Cancer Immunol Immunother (1989) 29: 87–92

Cancer Immunology Immunotherapy

Synergistic induction of lymphokine (IL-2)-activated killer activity by IL-2 and the polysaccharide lentinan, and therapy of spontaneous pulmonary metastases

Ken-ichiro Yamasaki, Saburo Sone, Takashi Yamashita and Takeshi Ogura

3rd Department of Internal Medicine, The University of Tokushima School of Medicine, Kuramoto-cho 3, Tokushima 770, Japan

Summary. Spleen cells of C57BL/6N mice bearing lung metastases were induced to the cytotoxic state by subcutaneous injection of recombinant human interleukin-2 (IL-2) at a minimum dose of 5×10^4 U/mouse three times a day for 3 consecutive days. A single intraperitoneal injection of lentinan alone at concentrations of up to 10 mg/kg body weight did not render spleen cells cytotoxic to P-29 cells, but a combination of subthreshold doses of these agents (5×10^4 U/ml IL-2 and 5 mg/kg lentinan) induced significant in vivo lymphokine-activated killer activity in spleen cells of tumor-bearing mice. Similarly, spleen cells from mice treated i.p. with lentinan became cytotoxic on in vitro treatment with IL-2. The in vitro responsiveness of spleen cells to IL-2 was maximal 3 days after i.p. injection of lentinan. Synergism between IL-2 and lentinan was also observed in mice bearing spontaneous lung micrometastases: neither IL-2 ($< 5 \times 10^4$ U/mouse) nor lentinan (< 2.5 mg/kg) alone had a therapeutic effect, but multiple injections of IL-2 with a single injection of lentinan resulted in significant inhibition of spontaneous pulmonary metastases. From these results we conclude that IL-2 and lentinan in combination are more effective than either one alone for inducing destruction of pulmonary metastases.

Introduction

Interleukin-2 (IL-2) has been shown to have anti-metastatic activity in animals [17, 18, 23] and also in preliminary clinical trials in humans [22, 29]. In animal studies, however, the antitumor activity was observed on treatment with either a high dose of IL-2 alone [23] or a lower dose of IL-2 in combination with lymphokine (IL-2)-activated killer (LAK) cells [18, 19]. IL-2 was found to have various effects on cytotoxic lymphoid cells, such as induction of LAK activity [8] and augmentation of natural killer (NK) cell activity [13, 26]. However, intravenous administration of a high dose of IL-2 alone does not seem to be sufficient for induction of the cytotoxic cells [6] that are responsible for eradication of malignant diseases, because injected IL-2 is rapidly cleared from the blood [5]. Moreover, IL-2 has undesirable side-effects, such as causing pulmonary edema with dyspnea as a result of the vascular leak syndrome [24]. There are several reports that in murine tumor models, a combination of IL-2 and another biological response modifier, such as interferon-α or the tumor necrosis factor are more effective in induction of cytotoxic activity with anti-metastatic activity than either IL-2 or other biological response modifiers alone [1, 30].

Lentinan is a β1,3-glucan with β1,6 branches that has been purified completely from *Lentinus edodes*, an edible mushroom in Japan [2]. Lentinan was found to have marked antitumor activity in murine allogeneic and syngeneic hosts [21, 27], and to have immunomodulatory effects on T cells [4, 11] and some macrophages [12]. It has also been found to enhance NK activity in vivo [14]. These results suggest that lentinan may be of therapeutic use in combination with IL-2.

As both lentinan and IL-2 alone have immunomodulatory and antitumor activities, they may be even more effective in combination. For example, combined therapy with lentinan and IL-2 could be effective against a primary and/or metastatic tumor where IL-2-activated killer cells are the main effector cells. This combination therapy could also avoid the severe side-effects associated with high doses of IL-2.

In the present study we examined the in vivo, anti-metastatic effects of combinations of lentinan and IL-2 in a murine 3LL lung metastasis model. In animals bearing pulmonary micrometastases, treatment with a combination of lentinan with IL-2 was found to be more effective than treatment with either alone. We also found that the anti-metastatic effect was correlated with induction of LAK activity in the spleen.

Materials and methods

Animals. Male specific pathogen-free C57BL/6N mice, 8–10 weeks old, were obtained from Shizuoka Experimental Animal Farm (Shizuoka, Japan).

