Effect of Combined Intrapleural Administration of *Lactobacillus casei* (LC9018) and Adriamycin on Experimental Malignant Pleurisy in Mice

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The combined effect of *Lactobacillus casei* YIT9018 (LC9018) and adriamycin (ADR) on malignant pleurisy was investigated using an experimental model in BALB/c mice in which Meth A fibrosarcoma cells were intrapleurally implanted. The control mice died from dyspnea due to pleural effusion, before significant growth of tumor nodules could be achieved in the thoracic cavity. Intrapleural (ipl) administration of LC9018 (20–200 μg/head) on days 1 and 5 reduced the effusion volume and induced pleural adhesions in a dose-related manner. A statistically significant and reproducible prolongation of survival was observed at a dose of LC9018 200 μg/head: increase of lifespan (ILS) values of 15–39% were obtained. An ipl administration of ADR (2–4 mg/kg) on day 1 was also effective in prolonging survival without severe toxicity (ILS values of 100–122%). The combined use of ADR and LC9018 induced a high incidence of pleural adhesions, a delay in effusion accumulation, and an additive prolongation of lifespan (ILS values of 133–178%), compared with ADR monotherapy. In the combination therapy group, a marked and continuous ipl exudation of neutrophils, macrophages, and lymphocytes was observed with a significant decrease in pleural tumor cells. These findings suggest that ipl administration of LC9018 enhances the effect of ADR, probably through both host-mediated tumoricidal activity and sclerosing effects on the pleura.

Key words: *Lactobacillus casei* YIT9018 — LC9018 — Adriamycin — Malignant pleurisy — Meth A fibrosarcoma

Pleural effusions occur commonly in patients with lung and breast cancers as well as other malignancies associated with lung metastasis, and are a common complication, causing chest pain and severe respiratory dysfunction, when inadequately treated.[1, 2] Thoracentesis is effective but the pleural effusion usually recurs after simple aspiration.[1, 2] To prevent recurrence due to progression of the disease, obliteration of the pleural space and inhibition of tumor cell proliferation have been reported to be of value.[1–4] The ipl administration of several sclerosing agents or anticancer drugs plus thoracostomy has been recommended as the technique of choice. Favorable clinical results have been obtained in patients given anticancer agents with a sclerosing action, such as ADR, mitomycin C and cisplatin.[1, 2, 4, 5]

Recently, local immunotherapy using microorganism preparations, such as Bacillus Calmette-Guérin cell wall skeleton (BCG-CWS),[6, 7] *Nocardia rubra* cell wall skeleton (N-CWS),[8, 9] and OK-432,[10, 11] have been evaluated experimentally and clinically, and their beneficial effects on malignant pleural effusion have been reported in several instances. Therefore, a combination of these immunotherapeutic and chemotherapeutic drugs with sclerosing properties is expected to yield clinically satisfactory results.[1, 2, 12, 13]

Preparations of heat-killed cells of *Lactobacillus casei* YIT9018 (LC9018) have been reported to exhibit potent antitumor activity[14–17] as well as antimetastatic effects[18]

in various experimental tumor systems. Inhibition of the growth of effusion tumor cells and prolongation of survival time were observed in some animal models[19] and in clinical trials.[20] The effect of LC9018 was considered to be host-mediated and mainly due to augmentation of macrophage function.[14–19, 21–23] It was also reported that LC9018 demonstrated a sclerosing effect on the pleura.[20]

In the present study, by using the experimental malignant pleurisy model in mice,[19] we confirmed the antitumor activity of ipl administration of LC9018, and further investigated the effect of a combination of ADR and LC9018 with special attention to the changes in the relative populations of the various thoracic exudate cells. The usefulness of this model is discussed in detail.

MATERIALS AND METHODS

Animals Inbred male BALB/c mice, 5 weeks old, were purchased from the Shizuoka Laboratory Animal Center (Hamamatsu), and were housed under specific pathogen-free conditions in our animal laboratory. They were given free access to commercial diet and water.

