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Antitumor Effect of PSK at a Distant Site: Inductions of Interleukin-8-like Factor and Macrophage Chemotactic Factor in Murine Tumor

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The antitumor effect of PSK, a *Coriolus* preparation, at a distant site was analyzed with the use of a double grafted tumor system in which male BALB/c mice received simultaneous intradermal inoculations of Meth-A tumor in the right (10^6 cells) and the left (2×10^5 cells) flanks and were then injected with PSK in the right tumor on the third day thereafter. The antitumor effect of intratumoral administration of PSK in the right tumor on days 3, 4 and 5 was compared with the effect of surgical resection of the right tumor on day 5. Three out of 8 mice given PSK intratumorally became tumor-free whereas no mouse tumor-free in the left flank was found among the surgically resected mice. As regards sinecomitant immunity, tumor inoculation into the right flank followed by intratumoral administration of PSK on days 3 and 5 and surgical excision of the primary tumor on day 6 resulted in complete rejection of a tumor challenge in the left flank on day 21. The combination of presurgical intratumoral injections of PSK (more than 2 times) and postoperative oral administration of PSK appeared to be most effective in eradicating secondary tumors. Isolated TILs (tumor-infiltrating lymphocytes), obtained from the right tumor (treated with PSK) and the left tumor on day 10 in the double grafted tumor system were cultured in RPMI1640 with 10% fetal calf serum for 24 h. The culture supernatants were harvested and tested for the presence of chemotactic activity for neutrophils or macrophages. Significant neutrophil chemotactic factor (NCF) and macrophage chemotactic factor (MCF) activities were detected in the culture media from PSK-treated TILs that had been cultured for 24 h. Neither significant neutrophil nor macrophage chemotactic activity was detected in the media from untreated TILs. NCF and MCF activities were also detected in the culture supernatant from PSK-treated tumor tissue on day 6. PSK-induced NCF in the murine tumor was neutralized by treatment with anti-human IL-8 IgG, and might be murine IL-8-like factor. Therefore, neutrophil and macrophage infiltrations of tumors following intratumoral injections of PSK are probably mediated by inductions of IL-8-like factor and MCF.

Key words: Biological response modifier — Neutrophil chemotactic factor — Macrophage chemotactic factor — Antitumor effect — Interleukin-8

In our previous papers,[1-5] the antitumor effects at a distant site of PSK, a protein-bound polysaccharide preparation, and recombinant human interleukin-1β (IL-1β)[2] were analyzed by using the double grafted tumor system in which male BALB/c mice received simultaneous intradermal inoculations of Meth-A tumor in the right (10^6 cells) and the left (2×10^5 cells) flanks and were then injected with PSK or IL-1β in the right-flank tumor on days 3, 4 and 5. Among several biological response modifiers (BRM) examined, PSK and IL-1β significantly inhibited the growth of not only the right but also the left (non-treated) tumor. The first purpose of the research described herein was to show that the intratumoral administration of PSK into the primary tumor has a superior antitumor effect against distant, residual tumors to the surgical removal of the primary tumor.

Recently, human neutrophil chemotactic factor (IL-8)[6] and monocyte chemotactic and activating factor (MCAF)[7] were cloned and sequenced. The second purpose of this work was to examine the induction of neutrophil chemotactic factor (NCF, supposed to be murine IL-8) and macrophage chemotactic factor (MCF, supposed to be murine MCAF) in tumor tissue and tumor-infiltrating lymphocytes (TIL) in response to the intratumoral injection of PSK. A previous immunohistochemical study[2] showed that intratumoral administration of PSK first induced polymorphonuclear leukocytes and macrophages in the right primary tumor and subsequently induced macrophages and Lyt-2 positive cells in the left (non-treated) residual tumor. The present paper shows that the induction of NCF and MCF is an important trigger of the antitumor cascade reaction caused by PSK treatment in the double grafted tumor system.

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[2] The abbreviations used are: NCF, neutrophil chemotactic factor; MCF, macrophage chemotactic factor; IL-1, interleukin-1; IL-8, interleukin-8; MCAF, monocyte chemotactic and activating factor; TIL, tumor-infiltrating lymphocytes; FCS, fetal calf serum; PEC, peritoneal exudate cells.

