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Antitumor Effect of PSK: Role of Regional Lymph Nodes and Enhancement of Concomitant and Sinecomitant Immunity in the Mouse

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PSK, a *Coriolus* preparation, inhibited the growth of not only the right but also the left, non-treated tumor in a double grafted tumor system. In order to examine the role of lymph nodes and the spleen in the antitumor activity of PSK, regional (axillary and inguinal) lymph nodes and spleen were resected. Since in resected mice the antitumor activity of PSK against the right and left tumors was weakened, the regional lymph nodes and the spleen probably have a very important role in the antimetastatic effect of intratumoral administration of PSK. TIL (tumor-infiltrating lymphocytes) obtained from left and right side tumors treated with PSK were examined by Winn assay for their antitumor activity against Meth-A sarcoma in BALB/c mice. TIL from both sides clearly inhibited the growth of admixed Meth-A cells, but control TIL did not. A primary growth of Meth-A sarcoma inoculated into the right flank resulted in the generation of concomitant immunity to the growth of a second graft of the same tumor cells in the left flank. A significant inhibitory effect on the proliferation of the tumor cells inoculated secondarily was shown in mice bearing a primary right tumor that had previously been inoculated with PSK 3 times. After surgical excision of the primary tumor on day 6, daily oral administration of PSK significantly inhibited the growth of the secondary tumor inoculated on day 21, that is, PSK treatment also enhanced sinecomitant immunity. These observations suggest that presurgical intratumoral injection and postoperative oral administration of PSK are highly effective in eradicating metastatic tumors.

Key words: Immunopotentiator — Antimetastatic effect — Tumor-infiltrating lymphocytes — Concomitant immunity — Sinecomitant immunity

In our previous papers,[1,2] the antitumor effect at a distant site of PSK, a *Coriolus versicolor* preparation, was analyzed with a double grafted tumor system in which BALB/c mice received simultaneous intradermal inoculations of Meth-A tumor in the right (10^6 cells) and left (2×10^5 cells) flanks and were then injected with PSK in the right-flank tumor on day 3. PSK, a protein-bound polysaccharide preparation, significantly inhibited the growth of not only the right but also the left (non-treated) tumor. Intratumoral administration of PSK first induces polymorphonuclear leukocytes and then macrophages in the right tumor. Lyt-1-positive cells are subsequently induced in the right regional lymph nodes and in the spleen. Finally, intratumoral administration of PSK in the right tumor induces macrophages and Lyt-2-positive cells in the left, non-treated tumor.[2]

The purpose of the present study was to clarify the roles of regional lymph nodes and tumor-infiltrating lymphocytes (TIL)[2] in PSK antitumor activity in the double grafted tumor system. This investigation has shown that PSK enhances two forms of tumor immunity: concomitant immunity displayed by hosts bearing a progressive primary tumor growth against a second tumor challenge; and sinecomitant immunity displayed to a second tumor challenge after excision of the primary tumor.

MATERIALS AND METHODS

Mice and tumors Six-week-old male BALB/c (+/+) and BALB/c (*nu/nu*) mice were obtained from Shizuoka Laboratory Animal Center, Hamamatsu, and Clea Japan, Tokyo, respectively. Meth-A, a methylcholanthrene-induced fibrosarcoma, was administered to syngeneic BALB/c mice in solid form by intradermal inoculation.

Drug PSK was purified from a hot water extract of cultured mycelia from *Coriolus versicolor*, which belongs to *Basidiomycetes*. The average molecular weight is about 100,000 and its protein content is about 38%. The main glycoside portion of PSK is β-D-glucan.[3] PSK was supplied by Kureha Chemical Industry Ltd., Tokyo.

Double grafted tumor system As described in a previous paper,[1] we devised the double grafted tumor system as a new experimental model for tumor metastasis. BALB/c mice receive simultaneous intradermal inoculations of Meth-A tumor cells in both the right (1×10^6 cells, primary region) and the left (2×10^5 cells, metastatic region) flanks. Drugs are injected into the right-flank tumor on day 3, and the left (non-treated) tumor is observed for 21 days.

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[2] The abbreviations used are: TIL, tumor infiltrating lymphocytes; FITC, fluorescein isothiocyanate; FACS, fluorescence-activated cell sorter; PBS, phosphate-buffered saline.

