

Antitumor Effect of Recombinant Human Interleukin-1 β Alone and in Combination with Natural Human Tumor Necrosis Factor- α

Mitsuhiro Ohkura, Sadanori Fuchimoto and Kunzo Orita

First Department of Surgery, Okayama University Medical School, 2-5-1, Shikata-cho, Okayama 700

In order to investigate the antitumor effect of recombinant human interleukin-1 β (IL-1 β) alone and in combination with natural human tumor necrosis factor- α (nHuTNF- α), we used female BDF₁ mice bearing Lewis lung carcinoma (3LL). IL-1 β showed an antiproliferative effect against pulmonary metastatic tumors of 3LL in a dose-dependent manner. We observed 19.6 ± 6.6 , 18.6 ± 5.3 , 14.1 ± 4.4 and 13.0 ± 6.0 metastatic tumors at doses of 0.5, 1.0, 2.5 and 5.0 μg IL-1 β /mouse/day by daily intravenous administration (the number of metastatic tumors of the control group was 26.3 ± 8.2). Similar results were obtained by intraperitoneal administration, but in this case, mice showed a marked decrease of body weight. When IL-1 β was administered in combination with nHuTNF- α , pulmonary metastatic tumors decreased much more than when IL-1 β was administered alone. When the control group had 18.6 ± 12.7 metastatic tumors, the nHuTNF- α group had 12.3 ± 3.9 and the IL-1 β group had 12.8 ± 8.0 , the group which was administered both cytokines had a significantly decreased number of 5.6 ± 3.3 metastatic tumors. This antiproliferative effect of IL-1 β in combination with nHuTNF- α was reduced by the intravenous administration of anti-asialo GM₁ antibody and carrageenan. The number of metastatic tumors was increased from 8.9 ± 8.0 to 18.8 ± 11.4 by anti-asialo GM₁ antibody and from 9.5 ± 6.8 to 28.0 ± 12.3 by carrageenan. It was suggested that asialo GM₁-positive cells and macrophage were two of the most important effectors of the antiproliferative effect of IL-1 β and TNF- α .

Key words: Interleukin-1 β — Tumor necrosis factor- α — Lewis lung carcinoma — Antitumor effect — Antiproliferative effect

Interleukin-1 (IL-1) is a cytokine, which is produced by various cells including macrophages. It has a number of biological activities which are relevant in various ways to immunological, infectious and inflammatory stimuli.¹⁾ Recently, Kikumoto *et al.*²⁾ accomplished the purification and characterization of recombinant human IL-1 β produced in *Escherichia coli*, and its multiple biological activities can now be investigated by utilizing highly purified recombinant IL-1 preparations.

Though IL-1 and tumor necrosis factor (TNF) do not bind to the same receptor, they have a number of similar biological activities: they induce endothelial tissue factor, suppress lipoprotein lipase activity,³⁾ stimulate collagenase and prostaglandin synthesis,⁴⁾ increase expression of HLA-A, B antigens,^{5,6)} induce fever,⁷⁾ activate neutrophils,^{8,9)} and promote growth of fibroblasts. Concerning the cytotoxicity of TNF- α and IL-1 *in vitro*, Ruggiero and Baglioni¹⁰⁾ and Last-Barney *et al.*⁴⁾ reported that TNF- α and IL-1 exhibit synergistic action in cytotoxicity assays on human melanoma and carcinoma cell lines.

This study was undertaken to examine the combined nHuTNF- α - and IL-1 β -mediated antiproliferative effect on pulmonary metastatic tumors of Lewis lung carcinoma (3LL). The purpose was to gain some insight into the mechanism of action of these cytokines. Our results showed that low concentrations of IL-1 β and nHuTNF- α , administered together, synergistically inhibit the pro-

liferation of metastatic tumors of 3LL. In addition, on some schedules of administration around the time of operation (amputation of tumor-bearing leg), IL-1 β showed contradictory effects against the lung metastasis of 3LL.

We wanted to examine the possible usefulness of IL-1 β in combination with nHuTNF- α to see whether such a combination is favorable or not in respect to antitumor effect. It was found in murine syngenic tumor (3LL) models that nHuTNF- α significantly augmented the antitumor effect of IL-1 β .