Tumor cells Meth A fibrosarcoma was maintained by serial intraperitoneal passage in syngeneic BALB/c mice. Ascitic fluid was collected 7 days after implantation, and tumor cells were washed twice with Hanks' balanced salt solution without calcium or magnesium (Gibco Lab., New York). Tumor cells were finally suspended in the

same medium at a concentration of 5×10^6 cells/ml. The cell suspension (0.1 ml) was inoculated into the right thoracic cavity of mice.[19]

Drugs LC9018[14] was provided by Yakult, Tokyo, and was suspended in sterile isotonic saline at concentrations of 4 to 4000 μg/ml. ADR (Kyowa Hakko Kogyo Co., Ltd., Tokyo) was dissolved in sterile isotonic saline. On days 1 and 5 after tumor cell implantation, 0.05 ml of LC9018 suspension or vehicle (phosphate-buffered saline containing maltose) was administered ipl. ADR (0.05 ml) or saline was also given ipl on day 1.

Evaluation of therapeutic effects on malignant pleurisy Survival times of mice (9 to 10 mice/group) were monitored and the increase of lifespan (ILS) was calculated using the following formula: ILS% = (median survival time of treated group in days/median survival time of control group in days $-1) \times 100$. In certain cases, mice (4 to 7 mice/group) were killed 7 to 15 days after tumor inoculation and the volume and characteristics of pleural effusion were examined.

Examination of thoracic exudate cells Thoracic exudate cells were collected on days 2 to 15 by washing the thoracic cavity of mice (5 mice/group) with phosphate-buffered saline containing 0.313% sodium citrate. An aliquot of cell suspension was diluted with balanced electrolyte solution (ISOTON II, Coulter Scientific Japan Co. Ltd., Tokyo), and then erythrocytes were depleted by treatment with 0.33% KCN solution (Zap-Oglobin II, Coulter Scientific Japan). The number of residual cells was counted with a Coulter counter (Coulter Scientific Japan). Smears were prepared from aliquots of thoracic exudate cell suspension and stained with Wright-Giemsa solution. The cell population of the pleural effusion was microscopically determined by means of a differential cell count.

Statistical analysis The statistical significance of differences between survival days of test groups was evaluated by means of a median test. Student's t test was used to test the statistical significance of differences between effusion volumes or numbers of thoracic exudate cells in the groups. The probability of a difference between incidences of pleural effusion or of pleural adhesion in the groups was determined by Fisher's exact probability test.

RESULTS

Effect of single treatment with LC9018 on malignant pleurisy As shown in Table I-Exp. 1, LC9018 at doses of 0.2 to 20 μg/head had no significant effect on the survival time of the mice with Meth A pleurisy. At a dose of 200 μg/head, however, LC9018 induced a significant prolongation of the survival period. The antitumor effect of this dose was reproducible (Table I-Exps. 2 and 3). Although pleural effusion was observed in all mice at the time of

Table I. Effects of Local Administration of LC9018 on Malignant Pleurisy Caused by Intrapleural Transplantation of Meth A Cells

	Dose (μg/head)	Number of mice	Survival (days)		
			Range	Median	ILS%[a]
Exp. 1	0	10	8–12	9	—
	0.2	10	8–16	9	0
	2	10	8–23	9	0
	20	9	8–25	11	22.2
	200	10	9–19	12.5	38.9*
Exp. 2	0	10	7–14	9	—
	200	10	11–17	12	33.3*
Exp. 3	0	10	8–10	10	—
	200	10	8–15	11.5	15.0***

Meth A (5×10^5 cells) was inoculated into the right thoracic cavity of male BALB/c mice on day 0. LC9018 at the indicated doses was administered ipl on days 1 and 5. Saline (Exp. 1 and 2) or vehicle (Exp. 3) was given to the control mice. The volume and characteristics of pleural effusion were examined after death.
a) ILS% (increase of lifespan, %) was calculated from median survival days (see "Materials and Methods").
*, ***: $P < 0.05$, 0.001 vs. control by median test.

death, at an earlier stage, 7 days after tumor cell inoculation, a dose-related decrease in the number of mice with pleural effusion was observed (Table II). The effusions of control mice were cell-rich, turbid and hemorrhagic without exception, while chylaceous and fibrinous, rather than bloody, effusions were observed in the LC9018-treated groups. An almost dose-dependent reduction of effusion volume was also observed in the latter. The incidence of pleural adhesion was significantly higher in the group treated with 200 μg/head of LC9018, while no pleural adhesion was observed in the control group. In mice that survived more than 10 days, tumor nodules from pin-point to millet seed size were observed. The nodules were confined to the right cavity and coincided in position with impalement injuries incurred during tumor cell implantation.

