EFFECT OF A POLYSACCHARIDE FRACTION FROM *GRIFOLA FRONDOSA* ON IMMUNE RESPONSE IN MICE

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(Received September 19, 1984)

The biological and immunomodulating activities of polysaccharide fraction (GF-1), an antitumor polysaccharide fraction from cultured fruiting bodies of *Grifola frondosa*, was examined in mice. GF-1 showed no cytotoxic effect on culturing tumor cells. However, GF-1 induced resistance against Sarcoma 180 in ICR mice which had completely regressed from the tumor by the effect of GF-1. The administration of GF-1 into mice increased the weights or cell numbers of spleen, and peritoneal cavity. GF-1 enhanced the antigen specific antibody response and carbon clearance activity, whereas GF-1 did not show polyclonal B cell activation and mitogenic activities, and the effect on delayed type hypersensitivity.

Keywords — *Grifola frondosa*; polysaccharide; antitumor activity; immunomodulating activity; fungi

INTRODUCTION

Grifola frondosa is a fungus belonging to Polyporaceae, Basidiomycetes, and the fruiting bodies have been used as an edible mushroom. In our previous studies,¹⁾ we have characterized the antitumor activity and physicochemical properties of a polysaccharide fraction (GF-1) extracted from the cultured fruiting bodies. GF-1 is not completely purified preparation, and contains a small amount of protein and phosphorus in addition to 93.0% of carbohydrate. However, it was strongly suggested that the material playing major role in the antitumor activity of GF-1 is 6-branched β -1,3-linked glucan from the results of examination on relationship between activity and structural feature.²⁾ We had presumed that GF-1 will probably show a similar antitumor effect as lentinan which is also a 6-branched β -1,3-linked glucan, whereas some differences between GF-1 and lentinan were observed in several items of antitumor activity. Namely, 1) the antitumor effect was observed not only intraperitoneally (*i.p.*), intravenously (*i.v.*) but also

intratumorally (*i.t.*) administrations, 2) the antitumor effect was not observed when GF-1 was administered before the tumor inoculation, and 3) GF-1 exhibited an antitumor activity against solid form of Meth A fibrosarcoma in BALB/c mice.

Many studies have shown that antitumor polysaccharides possess immunomodulating activity,^{3,4)} and their antitumor effects are exhibited based on their activities as immunomodulator.⁵⁻⁷⁾ In this study, we attempted to examine whether GF-1 has similar properties as other many antitumor polysaccharides concerning the antitumor and immunomodulating activities. In a previous report,¹⁾ the characterization of antitumor effect of GF-1 was performed mainly in allogeneic host-tumor system (ICR mice-Sarcoma 180). Therefore, we also assayed the immunomodulating activity using mainly ICR mice. In this paper, the immunomodulating activity of GF-1 is discussed.

MATERIALS AND METHODS

Mice — Male ICR, BALB/c, C3H/He and C57BL/6 mice obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka, were employed for the experiments. Mice used were 6–8 weeks of age.

Tumors — Sarcoma 180 tumor cells have been maintained serially in ascites form by weekly passage in ICR mice. Similarly, Meth A fibrosarcoma and MM46 adenocarcinoma were maintained in BALB/c and C3H/He mice, respectively. EL-4 leukemia has been subcultured in RPMI 1640 medium (Nissui Seiyaku Co., Tokyo) containing 10% heat-inactivated fetal calf serum (FCS, Grand Island Biological Co., Grand Island, N.Y.), 5 mM *N*-hydroxyethyl-piperazine-*N'*-2-ethanesulfonate (HEPES), 100 U/ml of penicillin and 100 μ g/ml of streptomycin.

Preparation of GF-1 — GF-1 was prepared in accordance with the previous description¹⁾ as follows: The cut fruiting bodies of *G. frondosa* (100 g) were refluxed with 80% ethanol, and was extracted with distilled water (2 l) in an autoclave (1 kg/cm², 121 °C) for 1 h. After filtration, the filtrate was dialysed against distilled water. The internal solution was concentrated to a small volume *in vacuo*, and four volumes of ethanol was added to the concentrate to form a precipitate. The precipitate was dissolved in 500 ml of 1% (w/v) NaOH, and to the solution 500 ml of 7% (w/v) CuSO₄ was added. The yielded precipitate was collected and dissolved in 100 ml of 10% (v/v) acetic acid, and dialyzed against distilled water. After deionization by a column of Dowex 50 (H⁺) following dialysis, the internal solution was lyophilized. The yield of material obtained was 3.4% of the starting materials. This material was named GF-1.

