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Antitumor Effector Mechanism at a Distant Site in the Double Grafted Tumor System of PSK, a Protein-bound Polysaccharide Preparation

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The antitumor effect at a distant site of PSK, a *Coriolus* preparation, was analyzed with the 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 inhibited the growth of not only the right but also the left (non-treated) tumor. Immunized spleen cells were taken from mice which had been cured by the intratumoral administration of 5 mg of PSK and were injected into the Meth-A tumor on day 3. Adoptive transfer of PSK immunized spleen cells caused the complete regression of Meth-A tumors. The effector cell activity was lost only after treatment with anti-Lyt-1 monoclonal antibody plus complement. Spleen cells and right and left regional lymph node cells prepared from PSK immunized mice were examined for Thy-1, Lyt-1, Lyt-2 and asialo GM1 phenotypes. The number of Lyt-1-positive lymphocytes increased in the right regional lymph nodes after intratumoral administration of PSK. A massive accumulation of macrophages and polymorphonuclear leukocytes was found in the right tumor and an infiltration of macrophages and Lyt-2-positive lymphocytes was found in the left (non-treated) tumor by immunohistochemical analyses. These results suggest that intratumoral administration of PSK induces Lyt-1-positive cells first in regional lymph nodes, then in the spleen, and subsequently induces macrophages and Lyt-2-positive cells in the left (non-treated) tumor, thus bringing about the regression of metastatic tumors.

Key words: Antimetastatic effect — Biological response modifier — Intratumoral administration — Tumor immunity

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. In our previous paper,[1] the antitumor effect at a distant site of PSK, isolated from *Coriolus versicolor*,[2] a mushroom belonging to the *Basidomyocetes* class, was examined in the double grafted tumor system, in which BALB/c mice received simultaneous intradermal inoculations of Meth-A tumor cells in the right (1×10^6 cells) and left (2×10^5 cells) flanks, and were injected with PSK in the right-flank tumor (proximal tumor, hereafter referred to simply as primary region) 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 (distant tumor, metastatic region). The purpose of the present paper is to clarify the effector mechanism of PSK in the double grafted tumor system by 1) an adoptive transfer experiment, 2) FACS[*2] analysis, 3) *in vitro* cytotoxicity assay and 4) immunohistochemical analysis.

MATERIALS AND METHODS

Mice and Tumors Six-week-old male BALB/c mice were obtained from Shizuoka Laboratory Animal Center, Hamamatsu. Meth-A fibrosarcoma was administered to syngeneic BALB/c mice in solid form by intradermal inoculation.

Drugs PSK was supplied by Kureha Chemical Industry Ltd., Tokyo. Cyclophosphamide was purchased from Sigma Chemical Co., St. Louis, MO.

Treatment of Spleen Cells with a Nylon Wool Column Nylon wool (Wako Pure Chemical Industry, Ltd., Osaka) was soaked, dried and packed into a 20 ml syringe. Spleen cells (2×10^8 in 4 ml of RPMI 1640 medium with 10% fetal calf serum) were loaded on the nylon wool column.

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[*2] The abbreviations used are: FITC, fluorescein isothiocyanate; FACS, fluorescence activated cell sorter; NK, natural killer; PBS, phosphate-buffered saline; PAP, peroxidase antiperoxidase; PMN, polymorphonuclear leukocyte.

Treatment of Spleen Cells with Antibody and Complement Monoclonal antibodies to Thy-1.2, Lyt-1.1 and Lyt-2.1 were purchased from Sera-lab, Compiegne, France. Rabbit anti-asialo GM1 serum was purchased from Wako Pure Chemical Industry, Ltd. Rabbit low cytotoxic complement was purchased from Cederlane Laboratories Ltd., Canada. Spleen cells (10^7/ml) were incubated at 4° for 60 min with each of the above antibodies at a dilution of 1:500 for anti-Thy-1.2, anti-Lyt-1.1 and anti-Lyt-2.1 and 1:100 for anti-asialo GM1 in a volume of 0.5 ml. Cells were then washed and incubated at 37° for 45 min with rabbit complement at a final dilution of 1:10.

Evaluation of Antitumor Activity Antitumor activity was assessed in terms of tumor weight 21 days after the inoculation of Meth-A cells. Each experimental and control group consisted of 8 mice. The significance of the difference in tumor growth between the control and experimental groups in terms of tumor weight was tested statistically using Student's *t*-test.

