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Potentiation of Host-Mediated Antitumor Activity by Orally Administered Mushroom (*Agaricus bispora*) Fruit Bodies

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When tumor-bearing mice were given diet containing fruit bodies of mushroom (*Agaricus bispora*) (M-feed), the tumor growth was suppressed to 66.8% in the case of MM-46 carcinoma in C3H mice and to 53.2% in the case of IMC carcinoma in CDF_1 mice. To elucidate the mechanism of this action, the effect of M-feed on the macrophages against syngeneic tumor cells was studied: it was found that the phagocytic activity was increased about 2.2 times and the cytotoxicity of macrophages was enhanced 1.4 times. The production of superoxide anion by macrophages from tumor-bearing mice was decreased in mice given the normal feed, while in mice maintained on the M-feed, it was increased by 1.3 times. Furthermore, the cytotoxic activities of lymphokine-activated killer cells and cytotoxic T cells were increased 1.4 and 1.5 times, compared with their counterparts from mice fed on the mushroom-free diet. These results suggest that mushroom powder given orally acts not only by activation of various effector cells to attack tumor cells, but also by potentiating the cellular functions and preventing a decrease of immune functions of the tumor-bearing host.

Keywords——mushroom; antitumor activity; macrophage; superoxide anion; lymphokine-activated killer cell; cytotoxic T cell

Oral administration of shiitake (*Lentinus edodes*) fruit bodies was indicated previously to inhibit the growth of both allogeneic and syngeneic tumors in mice.[1,2] These antitumor activities were suggested to be due to direct activation of effector cells such as macrophages (Mϕ), natural killer cells (NK cells), lymphokine-activated killer cells (LAK cells) and cytotoxic T cells (Tc cells), and, in addition to prevention of the decrease in the immunological function of the host cells due to the presence of the tumors. With the purpose of elucidating whether or not such action is peculiar to shiitake fruit bodies, the authors fed mice with fruit bodies of *Agricus bispora*, which is more commonly eaten than shiitake throughout the world.

Materials and Methods

Feeds——The experimental diet containing 10—30% powdered mushroom fruit bodies, which were supplied by the Mushroom Research Institute of Japan (Kiryu, Gunma), was prepared as described previously (M-feed).[1] Mice receiving mushroom-free diet (normal feed: N-feed) served as controls.

Animals——Male mice of C3H (5 to 6 weeks old) and CDF_1 (5 weeks old) strains, purchased from Japan Charles River Co., were raised for one week before being used for the experiment.

Tumor Growth Inhibition——Syngeneic MM-46 carcinoma cells (2×10^6) were implanted in male C3H mice (6 or 7 weeks old) and IMC carcinoma cells (2×10^6) in male CDF_1 mice (6 weeks old) in the right axillary region. On the 30th day after tumor transplantation, the solid tumor was extirpated and weighed to obtain the tumor growth inhibition ratio, which was evaluated as: [1 − (weight of tumor mass from mice treated with M-feed ÷ weight of tumor mass from mice receiving no treatment)] × 100.

Collection of Mϕ in Peritoneal Cavity——Peritoneal cells were obtained by washing with Hanks' solution from the peritoneal cavity of mice killed by vertebral dislocation. After centrifugation at 1200 rpm for 10 min, the precipitated cells were collected and adjusted with RPMI-1640 medium to 1×10^6 cells/ml. These cells were pipetted (1.5 ml) onto a plastic plate and cultured at 37 °C for 2h in a 5% CO_2 atmosphere in air. Mϕ were adsorbed selectively on the plate wall. The non-adherent cells were eliminated by washing with 10 ml of Hanks' solution and adherent Mϕ

were detached by pipetting with cold 5% ethylenediamine tetraacetic acid (EDTA) solution containing fetal calf serum.

Measurement of Superoxide Anion (SOA)——Male mice of the C3H strain (5 weeks old) and of the CDF_1 strain (6 weeks old) were maintained on the normal feed or the M-feed. Mϕ were obtained and SOA was measured by the method of Ito *et al.*[3] A 1.5 ml aliquot of phosphate-buffered saline (PBS) containing 10 mM glucose, 80 μM ferricytochrome C and 0.2 mg/ml opsonized zymosan was added to Mϕ adhering to a plate. The plate was incubated for 90 min at 37°C. After centrifugation at 3000 rpm for 5 min, the supernatant was transferred into an ice-chilled test tube, and the absorbance was measured at 550 nm. On the other hand, 1 ml of 0.5% sodium dodecyl sulfate (SDS) was added to the precipitated cells. After standing for 5 min, the cells were well dispersed. The amount of protein was measured by the Lowry–Folin method. The quantity (nmol) of ferricytochrome C was obtained from the absorbance at 550 nm, based on $\Delta E\ 550 = 2.1 \times 10^4\ M^{-1}$, and released SOA per unit protein was calculated.

