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Effect of a thymic factor, thymostimulin, on growth and pulmonary metastases of Lewis lung carcinoma*

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Summary. *The antitumor and antimetastatic activities of a thymic factor, thymostimulin (TP-1), with or without cyclophosphamide (CPA) were examined in C57BL/6 mice inoculated with Lewis lung carcinoma (3LL).*

Tumor growth was followed by determining the tumor diameter after tumor implantation. TP-1 given to mice every 2 days after tumor implantation significantly inhibited tumor growth without affecting the survival rate.

For induction of spontaneous pulmonary metastases, 3LL cells were implanted into the footpads of mice, and the implanted tumor was removed on day 9. The antimetastatic effect of TP-1 on pulmonary metastases after removal of the primary tumor was evaluated by counting the number of pulmonary surface nodules. TP-1 showed antimetastatic activity depending on its time of administration and dose. Combined therapy with TP-1 plus CPA significantly prolonged the survival of mice with pulmonary metastases.

The cytolytic activities of spleen cells on 3LL cells were enhanced in mice treated with TP-1 and/or CPA and the cytolytic activity of nonadherent spleen cells, the T-cell population, was enhanced.

The role of cytolytic spleen cells in inhibiting and preventing metastases was discussed.

Introduction

It is now well recognized that cell-mediated immunity plays a major role in the immune system in host defense against neoplasms, and that the thymic gland acts as a central station for developing and regulating cell-mediated immunity. These facts have led to various studies on the development and metastases of tumor using congenitally or experimentally thymus-defective animals. Some investigators have reported that the incidence and development of tumors and the numbers of metastases are increased in thymectomized animals [1, 4, 13], although others have obtained the opposite results [20, 23], and there were some reports that the incidences of tumors are similar in intact and thymectomized animals [1, 20, 25]. Thus the data obtained so far are inconclusive and contradictory. However, since it has been clearly demonstrated that cell-mediated immunity is attenuated in thymectomized or tumor-bearing animals, the effects of extracts of thymus or thymic hormone on cancers in animals and humans have been tested. Thymosin [6, 21], thymic humoral factor [12], fraction V of thymosin [8], and α-thymopoietin pentopeptide [19] have been shown to exert antitumor effects, to restore the immune competence of mammals, and to prolong the survival of tumor-bearing hosts. However, there have been few studies on the effects of these thymic factors on cancer metastases and these have given conflicting results [9, 12, 17].

In the present study we examined the effect of a thymic factor, thymostimulin, on tumor growth and spontaneous pulmonary metastases in relation to its influence on cell-mediated cytotoxicity against a syngeneic tumor.

Materials and methods

Animals. Male C57BL/6N mice 6–8 weeks old and weighing 20–25 g were obtained from Shizuoka Animal Farm Co. (Shizuoka, Japan). They were given standard diet and water ad libitum throughout experiments.

Tumor. 3LL is an undifferentiated squamous cell carcinoma that arose spontaneously in the lung of a C57BL mouse, and it has been maintained by serial biweekly SC passage in the same strain of mice. A local tumor grown in the thigh was removed aseptically and minced. The tumor fragments were stirred in RPMI 1640 medium (Microbiological Associates, Md., USA) containing 0.2% trypsin (1:250, Difco Lab., Detroit, Mich, USA) at 37 °C for 30 min. The isolated tumor cells were washed twice with RPMI 1640 medium containing 10% FCS (Gibco, Grand Island, NY, USA), resuspended in fresh RPMI 1640 medium and counted in a hemocytometer. The viability of these tumor cells was estimated as more than 90% by the trypan blue dye exclusion method. A suspension of 5×10^4 or 10^6 viable cells in 0.05 ml RPMI 1640 medium was implanted SC into one footpad of each mouse.