Effect of single treatment with ADR on malignant pleurisy As shown in Table III-Exp. 1, a single ipl injection of 4 to 8 mg/kg of ADR significantly prolonged survival time in mice. The median survival times were 9 and 20 to 24 days, respectively, in the control and ADR-treated groups. There was no statistically significant difference between the results with 4 mg/kg ADR and with 8 mg/kg ADR. Treatment with 8 mg/kg ADR caused severe body weight loss of more than 15% (>3 g/mouse) compared with the values before administration, while no body weight loss but rather a body weight gain of 15%

Table II. Effects of LC9018 on Pleural Adhesion, Pleural Effusion on Day 7

Dose (μg/head)	Pleural adhesion[a] (%)	Pleural effusion		
		Occurrence[b] (%)	Volume (ml/head)	Characteristics[c]
0	0	71.4	0.58 ± 0.16^{d}	Bloody
20	14.3	57.1	0.29 ± 0.12	Chylaceous
60	14.3	42.9	$0.10 \pm 0.05^{*}$	Chylaceous
200	$57.1^{(*)}$	28.6	0.17 ± 0.14	Chylaceous, fibrinous

Meth A (5×10^5 cells) was inoculated into the right thoracic cavity of male BALB/c mice (7 mice/group) on day 0. LC9018 at the indicated doses was administered ipl on days 1 and 5. Control mice were given the vehicle in the same manner. Seven days after tumor cell implantation, mice were sacrificed and gross examination of the pleural cavity was performed.

a) (Number of mice with pleural adhesion/number of mice used) × 100.

b) (Number of mice with pleural effusion/number of mice used) × 100.

c) "Bloody" indicates cell-rich, hemorrhagic, turbid effusion; "chylaceous" signifies white (occasionally faintly pink) cloudy effusion; "fibrinous" covers white- or pale yellow-colored, opaque, tenacious fluid or effusion with pseudomembrane-like appearance.

d) Mean ± SE.

(*): $P < 0.05$ vs. control by Fisher's exact probability test.

*: $P < 0.05$ vs. control by t test.

Table III. Effects of Intrapleural Administration of ADR on Malignant Pleurisy Caused by Intrathoracic Implantation of Meth A Cells

Dose (mg/kg)		Number of mice	Survival (days)			Toxicity	
			Range	Median	ILS%[a]	8th day survivors	BWI%[b]
Exp. 1	0	10	8–12	9	—	60^{c}	15.2
	4	10	13–50	19.5	117***	100	0.5
	8	9	20–60<	24	167***	100	−16.9
Exp. 2	0	10	7–11	8.5	—	50	14.4
	16	10	5–8	6	−29*	0	nd^{d}
	32	10	5–7	5	−41***	0	nd

Meth A (5×10^5 cell) was inoculated into the right thoracic cavity of male BALB/c mice on day 0. ADR at the indicated doses was administered ipl on day 1. The volume and characteristics of pleural effusion were examined after death.

a) See footnote a) in Table I.

b) BWI% (Body weight increase %) = (mean body weight of mice surviving on day 8/mean body weight of mice on day 1 − 1) × 100.

c) (Number of mice surviving on day 8/Number of mice used) × 100.

d) Not determined because there were no survivors on day 8.

*, ***: $P < 0.05$, 0.001 vs. control by median test.

was observed in the 4 mg/kg ADR-treated and control groups, respectively. Most mice with prolonged survival had progressive tumor masses with bloody or serous effusion in the thoracic cavity at the time of death. The tumors, which varied in size, had formed along the scar line of tumor implantation in the lung, pleura and muscles of the thorax. Administration of 16 to 32 mg/kg of ADR failed to prolong survival despite translucent serous effusion suggesting inhibition of tumor cell growth (Table III-Exp. 2). Marked decreases in body weight were observed in the mice receiving these high doses of ADR, which may account for the failure. In terms of lethality and severe body weight loss, therefore, the optimal dose by a single ipl injection was estimated to be

Table IV. Combined Effects of Intrapleurally Administered LC9018 and Adriamycin on Malignant Pleurisy Caused by Intrapleural Transplantation of Meth A Cells

