Oral Administration of a Novel Glycoprotein Derived from the Fruit Bodies of the Basidiomycete Tricholoma Matsutake Enhances the Gut-associated Immune Responses of Mice

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Abstract

Issues of the study: Some kinds of mushrooms have been traditionally used as functional foods for the prevention or treatment of malignant diseases. Although the immunological mechanisms by injection of active components derived from edible mushrooms have been thoroughly examined, few studies are available on the mechanisms by oral administration. Therefore, we investigated the effects of oral administration of edible mushroom extracts on immune responses in gut-associated lymphoid tissue of mice in order to identify the active structure and the mechanisms.

Methods: B7/P815 tumor cells were transplanted into the subserosal space of the cecum in DBA/2 mice. High molecular weight fractions derived from hot water extracts of fruit bodies of edible mushrooms were orally administered daily 10 times, and the activity of mesenteric lymph node (MLN) cells was measured by the use of in vitro mixed tumor cells lymphocyte reaction (MLR) and MLR-cell-mediated cytotoxicity.

Results: 1) Among 12 samples examined, only the extract from Tricholoma matsutake significantly enhanced the responses. 2) When the extract from T. matsutake was fractionated, the activity was recovered in the em2 fraction which was prepared by treating the DEAE column-adsorbed fraction of the extract with B-1,3 glucanase. The effects of em2 were dependent on the timing of administration and the dose administered. 3) The physicochemical analysis revealed that em2 is a glycoprotein.

Conclusion: These findings suggest that a glycoprotein derived from fruit bodies of T. matsutake enhances the activity of MLN cells by oral administration.

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Introduction

Many kinds of mushrooms have been used from ancient times as ingredients in Chinese medicine and folklore treatment because of their diverse biological activities[1-4]. Recently, polysaccharide preparations derived from mushrooms were clinically applied as anticancer immuno-therapeutics in Japan[5-8]. The active components of these mushrooms are estimated to be ß-glucan[5, 6, 9, 10], protein-bound polysaccharides[7, 8, 11, 12], proteins[13, 14], and low molecular weight (MW) substances[15,16], however, unidentified molecules may still be present. In addition, few studies are available on the mechanisms when they are administered orally, although the immunological mechanisms by injection of active components derived from edible mushrooms have been extensively examined[17, 18, 19].

In the process of our search for dietary components that modulate the function of gut-associated lymphoid tissue (GALT), we found that a novel glycoprotein derived from fruit bodies of the basidiomycete Tricholoma matsutake enhances immunomodulatory activities of mesenteric lymph node (MLN) cells by oral administration.

Materials and Methods

Materials: Fresh viable edible fruit bodies of 12 basidiomycete species, i.e. *Agaricus bisporus, Agaricus blazei, Auricularia auricula, Flammulina velutipes, Grifola albicans, Grifola frondosa, Lentinus edodes, Lyophyllum ulmarium, Pholoita nameko, Pleurotus eryngii, Pleurotus ostresatus,* and *Tricholoma matsutake* were purchased from each manufacturer or supplier (Table 1, below). After confirmation of their species by morphological findings,[20,21] they were freeze-dried and pulverized. At least three lots of samples were prepared for each basidiomycete species in the present study.

Carbohydrate analysis: Carbohydrate content was determined by the phenol-sulfuric acid method. Sugar components were determined by HPLC using a TSK-gel Sugar AXG column (Toso, Tokyo) and a fluorescence detector system after hydrolysis with 2 mol/L trifluoro acetic acid at 100˚C for 6 hours.

Protein analysis: Protein content was determined by the copper Folin method. Amino acid components except for tryptophan were determined using a Hitachi L-8500 amino acid analyzer after hydtolysis of samples with 6 mol/L hydrochloride at 110˚C for 22 hours in a sealed and evacuated tube. Tryptophan contents were determined using the same amino acid analyzer after hydrolysis of samples with 4.2 mol/L sodium hydroxide at 110˚C for 16 hours in a sealed and evacuated tube.