MATERIALS AND METHODS

Animals Specific-pathogen-free female BD (C57BL/6 \times DBA/2)F₁ mice, aged 7 weeks, were purchased from Shizuoka Laboratory Animal Center, Shizuoka. They were housed in a conventional environment and fed with normal mouse pellets and tap water *ad libitum* during the experiments. Eight-week-old mice were used for tumor inoculation.

Tumor 3LL cells were serially passaged subcutaneously in C57BL/6 mice at the First Department of Surgery, Okayama University Medical School. For the experiment, tumors were excised aseptically on the 10th day after the last transplantation, minced, washed three times with Hanks solution, treated with 0.25% trypsin (Difco Labs, Detroit, MI) at 37°C for 15 min, washed twice

with Eagle's minimal essential medium (MEM, GIBCO Labs, Grand Island, NY) supplemented with 10% fetal calf serum (GIBCO Labs), filtered twice through #150 wire mesh and prepared as a single cell suspension (4×10^7 cells/ml). The viability of the cells was over 95%.

Reagents Recombinant human interleukin-1 β (IL-1 β)²⁾ was supplied by Otsuka Pharmaceutical Co. Ltd., Tokushima (LAF activity: 2×10^7 units/mg protein). Natural human TNF- α was purified from human acute B lymphoblastic leukemia cells (BALL-1 cells) stimulated by HVJ and was supplied by Hayashibara Biochemical Laboratories, Inc., Okayama.¹¹⁾ Its specific activity was 7×10^8 U/mg protein (2×10^6 JRU). The cytotoxicity of this preparation was determined by means of the highly sensitive and rapid assay of Eifel *et al.* for lymphotoxin, using actinomycin D-treated mouse L929 cells as the target. One unit of activity is designated as the reciprocal of the dilution that has a cytopathic effect in 50% of the target cells. Lyophilized anti-asialo GM₁ antiserum (Wako Pure Chemical Industries, Ltd., Osaka) was reconstituted with saline prior to use. As reported previously, anti-asialo GM₁ antibody can completely eliminate natural killer activity *in vitro* when diluted in the presence of guinea pig complement (1:10).^{12,13)} For the *in vivo* experiments, 500 μ g of anti-asialo GM₁ antibody was administered into the tail vein of mice just before the first injection of IL-1 β and nHuTNF- α . Lambda carrageenan (CRG) (Sigma Chemical Co., St. Louis, MO), which is toxic to macrophages,^{14,15)} was suspended in saline prior to use at a concentration of 5 mg/ml and sterilized by autoclaving at 120°C for 15 min. CRG solution (0.2 ml) was injected slowly into a lateral tail vein on the day before amputation (day -1) of the tumor-bearing leg. **Spontaneous pulmonary metastasis model** 3LL cells ($1 \times 10^6/0.025$ ml) were inoculated into the left footpad of BDF₁ mice, and the tumors which formed were regarded as the primary tumors. They were removed by femoral amputation under ether anesthesia on the 10th day (day 0) after the inoculation. On day 11, the mice were killed with ether and weighed. After that, the metastatic pulmonary tumors were counted by Wexler's method.¹⁶⁾ In brief, their lungs were excised in one piece, dyed with Indian ink, washed for 5 min with tap water, bleached and fixed in Fekete's solution for 24 h, then the metastatic tumors were counted with the naked eye. The mean values and standard deviation (SD) of each group were calculated. Statistical analysis was carried out by the use of Student's *t* test.

RESULTS

Antiproliferative effect of IL-1 β on metastatic pulmonary tumors of 3LL in BDF₁ mice Table I shows the antiproliferative effect of IL-1 β at a dose of 0.5 to 5.0

Table I. Antiproliferative Effect of IL-1 β on Metastatic Pulmonary Tumors of 3LL in BDF₁ Mice