Agents — Heat-killed cells of *Propionibacterium acnes* C7 were kindly provided by Kowa Co., Tokyo. Lipopolysaccharide (LPS) from *Escherichia coli* 055:B5 prepared by the method of Westphal was purchased from Difco Laboratories, Detroit, Mich.

Assay of Direct Cytotoxic Effect of GF-1 on

Tumor Cells — Direct cytotoxic effect of GF-1 on tumor cells was assayed by following two methods. i) One ml of a suspension of tumor cells (Meth A fibrosarcoma, MM46 adenocarcinoma or EL-4 lymphoma, 2×10^5 cells/ml) and GF-1 (final concentration of 50 or 500 μ g/ml) were mixed in 24-well tissue culture plate (Nunc, Roskilde, Denmark). The mixture were incubated for 24, 48 or 96 h at 37 °C in a humid atmosphere containing 5% CO₂ in air. After the culture was completed, cells were collected, washed with saline and viable tumor cells were counted by a dye exclusion method. ii) Sarcoma 180 (2×10^5 cells) were mixed with GF-1 at a final concentration of 50 or 500 μ g/ml and incubated for 3 h at 37 °C in a CO₂ incubator. After these procedures, the viability of cells in each group was about the same. Then, 0.2 ml of the suspension (1×10^7 cells/ml in saline) was injected *i.p.* into ICR mice. The survival time of those mice after tumor inoculation was recorded.

Assay of Effect of Reinoculation of Tumor Cells into Tumor-Regressed Mice — Sarcoma 180 (5×10^6 cells) were inoculated *s.c.* into the right groins of mice (day 0). Four mg of GF-1 was injected *i.p.* for 5 times on every other days from 24 h after the tumor inoculation. Tumor regression was checked on day 40. Tumor regressed mice were reinoculated *s.c.* with 5×10^6 cells of Sarcoma 180 in their right groins. Twenty-nine days later, growth of the reinoculated tumors was observed.

Estimation of the Weights and Nucleated Cell Numbers of Some Lymphoid Organs — Mice were killed by cervical dislocation, and their peritoneal exudate cells (PEC) were collected by washing out peritoneal cavities with Hanks' solution (Nissui Seiyaku Co.). Then, their spleens and thymuses were removed, and weighed in a wet condition. Spleens were teased in Hanks' solution to prepare single cell suspensions. The PEC or spleen cell suspensions were diluted approximately with Türk solution, and the cells were counted with a hemocytometer.

Plaque-Forming Cell (PFC) Assay — The PFC responses to sheep erythrocytes (SRBC) and

SRBC coupled with 2,4,6-trinitrophenyl (TNP-SRBC) were determined by a modification of the hemolytic assay of Jerne and Nordin.⁸⁾ Mice were killed by cervical dislocation and cell suspensions from the spleens were prepared by teasing the spleen in the cold MEM medium (Nissui Seiyaku Co.). Each cell suspension (0.1 ml) was mixed with 0.05 ml of a 50% suspension of TNP-SRBC and 1.5 ml of soft agar (0.8% agar, lactalbumin hydrolysate and 0.05% diethylaminoethyl (DEAE) dextran in Hanks' solution) kept at 47 °C after boiling. Each mixture was then layered onto a plastic dish (7.5 cm diameter) which was previously layered with a base agar (1.4% agar, 0.5% lactalbumin hydrolysate and 0.05% DEAE dextran in Hanks' solution). After the incubation of dishes for 60 min at 37 °C, 1.0 ml of guinea pig serum, which had been absorbed with TNP-SRBC and diluted with MEM medium, were added as the complement source and the dishes were further incubated at 37 °C for 60 min. In the case of anti-SRBC PFC assay, intact SRBC was used in place of TNP-SRBC. The numbers of TNP-specific response were calculated by subtracting the response to SRBC from the TNP-SRBC response. Rabbit anti-mouse immunoglobulin (Ig) serum (Miles-Yeda, Ltd., Miles Laboratories, Inc., Kankakee, Ill.) was used for indirect PFC assay.