Estimation of molecular weight (MW): The sample was dissolved in purified water at 3.0 mg/mL, applied to HPLC using an Asahipack GS-620 column (Asahikasei, Tokyo) and a differential refractive index

Table 1. Physiochemical properties of high MW fractions derived from hot water extracts of freshly fruit-bodies of edible mushrooms

No.	Basidiomycete species	Manufacturing (or geographical) location	Yield %	Sugar : protein ratio	Optical rotation $[\alpha]_D^{20}$	MW (kDa) of major components	Sugar components (%)*					
							Glu	Gal	Man	Fuc	Xyl	Rib
1	*Agaricus bisporus* [Tukuritake]	Niigata Pref., Japan	6.3	45:55	- 58.1	1,880	47	34	11	4	4	0
2	*Agaricus blazei* [Himematsutake]	Okinawa Pref., Japan	7.3	74:26	+ 22.7	1,800 115	56	19	13	6	5	0
3	*Auricularia* auricula [Kikurage]	Saitama Pref., Japan	4.8	91: 9	- 7.7	750 16	21	2	62	2	13	0
4	*Flammulina velutipes* [Enokitake]	Nagano Pref., Japan	5.5	60:40	- 7.8	1,900	56	19	13	6	5	0
5	*Grifola albicans* [Shiromaitake]	Nagano Pref., Japan	4.6	73:27	- 10.4	1,800 220	72	18	10	0	0	0
6	*Grifola frondosa* [Maitake]	Niigata Pref., Japan	4.9	61:39	- 27.3	2,000 340	70	14	11	0	0	4
7	*Lentinus edodes* [Shiitake]	Ooita Pref., Japan	6.0	53:47	- 21.6	2,500	63	19	12	3	0	0
8	*Lyophyllum ulmarium* [Bunashimeji]	Nagano Pref., Japan	6.9	42:58	- 22.9	190 18	49	28	13	8	0	2
9	*Pholiota nameko* [Nameko]	Ibaragi Pref., Japan	7.0	77:23	+ 34.5	1,560	74	6	15	1	4	0
10	*Pleurotus eryngii* [Eringi]	Niigata Pref., Japan	5.8	69:31	+ 56.1	2,100 1,750	82	7	7	0	0	3
11	*Pleurotus ostreatus* [Hiratake]	Ibaragi Pref., Japan	5.3	50:50	+ 1.2	2,000	57	20	14	1	0	8
12	*Tricholoma matsutake* [Matsutake]	Kyoto Pref., Japan	5.6	61:39	+ 8.0	1,950	57	22	13	5	2	0

Details of experimental conditions were described in Materials & Methods. * Glu, Glucose; Gal, Galactose; Man, Mannose; Fuc, Fucose; Xyl, Xylose; Rib, Ribose.

detector system. Calibration curves were prepared using pullulan with known MW. The MW of the sample was estimated by extrapolating the retention time into a calibration curve.

Optical rotation: The sample was dissolved with purified water at 1 mg/mL, the solution was passed through a 0.45μm filter, and the optical rotation of the filtrate was measured at 20˚C using an optical rotation meter.

IR and NMR analysis: Infrared (IR) spectra were recoreded as potassium bromide tablets on an infrared spectrophotometer (JASCO, Tokyo). For ^{1}H-NMR and ^{13}C-NMR analysis, samples were dissolved with D_2O or 0.3 mol/L NaOD, and the spectra were recorded using a Unity plus model 500 NMR (Varian Inc., CA, USA).

Enzymatic hydrolysis of glycoprotein: Zymolyase-100T derived from culture supernatants of *Arthrobacter luteus* was purchased from Seikagaku Kougyo (Tokyo), and ß-1,3 glucanase fraction was purified in our institute by affinity chromatography. The sample was incubated with the ß-1,3 glucanase fraction at 45˚C for 24 hours in a 50 mmol/L acetate buffer (pH 6.0) in the presence of protease inhibitor cocktail (Sigma-Aldrich Co., MO, USA).

Animals: Specific-pathogen-free female ICR mice and DBA/2 mice purchased from Japan CLEA (Tokyo) and Japan SLC (Shizuoka), respectively, were acclimated and then used in the experiments at the age of five and eight weeks, respectively. The mice were kept, five per cage, in polycarbonate cages in which sterilized sawdust had been spread. They were allowed free access to food (CE-2, Oriental Yeast, Tokyo) and sterilized tap water. The cages were kept at a temperature of 25±2˚C and a humidity of 55±10% under luminary air flow and a lighting cycle: 5 lux from 8:00 a.m. to 8:00 p.m. To keep the environment constant, noise was avoided and only the animal caretakers and experimenters entered the animal room.

