Jpn. J. Cancer Res. 82, 1292–1298, November 1991

Antitumor Effector Mechanism of Interleukin-1β at a Distant Site in the Double Grafted Tumor System

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Recombinant human interleukin-1β (IL-1β) inhibited the growth of not only the right, but also the left non-treated tumor in a double grafted tumor system. Since the antitumor activity of IL-1β against the right and left tumors was not seen in nude mice, lymphocytes have a key role in the antitumor effect of intratumoral administration of IL-1β. TIL (tumor-infiltrating leukocytes) obtained from left and right side tumors treated with IL-1β were examined by Winn assay for their antitumor activity against Meth-A sarcoma in BALB/c mice. TIL from the right side clearly inhibited the growth of admixed Meth-A cells, but control TIL did not. Spleen cells and right and left regional lymph node cells prepared from IL-1-treated mice were examined for Lyt-1, Lyt-2 and L3T4 phenotypes. The number of Lyt-1-positive lymphocytes increased in the spleen and in the right regional lymph nodes after intratumoral administration of IL-1. Isolated tumor cells obtained from the right tumor treated with IL-1β and the left side tumor on day 6 were cultured in RPMI 1640 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 and macrophage chemotactic factor activities were detected in the culture media from IL-1-treated tumor tissues cultured for 24 h. Neither significant neutrophil nor macrophage chemotactic activity was detected in the media from untreated tumor tissues. These results suggest that intratumoral administration of IL-1 first induces neutrophils and macrophages in the right tumor, then Lyt-1-positive cells in the right regional lymph nodes and in the spleen, and subsequently induces macrophages in the left, non-treated tumor.

Key words: Interleukin-1 — Interleukin-8 — Monocyte chemotactic factor — Antitumor effect — Sinecomitant immunity

Interleukin-1 (IL-1)[2] was so designated at the Second International Lymphokine Workshop held in Switzerland in 1979. After more than ten years, IL-1 can now be regarded as quite well characterized.[1)] Its relations with other cytokines and differentiated tissues suggest that it is a basic mediator of intercellular communication both within the immune system and between the immune system and other organ systems. *In vivo* studies of IL-1 have revealed its physiologic and pathogenic significance.[2)] In our previous paper,[3)] it was first shown that recombinant human interleukin-1β (IL-1β) has an antitumor activity *in vivo*. That is, the antitumor effect at a distant site of IL-1β, 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 IL-1β in the right-flank tumor on day 3. IL-1β significantly inhibited the growth of not only the right but also the left (non-treated) tumor. The first purpose of the present study was to clarify the mechanism of action of IL-1β in the double grafted tumor system by 1) a nude mouse experiment, 2) evaluating the antitumor effect of tumor-infiltrating leukocytes (TIL), 3) a FACS (fluorescence-activated cell sorter) analysis and 4) an analysis of neutrophil and macrophage chemotactic activity. The second purpose of this investigation was to examine whether IL-1β 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 tumor 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 Recombinant human interleukin-1β 71Ser mutant[4)] (rIL-1β) was supplied by Otsuka Pharmaceu-

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[2] The abbreviations used are: IL-1, interleukin-1; IL-8, interleukin-8; MCAF, monocyte chemotactic and activating factor; TIL, tumor-infiltrating leukocytes; PEC, peritoneal exudate cells; FACS, fluorescence-activated cell sorter; PBS, phosphate-buffered saline; FCS, fetal calf serum; NCF, neutrophil chemotactic factor.

tical Co. Ltd., Tokushima (lymphocyte activating activity: 2×10^7 units/mg protein).

Double grafted tumor system As described in previous papers,[5-7] we devised the double grafted tumor system as a new experimental tumor model. 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, distant region) flanks. Drugs are injected into the right-flank tumor on days 3, 4 and 5, and the left (non-treated) tumor is observed for 21 days.

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. Each experimental and control group consisted of 7 to 10 mice. The significance of 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.

Preparation of TIL Preparation of tumor cell suspensions was carried out according to the method of Ferry *et al.*[8] with some 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 10 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. Leukocytes 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.