Flow Cytometric Analysis of Cell Surface Markers of Lymphocytes FITC (fluorescein isothiocyanate)-conjugated monoclonal antibodies, anti-Thy-1.2, anti-Lyt-1.1 and anti-Lyt-2.1 antibodies were purchased from Becton-Dickinson, Inc., Sunnyvale, CA. Anti-asialo GM1 antibody was purchased from Wako Pure Chemical Ind. Ltd. In direct immunofluorescence assay, the cell pellets (10^6) were incubated with 10 μl of FITC-conjugated antibody on ice for 30 min. In indirect immunofluorescence assay, 10 μl of anti-asialo GM1 antibody was added to the cell pellets. The pellets were incubated on ice for 30 min, and washed with PBS (phosphate-buffered saline, pH 7.2) three times, then 100 μl of FITC-conjugated anti-rabbit IgG (Tago, Inc., USA) was added to the cell pellets and incubation was continued for a further 30 min on ice. The cells were washed with PBS three times, and the staining pattern was analyzed using a Becton-Dickinson FACS analyzer.

***In vitro* Cell-mediated Cytotoxicity Assay** Target cells used for *in vitro* cytotoxicity test were syngeneic (Meth-A, BAMC-1 and RL♂1), allogeneic (EL4, B16, P815 and YAC-1) and xenogenic human cells (K562, Daudi and KATO3) of different NK (natural killer) sensitivities. Cell-mediated cytotoxicity was assayed by a 4 hr chromium release method at an E/T ratio of 100:1.[3] The percentage of specific cytotoxicity was calculated using the following formula: % specific cytotoxicity = (experimental cpm − spontaneous cpm) / (total incorporated cpm − spontaneous cpm) × 100.

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.

Immunohistochemistry Three mice in each group (control or PSK treated) were sacrificed at 7 days and 14 days after intradermal tumor implantation. Right and left tumors from each group were excised, frozen in OCT compound (Lab-Tek Products, Miles Laboratoties Inc., Naperville, IL) and stored at −80°. The peroxidase-antiperoxidase (PAP) method of immunoperoxidase staining was used to visualize antibodies in frozen sections. The sections were fixed in 100% acetone for 10 min and then incubated with normal goat serum for 20 min to block nonspecific binding, and washed with PBS. Rat IgG2b anti-mouse-Thy 1-2, anti-Lyt-1.1, anti-Lyt-2.1, anti-L3T4 and anti-Mac-1 monoclonal antibodies (Sera-lab) at a final dilution of 1:100 and rabbit anti-asialo GM1 polyclonal antibody at a final dilution of 1:200 were incubated as primary antibodies on the sections for 1 hr. After washing of the sections with PBS, either goat anti-rat IgG serum (Wako Pure Chemical Ind. Ltd.) at a final dilution of 1:100 or sheep anti-rabbit IgG serum (Ortho Pharmaceutical Corp., USA) was incubated as a linking antibody on the sections for 40 min. After another wash with PBS, either rat peroxidase-antiperoxidase complex(Wako Pure Chemical Ind. Ltd.) or rabbit peroxidase-antiperoxidase complex (Ortho Pharmaceutical Corp.) was incubated as a labeling reagent on the sections for 40 min at room temperature. Following the addition of PAP reagent, the reaction was visualized by the addition of amino-ethyl carbazole-H_2O_2. Sections were counterstained with hematoxylin for 5 min. Histologic interpretations were made by a pathologist (Dr. Makoto Nose, Department of Pathology, Tohoku University School of Medicine, Sendai).

RESULTS

Adoptive Transfer of PSK-immunized Spleen Cells As described in a previous paper,[1] on day 3 PSK-immunized spleen cells (2×10^7 cells/mouse) were injected 1 hr after intravenous injection of cyclophosphamide (2 mg/mouse) into Meth-A tumors, the complete regression of which was brought about by this intratumoral adoptive transfer. Characterization of the effector cell subpopulation that showed antitumor activity in the adoptive transfer was conducted with spleen cells obtained from mice 7 and 21 days after tumor inoculation. PSK (5 mg/mouse) was intra-