Thymostimulin. Thymostimulin (TP-1), consisting of a group of polypeptides with molecular weights of

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Abbreviations: TP-1, thymic factor, thymostimulin; 3LL, Lewis lung carcinoma; CPA, cyclophosphamide; CCNU, 1- (2-chloroethyl)-3-cyclohexyl-1-nitrosourea; BCNU, 1,3-bis (2-chloroethyl)-1-nitrosourea; FCS, fetal calf serum; SD, standard deviation; SE, standard error

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1000 – 12 000, is a soluble product from calf thymus originally isolated by Falchetti et al. [10] in 1976. TP-1 was supplied by Mochida Chemical Co. (Tokyo, Japan) from the Istituto Farmacologico Serono (Rome, Italy). It was prepared according to the following procedure. Calf thymuses were minced and extracted with 0.15 *M* ammonium acetate, pH 4.5. After ultrafiltration on an Amicon PM-10 membrane, the extract was desalted on Sephadex G-25 and subjected to gel filtration on Sephadex G-50. The fractions that gave bands at R_F 0.22 and 0.42 on polyacrylamide gel electrophoresis were combined and named TP-1. The lyophilized sterile endotoxin-free preparation was dissolved at a concentration of 1, 10, or 100 mg/kg in 0.9% NaCl solution and injected IP to mice.

Anticancer agent. Cyclophosphamide (CPA) was dissolved at a concentration of 20 or 50 mg/kg in 0.9% NaCl solution and injected IP to mice.

Assay of growth of the implanted tumor. When 5×10^4 3LL cells were implanted into a footpad, the implanted tumor appeared as a small visible nodule within 10 days at the site of implantation. Subsequent tumor growth was followed by measuring the tumor diameter every 2 – 3 days as the thickness of the foot with an implanted tumor minus that of the control foot. The foot thickness was measured with a vernier caliper (Ozaki Co., Tokyo, Japan).

Assay of pulmonary metastases. Implantation of 10^6 3LL cells SC into the footpads of mice led to progressive tumor growth and subsequent pulmonary metastases. When the implanted tumor in the footpad reached 5 – 6 mm in diameter, 9 – 10 days after implantation of tumor cells, the mice were anesthesized by IP injection of 50 mg/kg sodium pentobarbital (Nembutal®), and the tumor-bearing leg was amputated by a cautery clamp technique. The leg was amputated at the site of knee joint by electrosurgical knife (Monotizer, type MM-1, Minato Medical Science Co., Osaka, Japan) and the edge of the wound was closed with Michel clips under aseptic conditions. Mice were autopsied 12 days after removal of the implanted tumor, and their organs were examined.

Pulmonary metastases were estimated grossly by counting the numbers of metastatic nodules on the pulmonary surface after fixing the lungs in 10% formalin solution for 2 days.

Preparation of spleen cells. Spleens were removed aseptically from normal, tumor-bearing, and tumor-excised mice. The spleen cells were passed through a stainless steel mesh and washed thoroughly with RPMI 1640 medium containing 5% FCS. The suspension was centrifuged and the cell pellet was subjected to hypotonic shock to remove contaminating red blood cells. The cell suspension was stood to allow the cell clumps to settle and the cells were then washed twice with fresh medium and resuspended in RPMI 1640 medium supplemented with or without 10% FCS. The viability of the spleen cells was more than 90%.

Plastic adherence of spleen cells. The spleen cells in RPMI 1640 medium with 10% FCS were incubated in plastic flasks (Eiken Instruments Co., Tokyo, Japan) for 1 h. Nonadherent cells were collected, centrifuged, and suspended at an appropriate cell concentration for cytotoxicity assay.

Treatment of spleen cells with anti-Thy-1.2 antibody and complement. Rabbit anti-Thy-1.2 antibody was purchased from Olac Co. (Bichester, Oxon., England). Spleen cells suspended with RPMI 1640 medium were incubated with a final dilution of 1:100 of anti-Thy-1.2 antibody at 4 °C for 45 min. The spleen cells were washed twice with RPMI 1640 medium and then mixed with a final dilution of 1:10 of rabbit complement (Seibo Science Co., Tokyo, Japan), and the mixtures were incubated at 37 °C for 45 min. Several controls were included in all experiments, i.e., cultures with complement only and anti-Thy-1.2 antibody without complement. The spleen cells subjected to various treatments were washed twice with RPMI 1640 medium and resuspended at an appropriate cell concentration with RPMI 1640 containing 10% FCS for cytotoxicity assay.

