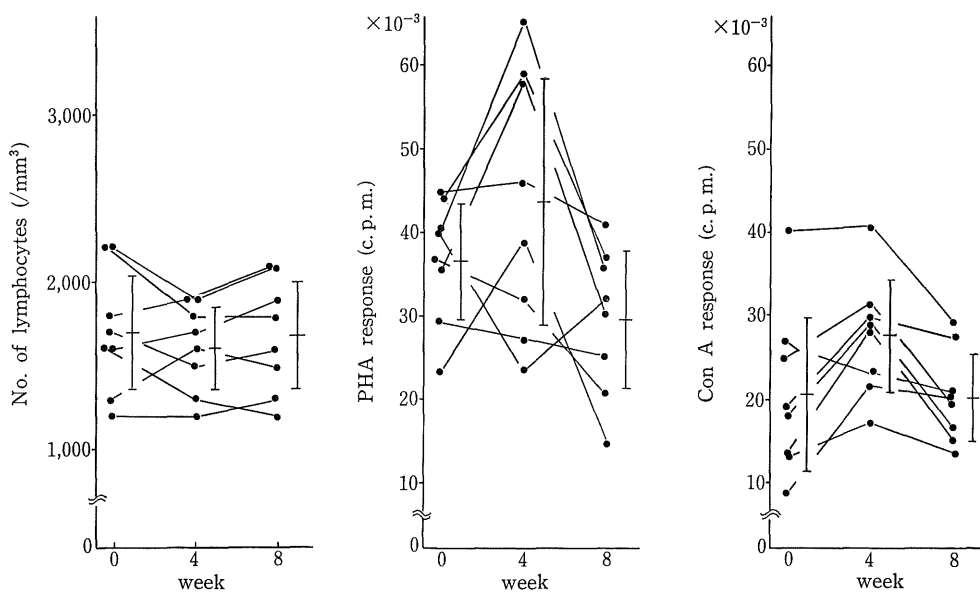


Fig. 1. Changes of number of lymphocytes and PHA, Con A response of lymphocytes in Group A by PSK administration

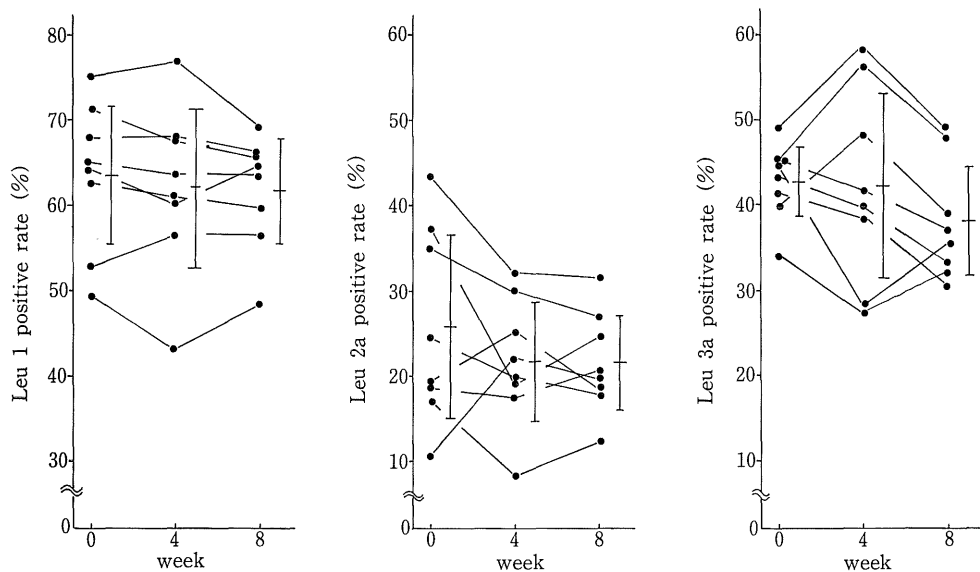


Fig. 2. Changes of lymphocyte subsets in Group A by PSK administration

thermore, no significant change could be demonstrated in α_1 -AT, α_1 -AG, α_2 -HS, C₃, IgG, IgA, and IgM.