MATERIALS AND METHODS

Mice and tumor Six-week-old male BALB/c mice were obtained from the Institute for Experimental Animals, Tohoku University School of Medicine. 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. 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 tumor model. BALB/c mice received 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, distant region) flanks. Drugs are injected into the right-flank tumor on day 3, and the left (non-treated) tumor is observed for 21 days.

Sinecomitant immunity Initially, primary tumor cells (10^6 cells) were inoculated intradermally in the right flank. On day 6 after tumor inoculation, the primary tumor was resected, and on day 21, rechallenge was made by inoculating 10^6 tumor cells into the left flank. The growth of the secondary tumor was followed for 21 days to evaluate the generation of sinecomitant immunity. PSK was administered intratumorally on days 3 and 5 into the primary tumor.

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 the long diameter × short diameter (mm). After 3 weeks, the animals were killed and each tumor was weighed to obtain the mean value (g) ± standard deviation. The significance of differences in tumor growth (tumor size or tumor weight) between the control and experimental groups was tested statistically by using Student's *t* test. The significance of differences in cure rates were statistically evaluated by means of the chi-square test.

Preparation of cell culture supernatant of tumor tissue and tumor-infiltrating lymphocytes Right- and left-flank Meth-A tumors in the double grafted tumor system were obtained from BALB/c mice 6 days or 10 days after tumor inoculation. Tumor tissue was cut into 2–3 mm^3 fragments in RPMI1640 medium (Nissui Pharmaceutical Co.), washed twice with RPMI1640 and then digested with 40 μg/ml 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 RPMI1640 by centrifugation for 5 min at 150*g*. Tumor cells from Meth-A-bearing mice 6 days after tumor inoculation were cultured at a density of 1×10^6 cells/ml in RPMI1640 with 10% fetal calf serum (FCS) at 37°C for 24 h. After centrifugation, the culture supernatants were stored at −80°C until the chemotactic assay. TIL were obtained from Meth-A bearing mice 10 days after tumor inoculation by the method previously reported,[3] and cultured at a density of 1×10^6 cells/ml in PRMI1640 with 10% FCS at 37°C for 24 or 48 h. After centrifugation, the supernatants were stored at −80°C until the chemotactic assay.

Preparation of neutrophils One milliliter of 3% proteose peptone (Difco, Detroit, MI) was injected intraperitoneally into BALB/c mice, and a booster injection of 1 ml of proteose peptone was administered 12 h later. At 2 h after the booster injection, peritoneal exudate cells (PEC) were obtained by peritoneal lavage and used as neutrophil-rich cell suspensions. PEC were washed with RPMI1640 medium and suspended in RPMI1640 with 10% FCS. The quantity of neutrophils in PEC was determined to be 85% by May-Giemsa staining. The above-mentioned PEC were used as a target source of neutrophil chemotactic activity.

Preparation of macrophages One milliliter of 3% thioglycolate (Nissui Pharmaceutical Co.) was injected intraperitoneally into BALB/c mice. At 3 days after injection, PEC were obtained by peritoneal lavage and used as macrophage-rich cell suspension. PEC were washed with RPMI1640 and suspended in RPMI1640 with 10% FCS. The quantity of macrophages in PEC was 90% as determined by May-Giemsa staining. The above-mentioned PEC were used as a target source of macrophage chemotactic activity.

Assay for chemotactic factor Neutrophil and macrophage chemotaxis was assayed in multiwell chambers. Two hundred microliters of a cell suspension containing 5×10^5 PEC was introduced into each chemotactic chamber (Chemotaxicell, Kurabo, Osaka) fitted with a 5 μm pore size polycarbonate filter. The filters separated the cells from the chemotactic factors contained in the lower compartment (24 multiwell plate, Sumilon, MS-80240, Sumitomo Bakelite Co., Tokyo) in 500 μl of cell culture supernatant. After incubation for 2 h (neutrophils) or 3 h (macrophages) at 37°C in a CO_2 incubator, the filters were removed, fixed with May-Grünwald solution for 5 min, stained with Giemsa solution for 30 min and then dried on a glass slide. The filters were mounted on cover glasses with xylol-balsam, and the migrated cells were counted under a microscope (×400 or ×1000).