Assay of in vitro cytolytic activity of spleen cells. The in vitro cytolytic activities of spleen cells, total and nonadherent spleen cells, were determined by a modification of the method of Rinehart et al. [16, 24]. The target 3LL cells, 2×10^6 cells, were labeled with 0.5 μCi/ml ^{3}H-thymidine (methyl-^{3}-thymidine, specific activity 2 Ci/10^{-3} *M*; Radiochemical Centre, Amersham, England) in 5 ml RPMI 1640 medium containing 10% FCS in 25 cm^2 plastic tissue culture flasks (Nunc, Roskilde, Denmark) for 24 h. The labeled tumor cells were washed three times with RPMI 1640 medium containing 10% FCS and 1×10^4 cells/0.1 ml were introduced into the wells of a tissue culture plate (no. 3042, Falcon Plastics, Oxnard, Calif, USA) with effector cells and incubated for 24 h in a 5% CO_2 incubator. Then the target cells labeled with ^{3}H-thymidine were washed vigorously and sucked out onto glass fiber paper (GF/B, Whatman, Maidstone, England) from the well, whereupon the paper was dried and transferred to glass vials containing toluene-based scintillation fluid. Radioactivity was counted in a scientillation counter. All experiments were done in triplicate. The percentage cytotoxicity was calculated from the following formula:

$$\textit{Cytolytic activity (\%)} = \frac{\textit{cpm in control group} - \textit{cpm in test group}}{\textit{cpm in control group}} \times 100$$

Statistical analysis. The statistical significance of difference between experimental values was analyzed by Student's *t*-test (two-tailed).

Results

Effect of TP-1 on tumor growth and survival of tumor-bearing mice

The inhibitory effect of TP-1 on the growth of 3LL tumors was examined. On days 9, 11, 13, 15 and 18 after tumor implantation the diameter of tumors was measured, but from day 18, since the tumors infiltrated into the lower portion or whole of the thigh and tended to show necrosis the tumor diameter was no longer measured, as it did not reflect the tumor size. As shown in Fig. 1, there was no significant difference between the growths of tumors in the TP-1-treated and control groups until day 9, but from day 11 tumor growth in the group treated with TP-1 at a dose of 100 mg/kg was significantly less than in the control

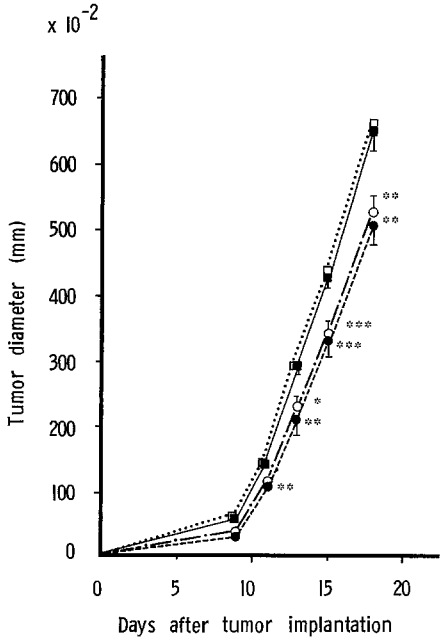

Fig. 1. Effect of TP-1 on tumor growth of Lewis lung carcinoma arising following implantation of 5×10^4 3LL cells into a footpad: thereafter 1, 10 or 100 mg/kg TP-1 was injected IP every other day from day 1.
* $p < 0.05$; ** $P < 0.01$ ***$P < 0.001$, compared with control group. (■———■), control group; experimental groups were treated with TP-1 at 1 mg/kg (□·····□), 10 mg/kg (○-·-·-○), and 100 mg/kg (●-----●)

group, and TP-1 at a dose of 10 mg/kg had a inhibitory effect from day 13.

The survival time of mice with implanted 3LL cells were measured. TP-1 was injected every 2 days from the day after implantation until day 19, when the first death occurred in control group. The mean survival time was 27.0 ± 4.1 days in the control group and 26.4 ± 6.2 days and 28.8 ± 6.2 days in the groups treated with TP-1 at 1 mg/kg and 100 mg/kg, respectively. No correlation was found between the growth curve and the survival time (data not shown). The results obtained indicated that TP-1 inhibited tumor growth, but that its effect on the primary tumor was not sufficient to prolong the survival.