Tumors. 3LL cells originated from an undifferentiated squamous cell carcinoma that arose spontaneously in the lung of a C57/BL6 mouse, and they have been maintained by serial bi-weekly s.c. passages in the same strain of mice [16]. A local tumor grown in the back was removed and minced aseptically. The tumor fragments in RPMI 1640 containing 0.2% trypsin (1:250; Difco Laboratories Inc., Detroit, Mich) were stirred at 37° C for 30 min. The resulting isolated tumor cells were washed twice with RPMI 1640 containing 10% fetal bovine serum (Grand Island Bi-

Offprint requests to: S. Sone

ological Co., Grand Island, NY), resuspended in fresh RPMI 1640, and counted in a hemocytometer. The viability of tumor cells was estimated as more than 90% by the trypan blue dye exclusion method. A suspension of 10^6 viable cells in 0.05 ml RPMI 1640 was implanted s.c. into hind footpad of each mouse. P-29 variant cells, which were subcloned from 3LL parent cells and adapted to growth in culture [28], were obtained from Dr. K. Takenaga (Chiba Cancer Center Research Institute, Chiba, Japan).

Reagents. Recombinant human IL-2 (specific activity, 5×10^7 U/mg) was a gift from Ajinomoto Co., Kawasaki, Japan. IL-2 was resuspended in distilled water containing 0.1% mouse albumin (Sigma Chemical Co., St. Louis) and frozen until use. Lentinan was prepared from *Lentinus edodes* at Ajinomoto Central Laboratory, Kawasaki, Japan, by the method of Chihara et al. [2]. It was dissolved in saline before use.

Preparation of effector cells. Spleen cells were harvested aseptically and gently crushed in RPMI 1640 with the flat end of a sterile syringe as described previously [31]. The cells were filtered through stainless-steel mesh and briefly rinsed in distilled water to lyze erythrocytes. The splenocytes were then washed three times with RPMI 1640 medium. Then various numbers of splenocytes resuspended in the same medium with 10% fetal bovine serum, were incubated for 4 days with or without IL-2, unless otherwise described.

Determination of LAK activity. LAK activity was assayed by measuring ^{51}Cr release [26]. Briefly, splenocytes were thoroughly washed twice and their cytotoxicity against 1×10^4 ^{51}Cr-labeled P-29 cells was measured at various effector/target (E/T) cell ratios. Incubations were terminated after 4 h, the supernatants (0.1 ml/well), obtained by brief centrifugation at 1500 rpm, were harvested, and their radioactivities were determined in a gamma counter. The percentage cytotoxicity was calculated as follows:

$$\text{Cytotoxicity (\%)} = 100 \times \frac{\text{experimental } ^{51}\text{Cr release (cpm)} - \text{spontaneous release (cpm)}}{\text{total release (cpm)} - \text{spontaneous release (cpm)}}$$

The spontaneous release observed with P-29 target cells ranged from 5% to 15% of the total lysis.

System for spontaneous pulmonary metastasis. Mice bearing spontaneous pulmonary and lymph node metastases of Lewis lung carcinoma (3LL) cells were used [16, 31]. To produce spontaneous metastases, 1×10^6 viable 3LL tumor cells in 0.05 ml RPMI 1640 were injected s.c. into one hind footpad of each mouse. This resulted in progressive tumor growth and subsequently in pulmonary metastases. When the local tumor had reached 10–12 mm in diameter, the tumor-bearing leg was amputated at the midfemur by the cautery clamp technique. The edge of the wound was then closed with Michel clips under aseptic conditions. Mice were autopsied 21 days after tumor implantation, and pulmonary metastases were estimated grossly by counting the numbers of metastatic nodules on the pulmonary surface after fixation of the lungs in 10% formaldehyde solution.

Statistical analysis. The statistical significance of differences was calculated by Student's *t*-test (two-tailed).

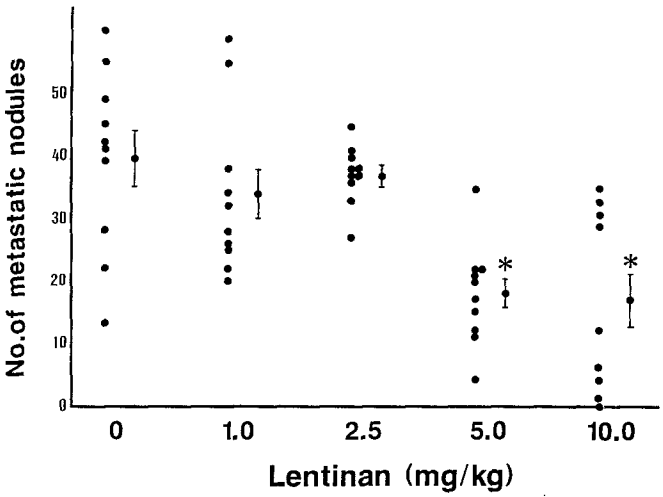

Fig. 1. Inhibition of spontaneous pulmonary metastases by intraperitoneal administration of lentinan alone. Inocula of 10^6 3LL cells were injected s.c. into the footpad. The implanted tumor was removed on day 12 after tumor implantation. Lentinan was injected i.p. once at the indicated doses 24 h later and all the mice were sacrificed on day 21. Bars show the mean numbers of surface nodules ± SE for ten mice. Data are representative of three separate experiments. $*P < 0.05$ versus value for untreated group by Student's *t*-test