Changes of immunological parameters in Group B

Number of peripheral blood lymphocytes, PPD skin reaction and PHA response of peripheral blood lymphocytes (Fig. 5)

No significant change could be demonstrated in number of peripheral blood lymphocytes, PPD skin reaction, and PHA response of peripheral blood lymphocytes.

Peripheral blood lymphocyte subsets (Fig. 6)

A study was made of Leu 1, Leu 2a, Leu 3a, and Leu 7 positive rate. Leu 1 positive rate was $43.1 \pm 3.4\%$ prior to PSK administration

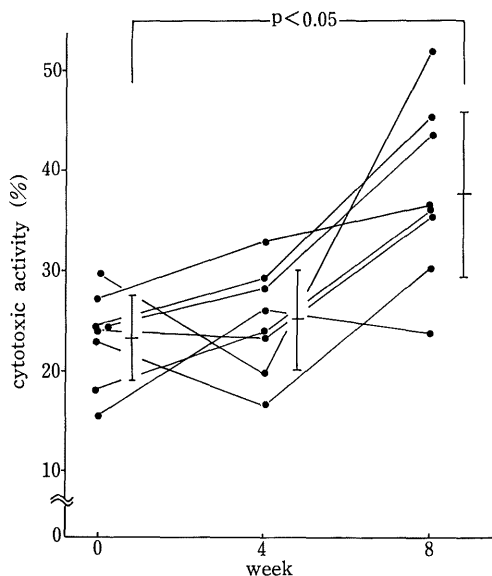


Fig. 3. Changes of NK cell activity in Group A by PSK administration

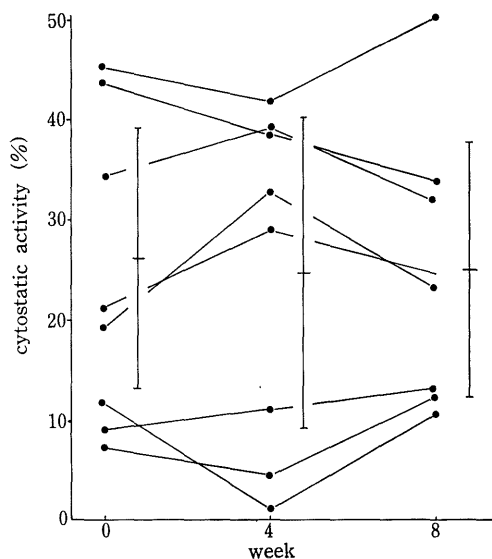


Fig. 4. Changes of macrophage mediated cytostatic activity in Group A by PSK administration

Table 1. Changes of interferon activity and various serum proteins in Group A by PSK administration

	before	4 W	8 W
Interferon (U/ml)	< 6	< 6	< 6
α_1 -AT (mg/dl)	298.3 \pm 37.0 ^{a)}	290.3 \pm 30.0	283.1 \pm 22.4
α_1 -AG (mg/dl)	98.6 \pm 31.1	93.3 \pm 19.5	115.9 \pm 18.2
α_2 -HS (mg/dl)	73.6 \pm 8.6	78.5 \pm 12.3	73.3 \pm 15.2
C ₃ (mg/dl)	100.5 \pm 16.9	91.1 \pm 11.2	87.5 \pm 15.7
IgG (mg/dl)	1705.4 \pm 328.1	1665.6 \pm 283.9	1597.0 \pm 189.0
IgA (mg/dl)	301.8 \pm 126.0	325.3 \pm 259.4	324.5 \pm 204.8
IgM (mg/dl)	191.9 \pm 131.8	215.8 \pm 78.2	147.0 \pm 70.7

a) Data present mean \pm S. D.