Dose of IL-1 β (μ g/mouse/day)	No. of metastases ^{a)}	Body weight (g) ^{a)}	Splenic weight (g) ^{a)}
Exp. 1 (iv) ^{a)}			
saline	26.3 \pm 8.2	20.4 \pm 1.1	0.20 \pm 0.02
0.5	19.6 \pm 6.6	19.8 \pm 0.7	0.28 \pm 0.04**
1.0	18.6 \pm 5.3	18.5 \pm 0.5*	0.31 \pm 0.04**
2.5	14.1 \pm 4.4*	18.5 \pm 0.7*	0.37 \pm 0.07**
5.0	13.0 \pm 6.0*	16.0 \pm 0.8**	0.40 \pm 0.01**
Exp. 2 (ip) ^{b)}			
Saline	36.6 \pm 15.5	17.9 \pm 1.2	0.21 \pm 0.02
1.0	20.7 \pm 10.5*	16.0 \pm 1.2**	0.32 \pm 0.04**
2.5	3.7 \pm 3.5**	14.5 \pm 0.8**	0.38 \pm 0.05**

a) IL-1 β was administered into the tail vein of 8 mice in each group at the indicated dose daily for 10 days. Saline (0.1 ml/mouse/day) was injected as a control.

b) IL-1 β was administered into the intraperitoneal cavity of 8 mice in the control group and 7 mice in the other groups at the indicated doses daily for 10 days.

c) Mean \pm standard deviation. *, $P < 0.05$; **, $P < 0.01$: Statistically significant difference from the control group (Student's *t* test)

μ g/mouse/day when intravenously administered for 10 consecutive days, and when intraperitoneally administered for 10 consecutive days. The number of metastatic tumors of the IL-1 β -treated mice was decreased in a dose-dependent manner. The number of metastatic tumors of the control group was 26.3 ± 8.2 , and 19.6 ± 6.6 , 18.6 ± 5.3 , 14.1 ± 4.4 and 13.0 ± 6.0 metastatic tumors were obtained at doses of 0.5, 1.0, 2.5 and 5.0 μ g IL-1 β /mouse/day, by intravenous administration (iv). When the number of metastatic tumors of the control group was 36.6 ± 15.5 , 20.7 ± 10.5 and 3.7 ± 3.5 were obtained at doses of 1.0 and 2.5 μ g/mouse/day, by intraperitoneal administration (ip). A significant antiproliferative effect was shown at dose levels of more than 2.5 μ g/mouse/day, iv, and more than 1.0 μ g/mouse/day, ip, but the mice injected with IL-1 β by the ip route showed a marked decrease in body weight. The body weight of mice given iv injection of 0.5, 1.0 and 2.5 μ g/mouse/day of IL-1 β was within 10% of the control value. Therefore we used a dose of 1.0 μ g/mouse/day in the following experiments. Injection of IL-1 β for 10 consecutive days gave more effective results than intermittent injection did (data not shown).

Effect of IL-1 β on the number of white blood cells (WBC) of 3LL-bearing BDF₁ mice The number of WBC of IL-1 β -treated 3LL-bearing mice increased to three times that of normal mice after 8 injections of 1.0 μ g/mouse/day of IL-1 β , though there was no increase after one or two injections (Fig. 1).

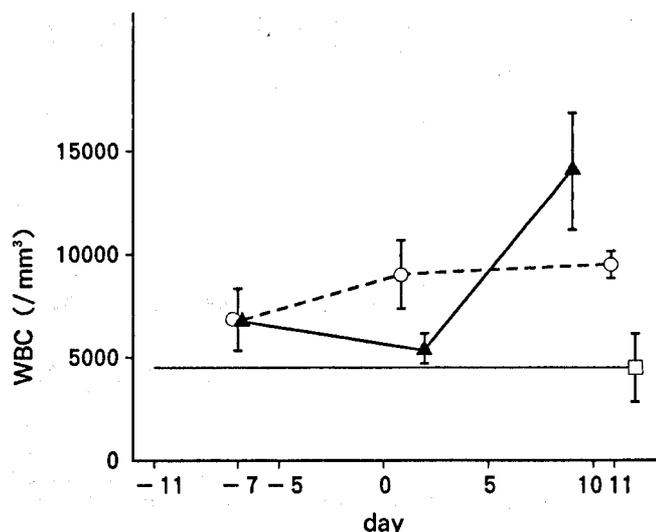


Fig. 1. The effect of IL-1β on the number of white blood cells (WBC) of 3LL-bearing BDF₁ mice. 3LL cells were inoculated into the left footpad of BDF₁ mice on day -11, and the left leg was amputated on day 0. The dose of 1.0 μg/mouse/day of IL-1β was injected on days 1 to 10. The number of WBC of 3LL-bearing mice injected with IL-1β (▲) was significantly increased compared to the mice which were not injected with IL-1β (○) or normal mice (□).