Neutralization by anti-human IL-8 IgG Anti-human IL-8 rabbit IgG (1 mg IgG/ml, P-801) and pre-immune rabbit control IgG (1 mg IgG/ml, C-100) were purchased from Endogen, Boston, MA. Two hundred micro-

liters of NCF sample was mixed with 300 μl of appropriately diluted anti-IL-8 IgG and incubated at 4°C for 45 min in the wells of 24 multiwell plates. The residual NCF activity was measured by the method described above.

Statistical studies Statistical analysis to determine the significance of differences of chemotactic activity was performed by the use of Student's t test.

RESULTS

Comparison of intratumoral administration of PSK with surgical operation In the double grafted tumor system the antimetastatic effect of intratumoral administration of 5 mg of PSK on days 3, 4 and 5 into the right, primary tumor was compared with the effect of surgical resection of the primary tumor on day 5. As shown in Table I, 3 out of 8 mice receiving PSK intratumorally became tumor-free on the left (metastatic tumor) flank on day 21. No mouse tumor-free in the left flank was observed among the surgically resected mice. Also, the tumor size in PSK-treated mice was significantly smaller than that in surgically operated mice, as shown in Fig. 1.

Enhancement of sinecomitant immunity by intratumoral administration of PSK In a previous report,[3] we showed that oral administration of PSK enhanced sinecomitant

Table I. Comparison of Intratumoral Administration of PSK with Surgical Operation

Group	Right tumor			Left tumor		
	Tumor-free /tested	Tumor diameter (mm±SD)	Tumor weight (g±SD)	Tumor-free /tested	Tumor diameter (mm±SD)	Tumor weight (g±SD)
Control	0/8	25.0±1.7	5.6±1.3	0/8	20.3±2.0	3.2±0.7
PSK (i.t.) (3, 4, 5d)	7/8	1.1±3.1	0.1±0.3	3/8	9.3±8.4*	1.1±1.3
Operation (5d)				0/6	18.1±3.0	2.4±1.0

* Significant difference from the 5d operation group: $P<0.05$.
SD: standard deviation.