Winn neutralizing assay The antitumor effect of TIL *in vivo* was investigated in normal recipient mice using the Winn neutralizing assay.[9] TIL were obtained from control and IL-1β 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 lymphocytes FITC (fluorescein isothiocyanate)-conjugated anti-Lyt-1, anti-Lyt-2 and anti Mac-1 antibodies and PE (phycoerythrin)-conjugated anti-L3T4 antibody were purchased from Becton-Dickinson, Inc., Sunnyvale, CA. The cell pellets (10^6) were incubated with 10 μl of FITC- or PE-conjugated antibody on ice for 30 min, and washed with PBS (phosphate-buffered saline, pH 7.2) containing 0.2% bovine serum albumin three times. The cells were then washed with PBS three times, and the staining pattern was analyzed using a Becton-Dickinson FACS analyzer.

Preparation of culture supernatant of tumor tissue Right- and left-flank Meth-A tumors in the double grafted tumor system were obtained from BALB/c mice 6 days after tumor inoculation. Tumor tissue was cut into 2–3 mm^3 fragments in RPMI 1640 medium (Nissui Pharmaceutical Co., Tokyo), washed twice with RPMI 1640 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 RPMI 1640 by centrifugation for 10 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 RPMI 1640 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 factor 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 RPMI 1640 medium and suspended in RPMI 1640 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 RPMI 1640 and suspended in RPMI 1640 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 2 h (neutrophils) or 3 h (macrophages) of incubation 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 canada-balsam, and the migrated cells were counted under a microscope ($\times$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 microliters of NCF (neutrophil chemotactic factor) 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 analysis of chemotactic activity data was performed by using Student's t test.

Concomitant immunity Primary Meth-A cells (10^6 cells) were inoculated intradermally at -9 to -3 days into the right flanks 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. IL-1β was injected intratumorally into 3-, 4- and 5-day primary tumors.

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, 10^6 secondary tumor cells were rechallenged into the left flank. The growth of the secondary tumor was followed for 21 days to evaluate the generation of sinecomitant immunity. IL-1β was administered intratumorally on days 3 and 5 into the primary tumor.

RESULTS

Double grafted tumor system in nude mice In the double grafted tumor system in BALB/c nude mice, IL-1β inhibited the growth of neither the right tumor nor the left tumor, as shown in Table I. That is, IL-1β has no direct cytotoxic activity. The antitumor activity of IL-1β in the double grafted tumor system, therefore, is associated with a sequential immune mechanism in which T cells may play an important role.

Antitumor effect of TIL TIL obtained from right- and left-flank tumors treated with IL-1β were examined by Winn neutralizing test for their antitumor activity

Table II. Winn Assay of TIL Obtained from rIL-1β-treated Mice in the Double Grafted Tumor System

Group	Tumor-Free /tested	Tumor diameter (mm±SD)	Tumor weight (g±SD)
Control	0/6	21.38±2.51	3.86±1.01
TIL. Control	0/7	17.73±1.40	2.56±0.58
TIL. IL-1 Right	4/7	4.07±5.46**	0.24±0.36**
TIL. IL-1 Left	0/7	17.93±1.92	2.67±0.83

TIL were obtained from 10-day Meth-A-bearing mice with or without IL-1β (0.2 μg/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: ** $P<0.01$. SD: standard deviation.

Table I. Antitumor Effect of IL-1β in the Double Grafted Tumor System of BALB/c Nude Mice

Tumor	Group	Tumor-free /tested	Tumor diameter (mm±SD)	Tumor weight (g±SD)
Right tumor (1×10^6)	Control	0/10	24.20±1.25	4.69±0.90
	IL-1β	0/10	23.78±2.19	4.38±0.96
Left tumor (2×10^5)	Control	0/10	19.72±1.56	2.63±0.63
	IL-1β	0/10	18.78±1.62	2.45±0.63

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. IL-1β (0.2 μg/0.1 ml/day, days 3, 4 and 5) was injected into the right tumor, and a 21-day observation period followed. SD: standard deviation.