Evaluation of antitumor activity Tumor diameter was measured 3 times a week with calipers and the tumor size was calculated as the square root of long diameter × short diameter (mm). Each experimental and control group consisted of 10 mice. After 3 weeks, the animals were killed and each tumor was weighed to obtain the mean value(g) ± standard deviation. The difference in tumor growth (tumor size or tumor weight) between the control and experimental groups was tested statistically by using Student's *t*-test. The differences in cure rates were statistically evaluated by means of the chi-square test.

Lymph nodes resection and splenectomy Three to 5 days before tumor inoculation into BALB/c mice of the double grafted tumor system, the right side and the left side regional (axillary and inguinal) lymph nodes were resected. Spleens were also resected 3 days before tumor inoculation.

Preparation of TIL Preparation of tumor cell suspensions was carried out according to the method of Ferry *et al.*[4] with a modification. Briefly, Meth-A tumor tissues obtained from BALB/c mice 10 days after tumor inoculation were cut into 2–3 mm^3 fragments in RPMI-1640 medium (GIBCO Lab., Grand Island, NY), washed twice with RPMI and then digested with 40 μg/ml of deoxyribonuclease (Sigma, Type 1) and 250 μg/ml of collagenase (Sigma, Type 1A) at 37°C for 45 min. The cell suspension was passed through a stainless steel wire sieve (100 mesh) and washed with RPMI by centrifugation for 5 min at 150*g*. The sedimented cells were then resuspended in RPMI containing 1% fetal calf serum and 0.1% heparin (Hep-medium) and washed twice by centrifugation. An aliquot of the cell suspension was allowed to sediment in a 4 and 6% stepwise gradient of Ficoll 400 (Pharmacia Fine Chemicals, Uppsala) for 10 min at 20°C. Lymphocytes were collected in the upper fraction and washed in Hep-medium. Cell number and viability were determined by trypan blue dye exclusion. Murine TIL obtained from Meth-A tumors by this method contain less than 10% tumor cells. Some aliquots of TIL were treated with anti-Thy-1 monoclonal antibody (Cedarlane Lab. Ltd., Canada) and rabbit low cytotoxic complement.

Winn neutralizing assay The antitumor effect of TIL *in vivo* was investigated in normal recipient mice using the Winn neutralizing assay.[5] TIL were obtained from control and PSK treatment groups of mice 10 days after tumor inoculation. TIL (1.25×10^6) mixed with 2.5×10^5 Meth-A cells were injected into the flank of syngeneic BALB/c mice. Antitumor activity was assessed in terms of the tumor weight 21 days after the injection of the mixture, and tumor diameter was serially measured with calipers to estimate the tumor size.

Flow cytometric analysis of cell surface markers of TIL Fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies (anti-Thy-1, anti-Lyt-1, anti-Lyt-2, anti-L3T4 and anti-Mac-1 antibodies) were purchased from Becton-Dickinson, Inc., Sunnyvale, CA. The cell pellets (10^6) were incubated with 10 μl of FITC-conjugated antibody on ice for 30 min. The cells were then washed with phosphate-buffered saline (PBS, pH 7.2) three times and the staining pattern was analyzed using a Becton-Dickinson FACS analyzer.

Immunofluorescence staining Indirect immunofluorescence staining of tumor sections from mice administered PSK intratumorally was performed. In the double grafted tumor system, the right and the left tumors at day 7 after tumor inoculation were removed and frozen at −80°C. Cryostat sections of fresh tumors were fixed in cold acetone for 8 min and washed with PBS 3 times. Sections were then preabsorbed with normal goat serum for 15 min and washed with PBS. Anti-PSK rabbit antibody[6] was supplied by Biomedical Research Laboratory, Kureha Chemical Industry Ltd., Tokyo, diluted to 1:50 and applied as drops on the sections, which were then incubated for 60 min at 4°C. The sections were rinsed in 3 changes of PBS and stained with goat FITC-labeled anti-rabbit IgG, diluted to 1:100, for 30 min at 4°C. After rinsing in PBS, the sections were mounted in mounting medium (Difco Lab. Detroit, MI). The stained tumor tissue was examined under a fluorescence microscope (FLUOPHOT, Nikon, Tokyo).

Concomitant immunity and sinecomitant immunity Primary Meth-A cells (10^6 cells) were inoculated intradermally at −9 to −3 days into the right flank and 2×10^5 Meth-A cells were inoculated on day 0 into the left flank. The growth of the second challenge tumor was observed for 21 days to evaluate the generation of concomitant immunity. It is the simultaneous inoculations of Meth-A cells into the right and left flanks that constitute our double grafted tumor system. PSK was injected intratumorally into 3-, 4- and 5-day primary tumors.