Results

Inhibition of spontaneous pulmonary metastases by lentinan and IL-2

For production of spontaneous pulmonary metastases, C57BL/6N mice received a s.c. injection in a footpad of 10^6 3LL cells and 12 days later the leg with the tumor was amputated. By this time, spontaneous pulmonary micrometastases had been established [16, 31]. In our first experiments, a single i.p. injection of lentinan (1.0, 2.5, 5.0 or 10 mg/kg body weight) was given 24 h after surgical removal of the primary s.c. tumors, and mice were killed 21 days after s.c. inoculation of tumor cells. As shown in Fig. 1, i.p. injection of lentinan at concentrations of more than 5.0 mg/kg inhibited spontaneous development of pulmonary metastases of 3LL cells. Next, mice bearing pulmonary metastases were given s.c. injections of IL-2 (10^4, 5×10^4, 10^5 or 2×10^5 U/mouse) three times a day from day 13 to day 19 after the s.c. implantation of tumor cells, and were killed on day 21. Significant inhibition of metastases was observed when mice bearing pulmonary micrometastases were treated with IL-2 at more than 10^5 U/mouse (Fig. 2).

Synergistic inhibitory effects of lentinan and IL-2 on pulmonary metastases

The effect of combined therapy with lentinan and IL-2 on pulmonary metastases was examined. The results of five experiments are shown in Table 1. A single dose of lentinan (2.5, 5 or 10 mg/kg) was given i.p. on day 13 after tumor implantation, and a dose of IL-2 was given s.c. three times a day from day 13 to day 19. All mice were killed on day 21 after tumor implantation. Development of pulmonary metastases was significantly inhibited by all the combinations of lentinan and IL-2 examined. In particular, results showed that suboptimal doses of lentinan (2.5 mg/kg) and IL-2 (5×10^4 U/mouse) had synergistic effects in therapy of pulmonary metastases.

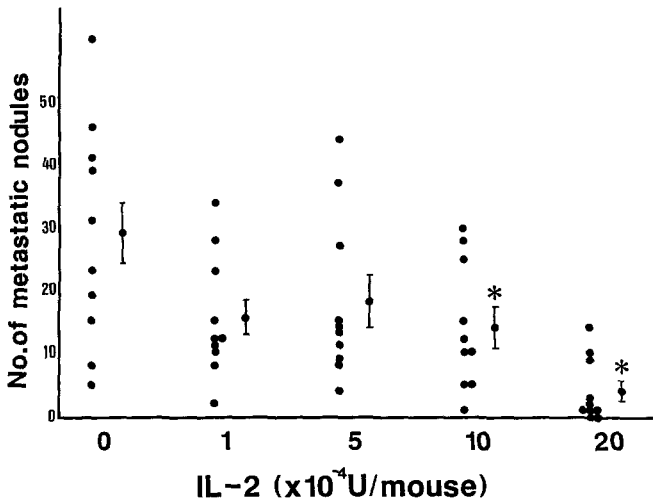

Fig. 2. Inhibitory effect of IL-2 on spontaneous pulmonary metastases. Inocula of 10^6 3LL cells were injected s.c. into the footpad. The implanted tumor was removed on day 12 after the implantation, and from the next day IL-2 was injected s.c. three times a day for 7 days. All the mice were killed on day 21 after tumor implantation. Bars show mean numbers of surface nodules ± SE for ten mice in each group. Data are representative of three separate experiments. $*P < 0.05$ versus value for untreated group by Student's *t*-test

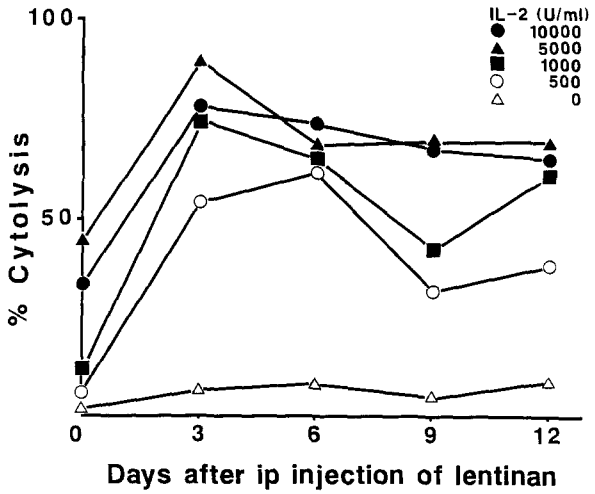

Fig. 3. Effect of pretreatment of mice with lentinan on in vitro induction of LAK activity by IL-2 in spleen cells of normal mice. Lentinan (10 mg/kg) was injected i.p. into mice and at the indicated time points spleen cells were collected. Then the cells (5×10^6) were incubated for 4 days in medium with the indicated concentrations of IL-2 before LAK assay against 1×10^4 ^{51}Cr-labeled P-29 cells. Data are representative of two separate experiments. The SD values were consistently < 10% of means