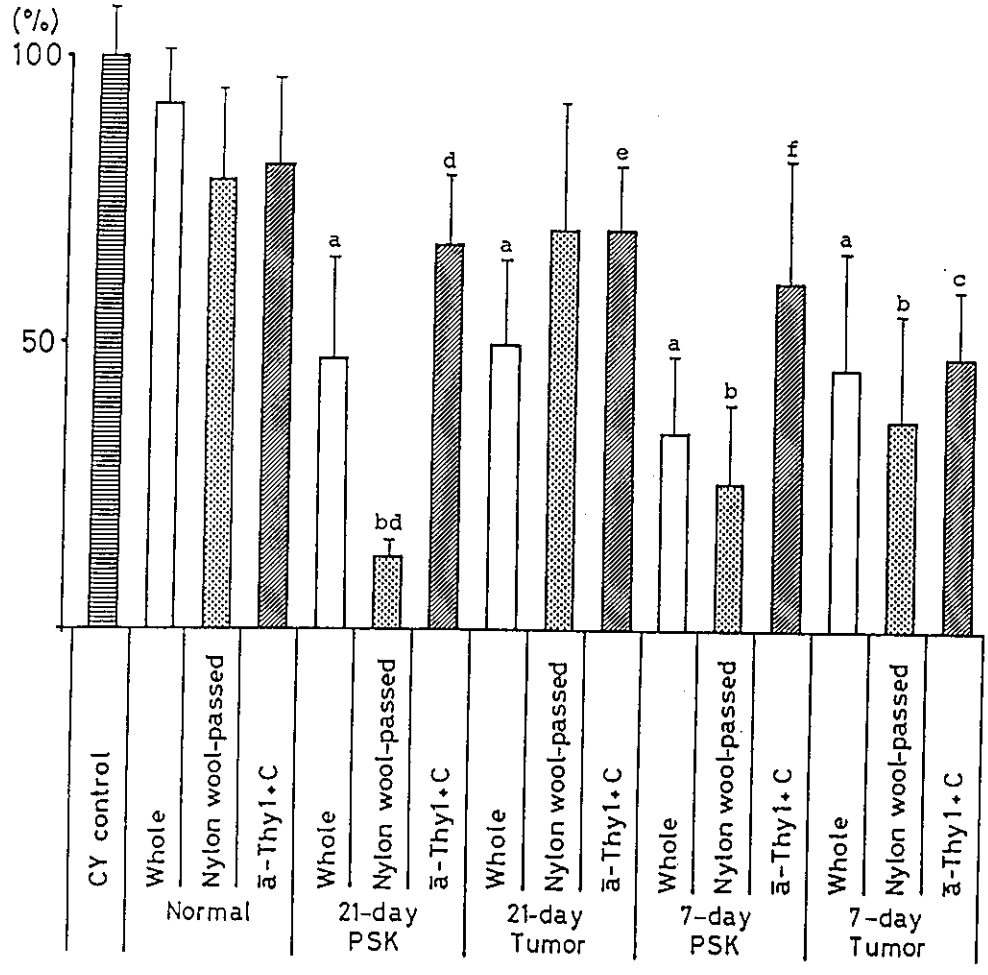

Fig. 1. Antitumor effect of activated spleen cells obtained after injection of PSK on the growth of Meth-A cells by intratumoral adoptive transfer. (CY), cyclophosphamide (2 mg/mouse) was injected intravenously 1 hr before spleen cells (2×10^7 cells/mouse) were injected into the Meth-A tumor on day 3. (Normal), normal mouse spleen; (7-day or 21-day), 7 days or 21 days after Meht-A tumor inoculation; (PSK), PSK-treated mice; (Tumor), Meth-A tumor-bearing mice; (Whole), whole spleen cells; (Nylon wool-passed), nylon wool column-passed spleen cells; (ā-Thy1+C), anti-Thy-1 monoclonal antibody and complement-treated spleen cells. Mean tumor weight of the CY control was 4.6 ± 0.4 g. Values are expressed as a percentage of the CY control and bars show standard deviation. Significant differences: a, vs. normal whole group ($P<0.01$); b, vs. normal nylon wool-passed group ($P<0.01$); c, vs. normal ā-Thy1+C group ($P<0.01$); d, vs. 21-day PSK whole group ($P<0.05$); e, vs. 21-day tumor whole group ($P<0.05$); f, vs. 7-day PSK whole group ($P<0.05$).

tumorally injected on days 3, 4 and 5. As shown in Fig. 1, antitumor activity was abolished by anti-Thy-1 monoclonal antibody treatment. When the adherent cells were removed by nylon wool column treatment, the antitumor activity was enhanced. The two experimental results clearly showed that it was nylon wool column-passed cells, i.e., T or NK cells, that were responsible for antitumor activity *in vivo*. Next, after PSK-immunized spleen cells were treated with anti-Lyt-1, anti-Lyt-2 or anti-asialo GM1 antibodies, adoptive transfer experiments were carried out. As shown in Fig. 2, the antitumor activity com-