Primary tumor cells (10^6 cells) were inoculated intradermally on day 0 into the right flank. On day 6 or day 10 after tumor inoculation, the primary tumor was resected and on day 21 10^6 secondary tumor cells were rechallenged into the left flank. The growth of the secondary tumor was observed for the following 21 days to evaluate the generation of sinecomitant immunity. PSK was administered orally every day from the day after primary tumor excision to the day before secondary tumor challenge.

RESULTS

Double grafted tumor system in nude mice In the double grafted tumor system in BALB/c nude mice, PSK inhibited the growth of neither the right tumor nor the left

tumor, as shown is Table I. The antitumor activity of PSK in the double grafted tumor system, therefore, is associated with a sequential immune mechanism in which T cells may play an important role.

Double grafted tumor system in mice with resected regional lymph nodes or spleen In order to examine the role of the regional lymph nodes and the spleen in the antitumor activity of PSK in the double grafted tumor system, regional (axillary and inguinal) lymph nodes were resected from BALB/c mice. As shown in Table II, the antitumor activity of PSK on the right tumor was weakened in mice with resected right regional lymph nodes. Furthermore, PSK antitumor activity on the left distant tumor became weaker in the following order: sham operation mice, resected right lymph node mice, resected right and left lymph node mice, as shown in Table III. The regional lymph nodes, therefore, play a very important role in the antimetastatic effect of intratumoral administration of PSK. A similar experiment using splenectomized mice revealed that the antitumor activity of PSK on the right and the left tumors in the double grafted tumor system was weakened, as shown in Fig. 1. Therefore, the spleen also has an important role in the antimetastatic effect of the intratumoral administration of PSK.

Antitumor effect of TIL TIL obtained from right- and left-flank tumors treated with PSK were examined by Winn neutralizing test for their antitumor activity against Meth-A sarcoma in BALB/c mice. As shown in Table IV, TIL from both flank tumors clearly inhibited the growth of admixed Meth-A cells but control TIL did not. The antitumor activity of TIL from neither tumor was lost after treatment with anti-Thy-1 monoclonal antibody plus complement as shown in Fig. 2. It is suggested, therefore, that macrophages in TIL of primary and metastatic tumors may play an important role in the antitumor activity of the intratumoral administration of PSK into a primary tumor.

Flow cytometric analysis of TIL TIL were obtained from Meth-A-bearing mice 10 days after tumor inocula-

Table I. Antitumor Effect of PSK in the Double Grafted Tumor System of BALB/c Nude Mice

Group		Right tumor (1×10^6)		Left tumor (2×10^5)	
		Tumor-free /tested	Tumor weight (g±SD)	Tumor-free /tested	Tumor weight (g±SD)
BALB/c(+/+)	Control	0/10	4.6 ± 0.65	0/10	2.3 ± 1.06
	PSK	1/10	$1.2\pm0.96^{a)}$**	4/10	$0.7\pm0.34^{a)}$**
BALB/c(*nu/nu*)	Control	0/10	$5.2\pm0.78^{b)NS}$	0/10	$2.4\pm0.58^{b)NS}$
	PSK	0/10	$5.1\pm0.79^{a)NSc)}$**	0/10	$2.9\pm0.56^{a)*c)}$**

BALB/c (+) and BALB/c (*nu/nu*) mice received simultaneous intradermal inoculations of Meth A fibrosarcoma cells in both the right (1×10^6 cells) and the left (2×10^5 cells) flanks on day 0. PSK (5 mg/0.1 ml/day, days 3, 4 and 5) was injected into the right tumor, and a 21-day observation period followed.
a) Significant difference from the corresponding control, *b*) BALB/c (+/+) control or *c*) BALB/c (+/+) PSK treated group; * $P<0.05$, ** $P<0.01$. NS, No significant difference.