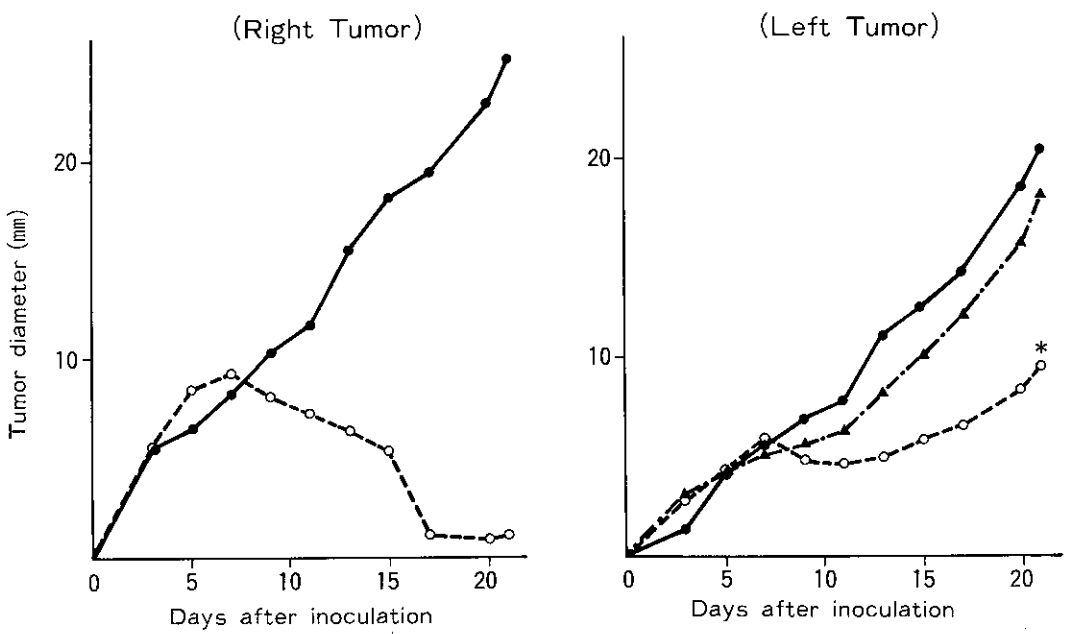

Fig. 1. Comparison of intratumoral administration of PSK with surgical operation. In the double grafted tumor system, PSK was injected in the right tumor on days 3, 4 and 5 (○). In the surgical operation group, the right tumor was resected on day 5 (▲). Control tumor (●). * Significant difference from the 5-day operation group, $P<0.05$.

Table II. Enhancement of Sinecomitant Immunity by Intratumoral Administration of PSK

Group	Tumor-free /tested	Tumor diameter (mm±SD)	Tumor weight (g±SD)
Control	0/10	21.85±2.91	3.95±0.75
Meth-A control	4/7	7.47±6.65**	0.47±0.68**
Meth-A, 3d PSK i.t.	5/8	8.83±4.54**	0.43±0.42**
Meth-A, 3d, 5d PSK i.t.	8/8**	0	0

** Significant difference from the control group: $P<0.01$.
SD: standard deviation.

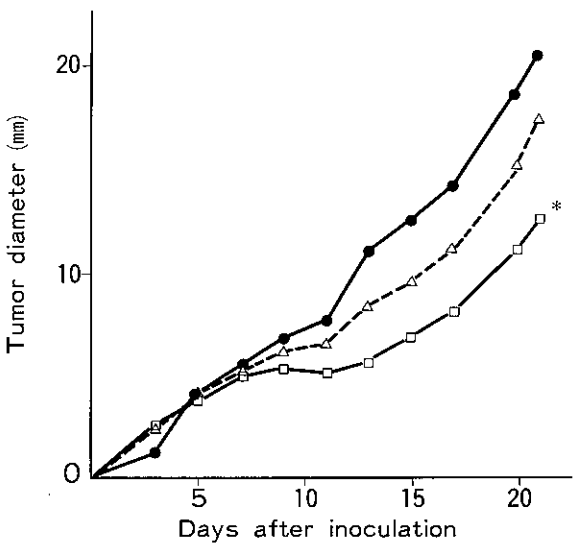

Fig. 2. Effect of presurgical intratumoral administration of PSK on antitumor activity. ●: After the surgical removal of the primary tumor on day 7, the challenge tumor was inoculated on day 21 and observed for 21 days thereafter. △: After the surgical resection of the primary tumor, PSK was administered orally every day from day 8 to day 20. □: Before surgical resection of the primary tumor, PSK was injected into the tumor on days 3, 4 and 5 and PSK was administered orally from day 8 to day 20. * Significant difference from the control, $P<0.05$.

immunity. As shown in Table II, intratumoral administration of PSK on days 3 and 5, before surgical excision of the primary tumor on day 6, resulted in complete rejection of the challenge on day 21. That is, the intratumoral administrations of PSK strongly enhanced sinecomitant immunity. Next, the effect of presurgical intratumoral administration of PSK on antimetastatic activity was investigated in mice orally given PSK. After the surgical excision of the primary tumor on day 7, PSK was administered orally every day a dose of 5 mg/day from day 8 to day 20. The mice were challenged on day 21 and subsequently observed for 21 days. As shown in Fig. 2, a significant inhibitory effect on the proliferation of tumor cells inoculated secondarily was observed in mice that had received intratumoral administration as compared with the mice not given intratumoral administration.