Phagocytic Activity Assay — The phagocytic function was measured by the method of Biozzi *et al.*⁹⁾ Mice were injected *via* the tail vein with colloidal carbon (Pelikan Drawing Ink, Günther Wagner, Germany) and 25 μ l blood samples were taken from the orbital veins of the mice at 5, 10, 15 and 20 min. The blood was discharged into 2 ml of 0.1% (w/v) Na_2CO_3 solution and the absorbance at 675 nm was determined. The phagocytic activity is expressed as the clearance rate ($t_{1/2}$: the half-life period of the injected carbon in the blood). The phagocytic index, K , was calculated by means of the following equation:

$$K = \frac{\ln \text{OD}_1 - \ln \text{OD}_2}{t_{1/2} - t_1} \quad t_{1/2} = \frac{0.693}{K}$$

where OD_1 and OD_2 are the optical densities at times t_1 and t_2 , respectively.

Delayed Type Hypersensitivity (DTH) Assay — DTH was measured as footpad swelling. An eliciting dose of 1×10^8 SRBC in a volume of 20 μ l was injected into subcutaneous tissue on the plantar surface of one hind footpad. The degree of swelling was measured after 24 h with dial thickness gauge calipers. Reactions were expressed as the difference in thickness between the feet that had received SRBC and those that had not.

Mitogenicity Assay — Mice were killed by cervical dislocation and the cell suspensions from the spleens were prepared by teasing the spleen in the cold RPMI 1640 medium (Nissui Seiyaku Co.) containing FCS, HEPES and antibiotics. The cell suspensions were passed through a 200-gauge stainless steel sieve and then let stand to remove tissue fragments. The cell suspensions were centrifuged ($600 \times g$ for 10 min), resuspended gently in a 0.83% (w/v) ammonium chloride solution to lyse red cells, and washed with fresh medium. Viability was assessed with the trypan blue dye exclusion test. The cell suspensions were adjusted to 5×10^6 viable cells per ml. Each sample solution (25 μ l) was placed in a flat bottomed 96-well tissue culture plate (Nunc) and 2-fold enriched medium (25 μ l) was added for osmotic stabilization. And then 100 μ l of the cell suspension prepared above was added to each well. The culture was incubated for 48 or 72 h at 37 °C in a CO_2 incubator. Twenty hours before harvesting, 0.5 μCi ^3H -TdR, sp. act: 22.5 Ci/mmol (New England Nuclear, Boston, Mass.), was added to the culture at a volume of 20 μ l. At the end of culturing, the cells were harvested and radioactivity of the cells was measured in a scintillation counter.

RESULTS

Direct Cytotoxic Effect of GF-1 on Tumor Cells

To examine the direct cytotoxic effect of GF-1 on tumor cells, the following two experiments were performed. First of all, tumor cells

(Meth A, MM46 or EL-4) were incubated with GF-1 (50 or 500 $\mu\text{g/ml}$) for 24, 48 or 72 h, and the viable cell numbers were counted. As shown in Fig. 1, both doses of GF-1 did not affect the growth of tumor cells in all cases. Next, mice were inoculated with Sarcoma 180 tumor cells pretreated with GF-1 (50 or 500 $\mu\text{l/ml}$) for 3 h, and the survival times of those mice after inoculation were recorded. The survival times of those mice were almost equal to those observed in the control mice (Table I). These results indicate that GF-1 does not possess a direct cytotoxicity on tumor cells.

Effect of Reinoculation of Tumor Cells into Tumor-Regressed Mice

The results described above raised the possibility that GF-1 has an ability to induce antitumor resistance in tumor-bearing hosts. Therefore, we examined this possibility. ICR mice were injected *i.p.* with 4 mg of GF-1 once a day for 5 successive days from the day following

tumor inoculation. The mice which showed complete regression of Sarcoma 180 on the 40th day after the tumor inoculation were further challenged with same cells (5×10^6) into their right groins. As shown in Table II, all of the mice tested were resistant to the challenge. This and above described results suggest that the antitumor activity of GF-1 is generated by host-mediated mechanism.