Treatment	Number of mice	Survival		
		Median (days)	ILS%[a]	20th day survivors (%)
Control	10	9	—	0[b]
LC9018	9	12	33***	0
ADR 2 mg/kg	9	18	100***	22.2
ADR 2 mg/kg +LC9018	10	21	133**, c)	50.0(*)
ADR 4 mg/kg	10	20	122***	40.0(**)
ADR 4 mg/kg +LC9018	10	25	178***	60.0(**)

Meth A (5×10^5 cells) was inoculated into the right thoracic cavity of male BALB/c mice (9–10 mice/group) on day 0. LC9018 at 200 μg/head or vehicle was administered ipl on days 1 and 5. ADR at the indicated doses or saline was injected ipl on day 1.
a) See footnote a) in Table I.
b) (Number of surviving mice on day 20/number of mice used) $\times 100$.
c) $P<0.05$ vs. ADR alone by the median test.
**, ***: $P<0.01$, 0.001 vs. control by the median test.
(*),(**): $P<0.05$, 0.01 vs. control by Fisher's exact probability test.

under 8 mg/kg for ADR in the tumor-bearing mice, and 2 and 4 mg/kg of ADR were used for further studies of combination therapy.

Combined effect of ADR and LC9018 on survival time
As shown in Table IV, LC9018 at a dose of 200 μg/head reproducibly showed an ILS value of 33%. The survival period was prolonged by a single ipl administration of ADR to a level about twice that of the control, and additive prolongation was observed in the combination therapy group. The median survival times were 18 and 21 days and the ILS values were 100% and 133%, respectively, in the groups treated with ADR 2 mg/kg alone and with ADR 2 mg/kg plus LC9018. The difference between the two was statistically significant. The median survival time and the ILS value in mice given ADR 4 mg/kg plus LC9018 were 25 days and 178%, while those in mice given ADR 4 mg/kg were 20 days and 122%. None of the control mice or those treated with LC9018 alone survived until day 20. The percentage of long-term survivors (those surviving at the 20th day and therefore having ILS values of over 125%) was 50% in the group treated with ADR 2 mg/kg plus LC9018, but only 22% in the 2 mg/kg ADR monotherapy group. Survival beyond 20 days was seen in 40% and 60% of mice treated with 4 mg/kg of ADR alone and with LC9018, respectively.

Table V. Effects of LC9018 and/or ADR on Pleural Adhesion and Pleural Effusion

Day	Treatment	N[a]	Pleural adhesion (%)	Pleural effusion		
				Occurrence (%)	Volume (ml/head)	Characteristics[b]
7	Control	5	0[c]	60[d]	0.52 ± 0.22[e]	Bloody, turbid
	LC9018	5	20	40	0.10 ± 0.06	Chylaceous, fibrinous
	ADR	5	0	20	0.22 ± 0.22	Bloody
	ADR+LC9018	5	40	20	0.06 ± 0.06	Serous
11	ADR	5	0	100	0.25 ± 0.07	Turbid, transluscent
	ADR+LC9018	5	50	25*	0.06 ± 0.06	Chylaceous
15	ADR	6	0	100	0.54 ± 0.07	Bloody, transluscent
	ADR+LC9018	4	50	100	0.50 ± 0.14	Bloody, chylaceous, serous

Meth A (5×10^5 cells) was inoculated into the right thoracic cavity of male BALB/c mice on day 0. ADR (2 mg/kg) was administered ipl on day 1, or LC9018 (200 μg/head) on days 1 and 5. Control mice were given saline and vehicle in the same manner. The mice were killed on days 7, 11, and 15, and a gross examination of the pleural cavity was carried out.
a) Number of mice used.
b) The terms "bloody," "chylaceous," and "fibrinous" are explained in footnote c) in Table II; "turbid" indicates a non-hemorrhagic cell-rich effusion; "transluscent," a non-hemorrhagic exudate with lower turbidity; and "serous," an amber- or straw-colored semitransparent effusion containing few cells.
c) (Number of mice with pleural adhesion/number of mice used) $\times 100$.
d) (Number of mice with pleural effusion/number of mice used) $\times 100$.
e) Mean $\pm$ SE.
*: $P<0.05$ vs. ADR alone by Fisher's exact probability test.