As a general rule, each experimental group consisted of five to ten animals and the same experiment was repeated at least twice. The experimental design was reviewed by the Committee of Ethics on Animal Experiments of the Biomedical Research Laboratories, Kureha Chemical Industry Company Limited (Tokyo) and conducted in accordance with the guidelines of the Institute.

Antitumor activity against sarcoma 180: Sarcoma 180 cells maintained in the peritoneal cavity of ICR mice at our institution were used. One x 10^6 sarcoma 180 cells were transplanted subcutaneously in the axillary region of ICR mice (10 animals/group), and 5 mg/kg or 10 mg/kg of the sample or 0.2 mL saline was injected intraperitoneally every other day ten times, starting the day after tumor transplantation. The mice were sacrificed 25 days after tumor transplantation, the number of tumor-free mice was examined, and the tumors were excised and weighed. The growth inhibition rate was calculated by the following formula; [S - C] x 100/C. In the formula, S is the mean tumor weight of the experimental group and C is the mean tumor weight of the saline-treated control group.

Mixed lymphocyte reaction (MLR) and MLR-cell-mediated-cytotoxicity (MLR-CMC) in MLN cells: The mouse mastocytoma cell lines P815 and B7/P815 were provided by Dr. Harada M. of Kyusyu University (presently at Kurume University School of Medicine, Fukuoka). The activities of MLN cells were evaluated by the method of Harada et al.[22,23] Briefly, DBA/2 mice (5 to 8 animals/group) were given a laparotomy under ether anesthesia, 1 x 10^6 B7/P815 tumor cells were transplanted in the cecal wall, and the abdomen was closed. Starting on the day after tumor transplantation, 250 mg/kg or 500 mg/kg of the sample or 0.2 mL of distilled water was orally administered daily 10 times. The mice were sacrificed on the day after the last administration, the MLN was excised, single cell

suspension of MLN was prepared using RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 5 x 10^{-5} mol/L of 2-mercaptoethanol, 20 mmol/L of N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid), 0.2% sodium bicarbonate and 30 μg/mL of gentamycin. In the MLR assay, these cells were mixed with MMC-treated P815 tumor cells at the specified ratio, cultured in a 96-well culture plate at 37˚ in 5% CO_2 atmosphere for three days. Eight hours before culture termination, ^{3}H-thymidine at 37 kBq was added to the culture. The MLN cells were harvested using a cell harvester and the radioactivity incorporated into the cells was measured using a scintillation counter. Mitomycin C (MMC)-treated tumor cells were prepared by incubating tumor cells with 50 μg/mL of MMC (Sigma-Aldrich Co.) at 37˚C for one hour.

On the other hand, in MLR-CMC assay, MLN cells were co-cultured with MMC-treated tumor cells in 24-well culture plates at 37˚C in 5% CO_2 atmosphere for three days to induce killer cells. Then, MLN cells were recovered from the culture, incubated with ^{51}Cr-labeled P815 tumor cells at the specified ratio at 37˚C in 5% CO_2 atmosphere for four hours. After the incubation, the supernatant of the culture was obtained by centrifugation and its radioactivity was measured using a gamma counter.

Statistical analysis: The significance of differences was analyzed using Student's t test, and $p < 0.05$ was regarded as significant.

Results

Preparation of High MW fractions from hot water extracts of edible mushrooms and the physicochemical analysis:

High MW fractions were prepared by the extraction of edible mushrooms with purified water. Briefly, freeze-dried and powered fruit bodies of 12 kinds of edible mushrooms were extracted twice with purified water at 98˚C for three hours while stirring, followed by centrifugation and extensive dialysis of the supernatant against purified water through a membrane with a fractioning MW of 3,500 kD (Spectrum Medical Ind., Inc., Texas, USA). The inner part of dialyzate was concentrated by a rotary evaporator and freeze-dried to analyze the physicochemical natures and to examine the biologic activities.

Table 1 (p.214) shows the representative data among three preparations for each basidiomycete species. The yields of the fractions ranged from 4.9% to 7.3%, and all fractions were glycoproteins with a sugar protein ratio 42:58～91:9, optical rotation -58.1～ +56.1, MW of major components 115～2,500 kD, respectively. Further, carbohydrate component mainly consisted of glucose, galactose and mannose. NMR spectra suggest the presence of glucan, galactan or heteropolysaccharide configuration of both ∝ and ß but quantitative estimation was impossible due to interference with protein signals in most samples (data not shown).