Table 1. Inhibitory effect of interleukin-2 (IL-2) and/or lentinan on pulmonary metastases of Lewis lung carcinoma

Expt.	Treatment schedule[a]		No. of pulmonary metastases[b]	Mice with visible metastases/ total
	Lentinan (mg/kg)	IL-2 (kU)		
1	0	0	19 (6–52)[c]	10/10[d]
	0	200	0[e] (0– 4)	3/9
	10	0	6[e] (0–30)	8/9
	10	200	0[e] (0– 1)	2/10
2	0	0	21 (8–66)	9/9
	0	100	2[e] (0–11)	6/9
	5	0	3[e] (0–31)	8/9
	5	100	1[e] (0–12)	5/9
3	0	0	14 (3–59)	13/13
	0	100	15 (0–45)	10/11
	5	0	3[e] (0–11)	7/10
	5	100	2[e] (0–26)	8/12
4	0	0	35 (22–52)	15/15
	0	50	24 (10–48)	12/12
	5	0	15[e] (9–35)	11/11
	5	50	7[e] (1–22)	11/11
5	0	0	25 (3–59)	13/13
	0	50	11 (0–52)	10/11
	2.5	0	30 (5–71)	10/10
	2.5	50	10[e] (1–26)	11/11

[a] Lentinan at the indicated dose was given i.p. on day 13 after tumor implantation and the indicated dose of IL-2 was given s.c. three times a day from day 13 to day 19

[b] Median number of pulmonary nodules counted om day 21 after tumor implantation

[c] Numbers in parentheses, range

[d] No. of mice without lung metastases/total no. of mice examined

[e] Significantly different from value for untreated control mice ($P < 0.05$, Mann-Whitney *U*-test)

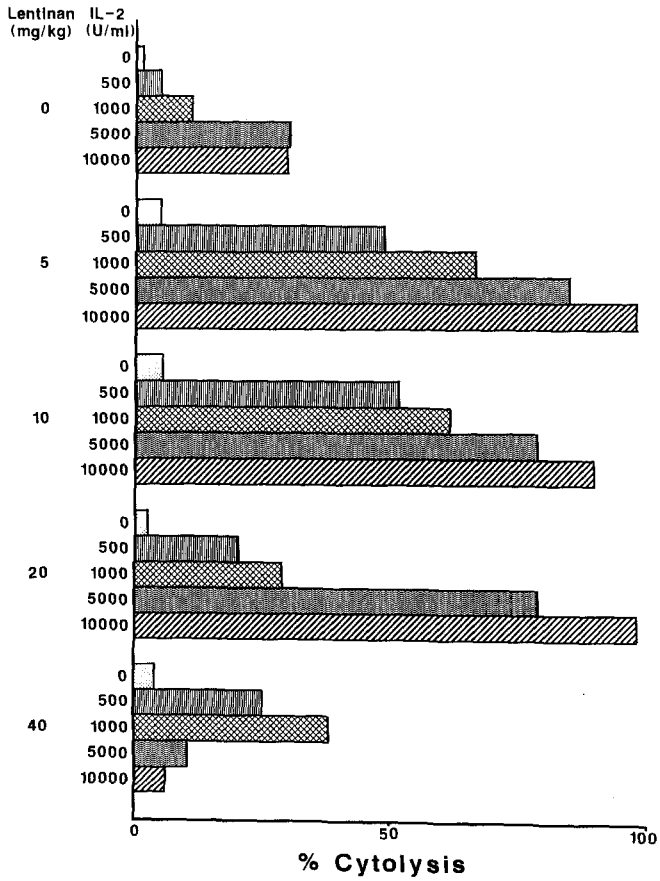

Fig. 4. Effect of previous injection of lentinan on in vitro induction of spleen LAK activity by IL-2. Three days after i.p. injection of the indicated doses of lentinan, spleen cells were harvested and incubated for 4 days with the indicated concentrations of IL-2. Then LAK activity was assayed with ^{51}Cr-labeled P-29 cells at an E/T ratio of 50:1. Data are representative of three separate experiments. The SD values were consistently < 10% of means