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Effects of ADR and/or LC9018 on pleural adhesion and pleural effusion Table V shows the gross findings in pleural cavities examined on days 7 to 15. No pleural adhesion was observed in the control mice or in those treated with ADR alone during this examination period. Pleural adhesion occurred in mice treated with LC9018 alone, and with ADR plus LC9018 at rates of 20% and as high as 40 to 50%, respectively. Significant amounts of bloody or turbid effusion were already observed in 60% of the control mice on day 7, while slight or moderate amounts of non-bloody effusion were observed in 40% of mice treated with LC9018 alone. The incidence of pleural effusion was lower (20%) in the groups treated with ADR alone and in those receiving ADR plus LC9018. On day 11, when most of the control and LC9018-treated mice had already died, the ratio of effusion-bearing mice reached 100% in the ADR-treated group, but only 25% in the ADR plus LC9018 group. Fifteen days after tumor cell implantation, no effusion-free mice were observed even in the combination therapy group, and most mice had progressing tumor nodules.

Effects of ADR and/or LC9018 on cell population in pleural effusion As shown in Fig. 1a, the number of Meth A cells in control mice increased markedly with the lapse of time. Tumor growth in the mice treated with LC9018 alone or with 2 mg/kg of ADR alone tended to be inhibited, but statistical significance was not obtained during the first 7 days. In contrast, the number of Meth A cells was significantly reduced during the first week in mice administered with both ADR and LC9018, indicating the effectiveness of combined therapy in inhibiting early tumor cell growth. The number of neutrophils (Fig. 1b) increased significantly after the administration of ADR and/or LC9018, especially 1 to 2 days after administration. A significant increase in the number of macrophages was also observed in the drug-treated groups on day 7 (Fig. 1c). The exudation of neutrophils and macrophages was most marked in the combined therapy group, and it persisted throughout the examination period. The increases induced by administration of

ADR alone were more transient and significantly lower than those induced by combined administration. On the other hand, a lasting increase of lymphocytes was observed in mice given ADR alone or ADR with LC9018 (Fig. 1d). Figure 2 indicates the effects of ipl administration of ADR and/or LC9018 on the proportions of constituent cells in pleural effusions 7 days after tumor implantation. The respective mean values of the total number of cells per thoracic cavity in the control, LC9018-treated, ADR-treated, and combination therapy groups were 2.3×10^7, 1.9×10^7, 1.9×10^7 and 2.0×10^7, respectively. The dominant cell population in the controls' pleural effusions consisted of tumor cells: Meth A cells accounted for more than 80%. In mice treated with LC9018 alone, the ratio of tumor cells was reduced
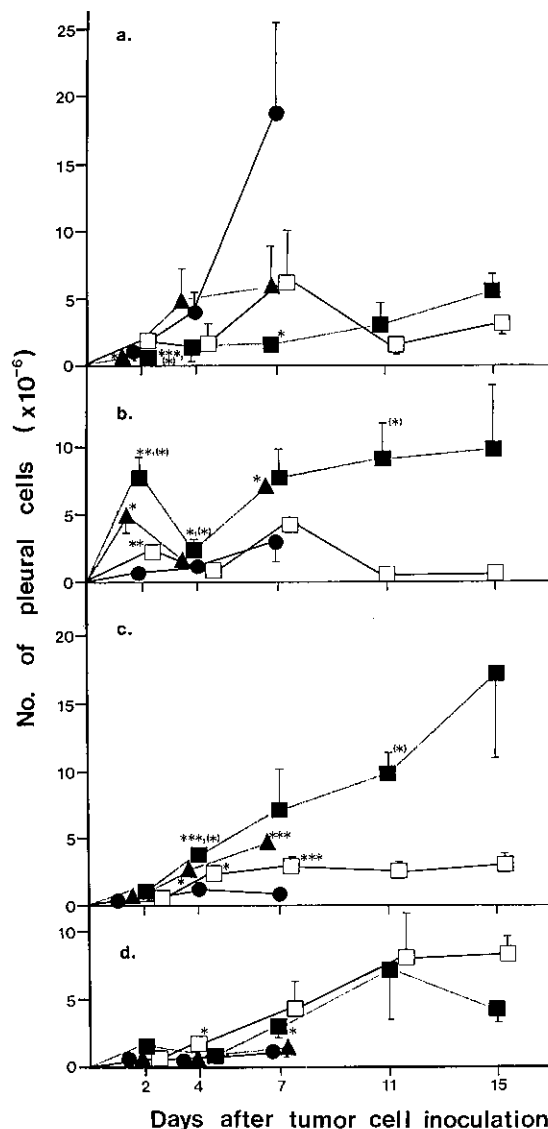