Induction of NCF from PSK-treated tumor tissue and TIL The culture supernatants of the 6-day tumor tissue were assayed for NCF activity. As shown in Table III, significant NCF activity was detected when the tumor was treated with PSK. This factor was found to be chemotactic and not chemokinetic by checkerboard analysis. No significant NCF activity was produced by untreated tumor tissue. The supernatants of TIL obtained from right- and left-flank tumors on day 10 were assayed for NCF. As shown in Table IV, significant NCF activity was detected when TILs were treated with PSK.

Characterization of PSK-induced NCF PSK-induced NCF was characterized by means of the neutralization

Table III. Neutrophil Chemotactic Factor Produced from PSK-treated Tumor Tissue

Culture supernatant	No. of migrated neutrophils±SD per field (×400)
Medium control	6.0±2.9
Meth-A tumor	5.7±2.9
PSK-treated Meth-A, right tumor	21.9±6.7**
PSK-treated Meth-A, left tumor	13.7±3.4**

** Significant difference from the Meth-A tumor group: $P<0.01$.
SD: standard deviation.

Table IV. Neutrophil Chemotactic Factor Induced from PSK-treated TIL

Culture supernatant	No. of migrated cells±SD per visual field (×1000)
Meth-A TIL	0.9±0.7
PSK-treated Meth-A, right tumor TIL	6.5±2.5**
PSK-treated Meth-A, left tumor TIL	3.7±1.8**

** Significant difference from the Meth-A TIL: $P<0.01$.
SD: standard deviation.

Table V. Neutralization of PSK-induced NCF by Anti-IL-8 IgG

Treatment[a] (IgG dilution)	No. of migrated neutrophils ± SD per visual field (×1000)
None	10.5±2.3**
ā IL-8 (1:500)	9.5±3.0**
ā IL-8 (1:250)	8.3±1.9
ā IL-8 (1:100)	7.1±3.0
ā IL-8 (1:50)	4.8±3.4
ā IL-8 (1:20)	3.5±1.6
Preimmune rabbit IgG (1:20)	12.8±3.2**
Medium control (RPMI, 10% FCS)	3.9±2.9

a) Anti-human interleukin-8 rabbit IgG (1 mg IgG/ml) was treated with neutrophil chemotactic factor for 45 min at 4°C. Culture supernatant of PSK-treated Meth-A right tumor tissue 24 h after incubation was used as NCF.
** Significant difference from the medium control: $P<0.01$.
SD: standard deviation.

Table VI. Macrophage Chemotactic Factor Produced from PSK-treated Tumor Tissue

Culture supernatant	No. of migrated macrophages ± SD per field (×100)
Medium control	2.9±2.3
Meth-A tumor	6.9±2.5
PSK-treated Meth-A, right tumor	11.6±3.9*
PSK-treated Meth-A, left tumor	11.8±2.5*

* Significant difference from the Meth-A tumor group: $P<0.05$.
SD: standard deviation.

test using anti-human IL-8 IgG. Neutralization was expressed in terms of the residual NCF activity. As shown in Table V, PSK-induced NCF activity was neutralized by a rabbit IgG against human IL-8 but was neutralized by a preimmune rabbit IgG. These results suggest that PSK-induced NCF in mice was cross-reactive with human IL-8 and might be an IL-8-like factor.

Induction of MCF from PSK-treated tumor tissue and TIL The culture supernatants of the 6-day tumor tissue were assayed for MCF activity. As shown in Table VI, significant MCF activity was detected when the tumor was treated with PSK. This factor was found to be chemotactic and not chemokinetic by checkerboard analysis. No significant MCF activity was produced by untreated tumor tissue. The supernatants of TIL obtained from the right- and left-flank tumors on day 10 were assayed for MCF activity. As shown in Table VII, significant MCF activity was detected when TILs were treated with PSK. No significant MCF activity was produced by unstimulated TIL.

Table VII. Macrophage Chemotactic Factor Induced from PSK-treated TIL

Culture supernatant	No. of migrated cells ± SD per visual field (×400)	
	24 h after incubation	48 h after incubation
Medium control	6.6±3.2	6.5±5.3
Meth-A TIL	10.4±4.5	6.5±3.2
PSK-treated Meth-A, right tumor TIL	30.2±12.4**	32.5±19.4**
PSK-treated Meth-A, left tumor TIL	15.6±6.2*	20.8±11.2**

Significant difference from the Meth-A TIL: ** $P<0.01$, * $P<0.05$.
SD: standard deviation.