Table II. Effect of Regional Lymph Nodes (LN) Resection on Antitumor Effect of PSK in the Double Grafted Tumor System (Right Tumor)

Group		Tumor-free /tested	Tumor diameter (mm±SD)	Tumor weight (g±SD)
Sham Op.	Control	0/10	19.76 ± 4.53	2.86 ± 1.36
	PSK	8/10**	8.99 ± 8.33**	0.64 ± 1.51**
2 right LN resection	Control	0/10	17.44 ± 3.11	2.12 ± 0.78
	PSK	3/10	9.63 ± 7.43*	0.96 ± 1.00*
2 right & 2 left LN resection	Control	0/10	18.66 ± 3.44	2.51 ± 1.02
	PSK	4/10	7.33 ± 6.34**	0.54 ± 0.36**

BALB/c mice were sham-operated or had the right regional (axillary and inguinal) lymph nodes or the right and the left regional lymph nodes resected 3 to 5 days before inoculation of Meth-A tumors. PSK (5 mg/day) was injected into the right tumor on days 3, 4 and 5 and a 21-day observation period followed. Significant difference from the corresponding control; ** $P<0.01$, * $P<0.05$.

Table III. Effect of Regional Lymph Node (LN) Resection on Antitumor Effect of PSK in the Double Grafted Tumor System (Left Tumor)

Group		Tumor-free /tested	Tumor diameter (mm±SD)	Tumor weight (g±SD)
Sham Op.	Control	0/10	19.43±3.61	2.76±1.44
	PSK	5/10[a)]*	9.59±6.57[a)]**	0.82±1.45[a)]**
2 right LN resection	Control	0/10	23.02±2.34	3.73±1.03
	PSK	2/10	14.18±9.08[a)]*	1.72±1.50
2 right & 2 left LN resection	Control	0/10	20.94±4.54	3.28±1.29
	PSK	0/10[b)]*	18.63±3.47[b)]**	2.63±1.36[b)]*

Experimental conditions were identical with those presented in Table II.
a) Significant difference from the corresponding control; *b*) significant difference from the corresponding sham op. group; ** $P<0.01$, * $P<0.05$.

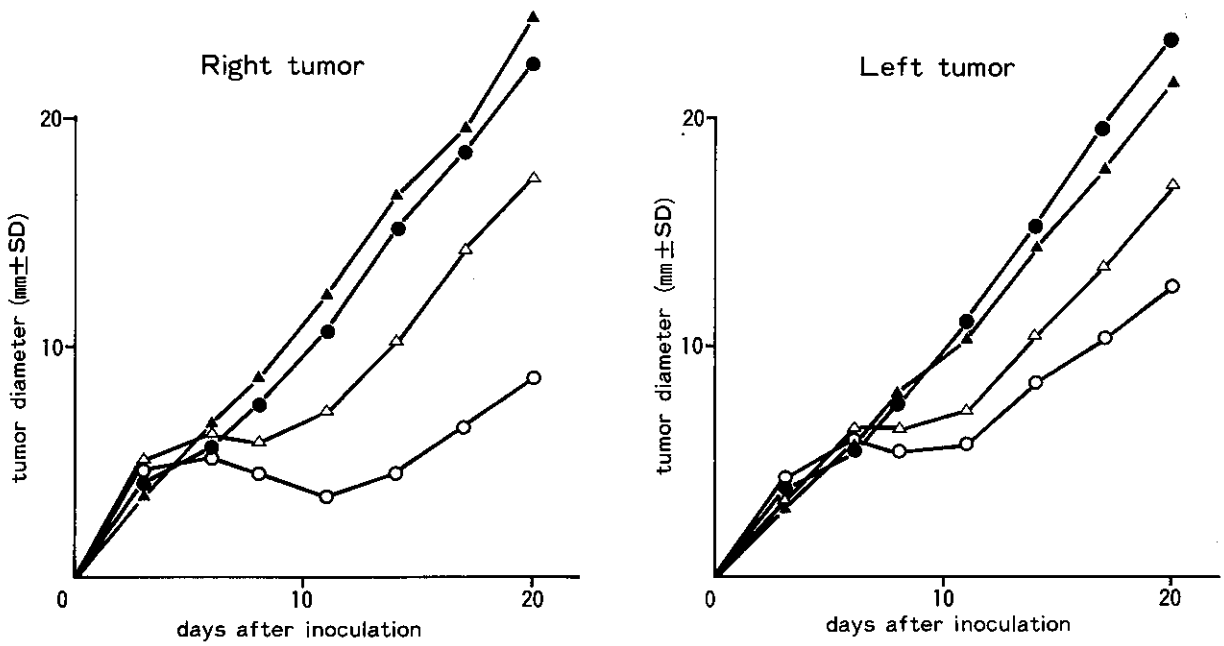

Fig. 1. Effect of splenectomy on the antitumor activity of PSK in the double grafted tumor system. Splenectomized BALB/c mice (△, ▲) or sham-operated mice (○, ●) 3 days before inoculation of Meth-A tumors. PSK (5 mg/day) was injected into the right tumor on days 3, 4 and 5 (○, △).