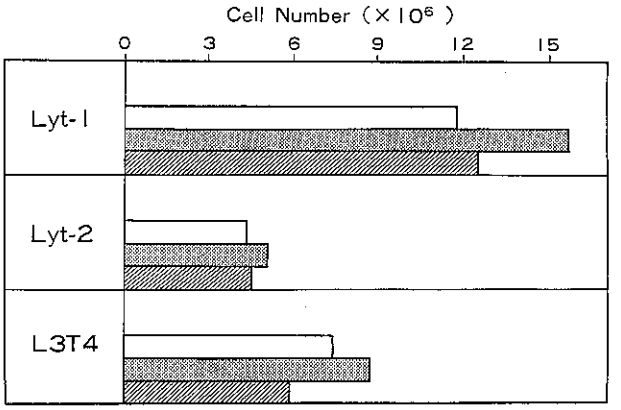

Fig. 1. Surface markers of lymph node cells obtained from IL-1-treated mice on day 10. ▭, control Meth-A bearing mice; ▨, right regional lymph node treated with IL-1; ▨, left regional lymph node treated with IL-1.

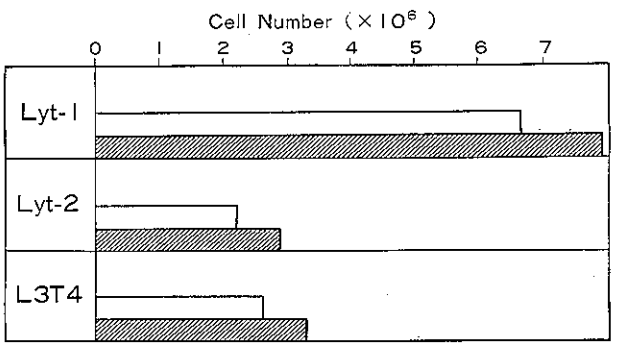

Fig. 2. Surface markers of spleen cells obtained from IL-1-treated mice on day 10. ▭, control Meth-A bearing mice; ▨, spleen cells treated with IL-1 on days 3, 4 and 5.

against Meth-A sarcoma in BALB/c mice. As shown in Table II, TIL from the right flank tumor clearly inhibited the growth of admixed Meth-A cells but control TIL did not. It is suggested, therefore, that TIL of the primary tumor may play an important role in the antitumor activity of the intratumoral administration of IL-1β into a primary tumor.

Flow cytometric analysis of IL-1-treated spleen and lymph node cells and TIL Spleen cells and right and left regional (axillary and inguinal) lymph node cells prepared from IL-1-treated mice 10 days after tumor inoculation were examined for Lyt-1, Lyt-2 and L3T4 phenotypes with the use of a FACS analyzer. Though the difference among groups is not statistically significant, the number of Lyt-1-positive lymphocytes was increased in the right regional lymph nodes (Fig. 1) and in the spleen (Fig. 2) after intratumoral injection of IL-1. TIL were obtained from Meth-A bearing mice 10 days after tumor inoculation and analyzed for Lyt-1 and Mac-1 phenotypes. It was found that Lyt-1-positive T cells and Mac-1-positive cells were increased in the right and left tumor TILs.

Table III. Neutrophil Chemotactic Factor Produced from rIL-1-treated Tumor Tissue

Culture supernatant	No. of migrated neutrophils ± SD/ visual field (×1000)
Medium control (RPMI 1640, 10% FCS)	2.4±1.5
Meth-A tumor	4.6±1.9
rIL-1-treated Meth-A, right tumor	11.2±5.1**
rIL-1-treated Meth-A, left tumor	6.7±2.5**

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

Table IV. Neutralization of IL-1β-induced NCF by Anti-IL-8 IgG

Treatment[a] (IgG dilution)	No. of migrated neutrophils ± SD /visual field (×1000)
None	17.3±4.1**
a IL-8 (1:250)	11.4±5.1
a IL-8 (1:50)	11.1±5.8
Preimmune rabbit IgG (1:25)	16.0±7.8**
Medium control (RPMI, 10% FCS)	4.0±2.3

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

Induction of neutrophil chemotactic factor from IL-1β-treated tumor tissue The culture supernatants of the 6-day tumor tissue were assayed for neutrophil chemotactic factor (NCF) activity. As shown in Table III, significant NCF activity was detected when the tumor was treated with IL-1β. This factor was found to be chemotactic but not chemokinetic by checkerboard analysis. No significant NCF activity was produced by untreated tumor tissue.