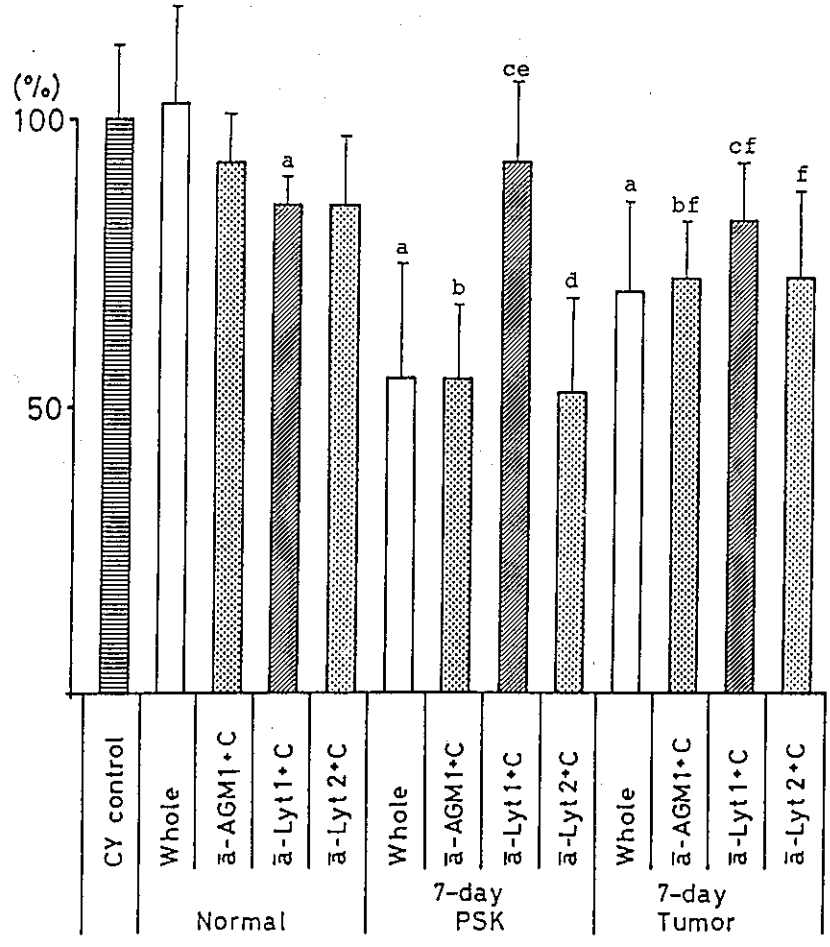

Fig. 2. Effect of various pretreatments on the capacity of spleen cells to suppress the growth of Meth-A tumors in intratumoral adoptive transfer. (CY), cyclophosphamide (2 mg/mouse) was injected intravenously 1 hr before spleen cells were injected into the Meth-A tumor on day 3. (Normal), normal mouse spleen; (7-day Tumor), Meth-A tumor-bearing mice 7 days after tumor inoculation; (7-day PSK), PSK-treated mice 7 days after tumor inoculation; (Whole), whole spleen cells; (ā-AGM1+C), anti-asialo GM1 plus complement treatment; (ā-Lyt1+C), anti-Lyt-1 monoclonal antibody plus complement treatment; (ā-Lyt2+C), anti-Lyt-2 monoclonal antibody plus complement treatment. Mean tumor weight of CY control was 4.0 ± 0.52 g and values are expressed as a percentage of the CY control. Bars show standard deviation. Significant differences: a, vs. normal whole group ($P<0.05$); b, vs. normal ā-AGM1+C group ($P<0.01$); c, vs. normal ā-Lyt-1+C group (not significant); d, vs. normal ā-Lyt-2+C group ($P<0.01$); e, vs. 7-day PSK whole group ($P<0.01$); f, vs. 7-day tumor whole group (not significant).

pletely disappeared after anti-Lyt-1 monoclonal antibody treatment. This suggests that the antitumor activity in PSK-immunized spleen might be due to Lyt 1^+2^- T cells.