tion and analyzed for Thy-1, Lyt-1, L3T4, Lyt-2 and Mac-1 phenotypes using a FACS flow cytometer. As shown in Fig. 3, Mac-1-positive cells and Lyt-1-positive lymphocytes increased in right tumor TIL, and Mac-1-positive cells increased in the left tumor TIL after intratumoral administration of PSK into the right tumor in the double grafted tumor system.

Immunofluorescence staining using antiserum against PSK Using anti-PSK serum as staining agent, indirect immunofluorescence stainings of the right and the left tumors were carried out on day 7 in the double grafted tumor system. Fluorescence was observed in macrophages of the left tumor from mice administered intratumorally with PSK into the right tumor 2 days earlier (Fig. 4). This observation suggests that intratumoral injection of PSK may allow leakage of PSK from the tumor to the blood stream, so macrophages of left side tumor may have chance to engulf PSK, or that macrophages in the right tumor engulf PSK, then reach the left tumor through the blood stream, showing anti-

Table IV. Winn Assay of TIL Obtained from PSK-treated Mice in the Double Grafted Tumor System

Group	Tumor-free /tested	Tumor weight (g±SD)	Tumor diameter (mm±SD)
Control	0/10	2.99±0.95	22.04±3.48
TIL·control	0/10	2.50±0.68	20.86±1.85
TIL·PSK right	1/10	1.76±1.32*	15.18±4.60**
TIL·PSK left	4/10	1.65±1.34*	14.62±6.26**

TIL were obtained from 10-day Meth-A-bearing mice with or without PSK (5 mg/day, days 3, 4 and 5) injected into the right tumor. Normal BALB/c mice were injected subcutaneously with a mixture of TIL (1.25×10^6 cells) and Meth-A (2×10^5 cells), and observed for 21 days.
Significant difference from the TIL control group: * $P<0.05$, ** $P<0.01$.