Then, experiments have been attempted to examine whether the administration of GF-1 into mice modulates their immune responses. Those experiments were performed using 1, 2 or 4 mg for once or 4 mg $\times$ 5 times of injection of GF-1. In these doses of GF-1, the *i.p.* administration of 1 or 2 mg once a day for 10 successive days from the day following tumor inoculation (Sarcoma 180 tumor cells) could induce significant antitumor activity which indicated over 90% of inhibition ratio. On the other hand, 4 mg of GF-1 was highly effective when it was admin-

FIG. 1. *Absence of Direct Cytotoxicity of GF-1 on Tumor Cells*

Each tumor cell was incubated with or without GF-1, and viable cell numbers were determined 24, 48 or 72 h thereafter. Assays were performed in triplicate with 2×10^5 tumor cells per culture.

GF-1: ○—○ 0 $\mu\text{g/ml}$, △—△ 50 $\mu\text{g/ml}$, ▲—▲ 500 $\mu\text{g/ml}$.

istered for not only 10 times but also 5 times.¹⁾

Effect of GF-1 on Weights and/or Nucleated Cell Numbers of Spleen, Thymus and Peritoneal Cavity

To examine the effect of GF-1 on weights and/or nucleated cell numbers of spleen, thymus and peritoneal cavity, mice were injected *i.p.* with 1, 2 or 4 mg of GF-1 at only once or for 5 successive days as shown in Fig. 2. The weight and/or cell number in each mouse was determined 2, 7 or 14 d after GF-1 injection. In these experiments, the effects of other two agents, thioglycollate and *P. acnes* which are able to induce inflammatory or antitumorally macrophages, respectively, were also tested. The administration of GF-1 increased the weight and nucleated cell number of spleen on day 7 and 14. As to the effect on thymus, increment of the weight was observed when 4 mg of GF-1 was injected at only once 7 d before. On the other

hand, the numbers of peritoneal exudate cells on all days tested were increased by the administration of GF-1, although their numbers were lower than that obtained by the injection of thioglycollate or *P. acnes*. All over these assays, there was no apparent relationship between the doses of GF-1 injected and the influence on weight and cell number. However, it is interesting that the administration of GF-1 at 4 mg for 5 times was scarcely effective.

Effect of GF-1 on Antibody Response

To examine the effect of GF-1 on antibody response, the following two experiments were performed. First, mice were injected *i.p.* with 1, 2 or 4 mg of GF-1 at only once (group A) or for 5 successive days (group B) as shown in Fig. 3. The mice of group A were immunized simultaneously with SRBC (1×10^7 or 1×10^8) *i.v.* On the other hand, the mice of group B were

TABLE I. Absence of Direct Cytotoxic Effect of GF-1 on Sarcoma 180 Tumor Cells

GF-1 $\mu\text{g/ml}$	Cell viability (%) ^{a)}	Survival days mean $\pm$ SD ^{b)}
50	98.2	18.4 $\pm$ 3.0
500	98.3	19.3 $\pm$ 3.1
Nil	98.4	18.4 $\pm$ 3.1

a) Sarcoma 180 tumor cells ($2 \times 10^5/\text{ml}$) were incubated with GF-1 (50 or 500 $\mu\text{g/ml}$) for 3 h, and the viable cell number was counted. b) After the above procedures, the tumor cells ($2 \times 10^6/\text{mouse}$) were inoculated *i.p.* into ICR mice, and the survival times of those mice were recorded. Results are expressed as arithmetic mean day $\pm$ standard deviation of 5 mice.