Effects of oral administration of high MW fractions on the activities of MLN cells in mice: Next, to screen the fractions that enhance the activity of GALT by oral administration, the high MW fractions were orally administered at the dose of 250 mg/kg or 500 mg/kg daily ten times to DBA/2 mice starting from the day after transplantation of B7/P815 tumor cells in the cecal wall, and the activities of MLN cells were determined on the day after the last administration.

Figure 1-A (p.217) shows that oral administration of high MW fractions derived from T. matsutake at the dose of 500 mg/kg significantly enhanced both the MLR and MLR-CMC activities to 142% and 155%, respectively, compared with those of the distilled water-administered control group, although the activities of 11 other kinds of fractions were marginal at all doses, being 120% or less of the distilled water-administered control group.

Next, in order to confirm whether the above fractions are effective or not in another immunological assay system, we ex-

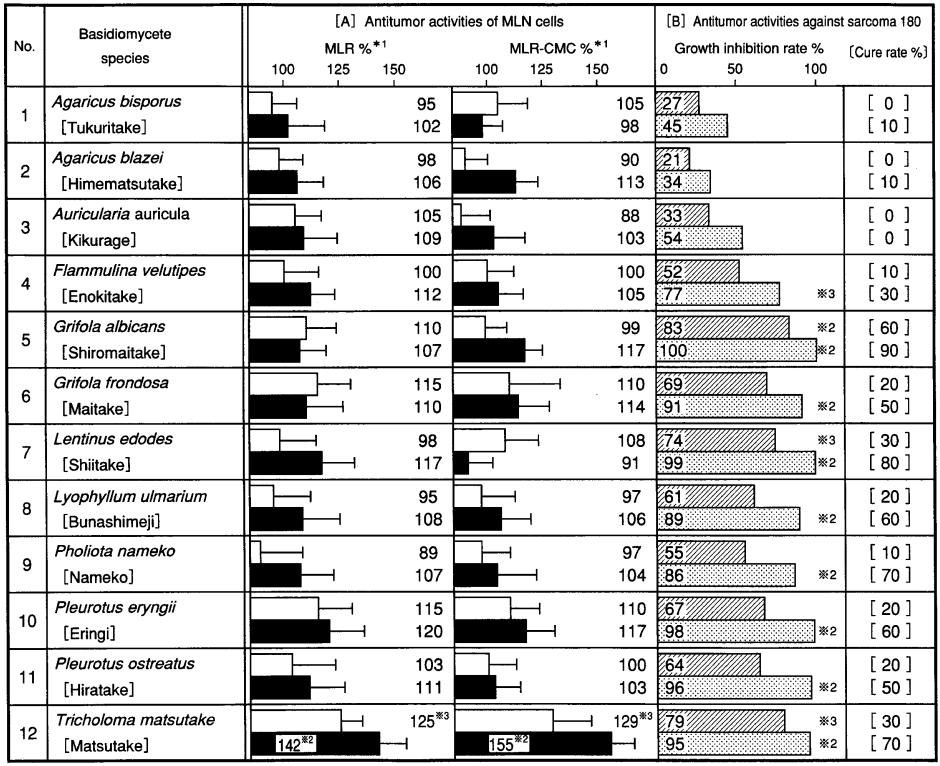
amined the effects of intraperitoneal injection of these fractions at the dose of 5 mg/kg or 10 mg/kg on the growth of sarcoma 180 cells in ICR mice transplanted subcutaneously. In contrast to Figure 1-A, (below), 75% (9/12) of samples significantly inhibited the growth of tumors and cured mice appeared when injected intra-peritoneally at the dose of 10 mg/kg (Figure 1-B, below). On the other hand, the activities of 3 other fractions derived from *A. bisporus*, *A. blazei*, and *A. auricula* were below 54% and there were no significant differences in the activities when compared that of the saline-injected control group.

These results suggest that among 12 kinds of fractions tested, only the high MW fraction derived from *T. matsutake* was effective for enhancing the activity of MLN cells via oral route in addition to suppressing the growth of sarcoma 180 cells by injection.