Effect of TP-1 on pulmonary metastases after removal of the primary tumor

As shown in Table 1, with doses of 10 and 100 mg/kg, the numbers of metastatic nodules were significantly decreased compared with those in controls, but there was no significant difference between the numbers in groups treated with doses of 10 or 100 mg/kg.

Combined effects of TP-1 and CPA on pulmonary metastases

Previous data indicated that 3LL cells are resistant to almost all chemotherapies, but are sensitive to CPA [16, 27]. Pulmonary metastases were significantly reduced by a single treatment with 20 mg/kg CPA (Table 2), and were reduced more by treatment with 50 mg/kg CPA. Combined therapy with TP-1 (10 mg/kg) and CPA (50 mg/kg) reduced metastases the most: after this treatment, no pulmonary metastatic nodules were observed in 3 of 11 mice. As shown in Fig. 2, the survival of mice was also significantly prolonged by this combination therapy, and after this therapy no metastases were seen in any organs of surviving mice killed on day 60.

In vitro cytolytic activity of spleen cells from mice treated with TP-1

The mechanism of activation of antimetastatic activity by TP-1 was studied by determining the in vitro cytolytic activity of spleen cells from 3LL tumor-excised mice treated with TP-1 (Fig. 3). Spleen cells were collected on day 16 after tumor implantation, and whole spleen cells and plastic-non-adherent cells were prepared as described in *Materials and Methods*.

TP-1 caused significantly increased cytolytic activity of spleen cells when injected into mice at 10 or 100 mg/kg. No difference was found in the cytolytic activities of whole spleen cells and plastic-non-adherent cells. To clarify the immunological properties of effector spleen cells, whole spleen cells from mice injected with TP-1 were treated with anti-Thy-1.2 antibody and complement, and then examined for cytolytic activity. As shown in Table 3, these spleen cells showed almost no cytotoxicity. This finding suggested that TP-1-activated Thy-1.2-positive cells and T cells, and thereby enhanced the cytolytic activity of spleen cells.

Table 1. Inhibition of pulmonary metastases by TP-1

Experimental group[a]	Dose (mg/kg × days)	Pulmonary metastases[b] No. of surface nodules (mean ± SE)	No. of mice with metastases/no. of mice treated
Control	–	25.0 ± 4.0	12/12
TP-1 treated	1×7	23.1 ± 3.6	12/12
	10×7	11.6 ± 1.8[c]	13/13
	100×7	10.2 ± 4.2[d]	12/12

[a] 1×10^6 3LL cells were implanted into a footpad. The implanted tumor was removed on day 9 after tumor implantation. A daily dose of 1, 10, or 100 mg/kg of TP-1 was injected IP for 7 days from day 9
[b] Pulmonary surface nodules were counted on day 21
[c] $P < 0.01$ compared with control group
[d] $P < 0.001$ compared with control group

Table 2. Effect of TP-1 in combination with cyclophosphamide on pulmonary metastases

Experimental group[a]	Dose (mg/kg × days) TP-1	CPA	Pulmonary metastases[b] No. of surface nodules (mean ± SE)	No. of mice with metastases/no. of mice treated
Control	–	–	35.5 ± 3.3	11/11
TP-1	10 × 7	–	19.5 ± 3.5[c]	10/10
CPA	–	20 × 1	16.0 ± 2.8[d]	10/10
CPA	–	50 × 1	8.5 ± 1.9[d]	10/10
TP-1 + CPA	10 × 7	20 × 1	13.8 ± 2.4[d]	11/11
TP-1 + CPA	10 × 7	50 × 1	3.5 ± 1.1[de]	8/11

[a] 1×10^6 3LL cells were implanted into a footpad. The implanted tumor was removed on day 9. TP-1 was injected IP for 7 days from day 9 and CPA was injected IP on day 10
[b] Pulmonary surface nodules were counted on day 21
[c] $P < 0.01$ compared with control group
[d] $P < 0.001$ compared with control group
[e] $P < 0.05$ compared with group treated with CPA at 50 mg/kg