TABLE II. Antitumor Resistance of Regressor Mice Induced by GF-1

GF-1 treatment ^{a)}	Tumor weight g, mean $\pm$ SD ^{b)}	Inhibition ratio (%) ^{c)}	Complete regression ^{d)}	Tumor-free mice after reinoculation ^{e)}
Yes	0.56 $\pm$ 0.86	94.7	6/13	6/6
No	10.46 $\pm$ 3.54	—	0/6	0/6

a) ICR mice were inoculated *s.c.* with Sarcoma 180 tumor cells ($5 \times 10^6/\text{mouse}$, day 0). A part of those mice were injected *i.p.* with 4 mg of GF-1 for 5 times. As controls, the other mice received saline instead of GF-1. b) Arithmetic mean weight of tumors $\pm$ standard deviation on day 40. c) The inhibition ratio was calculated from the mean weight of the treated group against that of the control group. d) The ratio of the number of mice showing complete regression to that of mice tested. e) The mice which showed complete regression were inoculated *s.c.* with Sarcoma 180 ($5 \times 10^6/\text{mouse}$) on day 40. The ratio of the number of tumor-free mice to that of mice tested 29 d after tumor inoculation.

immunized after the last injection of GF-1 with the same manner as group A. Two, 4 or 10 d after SRBC injection the mice were sacrificed and their spleens were removed and assayed for direct anti-SRBC PFC. In group A, the injection of GF-1 increased the direct anti-SRBC response, particularly in 1×10^8 SRBC-immunized mice, and the stimulating indices were about 2. However, the administration of GF-1 at 4 mg for 5 times was scarcely effective. Next, the ability of

GF-1 as an Polyclonal B cell Activator (PBA), which is able to increase the number of PFC against various antigens in unprimed B cell populations, were examined. Mice were injected *i.p.* with GF-1 in the same way as above experiments, and direct PFC responses to SRBC and TNP were determined 2 or 4 d thereafter. However, polyclonal B cell activation activity was not observed in all cases tested (date not shown).
Effect of GF-1 on Lymphocytes Blastogenic Trans-

FIG. 2. *Effects of GF-1 on Weights and/or Nucleated Cell Numbers of Some Lymphoid Organs*
ICR mice were injected *i.p.* with GF-1 (1, 2 or 4 mg), thioglycollate (TG, 40 μ g) or *P. acnes* (PA, 100 μ g), and the weight and/or nucleated cell number in each mouse was determined 2, 7 or 14 d after the injection. Results are expressed as arithmetic mean value (weight or number of cells) $\pm$ standard deviation of 5 mice. The mean values of control groups are as follows: spleen weights (mg/mouse) — 145 (day 2), 113 (day 7) and 132 (day 14); spleen cell numbers ($\times 10^7$ /mouse) — 17 (day 2), 35 (day 7) and 24 (day 14); thymus weights (mg/mouse) — 79 (day 2), 48 (day 7) and 64 (day 14); PEC numbers ($\times 10^5$ /mouse) — 14 (day 2), 12 (day 7) and 16 (day 14). Broken line show the control value in each experiment. The significance (*p* value) of differences between the control and treated group was evaluated according to Student's *t* test. a) *p* < 0.05, b) *p* < 0.01, and c) *p* < 0.001.

FIG. 3. Effect of GF-1 on Antibody Production in Antigen-Stimulated Mice

ICR mice were injected i.p. with GF-1 (1, 2 or 4 mg) only once (Group A) or 5 successive days (Group B). Those mice were immunized i.v. with 1×10^7 or 1×10^8 SRBC simultaneously (Group A) or one day after the last injection (Group B), and the PFC numbers of each group were determined 2, 4 or 10 d later. Results are expressed as arithmetic mean PFC $\pm$ standard deviation of 5 mice. The mean values of control groups are as follows: Group A, SRBC 10^7 ($\times 10^2$ /spleen)—1 (day 2), 201 (day 4), 16 (day 10, Ig M), 67 (day 10, Ig G), SRBC 10^8 ($\times 10^2$ /spleen)—3 (day 2), 587 (day 4), 28 (day 10, Ig M), 286 (day 10, Ig G); Group B, SRBC 10^7 ($\times 10^2$ /spleen)—2 (day 2), 142 (day 4), 25 (day 10, Ig M), 32 (day 10, Ig G), SRBC 10^8 ($\times 10^2$ /spleen)—12 (day 2), 1 471 (day 4), 27 (day 10, Ig M), 31 (day 10, Ig G). a) See Fig. 2.