Purification of active components derived from high MW fractions of fruit bodies of *T. matsutake.*

Next, we studied whether or not the active structures responsible for enhancing the activity of MLN cells via oral route is same as those for suppressing the growth of

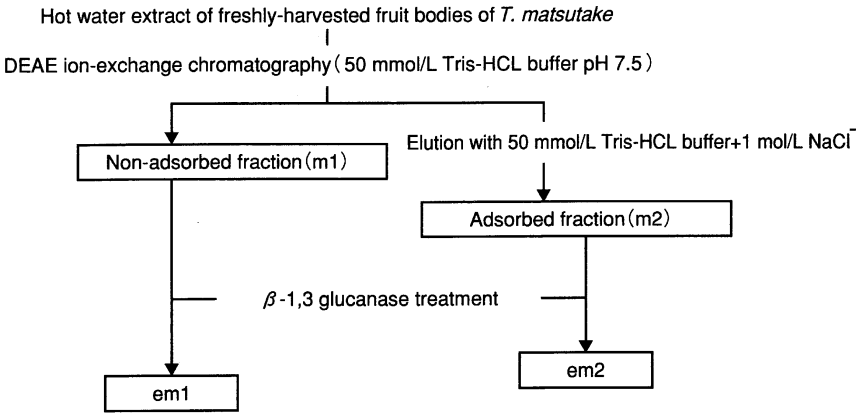
Figure 1. Effects of high MW fractions derived from hot water extracts of edible mushroom fruit bodies on antitumor activities of MLN cells by oral administration and antitumor activities against sarcoma 180 by intraperitoneal injection.
* 1 % activity of distilled water-treated control group; significant at *2 p< 0.01 and *3p<0.05 (vs. distilled water-treated control group) by Student's t test.
CM6271 was administered orally [A] at the dose of 250 mg/kg (□) and 500 mg/kg (■), or intraperitoneally [B] at the dose of 5 mg/kg (▨) and 10 mg/kg (▩).



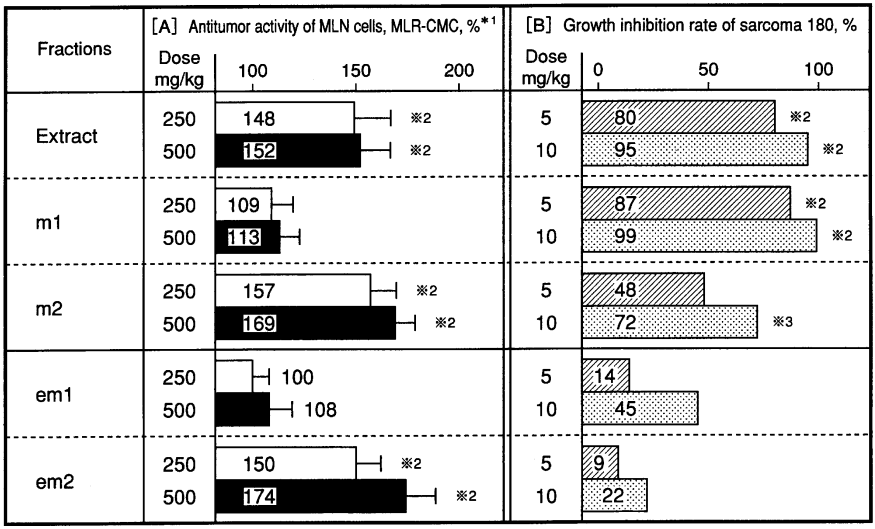
sarcoma 180 cells by injection. The high MW fraction derived from the hot water extract of fruit bodies of *T. matsutake* was fractionated using a DEAE Sephacel ion exchange chromatograph. Briefly, the fraction was dissolved with 50 mmol/L Tris HCL buffer (pH 7.5), the solution was applied on the column, and a fraction not adsorbed (m1) and a fraction

Figure 2. Fractionation of the high MW fraction derived from hot water extracts of *T. matsutake* and antitumor activities of the fractionates.
*1 % activity of distilled water-treated control group; significant at *2 $p < 0.01$ and *3$p<0.05$ (vs. distilled water-treated control group) by Student's t test. CM6271 was administered orally [A] at the dose of 250 mg/kg (□) and 500 mg/kg (■), intraperitoneally [B] at the dose of 5 mg/kg (▨) and 10 mg/kg (▩).