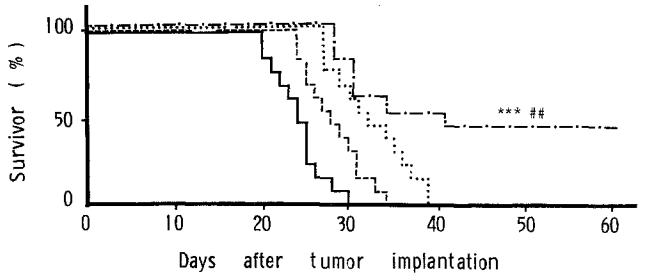

Fig. 2. Effect of TP-1 with or without cyclophosphamide on survival of mice with pulmonary metastases after removal of the implanted tumor (1×10^6 3LL cells were implanted into a footpad). The implanted tumor was removed on day 9. A daily dose of 10 mg/kg TP-1 was injected IP for 7 days from day 9. A single dose of 50 mg/kg CPA was injected IP on day 10. Each group contained 13 mice. *** $P < 0.001$ compared with the TP-1-treated group; ## $P < 0.01$ compared with the CPA-treated group. Groups: (———), control; (– – – – –), TP-1 treated; (·····), CPA-treated; (·—·—·—), TP-1 + CPA-treated

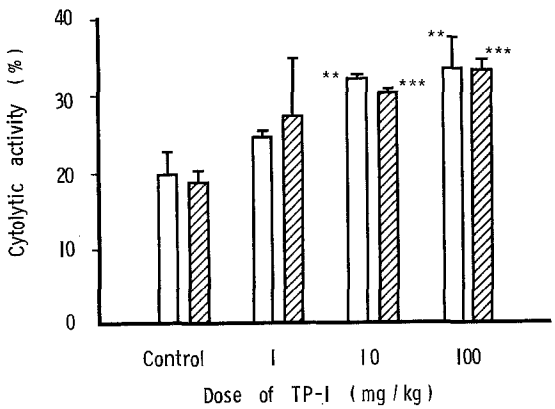

Fig. 3. Cytolytic activities of whole and nonadherent spleen cells from mice treated with TP-1.
3LL cells (1×10^6) were implanted into a footpad. The implanted tumor was removed on day 9. A dose of 1, 10, or 100 mg/kg TP-1 per day was injected IP for 7 days from day 9. Spleen cells were harvested on day 16. The ratio of effector cells to 3LL target cells was 50:1. *Columns*, means of three experiments, *bars*, SD. ** $P < 0.01$; *** $P < 0.001$ compared with the control group. □, whole spleen cells; ▨, nonadherent spleen cells

The antimetastatic effect of combined therapy with TP-1 and CPA was also examined by the same in vitro cytotoxicity test (Fig. 4). Considerable enhancement of the cytolytic activity of spleen cells was noted on treatment with 10 mg/kg TP-1 alone and 20 or 50 mg/kg CPA alone over the activity of cells from control mice treated with saline. Combined therapy of TP-1 and CPA resulted in far greater enhancement of the cytolytic activity of spleen cells than therapy with either TP-1 or 20 mg/kg of CPA alone.

In vitro cytolytic activity of spleen cells from tumor-bearing and tumor-excised mice

In vitro cytolytic activity of spleen cells from tumor-bearing mice was examined every 5 days after tumor implanta-

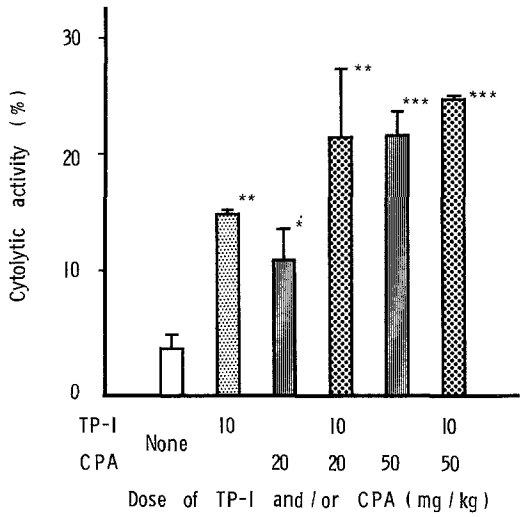

Fig. 4. Cytolytic activity of spleen cells from tumor-excised mice treated with TP-1 and cyclophosphamide. 3LL cells (1×10^6) were implanted into a footpad. The implanted tumor was removed on day 9. A daily dose of 10 mg/kg TP-1 was injected IP for 7 days from day 9. A single dose of 20 or 50 mg/kg CPA was injected IP on day 10. Spleen cells were harvested on day 16. The ratio of effector cells to 3LL target cells was 50:1. *Columns*, means of three experiments; *bars*, SD. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, compared with the control group