TABLE III. Effect of GF-1 on ^3H -TdR uptake of Spleen Cells

Sample $\mu\text{g/culture}$		48 h ^{a)}		72 h ^{a)}	
GF-1	0.01	8 328 $\pm$ 1 145 ^{b)}	(1.4) ^{c)}	2 810 $\pm$ 223	(1.5)
	0.1	6 847 $\pm$ 775	(1.1)	1 878 $\pm$ 558	(1.4)
	1	6 559 $\pm$ 375	(1.1)	1 973 $\pm$ 151	(1.0)
	10	6 300 $\pm$ 276	(1.0)	1 935 $\pm$ 198	(1.0)
	100	5 541 $\pm$ 254	(0.9)	2 167 $\pm$ 73	(1.1)
LPS	10	49 547 $\pm$ 3 775	(8.1)	31 244 $\pm$ 643	(16.2)
Con A	0.5	88 612 $\pm$ 5 686	(14.4)	164 769 $\pm$ 25 758	(85.2)
Nil		6 148 $\pm$ 1 226	(1.0)	1 933 $\pm$ 122	(1.0)

a) 5×10^5 spleen cells from ICR mice were incubated for 48 or 72 h. b) Arithmetic mean cpm $\pm$ standard deviation of triplicate cultures. c) Mean cpm in experimental culture/mean cpm in control culture.

formation

In order to examine whether GF-1 possesses a property as mitogen on murine lymphocytes, spleen cells from several strains of mice (ICR, BALB/c, C3H/He and C57BL/6) were cultured with various doses of GF-1, and the ^3H -TdR

uptake of the cells after 48 or 72 h of culturing was determined. As shown in Table III, GF-1 did not show mitogenic activity at any dose or culture time in all the strains of mice tested (data of only ICR mice were listed).

Effect of GF-1 on DTH

To examine the effect of GF-1 on DTH, mice were injected *i.p.* with 1, 2 or 4 mg of GF-1 at only once (group A) or for 5 successive days (Group B) as shown in Fig. 4. The mice of group A were immunized simultaneously with SRBC (1×10^8) *s.c.* On the other hand, the mice of group B were immunized after the last injection of GF-1 with the same manner as group A. The DTH reaction to SRBC was determined 8 d after the immunization. The administration of GF-1 did not enhance the reaction, preferably showed tendency to suppress that.

Effect of GF-1 on Carbon Clearance Activity

To examine the effect of GF-1 on carbon clearance activity, mice were injected *i.p.* with 1, 2 or 4 mg of GF-1 at only once or for 5 successive days as shown in Fig. 5. Their carbon clearance activities were determined 2, 7 or 14 d after the injection. On day 2, marked enhancing effect was observed in the cases of both one and

FIG. 4. Effect of GF-1 on DTH

ICR mice were injected *i.p.* with GF-1 (1, 2 or 4 mg) at only once (Group A) or 5 successive days (Group B). Those mice were immunized *s.c.* with 1×10^8 SRBC simultaneously (Group A) or one day after the last injection (Group B). Seven days after the immunization, 1×10^8 SRBC were injected into subcutaneous tissue of one hind footpad. The degree of swelling was measured after 24 h. Results are expressed as arithmetic mean swelling $\pm$ standard deviation (mm) of 5 mice. The mean value of control group is 0.42.

FIG. 5. Effect of GF-1 on Carbon Clearance Activity

ICR mice were injected *i.p.* with GF-1 (1, 2 or 4 mg) only once (Group A) or 5 successive days (Group B). Their carbon clearance activities were determined 2, 7 or 14 d after the (last) injection. Results are expressed as arithmetic mean half clearance time $\pm$ standard deviation (min) of 5 mice. The mean values of control groups are as follows: Group A, 16.2 (day 2), 10.1 (day 7), 9.9 (day 14); Group B, 12.8 (day 2), 9.5 (day 7), 9.6 (day 14). a, b, c) See Fig. 2.

5 times injections. Further, it was shown that the enhancing activity observed on day 2 was maintained until day 14 in the case of 5 times injection, although that disappeared thereafter in the case of single injection.