Table 2. Enhancing effect of lentinan on in vitro induction of lymphokine-activated killer activity (LAK) in splenocytes of normal mice, mice with pulmonary metastases and tumor-bearing mice

Treatment[a]			Cytotoxicity to P-29 cells (%) after incubation				
3LL tumor	Amputation	Lentinan[c]	0	500 U/ml	1000 U/ml	5000 U/ml	10000 U/ml
−	−	−	2.0±0.9[d]	37.5±4.1	42.1± 7.5	64.5±9.9	79.2± 8.3
−	−	+	2.3±1.4	44.0±4.9	72.0± 4.5	83.0±1.7	90.2± 2.3
+	+	−	1.5±3.2	16.5±4.4	32.7± 6.5	37.6±5.6	59.8± 6.9
+	+	+	1.2±6.7	37.9±6.2	45.2± 1.7	54.4±7.5	68.0±10.6
+	−	−	1.4±1.0	1.3±2.3	5.7± 8.2	13.5±3.3	31.9± 2.9
+	−	+	3.2±1.7	11.6±5.4	23.8±10.4	46.6±5.8	63.4± 6.1

[a] Lentinan (10 mg/kg body weight) was given i.p. on day 10 after tumor implantation
[b] 10^6 3LL cells were inoculated into the footpad on day 0, and amputation of the leg or a sham operation was carried out on day 12
[c] Spleen cells harvested on day 17 after tumor implantation were incubated for 4 days in medium with the indicated concentrations of IL-2 and then LAK activity on P-29 cells was assayed at an E/T ratio of 100:1
[d] Mean ± SD for triplicate cultures. Data are representative of three separate experiments

Priming effect of lentinan on in vitro induction of LAK activity by IL-2

Next, we examined the effect of the time of pretreatment of mice with lentinan on expression of LAK activity by mouse splenocytes in response to IL-2. For this, mice received a single i.p. injection of lentinan (10 mg/kg) and were then sacrificed at various times to harvest splenocytes. The splenocytes were incubated in medium containing IL-2 at concentrations of 500–10000 U/ml for 4 days and then LAK activity was assayed. As shown in Fig. 3, the priming effect of lentinan on expression of LAK activity induced by IL-2 was maximal when it was administered 3 days before harvesting splenocytes, and with a longer interval before treatment with IL-2 the LAK activity tended to decrease gradually. In a parallel experiment, we examined the optimal concentration of lentinan for increasing the susceptibility of mouse splenocytes to in vitro activation with IL-2. For the results in Fig. 4, mice received various doses of lentinan i.p. 3 days before harvesting splenocytes. Then, the splenocytes were incubated for 4 days in medium containing various concentrations of IL-2 and tested for LAK assay. IL-2 alone caused significant dose-dependent induction of LAK activity in splenocytes, but no significant induction was observed on a single i.p. injection of lentinan alone at a dose of up to 40 mg/kg. Under these conditions, mice that had received a single dose of lentinan (5 mg/kg and 10 mg/kg) showed maximal responsiveness to in vitro activation with IL-2 to become LAK cells. A high dose of lentinan (40 mg/kg) inhibited in vitro induction of LAK activity by IL-2. These results indicate that lentinan (5 mg/kg or 10 mg/kg) should be injected intraperitoneally at least 3 days before induction of LAK activity by IL-2.

In vitro induction of LAK activity in the spleen of lentinan-treated mice bearing pulmonary metastases

To determine whether a single i.p. injection of lentinan could augment the in vitro induction of LAK activity in the spleen of tumor-bearing mice, mice with or without primary or metastatic tumors were given a single i.p. injec-