Flow Cytometric Analysis of PSK-immunized Spleen and Lymph Node Cells Spleen cells and right and left regional (axillary and inguinal) lymph node cells prepared from PSK-immunized mice 7 and 14 days after tumor inoculation were examined for Thy-1, Lyt-1, Lyt-2 and asialo GM1 phenotypes with the use of a FACS analyzer. There were no differ-

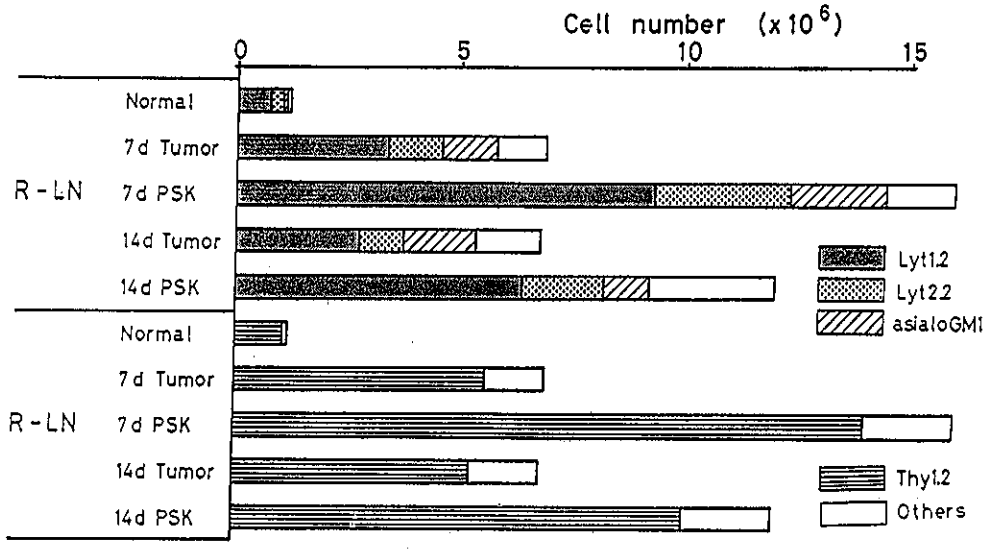

Fig. 3. Subpopulation of right regional (axillary and inguinal) lymph node cells with various cell surface markers. (Normal), normal mouse spleen; (7d or 14d), 7 days or 14 days after tumor inoculation; (Tumor), Meth-A tumor bearing mice; (PSK), Meth-A bearing mice treated with PSK on days 3, 4 and 5.