Characterization of IL-1-induced NCF IL-1-induced NCF was characterized by the neutralization test using anti-human IL-8 IgG. Neutralization was expressed in terms of the residual NCF activity. As shown in Table IV, IL-1-induced NCF activity was partially neutralized

by rabbit IgG against human IL-8 but was not neutralized by preimmune rabbit IgG. These results suggest that IL-1-induced NCF in mice was cross-reactive with human IL-8 and might be an IL-8-like factor.

Induction of macrophage chemotactic factor from IL-1β-treated tumor tissue The culture supernatants of the 6-day tumor tissue were assayed for macrophage chemotactic factor (MCF) activity. As shown in Table V, significant MCF activity was detected when the tumor was treated with IL-1β. No significant MCF activity was produced by untreated tumor tissue.

Enhancement of concomitant immunity by IL-1β 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. A significant inhibitory effect on the proliferation of tumor cells inoculated secondarily into the left flank was found in mice bearing a primary tumor that had previously been treated with IL-1β on days 3, 4 and 5. As shown in Table VI, IL-1 column, the maximum enhancement of concomitant immunity by IL-1β occurred on day 7 of primary tumor growth, that is, all 8 treated mice became tumor-free. These observations clearly show that intratumoral IL-1β administration enhances concomitant immunity and leads to the eradication of the left, distant, small tumor.

Enhancement of sinecomitant immunity by an intratumoral administration of IL-1β As shown in Table VII, before surgical excision of the primary tumor on day 6, intratumoral administration of IL-1β on days 3 and 5 resulted in complete rejection of the secondary tumor inoculated on day 21. That is, the intratumoral administration of IL-1β strongly enhanced sinecomitant immunity. These observations suggest that presurgical intra-

Table V. Macrophage Chemotactic Factor Produced from rIL-1β-treated Tumor Tissue

Culture supernatant	No. of migrated macrophages±SD/visual field (×1000)
Medium	1.0±1.0
Meth-A tumor	5.5±2.9
rIL-1-treated Meth-A, right tumor	10.9±3.3**
rIL-1-treated Meth-A, left tumor	9.3±2.4*

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

Table VII. Enhancement of Sinecomitant Immunity by Intratumoral Administration of rIL-1β

Group	Tumor-free /tested	Tumor diameter (mm±SD)	Tumor weight (g±SD)
Control	0/8	21.0±1.69	3.2±1.10
Meth-A control	0/8	21.4±1.22	3.3±1.30
Meth-A 3d, IL-1 i.t. (a)	3/7	9.2±8.13*	0.81±0.94**
Meth-A 3d, 5d IL-1 i.t. (b)	7/7**	0	0

Mice were inoculated intradermally with 1×10^6 Meth-A cells in the right flank on day 0. IL-1β (0.2 μg/day) was injected into the 3-day (a) or the 3- and 5-day (b) primary tumor, which was surgically excised on day 6. On day 21, 10^6 secondary tumor cells were challenged in the left flank and the mice were observed for another 21 days.

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

Table VI. Enhancement of Concomitant Immunity by Intratumoral Administrasion of rIL-1β

Inoculation day of primary tumor	Tumor-free/tested		Tumor diameter (mm±SD)	
	Control[b]	IL-1[c]	Control	IL-1[c]
−9 day	2/8	4/5	5.4±4.59	0.6±1.34*
−7 day	2/6	8/8*	5.2±5.23	0
−5 day	1/6	7/8	10.1±6.51	0.6±1.76*
−3 day	0/7	5/8	13.4±2.55	5.6±6.33*
0 day[a]	0/8	4/8	20.3±0.44	2.9±3.74**

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) IL-1β (0.2 μg/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: ** $P<0.01$, * $P<0.05$. SD: standard deviation.

tumoral injections of IL-1β (more than 2 times) would be effective in eradicating residual tumors.