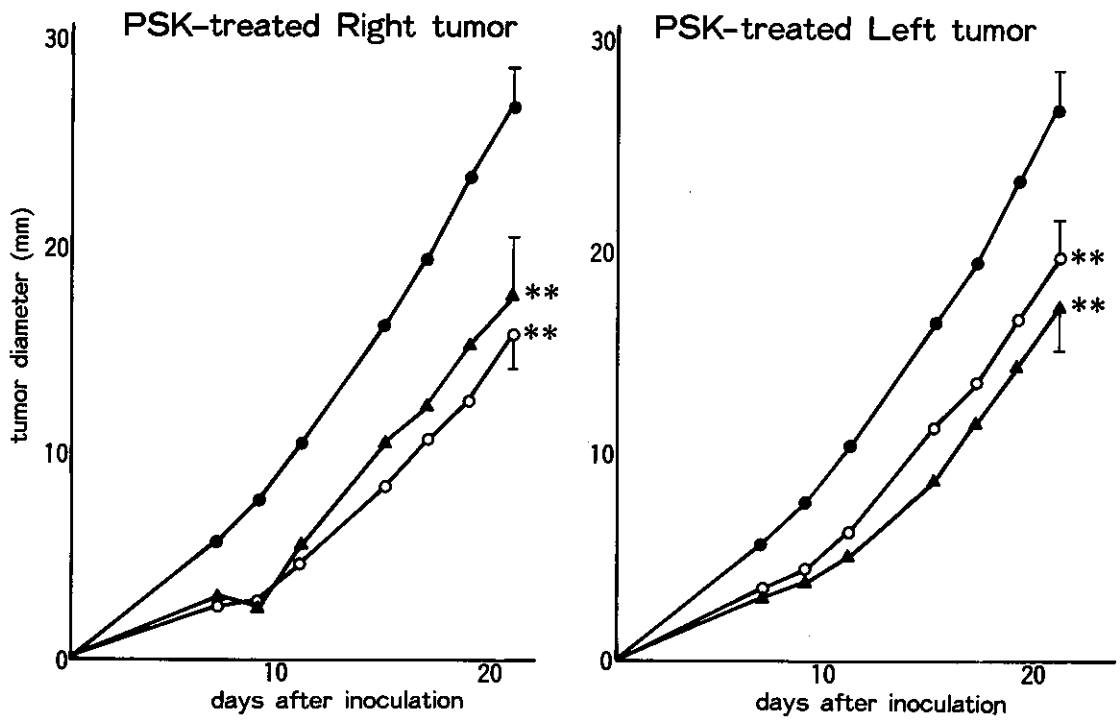

Fig. 2. Winn assay of TIL obtained from PSK-treated mice on day 10 in the double grafted tumor system. Normal mice were injected subcutaneously with a mixture of TIL (1.25×10^6 cells) and viable Meth-A (2.5×10^5 cells). An aliquot of TIL was treated with anti-Thy-1 antibody and complement (▲). Significant difference from the control group (●): ** $P<0.01$. Whole TIL (○).

tumor activity in the left tumor of the double grafted tumor system.

Enhancement of concomitant immunity by PSK treatment A primary growth of Meth-A sarcoma inoculated into the right flank of mice resulted in the generation of concomitant immunity to the growth of a second graft of the same tumor cells in the left flank with a peak intensity on day 7 of primary tumor growth, as shown in Table V, control column. A significant inhibitory effect on the proliferation of tumor cells inoculated secondarily in the left flank was shown in mice bearing a primary tumor that had been previously administered with PSK on days 3, 4 and 5. As shown in Table V, PSK column, the maximum enhancement of concomitant immunity by PSK treatment occurred on day 7 of primary tumor growth, that is, all 10 PSK-treated mice became tumor-free. These observations clearly show that intratumoral PSK administration enhances concomitant immunity

and leads to the eradication of the left, distant, small tumor.

Enhancement of sinecomitant immunity by oral administration of PSK Primary Meth-A tumor cells (10^6 cells) were inoculated intradermally on day 0 into the right flank, and the tumor was excised on day 6 or day 10. Secondary Meth-A cells (10^6 cells) were inoculated on day 21, and secondary tumor growth was observed for the following 21 days. PSK was administered orally every day at a dose of 5 mg/day from day 7 to day 20 in the day 6 tumor excision group and from day 11 to day 20 in the day 10 tumor excision group. As shown in Fig. 5, a significant inhibitory effect on the proliferation of tumor cells inoculated secondarily was observed in mice with primary tumors resected on day 6 and orally administered PSK. This observation suggests that PSK treatment enhances sinecomitant immunity and inhibits the regrowth of minute metastatic foci.

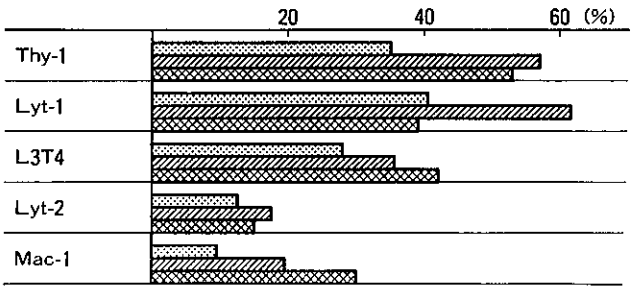

Fig. 3. Surface markers of TIL obtained from PSK-treated mice on day 10 in the double grafted tumor system. PSK was injected into the right tumor on days 3, 4 and 5. ▒, Control Meth-A TIL (day 10); ▨, right tumor TIL treated with PSK; ▩, left tumor TIL treated with PSK.