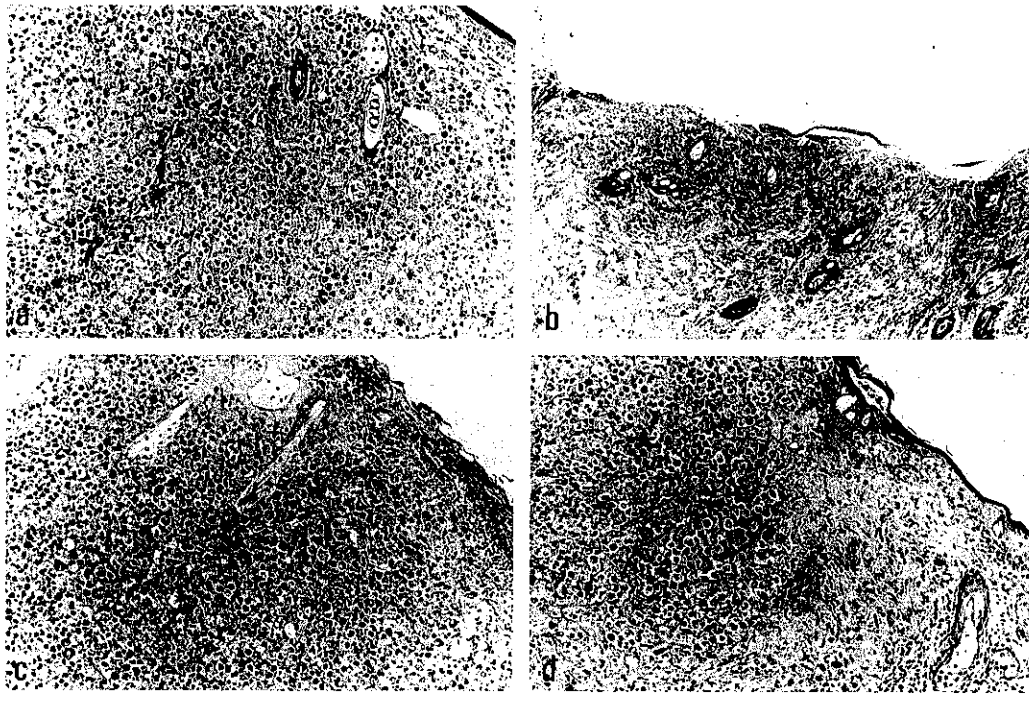

Fig. 4. Histologic appearance of murine Meth-A sarcoma. Hematoxylin and eosin, ×100. a) Control Meth-A on 6 days after intradermal inoculation. Note tumor cells infiltrating the whole dermis but not the epidermis. b) PSK-treated tumor on day 6, 1 day after intratumoral administration of PSK. Ulcer, necrosis and PMN infiltration are noted. c) Control Meth-A tumor on day 10. Tumor cells infiltrate the epidermis and ulceration is noted. d) PSK-treated tumor on day 10, 5 days after intratumoral administration of PSK. Reconstitution of epidermis and necrosis are noted.

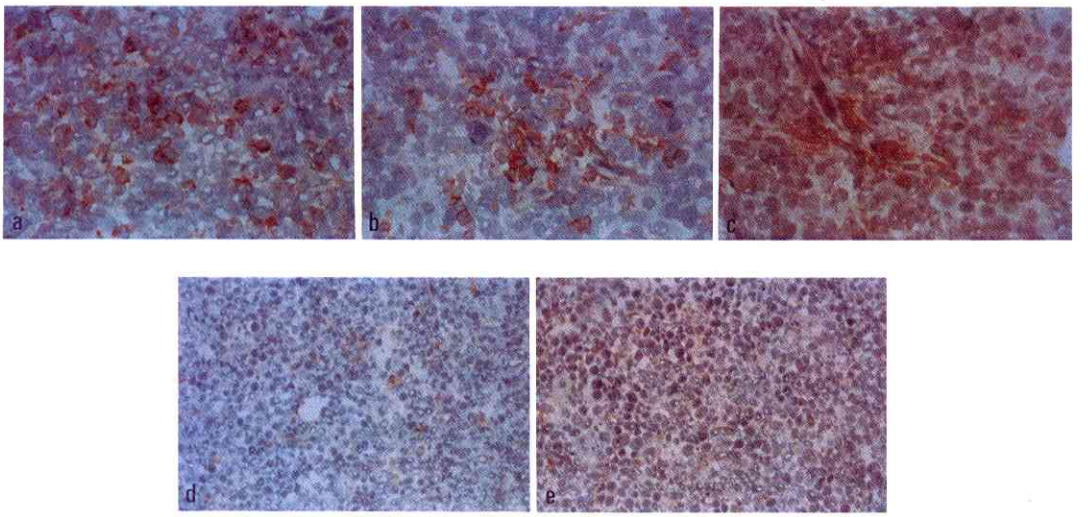

Fig. 5. Immunohistochemical appearance of Meth-A tumor. PAP immunocytochemical method with hematoxylin counterstain. Magnification of a, b, c, ×400; d, e, ×200. a) Abundant Mac-1 positive cells (macrophages) are detected in the PSK-treated, right tumor on day 7, 2 days after intratumoral administration of PSK. b) Increased numbers of Mac-1-positive cells (macrophages) are detected 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. c) Increased numbers of Lyt-2-positive (suppressor-cytotoxic) lymphocytes are detected 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. d) Control Meth-A right tumor on day 7. Few Mac-1-positive cells are detected. e) Control Meth-A left tumor on day 7. No Lyt-2-positive lymphocytes are detected.

ences in cell number and cell surface markers of spleen and left regional lymph node cells between the PSK-treated group and the tumor-bearing group. However, it is clear that the number of Lyt-1-positive lymphocytes increased in the right regional lymph nodes after intratumoral injection of PSK, as shown in Fig. 3.

***In vitro* Cytotoxicities of PSK-immunized Spleen and Lymph Node Cells** PSK-immunized spleen cells and regional lymph node cells were assayed for *in vitro* tumor cell killing activities 7 and 14 days after Meth-A tumor inoculation. Cytotoxicities *in vitro* against Meth-A tumor cells, NK-sensitive syngeneic RL♂1, NK-sensitive allogeneic YAC-1 cells and allogeneic tumor cells (EL-4, B16 and P815) were not enhanced by intratumoral injection of PSK. The most interesting aspect of the cytotoxicity data is that no significant effect against Meth-A is observed. Xenogenic killer cell activities against human K562, Daudi and KATO3 cells were also unchanged by PSK treatment. These results clearly showed that intratumoral injection of PSK did not enhance NK and broad-reactive killer (LAK) activities in spleen and lymph nodes.