Table 2. Changes of interferon activity and various serum proteins in Group B by PSK administration

	before	4 W
Interferon (U/ml)	< 6	< 6
α_1 -AT (mg/dl)	373.0 \pm 38.9 ^{a)}	407.5 \pm 38.9
α_1 -AG (mg/dl)	74.8 \pm 14.3	81.8 \pm 19.8
α_2 -HS (mg/dl)	38.8 \pm 6.1	48.8 \pm 6.1
C ₃ (mg/dl)	60.8 \pm 4.3	65.0 \pm 7.0
IgG (mg/dl)	1940.0 \pm 336.2	2055.0 \pm 352.5
IgA (mg/dl)	390.5 \pm 193.2	359.5 \pm 200.4
IgM (mg/dl)	191.5 \pm 39.3	193.5 \pm 41.6

a) Data present mean \pm S. D.

to increase to 52.2 \pm 2.1% 4 weeks after PSK administration, showing a significant elevation ($p < 0.01$). Furthermore, Leu 7 positive rate was 33.5 \pm 4.7% prior to PSK administration to increase to 42.3 \pm 1.1% 4 weeks after PSK administration, also showing a significant elevation ($p < 0.05$). However, Leu 2a and Leu 3a positive rate, including Leu 3a/Leu 2a ratio, did not demonstrate any significant change. *NK cell activity* (Fig. 7)

NK cell activity prior to PSK administration was 24.7 \pm 11.7% to elevate to 32.6 \pm 15.5% 4 weeks after PSK administration, but the difference did not reach the significant level.

Others (Table 2)

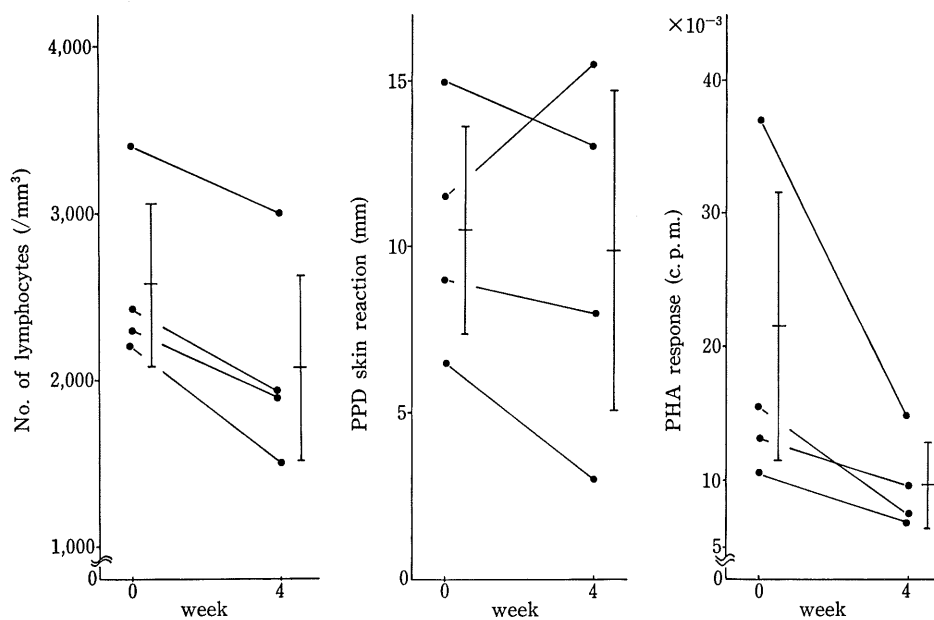


Fig. 5. Changes of number of lymphocytes, PPD skin reaction and PH A response of lymphocytes in Group B by PSK administration