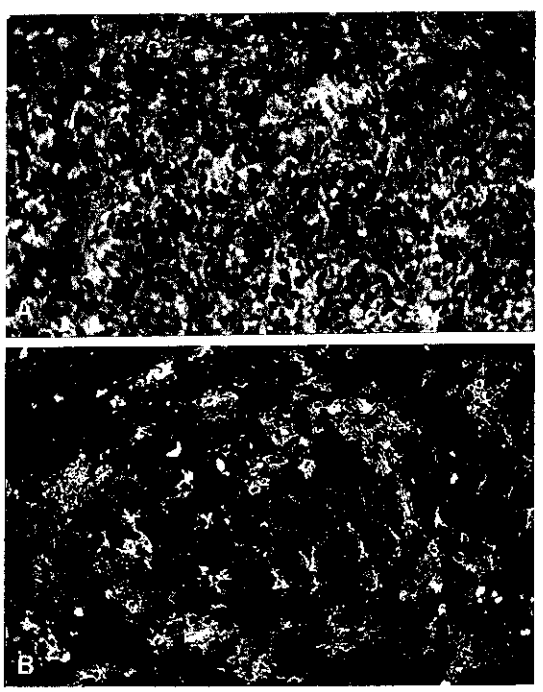

Fig. 4. Indirect immunofluorescence staining of Meth-A tumor. Indirect staining using anti-PSK rabbit serum and FITC-labeled anti-rabbit IgG. ×400. A) Control Meth-A tumor on day 7. B) Fluorescence-positive macrophages were observed in the left, non-treated tumor on day 7, 2 days after intratumoral administration of PSK into the right tumor of the double grafted tumor system.

DISCUSSION

In a previous immunohistochemical study,[2] it was shown that intratumoral administration of PSK induced first polymorphonuclear leukocytes and then macrophages in the right primary tumor. The present paper has shown that macrophages and Lyt-1-positive cells increased in the tumor-infiltrating lymphocytes (TIL) of the right tumor (Fig. 3) and that macrophages were involved in the antitumor activity *in vivo* (Fig. 2). We previously showed that intratumoral administration induced Lyt-1-positive cells first in the right regional lymph nodes and then in the spleen, and subsequently induced macrophages and Lyt-2-positive cells in the left (non-treated) tumor. The present paper has directly shown that Mac-1-positive cells increased in the left tumor TIL (Fig. 3) and had antitumor activity *in vivo* (Fig. 2). This suggests that the growth of a metastatic tumor phenotypically different from the primary tumor might be inhibited by the bystander effect[7,8] of tumor-infiltrating macrophages. Ferry *et al.*[4] reported that TIL from methylcholanthrene-induced sarcoma in rats have the cytotoxic properties of natural killer (NK) cells, not cytotoxic T lymphocytes (CTL). The effector cells had the morphology of large granular lymphocytes and the pattern of surface markers was identical with that of normal splenic NK cells. Holmes[9] reported that TIL from human pulmonary tumors were similar to peripheral blood lymphocytes (PBL) so far as their cell surface markers were concerned and that NK cell activity was markedly diminished in TIL, unlike in PBL. In addition, he reported that the direct injection of Bacille de Calmette et Guerin (BCG) into these tumors reverses this phenomenon by significantly increasing T cell and NK cell functional activity. Our previous immunohistochemical analysis and *in vitro* cytotoxicity assay showed that intratumoral injection of PSK did not enhance NK activity in spleen and lymph nodes or increase asialo-GM1-positive cells in either tumor.

Table V. Enhancement of Concomitant Immunity by PSK Treatment

Inoculation day of primary tumor	Tumor-free/10 mice		Tumor weight (g±SD)		Tumor diameter (mm±SD)	
	Control[b]	PSK[c]	Control	PSK	Control	PSK
−9 day	1	4	0.76±0.81	0.66±0.86	10.90±6.13	7.07±7.00
−7 day	5	10*	0.22±0.31	0	4.43±5.75	0
−5 day	2	4	2.21±2.13	0.95±1.28	14.70±8.68	8.79±8.79
−3 day	2	4	1.61±1.79	0.87±1.34	14.69±9.80	8.51±8.89
0 day[a]	0	3	2.69±1.41	0.73±1.66**	20.30±3.10	8.17±9.18**

Mice were inoculated intradermally with 1×10^6 Meth A cells in the right flank on the indicated day and further inoculated intradermally with 2×10^5 Meth A cells in the left flank on day 0, and the growth of the left tumor was monitored for 21 days.

a) 0 day refers to our "double grafted tumor system."

b) Suppression of the development of a second tumor in tumor-bearing mice was observed (concomitant immunity).

c) PSK (5 mg/day) was injected into the 3-, 4- and 5-day primary tumor. The secondary tumor was observed for 21 days.

Significant difference from the control group: ** $P<0.01$, * $P<0.05$.