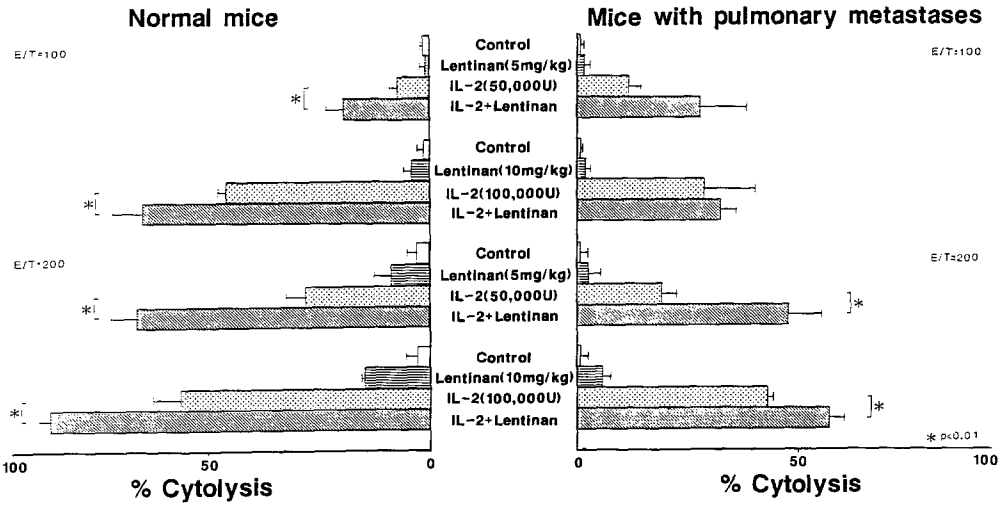

Fig. 5. Effect of combined therapy with lentinan and IL-2 on development of LAK activity in spleen cells of mice with or without pulmonary metastases. Inocula of 10^6 3LL cells were injected s.c. into the footpads of groups of five mice. Primary tumors were removed on day 12 after tumor implantation under the same conditions as for Fig. 1. On day 13, lentinan was injected i.p. IL-2 was injected s.c. on days 13, 14 and 15 after tumor implantation. Spleen cells were collected on day 16 and tested on 1×10^4 labeled P-29 cells for LAK activity at E/T ratios of 100:1 and 200:1. Columns and bars show means ± SD of representative results in three separate experiments

tion of lentinan (10 mg/kg) and then their splenocytes were harvested. After in vitro treatment with IL-2 for 4 days the splenocytes of lentinan-treated mice showed much higher LAK activity than those of untreated mice (Table 2).

In vivo induction of LAK activity in the spleen of mice bearing pulmonary metastases

The above results demonstrated that splenocytes harvested from the spleen of tumor-bearing mice expressed LAK activity when treated with IL-2 in vitro. A more meaningful finding, however, would be the demonstration that splenocytes of mice bearing lung metastases could be activated to express LAK activity after in vivo treatment with a combination of lentinan and IL-2. For study of this possibility, mice bearing pulmonary metastases (and normal mice) were given a single i.p. injection of 5 mg/kg or 10 mg/kg of lentinan on day 13 after tumor implantation, and a dose of 5×10^4 or 10×10^4 U/mouse of IL-2 s.c. three times on days 13, 14 and 15. On day 16 after tumor implantation, splenocytes were harvested from these mice and tested for LAK activity on P-29 target cells. As shown in Fig. 5, splenocytes of mice bearing pulmonary metastases showed less LAK activity than those of normal mice. Nevertheless, a synergistic effect of lentinan (5 mg/kg) and IL-2 (5×10^4 U/mouse) in combination was observed on induction of LAK activity in the spleen of mice bearing pulmonary metastases.

Discussion

Biological response modifiers seem to be therapeutically effective only with a relative small secondary tumor burden, such as micrometastases [7]. The present study showed that multiple s.c. injections of high doses of IL-2 resulted in significant inhibition of established pulmonary micrometastases of a syngeneic 3LL lung carcinoma, and that pretreatment of mice bearing micrometastases with suboptimal doses of lentinan was effective in augmenting the inhibition of spontaneous pulmonary metastases by suboptimal doses of IL-2 (experiments 4 and 5 in Table 1). Moreover, we showed that lentinan and IL-2 in combination acted synergistically to induce LAK activity in splenocytes.

Accumulating evidence that induction of LAK cells by IL-2 is important in host defense against primary and/or metastatic neoplasias has stimulated interest in agents that can increase LAK-mediated destruction of tumor cells in vivo. For example there are several reports that generation of LAK activity by IL-2 is enhanced by cytokines such as interferon-α or tumor necrosis factor α [1, 30] or bacterial preparations such as *Nocardia rubra* cell-wall skeletons [32]. We found that after a single i.p. injection of lentinan, primed splenocytes of mice expressed much higher LAK activity induced by IL-2 in vitro and in vivo than that of splenocytes from unprimed mice. The priming effect of lentinan on expression of LAK activity by IL-2 was maximal when lentinan was injected 3 days before the initiation of culture with IL-2, and this increased level was maintained for up to 12 days (Fig. 3), suggesting that for continuous in vivo induction of LAK activity, lentinan should be injected into mice once or twice weekly.