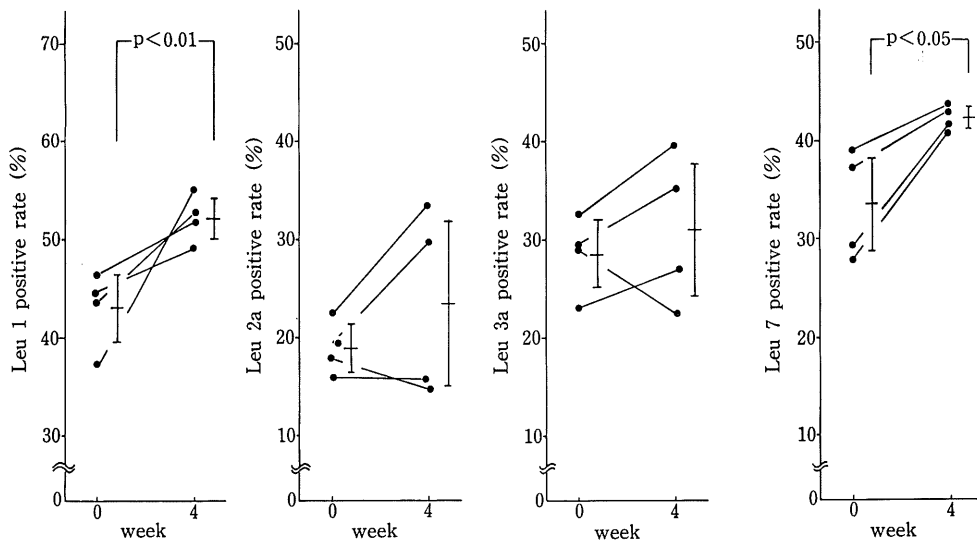


Fig. 6. Changes of lymphocyte subsets in Group B by PSK administration

No significant change in serum interferon activity was observed by PSK administration. Furthermore, no significant change could be

seen in α_1 -AT, α_1 -AG, α_2 -HS, C₃, IgG, IgA, and IgM.

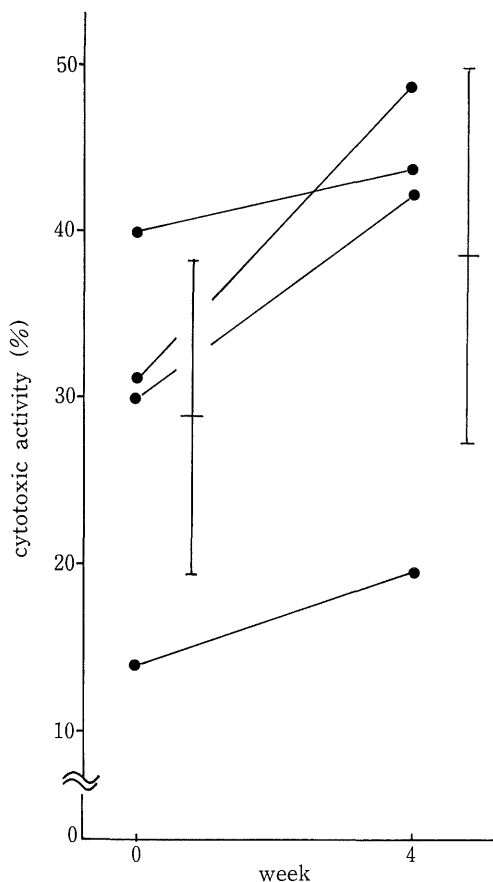


Fig. 7. Changes of NK cell activity in Group B by PSK administration

DISCUSSION

In recent years various types of non-specific immunotherapeutic drugs have been developed and a good number of reports have been published on their basic action mechanism and clinical effects. It has been reported that PSK has anti-tumor effect against experimental tumors^{3, 20, 21}, that its administration prior to tumor transplantation in particular has an inhibitory effect on the growth of the transplanted tumor^{20, 21}, and that its anti-tumor effect is of the host mediated type, that is, it possesses the property of BRM.