〔Fractionation〕



〔Activities〕



adsorbed on column followed by elution with the same buffer containing 1 mol/L NaCL (m2) were recovered, and their activities were compared. As shown in Figure 2, (p.218) fraction m1 significantly inhibited the growth of sarcoma 180 by injection, but had no effects on the activities of MLN cells by oral administration. In contrast, fraction m2 enhanced the activities of MLN cells by oral administration, but showed a weaker effect on the growth inhibition of sarcoma 180 by injection when compared to that of m1. Furthermore, treatment of these fractions with ß-1,3 glucanase (fractions em1 and em2) significantly reduced the growth inhibitory activity against sarcoma 180, but the treatment did not affect the activities of MLN cells. These results suggest that em2 derived from the DEAE column-adsorbed fraction predominantly enhances the activity of MLN cells by oral administration, on the other hand, m1 derived from the non-adsorbed fraction inhibits the growth of sarcoma 180 by injection.

Physicochemical and immunological nature of active fraction em2: As shown in Table 2, (below) em2 is a glycoprotein with a sugar protein ratio 34:66, and the main portion of the sugar component is glucose. The

Table 2. Physicohemical properties of em2 fraction derived from extracts of freshly harvested fruit-bodies of T. Matsutaki.

Parameters	em2 fraction
Sugar protein ratio	34 : 66
MW of major component	2,000 kDa
Optical rotation	$[\alpha]_D^{20}$ 38
Constitutive sugars	Glucose 43% Mannose 14% Galactose 35% Fucose 8%
Constitutive amino acids	Glutamic acid 13% Aspartic acid 13% Glycine 11% Alanine 9% Leucine 7% Thirteen other 13 kinds of amino acids 43%
NMR spectra analysis	Characteristics of α and β configuration of carbohydrates Characteristics of proteins
IR spectra analysis	Characteristics of carbohydrates and proteins

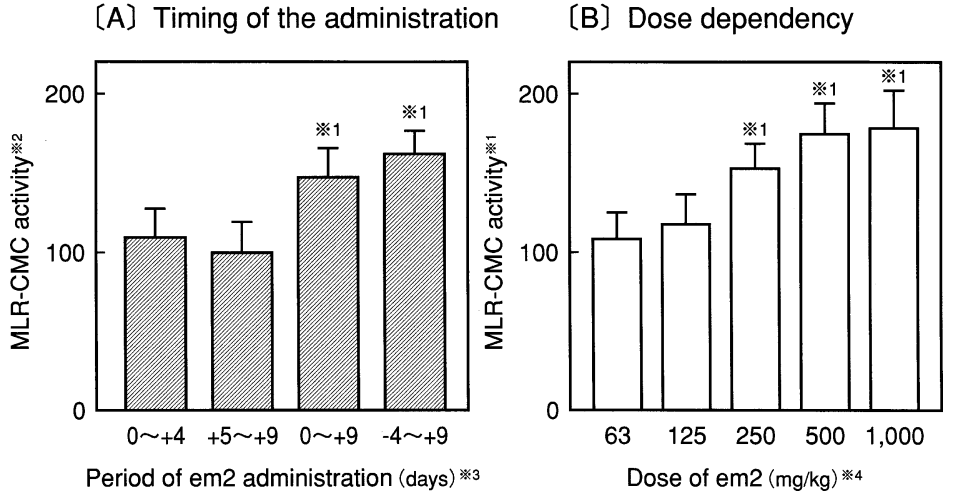
Details of experimental conditions were described in Materials & Methods.

protein portion consisted of 18 kinds of amino acids, rich in the acidic group i.e. glutamic acid, aspartic acid and glycine. Further, as shown in Figure 4-A (p.221) adsorptions at 3,600～3,200 cm^{-1}, 1,200～1,000 cm^{-1}, 890 cm^{-1} and 840 cm^{-1} in IR spectra suggest functional groups characteristic for carbohydrates, and adsorption at 1,600 cm^{-1} and 1,530 cm^{-1} suggest functional groups characteristic for proteins. ^{1}H-NMR spectra in Figure 4-B (p.221) suggest a signal at 5.0～5.4 ppm as the ∝1 configuration and a signal at 4.6～4.8 ppm as the ß1 configuration respectively, however, further analysis was impossible since the signals derived from protein and solvent interfered with the signals of the carbohydrates (Figure 4-B). The signals derived from the protein also interfered with the signals derived from the carbohydrate in ^{13}C-NMR spectra (data not shown).

The effects of oral administration of em2 on the activities of MLN cells were dependent on the duration and timing of administration; em2 significantly enhanced in vitro MLR and MLR-CMC when administered from the day of tumor transplantation to after 10 days, compared with the administration from the day of tumor transplantation to 5 days after tumor transplantation or from 5 days to 10 days after tumor transplantation (Figure 3-A, below). Further, em2 enhanced dose dependency of the activities of MLN cells when administered from the first day to 10 days after the tumor transplantation (Figure 3-B, below).