The mechanism by which lentinan and IL-2 in combination eradicate pulmonary micrometastases in vivo is unknown, but may include the participation of LAK cells, because we found in this work that in vitro and in vivo inductions of LAK activity by IL-2 were increased synergistically by in vivo pretreatment with lentinan and that the anti-metastatic activity correlated well with the generation of LAK activity in spleen cells after treatment with these biological response modifiers. As lentinan did not enhance induction of LAK activity by IL-2 in vitro, humoral factors or cellular events induced in vivo by lentinan may contribute to the synergistic effects observed in vitro and in vivo. For example, in vivo administration of lentinan was previously found to augment production of interleukin-1 (IL-1), IL-3 and colony-stimulating factor [15, 33]. These findings suggest that these cytokines may be involved in augmentation of IL-2-activated LAK induction. There is also evidence that precursors of LAK cells are heterogeneous lymphoid cell subpopulations consisting of NK cells and T cells [3, 9, 20, 26]. This finding, and previous observations that lentinan may augment both the generation of cytotoxic lymphocytes and NK cell activity in murine systems [14, 33] suggest that on combined therapy with lentinan and IL-2, LAK activity induced from both T cells and NK cells may be responsible for destruction of pulmonary metastases. Our recent finding that monocytes enhance induction of LAK activity by IL-2 from T cells and a subpopulation of NK cells [26] also raise the possibility that monocyte-macrophages primed by lentinan may be involved in increase in the LAK induction. Nevertheless, the present data do not exclude the possibility that the effector cell responsible for inhibition of lung metastases is macrophage, since IL-2 stimulates T cells to produce IFN-γ [16], which is capable activating macrophages to the antitumor state [25]. This is also supported by the previous finding that administration of lentinan may augment the generation of tumoricidal macrophages [10]. Such a study is in progress to elucidate the antitumor effector mechanism(s) induced by IL-2 and lentinan.

In this animal model, a single i.p. injection of lentinan was found to prime splenocytes for efficient induction of LAK activity by IL-2, and this synergism between lentinan and IL-2 in therapy of pulmonary metastases seems significant because, without causing undesirable side-effects, lentinan can be used in combination with IL-2 to reduce the dose of the latter required for in vivo expression of the LAK activity responsible for anti-metastatic activity.

Acknowledgements. This research was supported in part by grants from the Ministry of Health and Welfare of Japan, and the Japanese Foundation for Clinical Pharmacology.

References

1. Brunda MJ, Bellantoni D, Sulich V (1987) In vivo anti-tumor activity of combinations of interferon alpha and interleukin-2 in a murine model. Correlation of efficacy with the induction of cytotoxic cells resembling natural killer cells. Int J Cancer 40: 365
2. Chihara G, Hamuro J, Maeda YY, Arai Y, Fukuoka F (1970) Fractionation and purification of the polysaccharides with marked antitumor activity, especially lentinan, from *Lentinus edodes* (Berk.) Sing. (an edible mushroom). Cancer Res 30: 2776
3. Damle NK, Doyle LV, Bradley EC (1986) Interleukin-2-activated human killer cells are derived from phenotypically heterogeneous precursors. J Immunol 137: 2814