DISCUSSION

Fisher and Fisher[10] 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. Our present data indicates that the intratumoral injection of IL-1 on days 3, 4 and 5 in the primary tumor causes complete eradication of the secondary (challenge) tumor inoculated on day 7 (Table VI, IL-1 column). That is, IL-1 treatment enhances concomitant immunity and leads to the eradication of a distant, metastatic, small tumor. Before surgical excision of the primary tumor on day 6, intratumoral administration of IL-1β on days 3 and 5 resulted in complete rejection of the secondary tumor inoculated on day 21 (Table VII). That is, IL-1 treatment also enhances sinecomitant immunity. These observations suggest that presurgical intratumoral injection of IL-1 and surgical excision of the primary tumor would be highly effective in eradicating distant tumors.

It was reported that IL-1 is chemotactic for human neutrophils and monocytes *in vivo*.[11, 12] However, highly purified or recombinant IL-1 has no chemotactic activity for neutrophils and monocytes *in vitro*.[13] Larsen *et al.*[14] clearly showed that cultured human fibroblasts were stimulated to produce IL-8 in response to IL-1 (0.1–1000 U/ml). Induction of mRNA for IL-8 in fibroblasts was rapid (within 30 min) and expression of mRNA for IL-8 was accompanied with the production of high levels of neutrophil chemotactic activity. Therefore, the intradermal injection of IL-1 caused infiltration of neutrophils and lymphocytes at sites of injection, and *in vivo* attraction of neutrophils was mediated by locally produced IL-8. Schroder *et al.*[15] also showed that IL-lα stimulated release of NAP-1/IL-8 related neutrophil chemotactic protein in human dermal fibroblasts. Thornton *et al.*[16] demonstrated that human hepatocyte lines were capable of expressing mRNA and biological activity for a NCF/IL-8 in response to the inflammatory mediators IL-1α and IL-1β. On the other hand, Sica *et al.*[17] showed that monocyte chemotactic and activating factor (MCAF) gene expression was induced in human endothelial cells by IL-1. Also, it has been reported that various types of malignant cells produce MCF *in vitro*[18]; such tumors are often heavily infiltrated with monocytes *in vivo* and this is associated with a better prognosis.[19] The present report shows that murine NCF and MCF are induced in tumor tissue (Tables III and V) by intratumoral injection of IL-1β. We also found that IL-1β-induced NCF was neutralized by anti-human IL-8 IgG (Table IV). Recently, murine macrophage inflammatory protein 2 (MIP-2) was found to be a potent chemotactic factor for human neutrophils and to have considerable sequence homology with IL-8.[20, 21] Therefore, intratumoral administration of IL-1 first induces neutrophils and macrophages in the right tumor. As IL-1 did not inhibit the growth of the right tumor in nude mice (Table I), the antitumor effect of IL-1 on the right tumor of the double grafted tumor system is not a direct cytotoxic effect. In the right tumor, a non-specific bystander killing by attracted neutrophils and macrophages seems to be the main killing activity, due to an inflammatory process. Mac-1-positive cells and Lyt-1-positive cells increased in the TIL of the right tumor and were involved in the antitumor activity *in vivo* (Table II). Then Lyt-1-positive cells were induced in the right regional lymph nodes (Fig. 1) and in the spleen (Fig. 2). Subsequently, Lyt-1-positive cells reached the left tumor through the blood stream, came into contact with Meth-A tumors and produced macrophage chemotactic and activating factor (Table V). Then Mac-1 positive cells increased in the left tumor TIL. As IL-1β did not inhibit the growth of either the right tumor or the left tumor in the double grafted tumor system of BALB/c nude mice (Table I), the antitumor activity of IL-1 on the left tumor is associated with a sequential immune mechanism in which Lyt-1-positive T cells may play an important role. MCAF is not only chemotactic for monocytes, but also activates monocytes to be cytostatic toward several types of tumor cell lines, to generate and release monocyte superoxide anions, and to release monocyte lysosomal enzymes *in vitro*.[18, 22] In the left tumor, therefore, a non-specific bystander killing induced by infiltrated macrophages seems to be the main mechanism of cell killing rather than an effect of the specific T cytotoxic cells.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Miss Eiko Ohkubo for editorial help. This work was supported in part by a grant from the Sendai Institute of Microbiology.