DISCUSSION

Generally, it has been considered that the materials possessing antitumor activity exhibit it by their direct cytotoxic effects on tumor cells and/or host-mediated effects. We have previously reported that a polysaccharide fraction (GF-1) obtained from *G. frondosa* exhibits an antitumor activity in both allogeneic and syngeneic tumor-host systems.¹⁾ Then, we examined whether GF-1 possesses direct cytotoxic effect on tumor cells or not. As shown in Fig. 1 and Table I, GF-1 did not show any cytotoxic effect on culturing tumor cells, and *in vitro* treatment of Sarcoma 180 tumor cells with GF-1 did not affect on their abilities of growth *in vivo*. Furthermore, GF-1 had induced resistance against Sarcoma 180 tumor cells in ICR mice which completely regressed the tumor (Table II). These findings suggest that the antitumor activity of GF-1 is exhibited by host-mediated mechanism although the strict mechanism in allogeneic or syngeneic tumor-host system is probably different from each other.

In the case of host-mediated antitumor action of many materials, the basal mechanisms were reported to be dependent on their immunomodulating activities.^{5-7,10-13)} Therefore, we examined the immunomodulating activities of GF-1 under some experimental conditions. On antibody formation, GF-1 enhanced antigen specific direct PFC response (IgM response) though the degree was very low (stimulating indices were about 2) (Fig. 3). It is interesting that the adjuvant activity was observed mainly in the case of single injection of GF-1. This finding seems to suggest a possibility that there is an optimal dose-range for the generation of adjuvant activity of GF-1. On the other hand, GF-1 showed neither PBA activity nor mitogenicity (Table III), and this indicates that GF-1 does not

stimulate murine B cells polyclonally.

The administration of GF-1 into mice did not affect the DTH reaction to SRBC in all cases tested (Fig. 4). Akiyama *et al.* reported that the enhancing effect of lentinan on DTH reaction to SRBC were observed in DBA/2, C57BL/6 and C3H/He mice, but did not in ICR mice.¹⁴⁾ Therefore, it may safely be said that the effects on DTH reaction of GF-1 and lentinan are similar in ICR mice.

As shown in Fig. 5, GF-1 enhanced the carbon clearance activity of mice in the case of both single and 5 times of GF-1 injections. The enhancing activity observed when 4 mg of GF-1 was administered into mice for 5 successive days was maintained until day 14 from day 2 after the first injection. This mode of action of GF-1 on carbon clearance activity is interesting in comparison with that on antibody response in antigen-injected mice since the adjuvant activity was observed mainly in the case of single injection of GF-1 (Fig. 3). It was reported that lentinan does not stimulate the carbon clearance activity of mice. Therefore, the effect of GF-1 on this activity differ remarkably from that of lentinan. The phagocytic function including carbon clearance activity is one of those of macrophages. In our preliminary experiment, GF-1 induced cytotoxic macrophages against syngeneic tumor cells when GF-1 was injected *i.p.* into mice. In addition, the injection of GF-1 gave significant effect on the number of PEC (Fig. 2). These findings may suggest a possibility that macrophages play major role in antitumor activity of GF-1.

Recently, we found that 1) GF-1 was separated into neutral and acidic fractions possessing potent antitumor activity, and 2) the former contained mainly α -1,4 and 6-branched β -1,3-linkages, and the latter contained mainly β -1,6- and 6-branched β -1,3-linkages.¹⁵⁾ It was also found that the α -1,4- and β -1,6-linked glucans do not participate in the antitumor activity of GF-1 since the antitumor activity of GF-1 was not lowered both by the α -amylase digestion and periodate oxidation.²⁾ Therefore, the differences in antitumor and immunomodulating

activities between GF-1 and lentinan, which is 6-branched β -1,3-linked glucan, is considered to be dependent on the presence of acidic component(s) in GF-1. However, there is a possibility that the difference of biological activities is generated by that in their fine structures such as their physicochemical properties including molecular weights, and numbers of branched parts. Now, a detailed examination on the anti-tumor and immunomodulating activities, particularly in the effects on the functions of effector cells such as macrophages, natural killer cells and killer T cells in tumor-bearing mice, of purified polysaccharides is in progress.

Acknowledgment We wish to thank Misses Y. Jindo and K. Sudo for their technical assistances.

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