These results suggest that em2 is a glycoprotein and that the fraction enhances time- and dose-dependency of the activity of MLN cells by oral administration.

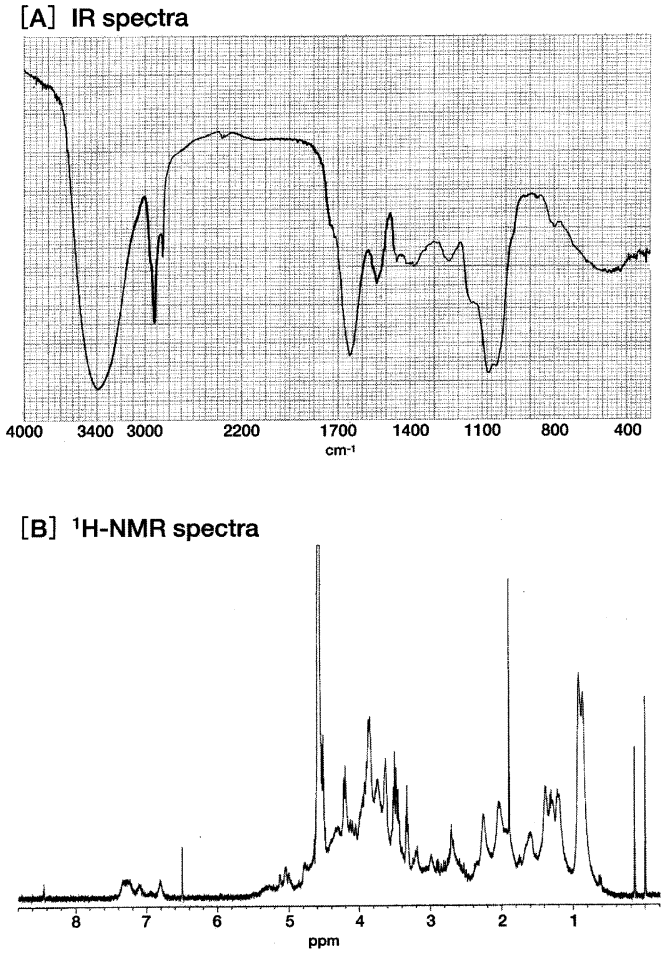
Figure 3. Effects of oral administration of the em2 fraction on antitumor activities of MLN cells in mice.
Detailed experimental conditions were described in Materials & Methods. Significant at *1 $p < 0.01$ (vs. distilled water-treated control group) by Student's t test; *2% activity of distilled water-treated control group; [A] Tumor cells were transplanted to the cecal wall of DBA/2 mice on day 0, and CM6271 was administered orally daily *3 the specified periods at the dose of 250 mg/kg. [B] CM6271 was administered orally daily ten times at *4the specified dose from the day after tumor transplantation.



Discussion

In the microenviroment of cancer tissue, there exists various physiologically active molecules and cells affecting the interaction between cancer and host cells. Therefore, it is important to evaluate biological responses of the host to tumor cells using an orthtropic tumor model which shows both antitumor reactions and organ-specific reactions.[24] In particular, in the GALT where a tolerance-inducing mechanism acts to prevent food allergy, antitumor immune responses are often impaired by bystander action of TGF-ß-producing CD8-positive cells in addition to induction of anergy.[25] So, biological re-

Figure 4. IR and 1H-NMR spectra of em2 fraction. Experimental conditions were described in Materials & Methods. [A] IR spectra were recorded as KBr tablet on an infrared spectrophotometer. [B] Samples were dissolved in D_2O, and the spectra were recorded at 25˚C using a Unity plus model 500 NMR.



sponse modifiers (BRM) which enhance the antitumor response in GALT via the oral route are suggested to be required for a possibly different active structures and mechanisms from those of BRM by injection. In the present study, we transplanted B7/P815 tumor cells to the cecal wall of DBA/2 mice, and examined effects of oral administration of hot water extracts derived from fruit bodies of edible mushrooms on the antitumor responses of draining MLN cells. As a result, from the extracts derived from 12 mushrooms species, only the extracts derived from freshly-harvested fruit bobies of *T. matsutake* showed a significantly enhanced antitumor response, and its active component was estimated to be a glycoprotein.