Table II. Antiproliferative Effect of IL-1β Combined with nHuTNF-α on Metastatic Pulmonary Tumors of 3LL in BDF₁ Mice

Exp.	Dose of reagents ^{a)}		No. of metastases ^{b)}	Body weight (g) ^{b)}
	IL-1β (μg/mouse/day)	nHuTNF-α (U/kg/day)		
Exp. 1	saline	—	18.6 ± 12.7	19.7 ± 0.7
	—	10 ²	12.3 ± 3.9	19.3 ± 0.5
	1.0	—	12.8 ± 8.0	17.8 ± 0.9
	1.0	10 ²	5.6 ± 3.3*	18.4 ± 0.9
Exp. 2	saline	—	36.9 ± 17.7	19.7 ± 1.1
	—	10 ²	27.4 ± 15.4	19.7 ± 1.5
	2.5	—	30.1 ± 21.4	19.4 ± 1.4
	2.5	10 ²	20.3 ± 15.2**	19.8 ± 1.7

a) In experiment 1, IL-1β and nHuTNF-α were administered into the tail vein of 7 mice in each group at the indicated dose daily for 10 days. In experiment 2, IL-1β was injected into the tail vein of 9 mice on days 1, 2, 5, 6, in order to administer the same total dose as in experiment 1. nHuTNF-α was injected in the same way as in experiment 1. Saline (0.1 ml/mouse/day) was injected into the tail vein of 7 mice from each group, daily for 10 days as a control group.
 b) Mean ± standard deviation. *, P < 0.05 from all other groups, and **, P < 0.05 from the control group and the IL-1β-injected group.

Table III. Antiproliferative Effect of IL-1β Combined with nHuTNF-α on 3LL in Anti-asialo GM₁ Antibody-pretreated Mice

No. of group	Reagents ^{a)}	No. of metastases ^{b)}	Body weight (g) ^{b)}
1	saline	30.1 ± 18.2	18.9 ± 0.8
2	anti-asialo GM ₁ antibody	36.8 ± 19.5	18.0 ± 1.7
3	IL-1β + nHuTNF-α	8.9 ± 8.0*	18.0 ± 1.1
4	IL-1β + nHuTNF-α + anti-asialo GM ₁ antibody	18.8 ± 11.4**	17.9 ± 0.9

a) Anti-asialo GM₁ antibody (500 μg/mouse) was given intravenously on day 1, before the first administration of IL-1β and nHuTNF-β. IL-1β and nHuTNF-α were administered into the tail vein of 8 mice in each group at doses of 1.0 μg/mouse/day and 10² U/kg/day daily for 10 days. Saline (0.1 ml/mouse/day) was injected as a control.
 b) Mean ± standard deviation. *, P < 0.05 from group 1. **, P < 0.05 from group 3.

Table IV. Antiproliferative Effect of IL-1β Combined with nHuTNF-α on 3LL in Carrageenan-pretreated Mice

No. of group	Reagents ^{a)}	No. of metastases ^{b)}	Body weight (g) ^{b)}
1	saline	37.8 ± 11.0	19.7 ± 0.8
2	carrageenan	43.2 ± 15.1	18.5 ± 1.4
3	IL-1β + nHuTNF-α	9.5 ± 6.8*	19.0 ± 1.4
4	carrageenan + IL-1β + nHuTNF-α	28.0 ± 12.3**	19.3 ± 1.6

a) Carrageenan (1 mg/mouse) was given intravenously on day -1. The doses of 1.0 μg/mouse/day of IL-1β and 10² U/kg/day of nHuTNF-α were administered into the tail vein of 6 mice in groups 1 and 3, and 5 mice in groups 2 and 4 daily for 10 days. Saline (0.1 ml/mouse/day) was injected as a control.
 b) Mean ± standard deviation. *, P < 0.05 from group 1. **, P < 0.05 from group 3.