The authors have reported that the retired workers of the Poison Gas factory are a high risk group of malignant neoplasms such as lung cancer^{23, 24} and that they show such immunological abnormalities as depression in cell mediated immunity¹⁸. In the present study the authors using as subjects the retired workers of the Poison Gas factory examined the mech-

anism of immunopotential of PSK in non-tumor bearing individuals with immune depression.

Among the retired workers of the Poison Gas factory, included in Group A were eight individuals whose immune function was comparatively well maintained and included in Group B were four individuals whose Leu 1 positive rate was found to have a low value as determined by examination of their peripheral blood lymphocyte subsets. The effect of PSK administration was studied in these two groups.

Kondo et al. administered PSK on aged non-tumor bearing individuals with depressed immunity and studied both the short term and long term effects of PSK administration using as indices various skin reactions and PHA response of peripheral blood lymphocytes. They have reported as results of their studies that PPD skin reaction was enhanced with initiation of PSK administration, that PHA response of peripheral blood lymphocytes using stimulation index (S.I.) began to elevate from the first month after PSK administration, and that these effects continued during the period of PSK administration^{14, 25}. In the author's present study, no significant change could be observed in both PPD skin reaction and PHA response of peripheral blood lymphocytes and furthermore no consistent tendency could be seen in cases with low values prior to PSK administration.

The most remarkable finding in the present study was the significant elevation in Leu 1 positive rate observed four weeks after PSK administration in Group B which had a low Leu 1 positive rate. According to Oguchi, Tsuru et al. who administered PSK to mice, PSK by activating cell division in the thymus demonstrated the action of bringing about recovery from depression of thymus function in tumor bearing state prior to its inhibitory effect on tumor proliferation and furthermore by preventing atrophy of the thymus PSK displayed its effect of inhibiting tumor proliferation^{16, 22}. The significant elevation of Leu 1 positive rate of the peripheral blood lymphocytes observed in the present study by the authors is considered to be one of the dominant properties of PSK as BRM.

On the other hand, Ogoshi et al. have re-

ported in their study of pre-operative and post-operative cases of gastric cancer that PSK administration inhibits the significant increase of IgG-FcR⁺ T cells during the post-operative period¹⁵), but in the present study of the authors no consistent trend was observed in Leu 2a and Leu 3a positive rate, including Leu 3a/Leu 2a ratio, by PSK administration and no significant change could be seen.

In examining the changes in NK cell activity, a significant increase was observed eight weeks after PSK administration when compared to that prior to PSK administration in Group A. Furthermore, in Group B no significant difference could be observed, but four weeks after PSK administration NK cell activity tended to elevate. Study of Leu 7 positive rate in Group B showed a significant elevation four weeks after PSK administration and it was considered that elevation of NK cell activity and increase in Leu 7 positive rate were closely related to each other. Oguchi et al. have reported that IL-2 production of mouse spleen cells is elevated by PSK administration¹⁷), but though not examined in the present study, there is a possibility that NK cell activity is enhanced through the elevation of IL-2 production.

With regard to macrophage function, Inokuchi, Kumashiro et al. have reported that PSK activates macrophage by the fact that O₂⁻ production of peritoneal macrophage is enhanced by PSK administration⁷), but in the *in vitro* study of Konishi, it was observed that PSK did not enhance monocyte mediated cytostatic activity¹²). The authors examined monocyte mediated cytostatic activity using MeWo as target cells, but no significant change could be observed by PSK administration.

In an *in vivo* study of tumor bearing mice Kitani et al. have observed that PSK administration brought about a recovery of depressed interferon activity⁹), but in the present study made by the authors no significant change in serum interferon activity could be observed.

In a detailed study on the effect of PSK administration on humoral immunity Nomoto et al. have reported that PSK restores antibody production in tumor bearing mice and reactivates humoral immunity¹⁴), while Tanaka et al. have observed in cases of gastric cancer received curative operation that serum IgG and IgM were elevated by PSK administration¹⁹). In the

present study the authors could not observe any significant change in serum proteins, including immunoglobulins.

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