Fig. 1. Effects of intrapleural administration of ADR and/or LC9018 on the number of exudate cells in the pleural cavity of mice with malignant pleurisy. Meth A cells (5×10^5 cells) were transplanted into the pleural cavity of male BALB/c mice on day 0. The mice were administered 2 mg/kg of ADR or saline on day 1, and 200 μg/head of LC9018 or vehicle on days 1 and 5. Thoracic exudate cells were collected on the days indicated, and the numbers of tumor cells (a), neutrophils (b), macrophages (c) and lymphocytes (d) were determined as described in "Materials and Methods." Each plot represents the mean and the standard error. ●, control; ▲, LC9018; □, ADR; ■, ADR+LC9018. *, **, ***, $P<0.05$, 0.01, 0.001 vs. control by t test. (*), $P<0.05$ vs. ADR alone by t test.
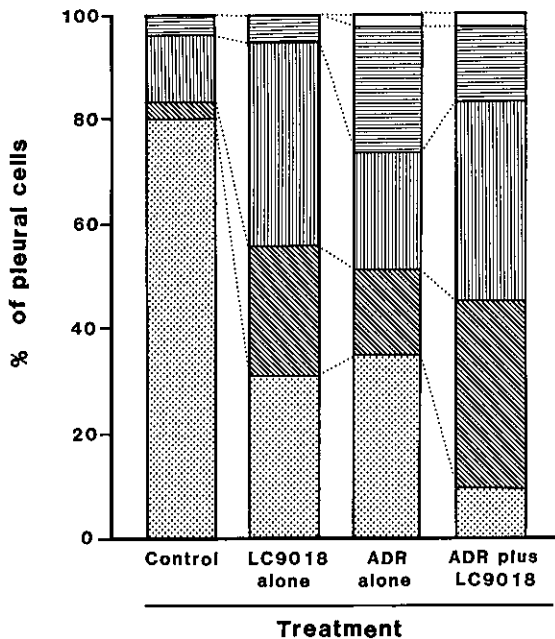
Fig. 2. Constituent proportions of the various thoracic exudate cells in mice treated with LC9018 and/or ADR at 7 days after ipl tumor implantation. Meth A cells (5×10^5 cells) were inoculated into the pleural cavity of mice. Drugs or vehicles were administered intrapleurally according to the same schedule as noted in the legend for Fig. 1. Seven days after implantation, pleural effusion smears were examined microscopically. ▨, Meth A cells; ▩, macrophages; ▥, neutrophils; ▤, lymphocytes; ☐, others (eosinophils, mast cells, etc.).

to 31%, and that of neutrophils and macrophages was increased to 38% and 25%, respectively. In mice treated with 2 mg/kg of ADR alone, the ratio of tumor cells was 35%, and a characteristically high population of lymphocytes (24%) was observed. The combined administration of ADR and LC9018 induced a marked decrease in the ratio of tumor cells: Meth A cells amounted to only 9% of the total. In contrast, the ratios of neutrophils and macrophages were high (38 and 36%, respectively), and that of lymphocytes was relatively high (14%).

DISCUSSION

Matsuzaki et al.[19] reported in relation to the antitumor effect of immunotherapeutic agents in this experimental model that multiple ipl administration of LC9018 at doses of 100 to 500 μg/head significantly prolonged the survival time, and this activity was almost equal or even superior to that of OK-432, Corynebacterium parvum (C. parvum), or BCG. In the present study, two injections of LC9018 were effective in controlling pleural effusion at

doses as low as 20 to 60 μg/head, as judged from the reduction in effusion volume, decrease in incidence of hemorrhagic effusion, and increase in effusion-free mice at an early stage. In addition, a statistically significant and reproducible prolongation of survival was obtained at a dose of 200 μg/head. These data strongly suggest the effectiveness of a single use of LC9018 in patients with malignant pleurisy. In fact, Nakata et al.[20] administered LC9018 at ipl doses of 0.5 to 1 mg/man to 4 patients with malignant pleurisy, and obtained favorable results in 3 of them.