DISCUSSION

Many chemotactic cytokines have been reported. Monocyte-derived neutrophil chemotactic factor (MDNCF),[8] T lymphocyte chemotactic factor (TCF) and neutrophil-activating protein (NAP-1)[9] have been reported to have neutrophil chemotactic properties. Recently, these human chemotactic factors have been purified and gene-cloned.[6] In view of the effect on neutrophils and the production by multiple cell types, these cytokines were renamed interleukin-8 (IL-8). Monocyte chemotactic and activating factor (MCAF),[10] monocyte or macrophage chemotactic factor (MCF)[11] and monocyte chemoattractant protein (MCP-1)[12] have been reported to have monocyte chemotactic properties. Recently, the cDNA for human MCAF was cloned and sequenced.[7] It has been reported that human fibroblasts and endothelial cells were stimulated to produce IL-8 and MCAF in response to IL-1 or tumor necrosis factor[13, 14] and that human peripheral blood mononuclear cells were stimulated to produce IL-8 and MCAF in response to phytohemagglutinin or lipopolysaccharide.[15] Larsen *et al.*[14] reported that intradermal injection of IL-1 caused infiltration of leukocytes at the sites of injection and that the *in vivo* attraction of neutrophils was mediated by locally produced IL-8. Also, it has been reported that various types of malignant cells produce MCF *in vitro*.[16] Such tumors are often heavily infiltrated with monocytes

in vivo, and this is associated with a better prognosis.[17] The present report shows that murine NCF and MCF are induced in tumor tissue (Tables III and VI) and TILs (Tables IV and VII) by intratumoral injection of PSK, a protein-bound polysaccharide preparation. We also found that PSK-induced NCF was neutralizaed by anti-human IL-8 IgG (Table V). Recently, murine macrophage inflammatory protein 2 (MIP-2) was found to be a potent chemotactic agent for human neutrophils and to have considerable sequence homology with IL-8.[8, 18] Hirose *et al.* showed that IL-8 and MCAF mRNA in human peripheral blood mononuclear cells were induced by PSK treatment *in vitro* (personal communication). Therefore, neutrophil and macrophage infiltrations of tumors caused by intratumoral injection of PSK[2] may be mediated by the induction of NCF (supposedly murine IL-8) and MCF (supposedly murine MCAF). Murine NCF (IL-8-like factor) may be the first vital paticipant in the cascade of interacting cytokines and effector cells that is induced by intratumoral administration of PSK in the double-grafted tumor system. Our present and previous studies[2, 3] on the antitumor effect of PSK in the double grafted tumor system show that the intratumoral administration of PSK first induces neutrophils in the right tumor via an IL-8-like factor (Tables III and V) and then macrophages are induced by an MCF (Table VI) which might be produced from tumor cells and neutrophils. Then Lyt-1 (L3T4)-positive cells[5] are induced in the right regional lymph nodes and in the spleen, probably via IL-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 produce MCF (Table VII) and interleukin-2. Intratumoral administration of PSK in the right tumor thus induces activated macrophages and Lyt-2-positive killer T cells in the left, non-treated tumor, thereby bringing about the regression of the distant tumor.