4. Dennert G, Tuckor D (1973) Antitumor polysaccharide lentinan – a T cell adjuvant. J Natl Cancer Inst 51: 1727
5. Donohue JH, Rosenberg SA (1983) The fate of interleukin-2 after in vivo administration. J Immunol 130: 2203
6. Ettinghausen SE, Rosenberg SA (1986) Immunotherapy of murine sarcomas using lymphokine activated killer cells: optimization of the schedule and route of administration of recombinant interleukin-2. Cancer Res 46: 2784
7. Fidler IJ (1985) Macrophages and metastases-a biological approach to cancer therapy. Presidential address. Cancer Res 45: 4714
8. Grimm EA, Mazumder A, Zhang HZ, Rosenberg SA (1982) Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin-2-activated autologous human peripheral blood lymphocytes. J Exp Med 155: 1823
9. Grimm EA, Ramsey KM, Mazumder A, Wilson DJ, Djeu JY, Rosenberg SA (1983) Lymphokine-activated killer cell phenomenon II: Precursor phenotype is serologically distinct from peripheral T lymphocytes, memory cytotoxic thymus-derived lymphocytes and natural killer cells. J Exp Med 157: 884
10. Hamuro J, Chihara G (1985) Lentinan, a T-cell oriented immunopotentiator: its experimental and clinical applications and possible mechanism of immune modulation. In: Immune modulating agents and their mechanisms. Marcel Dekker, New York, p 409
11. Hamuro J, Wagner H, Rollinghoff M (1978) β(1–3)-Glucans as a probe for T cell specific immune adjuvants. II. Enhanced in vitro generation of cytotoxic T lymphocytes. Cell Immunol 38: 328
12. Hamuro J, Rollinghoff M, Wagner H (1980) Induction of cytotoxic peritoneal exudate cells by T-cell immune adjuvants of the β(1,3) glucan-type lentinan and its analogues. Immunology 39: 551
13. Henney CS, Kuribayashi K, Kern DE, Gills S (1981) Interleukin-2 augments natural killer cell activity. Nature 291: 335
14. Herberman RB, Nuun-Hargrove ME (1981) Augmentation of natural killer (NK) cell activity by lentinan. In: Aoki T, Urushizaki I, Tsubura E (eds) Manipulation of host defense mechanisms. Excerpta Medica, Amsterdam, p 188
15. Izawa M, Ohno K, Amikura K, Hamuro J (1983) Lentinan augments the production of interleukin 3 and colony stimulating factor(s) by T cells. In: Aoki T, Tsubura E, Urushizaki I (eds) Manipulation of host defence mechanisms. Excerpta Medica, Amsterdam, p 59
16. Kawase I, Brooks CG, Kuribayashi K, Olabuenaga S, Newman W, Gillis S, Henney CS (1983) Interleukin 2 induces γ-interferon production: participation of macrophages and NK-like cells. J Immunol 131: 288
17. Lafreniere R, Rosenberg SA (1985) Successful immunotherapy of murine experimental hepatic metastases with lymphokine-activated killer cells and recombinant interleukin 2. Cancer Res 45: 3735
18. Mule JJ, Shu S, Schwarz SL, Rosenberg SA (1984) Adoptive immunotherapy of established pulmonary metastases with LAK cells and recombinant interleukin-2. Science 225: 1487
19. Mule JJ, Shu S, Rosenberg SA (1985) The anti-tumor efficacy of lymphokine-activated killer cells and recombinant interleukin-2 in vivo. J Immunol 135: 646
20. Ortaldo JR, Mason A, Overton R (1986) Lymphokine-activated killer cells: analysis of progenitors and effectors. J Exp Med 164: 1193
21. Rose WC, Reed III FC, Siminoff P, Bradner WT (1984) Immunotherapy of Madison 109 lung carcinoma and other murine tumors using lentinan. Cancer Res 44: 1368
22. Rosenberg SA, Lotze MT, Muul LM, Leitman S, Change AE, Ettinghausen SE, Matory YL, Skibber JM, Shiloni E, Vetto JT, Seipp CA, Simpson C, Reichert CM (1985) Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N Engl J Med 313: 1485
23. Rosenberg SA, Mule JJ, Spiess PJ, Reichert CM, Schwarz SL (1985) Regression of established pulmonary metastases and subcutaneous tumor mediated by the systemic administration of high-dose recombinant interleukin-2. J Exp Med 161: 1169
24. Rosenberg SA, Lotze MT, Muul LM, Chang AE, Avis FP, Leitman S, Linehan WM, Robertson CN, Lee RE, Rubin JT, Seipp CA, Simpson CG, White DE (1987) A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N Engl J Med 316: 889
25. Sone S (1986) Role of alveolar macrophages in pulmonary neoplasias. Biochim Biophys Acta 823: 227
26. Sone S, Inamura N, Nii A, Ogura T (1988) Heterogeneity of human lymphokine (IL-2)-activated killer (LAK) precursors and regulation of their LAK induction by blood monocytes. Int J Cancer 42: 428
27. Suga T, Shiio T, Maeda YY, Chihara G (1984) Antitumor activity of lentinan in murine syngeneic and autochthnous hosts and its suppressive effect on 3-methylcholanthrene-induced carcinogenesis. Cancer Res 44: 5132
28. Takenaga K (1984) Enhanced metastatic potential of cloned low-metastatic Lewis lung carcinoma cells. Cancer Res 44: 1122
29. West WH, Tauer KW, Yannelli JR, Marshall GD, Orr DW, Thurman GB, Oldham RK (1987) Constant-infusion of recombinant interleukin-2 in adoptive immunotherapy of advanced cancer. N Engl J Med 316: 898
30. Winkelhake JL, Stampfl S, Zimmerman RJ (1987) Synergistic effects of combination therapy with human recombinant interleukin-2 and tumor necrosis factor in murine tumor models. Cancer Res 47: 3948
31. Yagi M, Yamashita T, Tsubura E (1985) Effect of a thymic factor, thymostimulin, on growth and pulmonary metastases of Lewis lung carcinoma. Cancer Immunol Immunother 19: 198
32. Yokota S, Shirasaka T, Nishikawa H, Hosoe S, Ikeda T, Komuta K, Kawase I, Masuno T, Ogura T, Kishimoto S (1988) Augmentative effect of *Nocardia rubra* cell-wall skeleton (N-CWS) on lymphokine-activated killer (LAK) cell induction. Cancer Immunol Immunother 26: 11
33. Yoshimoto R, Akiyama Y, Hayami T, Iguchi Y, Izawa M, Hamuro J (1982) Augmentation of interleukin 1 production by antitumor polysaccharide, lentinan and its role in the generation of effector T cells against tumors. Int J Immunopharmacol 4: 267

Received September 7, 1988/Accepted December 12, 1988