Different mechanisms of action may be responsible for the control of effusions by different agents. Sclerosing agents, such as tetracycline and quinacrine, may act by creating a fibrous pleuritis,[1-3] whereas 5-FU and bleomycin may act exclusively through their cytotoxic effect on cancer cells.[1,2] ADR, mitomycin C and cisplatin also have a local effect on the serous membranes in addition to their direct cytotoxic effect on cancer cells.[1,2,4,5] In the case of LC9018, the role of effector cells induced into the thoracic cavity was considered to be very important. The changes in amount and composition of the pleural effusion in tumor-bearing mice indicated that, after LC9018 administration, at first neutrophils rapidly increased, and subsequently macrophages became dominant. These changes closely resembled those in normal mice given LC9018 intrapleurally.[18,19] Matsuzaki and coworkers[18,19] demonstrated that ipl administration of LC9018 to normal mice resulted in enhancement of the phagocytic function and cytolytic activity of thoracic macrophages, as well as the NK cell activity of thoracic exudate cells and the subsequent appearance of Ia-positive macrophages. These results suggest that the effectiveness of ipl administration of LC9018 on malignant pleurisy is mainly attributable to the augmentation of non-specific as well as specific host defense systems, which is triggered by the phagocytosis of LC9018 by macrophages. On the other hand, the characteristic findings in mice given ipl LC9018 were an extreme thickening and fibrosis of the serous membranes as well as adhesions of the pleura. The effect of LC9018 on mouse pleura, grossly evaluated, was greater than that of ADR. In previous histological studies,[5,12,13,20] the sclerosing effect of LC9018 on rabbit pleura was shown to be inferior to that of ADR, but almost equal to those of other microorganism preparations, such as OK-432, N-CWS and BCG, and probably superior to those of antitumor polysaccharides, schizophyllan and lentinan. Therefore, the beneficial response was partly due to a local serositis leading to partial obliteration of the serous cavity.

Combined administration of LC9018 and ADR resulted in enhancement of pleural effusion control and an additive prolongation of survival: the survival of mice treated with LC9018 as a supplement to ADR treatment

was superior to that of mice receiving ADR alone. Although the mechanisms of action of combination therapy with ADR and LC9018 are still obscure, several possibilities are consistent with the present results. First, the direct antitumor effect of ADR and the host-mediated antitumor action of LC9018[14-17, 21-23] inhibited tumor cell proliferation in pleural fluid to a great extent. Secondly, obliteration of the pleural space was enhanced through the sclerosing effects of both agents.[5, 13, 20] Komuro et al.[13] studied the histological changes induced in pleura by various immunostimulants given in combination with ADR, and noted that there were marked fibrin exudation and adhesion of the pleura, accompanied by infiltration of lymphocytes into the connective tissue layers. ADR has recently been shown to have a significant influence on non-specific cytotoxic effector cells[24-27]: for example, Salazar and Cohen[24] reported that peritoneal exudate cells from mice given ip injections of ADR were cytotoxic to both macrophage- and NK-sensitive tumor cells. The present cytological examination of effusion obtained from mice treated with ADR showed a moderate increase in neutrophils and macrophages, and a subsequent increase in lymphocytes. This is a strong indication of a third mechanism of action, in which synergistic augmentation of these non-specific effector cells by LC9018 and ADR plays an important role in the inhibition of tumor cells.

The strong similarities between this experimental model and clinical cases are that, without control of the pleural effusion, the tumor-bearer died from dyspnea before significant tumor progression, and that re-accumulation of effusion was associated with disease progression elsewhere.[1-4] Furthermore, in this model, control of the effusion as a temporizing treatment was capable of increasing the survival period, but the response was terminated by death when measures to prevent tumor progression were inadequate. This reflected both the usefulness and the limitation of this therapy, as previously observed in clinical trials.[1-4] OK-432,[2, 10, 11] C. parvum[1, 2] and BCG,[2, 28] whose beneficial effects on pleurisy have already been confirmed by clinical trials, were reported to demonstrate their antitumor activities clearly in this model.[19] The therapeutically effective but low-toxicity dose of ADR in this model was 6 to 12 mg/m^2, which is within the range of clinical dose reported previously: Harada et al.[4] concluded that the appropriate intrathoracic dose of ADR in the treatment of malignant pleural effusion was about 20 to 30 mg/man (11 to 18 mg/m^2). Therefore, we consider that this experimental model has potential as an evaluation method of the therapeutic effects of pharmacological agents on malignant pleurisy.

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