Table 3. Loss of cytolytic activitly of TP-1 treated spleen cells[a] by treatment with anti-Thy-1.2 antibody and complement

Treatment	Cytolytic activity (%)[b]	
	Control	TP-1 treatment
(–)	10.3 ± 1.4	24.4 ± 10.1
Complement	13.8 ± 0.2	20.8 ± 1.7
Anit-Thy-1.2	12.2 ± 1.5	20.8 ± 2.4
Anit-Thy-1.2 + complement	13.8 ± 2.1	5.6 ± 2.4[c]

[a] 1×10^6 3LL cells were implanted into a footpad. A daily dose of 10 mg/kg of TP-1 was injected IP for 7 days from the day of removal of the implanted tumor on day 9

[b] Cytolytic activity of spleen cells was assayed on day 16. The ratio of effector cells to 3LL target cells was 50:1

[c] $P < 0.01$ compared with other TP-1 treated groups

tion. Cytolytic activity of spleen cells was increased by degrees as the tumors grew, and reached maxima on day 15 after tumor implantation. Thereafter, their activities declined. Cytolytic activity of spleen cells from mice from which the implanted tumor was excised on day 9 was significantly decreased on day 15, compared with their activity from tumor-bearing mice (Fig. 5). Nonadherent cells were responsible for the cytolytic activity of spleen cells (Table 4).

Discussion

TP-1 was extracted from calf thymus by Falchetti et al. [10] in 1976. Since then it has been tested in vitro [2] and in vivo [22, 26]. This extract has been shown to have the capacity to increase the mitogen response of mouse spleen lymphoid cells, to stimulate an increase in the theta-positive cell population in mouse spleen and human cord blood, and to stimulate the ability of mouse marrow cells to induce a graft-versus-host response in x-ray-irradiated mice [11].

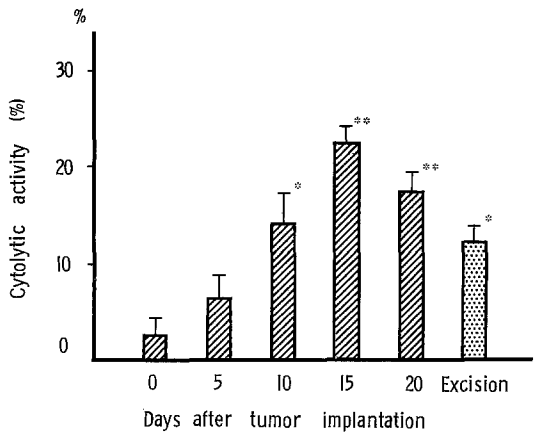

Fig. 5. Change in cytolytic activity of spleen cells from tumor-bearing and tumor-excised mice. 3LL cells (1×10^6) were implanted into a footpad. Cytolytic activity of spleen cells was assayed on various days. In mice from which the implanted tumor was excised on day 9, the cytolytic activity was assayed on day 15. The ratio of effector cells to 3LL target cells was 50:1 *Columns*, means of three experiments; *bars*, SD * $P < 0.05$; ** $P < 0.01$ compared with the control group

Table 4. Cytolytic activity of subpopulations of spleen cells from tumor-bearing and tumor-excised mice[a]

Experimental group	Cytolytic activity (%)		
	Whole spleen cells	Nonadherent spleen cells	Adherent spleen cells
Control	10.3 ± 2.1	8.1 ± 0.5	1.2 ± 16.9
3LL-excised	15.5 ± 0.02[b]	15.3 ± 0.3[c]	6.0 ± 6.1
3LL-bearing	24.0 ± 0.3	19.3 ± 7.1	7.1 ± 7.6[d]

[a] 1×10^6 3LL cells were implanted into a footpad. Cytolytic activity of spleen cells was assayed 7 days after removal of the implanted tumor on day 9 in tumor-excised mice and 16 days after tumor implantation in tumor-bearing mice. The ratio of effector cells to 3LL target cells was 50:1

[b] $P < 0.05$ compared with control group

[c] $P < 0.001$ compared with control group

[d] $P < 0.05$ compared with whole spleen cells from 3LL-bearing group

The present results indicate that TP-1 significantly inhibited both growth of the primary tumor and pulmonary metastases following implantation of 3LL cells, but that its inhibitory effect on tumor growth was not sufficient to increase the survival of mice with a primary tumor. We then tested whether TP-1 alone or in combination with CPA could prevent pulmonary micrometastases after removal of the primary tumor. TP-1 alone inhibited pulmonary metastases, and this effect was significantly greater with combination therapy.