The antitumor immune responses of MLN cells are mainly exerted by CD4-positive T cells, the activity of which is affected by activities of TGF-ß-producing CD8-positive cells and the local level of TGF-ß.[23] The results of the present study can be explained by improvement in the responses by the glycoprotein present in the extracts of *T. matsutake* fruit bodies. The fruit bodies of *T. matsutake* have been reported to contain high MW substances that inhibit in vivo tumor growth by injection,[26,27] and a protein that induces apoptosis in oncovirus-transformed cells *in vitro*.[13] Thus, a glycoprotein that shows enhanced responses in GALT after oral administration is a new finding. However, it could not be clarified in the present study whether active substances are present only in the fruit bodies of *T. matsutake* and not in the other 11 species of edible mushrooms, or the amounts of the substances for the expression of the activities are adequate only in the fruit bodies of *T. matsutake*. Previous studies have reported that oral administration of edible mushroom fruit body powders,[28,29] Proflamin as an acidic glycoprotein derived from *Flammulina veltipes*,[14] polyphenol-ß-1,3 glucan complex derived from the fruit bodies of *Ganoderma lucidum*,[9] a lignin-like substance[30] and KS-2 as a mannnan-peptide complex[11] derived from *Lentinus edodes*, or glycoproteins derived from *Agaricus blazei*[29,30] promotes the activities of mouse peritoneal macrophages and natural killer cells, suppressing the growth of subcutaneously-transplanted tumor cells. In interpreting the results of the present study in comparison with those of other studies, consideration should be given not only to the differences in lymphatic organs and immune parameters but also to possible different stabilities in the intestine among the species and properties of mushrooms, and influences of the differences in fine structures on the distribution and excretion of active components in the body after intestinal absorption in order to develop and express the activities.

As Figure 1 (p.214) shows, promoting activity of MLN cells after oral administration was not consistent with sarcoma 180 growth inhibitiory activity after intraperitoneal injection, which supports a difference in active structures between the two evaluation systems; the extract from fruit bodies of *T. matsutake* was effective in both systems, but the extracts from 8 other species of mushrooms were effective in only the evaluation system of sarcoma 180 growth inhibition while those from the other three species were not effective in either system. The antitumor responses of mice to the transplantated allogeneic sarcoma 180 cells are primarily exerted by the cooperative action of lymphokine-producing lymphocytes and cytostatic macrophages.[31] Components such as ß-glucan contained in the fruit bodies or mycelia of edible mushrooms have been suggested to inhibit the growth of sarcoma 180 cells through the enhanced activities of macrophages.[2,4] These previous findings concur with the results of the present study that sarcoma 180 growth inhibitory activity was significantly reduced in the em1 fraction derived from ß-1,3 glucanase-treated fraction derived from *T. matsutake* when compared with that in the untreated m1 fraction.

Enhancement of activity in MLN cells

was similar between the *T. matsutake*-derived m2 fractions untreated and treated (em2 fraction) with ß-1,3 glucanase, which suggests that the active structure is a glycoprotein resistant to the ß-1,3 glucan degradation enzyme. We observed that the protein portion of *T. matsutake*-derived em2 fraction directly binds to active form of TGF-ß and inhibits its immunosuppressive activity (unpublished data). The TGF-ß inhibitiory activity of the fraction may be effective from the release of immunosuppression in the GALT of mice, but further detailed studies are necessary.

In addition to edible mushrooms, there are foods and their components that modify the responses of GALT or non-GALT by oral administration. Feeding of diets with certain cheese products activate the function of mouse T cells and inhibit the growth of transplanted tumors by iron-saturated transferrin as their component.[32] Oral administration of intestinal bacterial preparations enhances antitumor immune responses of mice through enhanced production of IL-12 and IFN-γ.[33,34] Lactoferrin, a cow milk-derived glycoprotein, affects the immune resonses of GALT by enhancing the production of IL-18 in the intestinal epithelium, inhibiting pulmonary metastasis of the tumor.[35, 36] These activities and mechanisms appear to differ from those of the glycoprotein em2 derived from the fruit bodies of *T. matsutake.*

The use of functional foods and supplements for the maintenance of homeostasis in the intestinal environment may be useful not only for treatment of gastrointestinal cancer, but also for cancer prevention and inhibition of cancer progression, maintaining and promoting health. Further detailed studies on the elucidation of fine structures involved in the development of the activity and its action mechanism are now in progress.

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