Immunohistochemical Observations First, on days 6 and 10 after Meth-A tumor inoculation, histological analysis was carried out of the tumor into which 5 mg of PSK had been injected intratumorally on days 3, 4 and 5. By day 6, Meth-A tumor cells had infiltrated into the dermis, but the epidermis was intact (Fig. 4a). On the other hand, in PSK-injected tumors, ulceration, necrosis, and infiltration of polymorphonuclear leukocytes (PMN) were observed (Fig. 4b). Macrophages had surrounded residual tumor cells and filled the site of the previous tumor bed. Necrosis and karyoklasis were also observed. On day 10, Meth-A tumor cells infiltrating into the epidermis and ulcer were observed (Fig. 4c). In PSK-injected tumors, necrosis and reconstitution of the epidermis were observed (Fig. 4d).

Next, on days 7 and 14, immunohistochemical analyses of the right and left tumors in the double grafted tumor system were carried out with PAP staining. On day 7 (2 days after PSK intratumoral injection into the right tumor), massive infiltration of macrophages was seen in the right tumor (Fig. 5a). In the left, non-treated tumor, an infiltration of both macrophages (Fig. 5b) and Lyt-2-positive lymphocytes (Fig. 5c) was also seen. By contrast, few macrophages (Fig. 5d) and no Lyt-2-positive cells (Fig. 5e) were found in the control tumors. On day 14, tumor regression was clearly observed and a massive accumulation of macrophages was observed in the PSK-treated, right tumor. In the left, non-treated tumor, the numbers of macrophages and Lyt-2-positive cells were found to have increased.

Discussion

Intratumoral administration of PSK first induces polymorphonuclear leukocytes (PMN) in the right tumor (Fig. 4b) and then macrophages are induced (Fig. 5a) by a chemotactic factor, which might be produced by PMNs. Then Lyt-1-positive cells are induced in the right regional lymph nodes (Fig. 3) and in the spleen (Fig. 2), probably by interleukin-1, which might be produced from macrophages in contact with tumor cells. Subsequently, Lyt-1-positive cells reach the left tumor through the blood stream, come into contact with Meth-A tumors and then possibly produce macrophage activating factor[4] and interleukin-2. Intratumoral administration of PSK in the right tumor thus induces macrophages and Lyt-2 positive cells in the left, non-treated tumor (Figs. 5b and 5c).

By day 21 of our investigation, both the right tumor (primary region) and the left tumor (metastatic region) were cured and Lyt-1-positive memory cells were left in the spleen (Fig. 1), possibly causing the rejection of reinoculated tumor cells.[1]

Fisher *et al.*[5] proposed two forms of tumor resistance: 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. Suppression by a primary tumor of the growth of a metastatic tumor seems to be an instance of concomitant immunity. Bursuker and North[6,7] showed that when primary Meth-A (1×10^6) cells were inoculated intradermally on day -9 to -6 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 inhibited. These findings suggest that our PSK treatment might enhance concomitant immunity and lead to the eradication of the left distal tumor. Ogura *et al.*[8] showed that AC1/N rats bearing AMC 60 fibrosarcoma into which *Nocardia rubra* cell wall skeleton (N-CWS) was repeatedly injected showed a significant resistance to the proliferation of tumor cells inoculated secondarily into the peritoneal cavity. Peritoneal macrophages were found to have their tumoricidal activity against AMC tumor cells significantly augmented. These results suggested that the augmentation of concomitant immunity by injection of N-CWS into primary solid tumors was mainly due to potentiation of the tumoricidal activity of macrophages in the peritoneal cavity. However, in the system of these authors the growth of primary solid tumors was not inhibited. On the other hand, Meiss *et* al.[9] showed that concomitant resistance to a second tumor implant might be mediated by soluble factors secreted from systemic host cells. Recently, Nomi *et al.*[10] assessed the roles of concomitant and sinecomitant antitumor resistance in the regulation of metastatic outgrowth. Intravenous challenge of mice bearing antigenically different tumors revealed that concomitant inhibition was antigen-specific in the case of small tumor burdens, but nonspecific in the case of large tumor burdens. Nonimmunological inhibition of metastatic seedings might have been due to the production of metastatic inhibition factor by the large primary tumor. These authors also found that sinecomitant inhibition after excision of a large tumor was antigen-specific, probably representing an extension of specific concomitant immunity. However, sinecomitant inhibition after excision of a small tumor was not observed. Gorelik *et al.*[11–13] have reported an experimental metastasis model using Lewis lung carcinoma in C57BL/6 mice and Fidler[14] has reported a spontaneous metastasis model using B16-BL6 melanoma in C57BL/6 mice. Our new model using Meth-

A fibrosarcoma in BALB/c mice provides a useful tool for the investigation of the effector mechanisms of antimetastatic drugs.