Antiproliferative effect of IL-1β combined with nHuTNF-α on metastatic pulmonary tumors of 3LL in BDF₁ mice IL-1β was administered at doses of 1.0 and 2.5 μg/mouse/day, and nHuTNF-α was administered at the dose of 10² U/kg/day, from day 1 to day 10.¹⁷⁾ These doses of each reagent separately did not decrease the numbers of metastatic pulmonary tumors significantly, and did not decrease the weight of the mice. The combined antiproliferative effects of these reagents against metastatic tumors are shown in Table II. When the number of the metastatic tumors of the control group was 18.6 ± 12.7, the group injected with nHuTNF-α

alone had 12.3 ± 3.9 metastatic tumors, and the group which was injected with IL-1 β (1.0 $\mu\text{g}/\text{mouse}/\text{day}$) alone had 12.8 ± 8.0 metastatic tumors. The group which was injected with both cytokines simultaneously had a significantly ($P < 0.05$) decreased number of 5.6 ± 3.3 metastatic tumors compared with each group injected with a single cytokine. A similar result was obtained when we used a dose of 2.5 $\mu\text{g}/\text{mouse}/\text{day}$ of IL-1 β and a dose of 10^2 U/kg/day of nHuTNF- α .

Antiproliferative effect of IL-1 β combined with nHuTNF- α on 3LL in anti-asialo GM $_1$ antibody-pretreated mice As shown in Table III, the number of metastatic tumors of group 3 (IL-1 β + nHuTNF- α) was 8.9 ± 8.0 , which was a considerable reduction compared with group 1 (control group). Treatment with anti-asialo GM $_1$ antibody on day 1 (group 2) increased the number of metastatic tumors (36.8 ± 19.5). Group 4 (treated with anti-asialo GM $_1$ antibody just before the first injection of both cytokines) showed 18.8 ± 11.4 metastatic tumors. This differs from group 3 ($P < 0.05$). No significant variation in murine body weight was recognized.

Antiproliferative effect of IL-1 β combined with nHuTNF- α on 3LL in carrageenan-pretreated mice As shown in Table IV, the number of metastatic tumors of group 3 (IL-1 β + nHuTNF- α) was 9.5 ± 6.8 , which was a considerable reduction compared with group 1 (control group). Group 2 (treated with CRG on day -1) had an increased number of metastatic tumors (43.2 ± 15.1). Group 4 (treated with CRG before the injection of both cytokines) showed 28.0 ± 12.8 metastatic tumors. This differed from group 3 ($P < 0.05$).

DISCUSSION

The antitumor effects of IL-1 are both direct and indirect. The latter effect arises from actions on the immunosystem and the inflammatory system. It has been reported that IL-1 has a direct antiproliferative effect against several human carcinoma cell lines, which derives from the reduction of ornithine decarboxylase activity.¹⁸⁾ There are reports of antitumor effects *in vivo*, such as an antiproliferative effect and a survival-enhancing effect, using Meth A sarcoma, sarcoma 180, X5563 myeloma, MH134 hepatoma, Lewis lung carcinoma and L1210 lymphoma. Ebina and Ishikawa¹⁹⁾ observed an anti-metastatic effect of IL-1 β in the double grafted tumor system of Meth-A when IL-1 β was injected intratumorally. Nakamura *et al.*²⁰⁾ reported that IL-1 α showed a strong antitumor effect against Meth A sarcoma, B16 melanoma, Colon 26 adenocarcinoma and Lewis lung carcinoma *in vivo*.