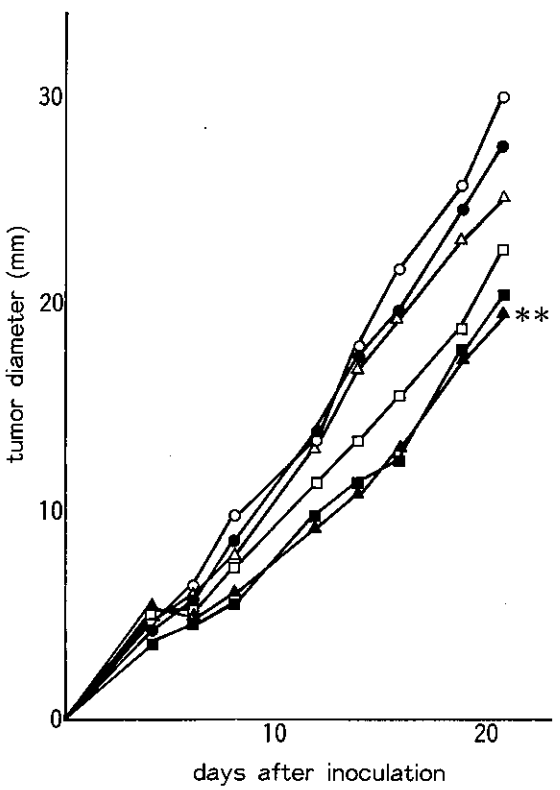

Fig. 5. Enhancement of sinecomitant immunity by oral administration of PSK. Removal of a 6-day (△, ▲) or 10-day (□, ■) primary tumor left the host with immunity to growth of the secondary (challenge) tumor on day 21. PSK (5 mg/day) was given orally every day from the day after excision of the primary tumor to the day before challenge with the secondary tumor (solid symbol: ●, ■, ▲). Significant difference between 6-day excision control group (△) and PSK-treated 6-day excision group (▲): ** $P<0.01$. ○, Sham-operated control.

Metastasis is one of the most serious problems in cancer, and its prevention is obviously of great importance for improving the prognosis of cancer patients. It may be suggested[10] that tumor cells and TIL in the primary tumor migrate to the regional lymph nodes through lymphatics and capillaries, then to the thoracic duct via the post capillary venules of lymph nodes and finally to the spleen and distant metastatic foci through arteries. The immunological role of the regional lymph node cells in tumor-bearing animals is still controversial.[11] The regional lymph node is the organ which initially recognizes antigens from the primary tumor and in which Lyt-1-positive lymphocytes, which also recognize the antigen, proliferate.[2] The spleen is the organ in which effector T cells are generated, which migrate to distant metastatic tumor(s) and show antitumor activity.[2] The present data clearly show that the regional lymph nodes play a very important role in antitumor activity against both the primary tumor (Table II) and metastatic tumor (Table III) after intratumoral administration of PSK into the primary tumor. These findings support the view that the extensive resection of regional lymph nodes should not be performed.

Fisher *et al.*[12] proposed two forms of tumor resistance: concomitant immunity displayed by hosts bearing a growing primary tumor against a second tumor challenge, and sinecomitant immunity displayed to a second tumor challenge after excision of the primary tumor. Suppression by a primary tumor of the growth of a metastatic tumor seems to be an instance of concomitant immunity.[13-18] North *et al.*[19-22] showed that when primary Meth-A cells were inoculated intradermally between −14 and −3 days into the right flank and Meth-A cells were inoculated on day 0 into the left flank, the growth of the second (challenge) tumor was inhibited. The intensity

of concomitant immunity showed a peak on days 6 to 9 after inoculation of primary tumor. Our present data confirm their data (Table V, control column) and show that the intratumoral injection of PSK on days 3, 4 and 5 in the primary tumor causes complete eradication of the secondary (challenge) tumor inoculated on day 7 (Table V, PSK column). That is, PSK treatment enhances concomitant immunity and leads to the eradication of distant, metastatic, small tumors.

In current clinical use,[3] PSK is administered orally after surgical operation. Thus, the effect of the oral administration of PSK on sinecomitant immunity was examined. It is shown in Fig. 5 that oral PSK treatment enhances sinecomitant immunity. That is, after the excision of a primary tumor on day 6, daily oral administration of PSK inhibits the growth of a secondary tumor inoculated on day 21. These concomitant and sinecomitant immunity experiments indicate that PSK can be administered for the treatment of cancer by first injecting it before surgical operation, and then administering it orally. It seems that this combination of PSK immunotherapy and surgical removal of a primary tumor is capable of bringing about the complete inhibition of metastatic tumor growth or the eradication of the tumor. Following our animal experiments, clinical studies in which primary breast, gastric and colon cancers are removed surgically after intratumoral PSK administration are in progress.

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