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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.*, **77**, 1034–1042 (1986).
2) Ebina, T. and Kohya, H. Antitumor effector mechanism at a distant site in the double grafted tumor system of PSK, a protein-bound polysaccharide preparation. *Jpn. J. Cancer Res.*, **79**, 957–964 (1988).
3) Ebina, T., Kohya, H. and Ishikawa, K. Antitumor effect of PSK: role of regional lymph nodes and enhancement of concomitant and sinecomitant immunity in the mouse. *Jpn. J. Cancer Res.*, **80**, 158–166 (1989).
4) Ebina, T. and Ishikawa, K. Antitumor effect of interleukin-1β in the double grafted tumor system. *Jpn. J. Cancer Res.*, **80**, 570–576 (1989).
5) Kohya, H., Ebina, T., Yamaguchi, T. and Ishida, N. The "double grafted tumor system," proposed to find effector cells in the analyses of antitumor effect of BRMs. *Biotherapy*, **1**, 139–151 (1989).
6) Mukaida, N., Shiroo, M. and Matsushima, K. Genomic structure of the human monocyte-derived neutrophil chemotactic factor IL-8. *J. Immunol.*, **143**, 1366–1371 (1989).
7) Furutani, Y., Nomura, H., Notake, M., Oyamada, Y., Fukui, T., Yamada, M., Larsen, C. G., Oppenheim, J. J. and Matsushima, K. Cloning and sequencing of the cDNA for human monocyte chemotactic and activating facotr (MCAF). *Biochem. Biophys. Res. Commun.*, **159**, 249–255 (1989).
8) Yoshimura, T., Matsushima, K., Tanaka, S., Robinson, E. A., Appella, E., Oppenheim, J. J. and Leonard, E. J. Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. *Proc. Natl. Acad. Sci. USA*, **84**, 9233–9237 (1987).
9) Larsen, C. G., Anderson, A. O., Appella, E., Oppenheim, J. J. and Matsushima, K. The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. *Science*, **23**, 1464–1466 (1989).
10) Larsen, C. G., Zachariae, C. D., Oppenheim, J. J. and Matsushima, K. Production of monocyte chemotactic and activating factor (MCAF) by human dermal fibroblasts in response to interleukin-1 or tumor necrosis factor. *Biochem. Biophys. Res. Commun.*, **160**, 1403–1408 (1989).
11) Yoshizuka, N., Yoshimura, M., Tsuchiya, S., Okamoto, K., Kobayashi, Y. and Osawa, T. Macrophage chemotactic factor (MCF) produced by a human T cell hybridoma clone. *Cell. Immunol.*, **123**, 212–225 (1989).
12) Yoshimura, T., Yuhki, N., Moore, S. K., Appella, E., Lerman, M. I. and Leonard, E. J. Human monocyte chemoattractant protein-1 (MCP-1), full-length cDNA cloning, expression in mitogen-stimulated blood mononuclear leukocytes, and sequence similarity to mouse competence gene JE. *FEBS Lett.*, **244**, 487–493 (1989).
13) Matsushima, K., Morishita, K., Yoshimura, T., Lavu, S., Kobayashi, Y., Lew, W., Appella, E., Kung, H. F.,

Leonard, E. J. and Oppenheim, J. J. Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. *J. Exp. Med.*, **167**, 1883–1893 (1988).

14) Larsen, C. G., Anderson, A. O., Oppenheim, J. J. and Matsushima, K. Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumor necrosis factor. *Immunology*, **68**, 31–36 (1989).

15) Yoshimura, T., Matsushima, K., Oppenheim, J. J. and Leonard, E. J. Neutrophil chemotactic factor produced by lipopolysaccharide (LPS)-stimulated human blood mononuclear leukocytes: partial characterization and separation from interleukin 1 (IL-1). *J. Immunol.*, **189**, 788–793 (1987).

16) Matsushima, K., Larsen, C. G., Du Bois, G. C. and Oppenheim, J. J. Purification and characterization of a novel monocyte chemotactic and activating factor produced by a human myelomonocytic cell line. *J. Exp. Med.*, **169**, 1485–1490 (1989).

17) Benomar, A., Ming, W. J., Taraboletti, G., Ghezzi, P., Balotta, C., Cianciolo, G. J., Snyderman, R., Dore, J. F. and Mantovani, A. Chemotactic factor and P15E-related chemotaxis inhibitor in human melanoma cell lines with different macrophage content and tumorigenicity in nude mice. *J. Immunol.*, **138**, 2372–2379 (1987).

18) Wolpe, S. D., Sherry, B., Juers, D., Davatelis, G., Yurt, R. W. and Cerami, A. Identification and characterization of macrophage inflammatory protein 2. *Proc. Natl. Acad. Sci. USA*, **86**, 612–616 (1989).