This report presents data concerning the antiproliferative effects of IL-1 β alone and in combination with nHuTNF- α against 3LL on BDF $_1$ mice. We know of no

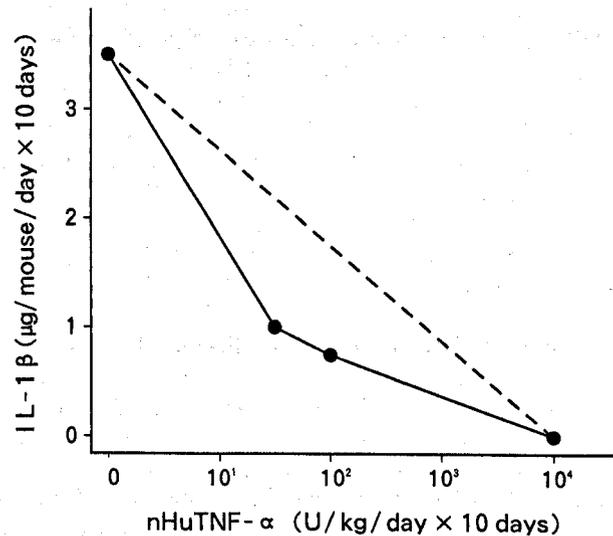


Fig. 2. Isobologram of the antiproliferative effect of IL-1 β and nHuTNF- α . Points connected by the solid line show doses of cytokines that cause in combination a 50% reduction of number of metastatic tumors relative to the control group. The dashed line represents the theoretical doses required to obtain the same inhibition of tumor proliferation if the interaction between IL-1 β and nHuTNF- α were merely additive.

previous report of an *in vivo* experiment on the antitumor effect of IL-1 β combined with nHuTNF- α . The dose of IL-1 β needed to obtain a high antitumor effect caused a severe body weight loss. So we examined the combined effect at a very low dose of IL-1 β and nHuTNF- α . The number of metastatic tumors was decreased only about 30% compared to the control group when the mice were injected with each cytokine alone.¹⁷⁾ The combination of both cytokines at the same dose as above decreased the number of metastatic tumors about 70% compared with that of the control group.

To determine whether the antiproliferative activity of IL-1 β was truly synergistic with that of nHuTNF- α , the data were plotted in an isobologram. The concentrations of these cytokines which individually or in combination decreased the number of the metastatic tumors by 50%, are plotted in Fig. 2. In this graphic analysis, a marked departure of the line connecting the experimental points below the diagonal is indicative of a synergistic interaction. The results of this analysis indicated that IL-1 β and nHuTNF- α decreased the number of metastatic tumors in a truly synergistic way, and suggested that these cytokines activated different antiproliferative mechanisms.

This antiproliferative effect of IL-1 β and nHuTNF- α was reduced by the intravenous administration of anti-asialo GM $_1$ antibody and carrageenan. Thus it was

suggested that asialo GM₁-positive cells, including natural killer cells, and macrophages are important effectors of the antiproliferative activities of IL-1 β and nHuTNF- α . Concerning the antitumor effect of asialo GM₁-positive cells, Nishiyama *et al.*²¹⁾ reported, using the colon 26 hepatic metastasis model, that histological examination of liver specimens in mice pretreated with nHuTNF- α revealed considerable lymphocytic infiltration around the tumor nodules, and that most of the lymphocytes proved to be asialo GM₁-positive.

Several reports have suggested possible mechanisms of the antitumor effect of IL-1 and TNF- α . Lowenthal *et al.*²²⁾ showed that recombinant IL-1 can induce both the secretion of IL-2 and the expression of IL-2 receptors by EL4 thymoma cells. Eisenthal and Rosenberg²³⁾ suggested that lymphokines such as IL-1 and TNF may synergize with IL-2 in the induction of antibody-dependent cellular cytotoxicity and could thus potentially be useful for immunotherapy of established tumors when combined with the administration of specific anti-tumor antibodies. Myers *et al.*²⁴⁾ demonstrated that mRNA expression and production of both TNF- α and IL-1 β in bone marrow-derived macrophages correspond to the stage of differentiation at which the cells acquire tumoricidal and antigen presentation activities. Ichinose *et al.*²⁵⁾ suggested that IL-1 and TNF are major cytotoxic molecules for activation of monocyte-macrophage-mediated tumor lysis. In addition, Sayers *et al.*²⁶⁾ reported that the synergy of IL-1 and TNF in promoting

polymorphonuclear neutrophil influx into the injected sites suggested that the local production of very low concentrations of these cytokines *in situ* could play a critical role in the emigration of PMN. IL-1 and TNF augment neutrophil chemotaxis through the induction of monocyte-derived neutrophil chemotactic factor.²⁷⁾ These reports suggest that IL-1 might show an antitumor effect through neutrophil activation. There have been few reports on the cytotoxic or cytostatic activity of neutrophils, so we should investigate the cytotoxic activity of neutrophils as well as other effectors on tumor cells.