As reported for other immunomodulators [16, 17, 27], the time of treatment was critical for obtaining good results with TP-1. In the present study, 3LL cells, which metastasize specifically to the lung, were implanted into the footpad, and the primary tumor was resected when it had grown to a predetermined size. When the primary tumor was removed at this time, all untreated mice died from pulmonary metastases without recurrence of the tumor at the original site. Our results suggest that TP-1 should be administered as near as possible to the time of formation of micrometastases in the lung. Two reasons are thought to be important for administration of TP-1 at this time. One is that micrometastases with a small tumor burden are easily eradicated by immunomodulators. The other is that the cytolytic activity of spleen cells increased by degrees after tumor implantation, reaching maxima in about 2 weeks. However, further stronger cytolytic activity of spleen cells was not induced after removal of primary tumor. This activity existed in the nonadherent fraction of spleen cells. Therefore, TP-1 was effective in micrometastases owing to an enhancing effect of the decreased cytolytic activity of cytotoxic T cells after removal of primary tumor.

Dosage is very important in studying the effect of thymic factors including TP-1 and various other immunomodulators. At high doses, some immunomodulators were reported not to be effective on tumor growth or even to cause immunosuppression resulting in promotion of tumor growth [26]. In the present study the highest dose of 100 mg/kg did not cause immunosuppression, but its inhibition of pulmonary metastases was the same as that of a dose of 10 mg/kg. This suggests that there is an optimal dose of TP-1.

Combined therapy with TP-1 and the anticancer agent CPA was very effective on lung metastases, resulting in a high rate of complete cure. Good therapeutic effects have also been observed with other combinations of thymic factors and anticancer chemotherapeutic agents. Chirigos [5] used thymosin with BCNU and CPA against Moloney lymphoid leukemia transplanted into BALB/c × DBA/2 mice, and Klein and Shoham [17] used TP-1 and CCNU against 3LL cells implanted into C57BL/6 mice. In human, success in treatment of leukemia [28], melanoma [3], and lung cancer [7] was reported following chemotherapy in combination with thymus humoral factor, TP-1, and thymosin fraction V.

To elucidate the antimetastatic mechanism of TP-1, we carried out a cytotoxicity test using spleen cells from mice treated with TP-1 as effector cells and 3LL cells as target cells. The results showed a significant increase in the cytotoxic activity of the effector cells. Enhancement of the cytolytic activity of spleen cells was not affected by selection of cells by plastic adherence, but was markedly reduced by treatment with anti-Thy-1.2 antibody and complement. This indicated that TP-1-activated Thy-1.2-positive cells, T cells, and thereby generated cytotoxic T lymphocytes.

The cytotoxicity of spleen cells from tumor-excised mice was enhanced by administration of CPA at 20 or 50 mg/kg, and a combination of TP-1 and CPA caused further enhancement of cytotoxicity. It was supposed that CPA increased antitumor cytotoxicity by eliminating suppressor elements in the spleen as well as by a direct antitumor effect. There are several reports that effective CPA therapy at a suitable time have resulted in elimination of suppressor elements in the spleen [14–16]. Various immunomodulators, such as thymic factors, are throught to induce not only cytotoxic cells but also suppressor cells at the same time [17, 18]. Thus if immunomodulators are used together with an anticancer agent such as CPA, which has inhibitory effect on activity of suppressor cells, the therapeutic effects should be greater than those expected from the additive effects of immunomodulators and anticancer chemotherapy.

It is concluded that TP-1 has potent antimetastatic activity at the stage of micrometastasis in the lung, which it exerts by causing activation of cytotoxic T cells in the target organ, and that its effect depends on time of its injection and dose. This suggests that TP-1 should be effective for inhibition and prevention of cancer metastases in humans.

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