Further analyses of both the cytotoxicities of tumor-infiltrating lymphocytes[15] in the left tumor and the soluble factors involved in antimetastatic effects in the double grafted tumor system are being carried out.

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REFERENCES

1) Ebina, T., Kohya, H., Yamaguchi, T. and Ishida, N. Antimetastatic effect of biological response modifiers in the "double grafted tumor system." *Jpn. J. Cancer Res. (Gann),* **77,** 1034–1042 (1986).
2) Tsukagoshi, S., Hashimoto, Y., Fujii, G., Kobayashi, H., Nomoto, K. and Orita, K. Krestin (PSK). *Cancer Treat. Rev.,* **11,** 131–155 (1984).
3) Saito, M., Ichimura, O., Kataoka, M., Moriya, Y., Ueno, K., Sugawara, Y., Nanjo, M. and Ishida, N. Pronounced antitumor effect of LAK-like cells induced in the peritoneal cavity of mice after intraperitoneal injection of OK-432, a killed Streptococcal preparation. *Cancer Immunol. Immunother.,* **22,** 161–168 (1986).
4) Sakamoto, K., Fujiwara, H., Nakajima, H., Yoshioka, T., Takai, Y. and Hamaoka, T. Requirements of adherent cells for activating Lyt-1^+2^- T cells as well as for functioning as antitumor effectors activated by factor(s) from Lyt-1^+2^- T cells. *Jpn. J. Cancer Res. (Gann),* **77,** 1142–1152 (1986).
5) Fisher, B., Saffer, E. A. and Fisher, E. R. Comparison of concomitant and sinecomitant tumor immunity. *Proc. Soc. Exp. Biol. Med.,* **135,** 68–71 (1970).
6) Bursuker, I. and North, R. J. Suppression of generation of concomitant antitumor immunity by passively transferred suppressor T cells from tumor-bearing donors. *Cancer Immunol. Immunother.,* **19,** 215–218 (1985).
7) North, R. J. The therapeutic significance of concomitant antitumor immunity. I. Lyt-1^+2^- T cells from mice with a progressive tumor can cause regression of an established tumor in γ-irradiated recipients. *Cancer Immunol. Immunother.,* **18,** 69–74 (1984).
8) Ogura, T., Hara, H., Yokota, S., Hosoe, S., Kawase, I., Kishimoto, S. and Yamamura, Y. Effector mechanism in concomitant immunity potentiated by intratumoral injection of *Nocardia rubra* cell wall skeleton. *Cancer Res.,* **45,** 6371–6375 (1985).
9) Meiss, R. P., Bonfil, R. D., Ruggiero, R. A. and Pasqualini, C. D. Histologic aspects of concomitant resistance induced by non-immunogenic murine tumors. *J. Natl. Cancer Inst.,* **76,** 1163–1175 (1986).
10) Nomi, S., Naito, K., Kahan, B. D. and Pellis, N. R. Effects of concomitant and sinecomitant immunity on postsurgical metastatis in mice. *Cancer Res.,* **46,** 6111–6115 (1986).
11) Gorelik, E., Segal, S. and Feldman, M. Growth of a local tumor exerts a specific inhibitory effect on progression of lung metastases. *Int. J. Cancer,* **21,** 617–625 (1978).
12) Gorelik, E., Segal, S. and Feldman, M. Control of lung metastasis progression in mice: role of growth kinetics of 3LL Lewis lung carcinoma and host immune reactivity. *J. Natl. Cancer Inst.,* **65,** 1257–1264 (1980).
13) Gorelik, E., Segal, S. and Feldman, M. On the mechanism of tumor "concomitant immunity." *Int. J. Cancer,* **27,** 847–856 (1981).
14) Fidler, I. J. Optimization and limitations of systemic treatment of murine melanoma metastases with liposomes containing muramyl tripeptide phosphatidylethanolamine. *Cancer Immunol. Immunother.,* **21,** 169–173 (1986).
15) De Weger, R. A., Wilbrink, B., Moberts, R. M. P., Mans, D., Oskam, R. and Otter, W. D. Immune reactivity of SL2 lymphoma-bearing mice compared with SL2-immunized mice. *Cancer Immunol. Immunother.,* **24,** 25–36 (1987).