We also got contradictory results when IL-1 β was administered at different times (data not shown). These data revealed that when mice were injected with IL-1 β in the preoperative period and postoperative period, there was an antitumor effect. But perioperative injections of IL-1 β tended to have a proliferative rather than an antiproliferative effect. A similar phenomenon was found with nHuTNF- α by Nishiyama *et al.*²¹⁾ in the colon 26 hepatic metastasis model. These results may suggest the difficulty of the clinical use of these cytokines as adjuvant immunotherapy for cancer therapy. But considering that the preoperative injections of IL-1 β showed an antitumor effect, IL-1 β may have an antimetastatic effect. Our next study will be designed to investigate the mechanism of the antimetastatic effect of IL-1 β and to determine whether it really has a strong antimetastatic effect or not, because metastasis is a major problem in cancer therapy.

(Received May 1, 1990/Accepted July 18, 1990)

REFERENCES

- 1) Dinarello, C. A. Multiple biological properties of recombinant human interleukin 1 (beta). *Immunology*, **172**, 301-315 (1986).
- 2) Kikumoto, Y., Hong, Y. M., Nishida, T., Nakai, S., Masui, Y. and Hirai, Y. Purification and characterization of recombinant human interleukin-1 produced in *Escherichia coli*. *Biochem. Biophys. Res. Commun.*, **147**, 315-321 (1987).
- 3) Beutler, B. A. and Cerami, A. Recombinant interleukin 1 suppresses lipoprotein lipase activity in 3T3-L1 cells. *J. Immunol.*, **135**, 3969-3971 (1985).
- 4) Last-Barney, K., Komon, C. A., Faanes, R. B. and Merluzzi, V. J. Synergistic and overlapping activities of tumor necrosis factor- α and IL-1. *J. Immunol.*, **141**, 527-530 (1988).
- 5) Lovett, D., Kozan, B., Kadam, M., Resch, K. and Gemsa, D. Macrophage cytotoxicity: interleukin 1 as a mediator of tumor cytostasis. *J. Immunol.*, **136**, 340-347 (1986).
- 6) Collins, T., Lapiere, L. A., Fiers, W., Strominger, J. L. and Pober, J. S. Recombinant human tumor necrosis factor increases mRNA levels and surface expression of HLA-A, B antigens in vascular endothelial cells and dermal fibroblasts *in vitro*. *Proc. Natl. Acad. Sci. USA*, **83**, 446-450 (1986).
- 7) Dinarello, C. A., Cannon, J. G., Wolff, S. M., Bernheim, H. A., Beutler, B., Cerami, A., Figari, I. S., Palladino, M. A., Jr. and O'Connor, J. V. Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. *J. Exp. Med.*, **163**, 1433-1450 (1986).
- 8) Klempner, M. S., Dinarello, C. A. and Gallin, J. I. Human leukocytic pyrogen induces release of specific granule contents from human neutrophils. *J. Clin. Invest.*, **61**, 1330-1336 (1978).
- 9) Klebanoff, S. J., Vadas, M. A., Harlan, J. M., Sparks, L. H., Gamble, J. R., Agosti, J. M. and Waltersdorff, A. M. Stimulation of neutrophils by tumor necrosis factor. *J. Immunol.*, **136**, 4220-4225 (1986).
- 10) Ruggiero, V. and Baglioni, C. Synergistic anti-proliferative activity of interleukin 1 and tumor necrosis factor. *J. Immunol.*, **138**, 661-663 (1987).
- 11) Fukuda, S., Ando, S., Sanou, O., Taniai, M., Fujii, M., Masaki, N., Nakamura, K., Andou, O., Torigoe, K., Sugimoto, T. and Kurimoto, M. Simultaneous production of natural human tumor necrosis factor- α , - β and inter-