(Received March 4, 1991/Accepted August 6, 1991)

REFERENCES

1) Dinarello, C. A. Interleukin-1 and its biologically related cytokines. *Adv. Immunol.*, **44**, 153–205 (1989).
2) Di Giovine, F. S. and Duff, G. W. Interleukin 1: the first interleukin. *Immunol. Today*, **11**, 13–20 (1990).
3) Ebina, T. and Ishikawa, K. Antitumor effect of interleukin-1β in the double grafted tumor system. *Jpn. J. Cancer Res.*, **80**, 570–576 (1989).
4) Kamogashira, T., Masui, Y., Ohmoto, Y., Hirano, T., Nagamura, K., Mizuno, K., Hong, Y. M., Kikumoto, Y., Nakai, S. and Hirai, Y. Site-specific mutagenesis of the human interleukin-1β gene: structure-function analysis of the cysteine residues. *Biochem. Biophys. Res. Commun.*, **150**, 1106–1114 (1988).
5) 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).
6) 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).
7) 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).
8) Ferry, B. L., Flanner, G. R., Robins, R. A., Lawry, J. and Baldwin, R. W. Phenotype of cytotoxic effector cells infiltrating a transplanted, chemically induced rat sarcoma. *Immunology*, **53**, 243–250 (1984).
9) Winn, H. J. Immune mechanisms in homotransplantation. II. Quantitative assay of the immunologic activity of lymphoid cell stimulated tumor homografts. *J. Immunol.*, **86**, 228–239 (1961).
10) Fisher, B. and Fisher, E. R. The interrelationship of hematogenous and lymphatic tumor cell dissemination. *Surg. Gynecol. Obstet.*, **122**, 791–798 (1966).
11) Luger, T. A., Charon, J. A., Colot, M., Micksche, M. and Oppenheim, J. J. Chemotactic properties of partially purified human epidermal cell-derived thymocyte-activating factor (ETAF) for polymorpho-nuclear and mononuclear cells. *J. Immunol.*, **131**, 816–820 (1983).
12) Sauder, D. N., Mounessa, N. L., Katz, S. I., Dinerello, C. A. and Gallin, J. K. Chemotactic cytokines: the role of leukocytic pyrogen and epidermal cell thymocyte-activating factor in neutrophill chemotaxis. *J. Immunol.*, **132**, 828–832 (1984).
13) 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).
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) Schroder, J. M., Sticherling, M., Henneicke, H. H., Preissner, W. C. and Christophers, E. IL-1α or tumor necrosis factor-α stimulate release of three NAP-1/IL-8-related neutrophil chemotactic proteins in human dermal fibroblasts. *J. Immunol.*, **144**, 2223–2232 (1990).
16) Thornton, A. J., Strieter, R. M., Lindley, I., Baggiolini, M. and Kunkel, S. L. Cytokine-induced gene expression of a neutrophil chemotactic factor/IL-8 in human hepatocytes. *J. Immunol.*, **144**, 2609–2613 (1990).
17) Sica, A., Wang, J. M., Colotta, F., Dejana, E., Mantovani, A., Oppenheim, J. J., Larsen, C. G., Zachariae, C. O. C. and Matsushima, K. Monocyte chemotactic and activating factor gene expression induced in endothelial cells by IL-1 and tumor necrosis factor. *J. Immunol.*, **144**, 3034–3038 (1990).
18) 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).
19) 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).
20) 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).
21) 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).
22) Zachariae, C. O. C., Anderson, A. O., Thompson, H. L., Appella, E., Mantovani, A., Oppenheim, J. J. and Matsushima, K. Properties of monocyte chemotactic and activating factor (MCAF) purified from a human fibrosarcoma cell line. *J. Exp. Med.*, **171**, 2177–2182 (1990).