- feron- α from BALL-1 cells stimulated by HVJ. *Lymphokine Res.*, **7**, 175-185 (1988).
- 12) Hanna, N. and Burton, R. C. Definitive evidence that natural killer (NK) cells inhibit experimental tumor metastasis *in vivo*. *J. Immunol.*, **127**, 1754-1758 (1981).
 - 13) Habu, S., Fukui, H., Shimamura, K., Kasai, M., Nagai, Y., Okumura, K. and Tamaoki, N. *In vivo* effects of anti-asialo GM1: reduction of NK activity and enhancement of transplanted tumor growth in nude mice. *J. Immunol.*, **127**, 34-38 (1981).
 - 14) Chaouat, G. and Howard, J. G. Influence of reticuloendothelial blockade on the induction of tolerance and immunity by polysaccharides. *Immunology*, **30**, 221-227 (1976).
 - 15) Sarwar, G. The effect of carrageenan on macrophages of mice. *Acta Med. Univ. Kagoshima*, **29**, 111-127 (1987).
 - 16) Wexler, H. M. A. Accurate identification of experimental pulmonary metastasis. *J. Natl. Cancer Inst.*, **36**, 641-645 (1966).
 - 17) Itano, S., Fuchimoto, S. and Orita, K. Antitumor effects of natural human TNF on BDF1 mice bearing Lewis lung carcinoma. *Hiroshima J. Med. Sci.*, **36**, 105-111 (1987).
 - 18) Endo, Y., Matsushima, K. and Oppenheim, J. J. Mechanism of *in vitro* antitumor effects of interleukin 1 (IL1). *Immunobiology*, **172**, 316-322 (1986).
 - 19) Ebina, T. and Ishikawa, K. Antitumor effect of interleukin-1 β in the double grafted tumor system. *Jpn. J. Cancer Res.*, **80**, 570-576 (1989).
 - 20) Nakamura, S., Nakata, K., Kashimoto, S., Yoshida, H. and Yamada, M. Antitumor effect of recombinant human interleukin alpha against murine syngeneic tumors. *Jpn. J. Cancer Res.*, **77**, 767-773 (1986).
 - 21) Nishiyama, N., Fuchimoto, S. and Orita, K. Preventive and antiproliferative effects of tumor necrosis factor against experimental hepatic metastasis of mouse colon-26 tumor. *Jpn. J. Cancer Res.*, **80**, 366-372 (1989).
 - 22) Lowenthal, J. W., Cerottini, J. C. and MacDonald, H. R. Interleukin 1-dependent induction of both interleukin 2 secretion and interleukin 2 receptor expression by thymoma cells. *J. Immunol.*, **137**, 1226-1231 (1986).
 - 23) Eisenthal, A. and Rosenberg, S. The effect of various cytokines on the *in vitro* induction of antibody-dependent cellular cytotoxicity. Enhancement of IL-2-induced antibody-dependent cellular cytotoxicity activity by IL-1 and tumor necrosis factor- α . *J. Immunol.*, **142**, 2307-2313 (1989).
 - 24) Myers, M., Pullen, J. K., Ghidyal, N., Eustis-Turf, E. and Schook, L. B. Regulation of IL-1 and TNF- α expression during the differentiation of bone marrow derived macrophage. *J. Immunol.*, **142**, 153-160 (1989).
 - 25) Ichinose, Y., Bakouche, O., Tsao, J. Y. and Filder, I. J. Tumor necrosis factor and IL-1 associated with plasma membranes of activated human monocytes lyse monokine-sensitive but not monokine-resistant tumor cells whereas viable activated monocytes lyse both. *J. Immunol.*, **141**, 512-518 (1988).
 - 26) Sayers, T. J., Wiltout, T. A., Bull, C. Y., Denn, A. C., Pilaro, A. M. and Lokesh, B. Effect of cytokines on polymorphonuclear neutrophil infiltration in the mouse. Prostaglandin- and leukotriene-independent induction of infiltration by IL-1 and tumor necrosis factor. *J. Immunol.*, **141**, 1670-1677 (1988).
 - 27) Matsushima, K., Morishita, K., Yoshimura, T., Lavu, S., Kobayashi, Y., Lew, W., Appella, E., Kung, H. F., Leonard, E. 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).