Opsonization of Zymosan[4]——Zymosan A was adjusted with PBS to 10 mg/ml, boiled for 1 h, washed 3 times with 30 ml of PBS and resuspended in PBS at 50 mg/ml. Four volumes of human serum were added to 1 volume of zymosan and the mixture was incubated at 37°C for 30 min. Then, after centrifugation, 10 mg/ml of opsonized zymosan was obtained by dilution with PBS.

Phagocytic Activity Assay——MM-46 carcinoma tumor cells, preliminarily cultured for 14 d in the peritoneal cavity of C3H mice (IMC carcinoma tumor cells in CDF_1 mice), were obtained and washed with Hanks' solution by centrifugation (1200 rpm, 5 min), and 2×10^7 tumor cells were resuspended in 1 ml of RPMI-1640 medium containing 1.25 μCi (30 μl) of ^{3}H-uridine. The free ^{3}H-uridine was rinsed away with about 700 ml of Hanks' solution after 2 h.[5] The labelled tumor cells were submitted to phagocytic assay at an E/T ratio of 10 : 1 for 1 h at 37°C. After incubation, adherent Mϕ were collected and dissolved in the scintillator (Univergel II, Nakarai Chemicals Co., Kyoto). The radioactivity (dpm) incorporated into Mϕ was counted with a liquid scintillation counter (Aloka LSC-700). The absence of non-phagocytosed tumor cells on the outer membrane of Mϕ was checked under a microscope.

Preparation of Spleen Cell Suspension[6]——A male C3H mouse (6 weeks old) was killed by vertebral dislocation. The femoral vein was cut, and the spleen was extirpated after exsanguination. After being washed with Eagle's minimum essential medium (MEM), the spleen was sufficiently untangled and passed through an 80 mesh stainless steel sieve. Cells were collected by centrifugation at 1100 rpm for 10 min, then 2 ml of 10-fold dilution of Eagle's MEM was added. Immediately after hypotonic elimination of erythrocytes mixed therein for 10 s, 2 ml of Eagle's MEM (2-fold concentration) was added and mixed. The whole was centrifuged at 1200 rpm for 10 min, and the cells thus obtained were adjusted to 1×10^7 cells/ml with RPMI-1640 medium. This was used as the whole spleen cell suspension and was placed in a plastic plate for 60 min. Non-adherent cells were collected and adjusted to 1×10^7 cells/ml with PRMI-1640 medium, and used as non-adherent spleen cells.

Elimination of T Cells[7]——Non-adherent spleen cell suspension (1 ml) obtained from male C3H mice (8 weeks old) was mixed with 20 μl of 5% Thy-1.2F7D5 monoclonal immunoglobulin M (IgM) cytotoxic antibody (Serotec Ltd., England) and incubated for 30 min, then washed once with 3 ml of RPMI-1640 medium. After that, 1 ml of RPMI-1640 medium with fetal bovine serum containing 5% guinea-pig complement was added and the whole was further incubated for 30 min. The cells that reacted with antibody were selectively destroyed. The remaining cells were sedimented by centrifugation at 1200 rpm for 10 min and rinsed 3 times with RPMI-1640 medium. The cells were adjusted to 1×10^7 cells/ml.

Labelling of Target Cells[8]——After being washed with RPMI-1640 medium, YAC-1 tumor cells were adjusted to 1×10^6 cells/ml. Then 25 μl of 45 μCi/ml ^{3}H-uridine solution was added to 1 ml of the cell suspension and after incubation for 55 min, cells were collected by centrifugation at 1200 rpm for 10 min. The cells were washed with RPMI-1640 medium and adjusted to 2×10^5 cells/ml (1.3×10^5—2.7×10^5 dpm/2.5×10^5 cells).

Cytotoxicity[8]——A 1 ml aliquot of the labelled target cell suspension (2×10^5 cells/ml) was mixed with 1 ml of lymphocyte suspension (1×10^7 cells/ml). Portions of 0.5 ml each were incubated for 4 h, then 0.4 ml was dropped on a Millipore filter (0.45 nm in pore size) and suction-filtered. The filter was washed with 25 ml of ice-cold 5% trichloroacetic acid (TCA) solution, dried and mixed with 10 ml of lipophilic scintillator. Radioactivity was measured with a scintillation counter. The cytotoxicity (P%) was calculated according to the following formula:

$$P(\%) = \left(1 - \frac{\text{dpm with immunolymphocytes} - \text{background dpm}}{\text{dpm when target cells only were incubated} - \text{background dpm}}\right) \times 100$$

Statistical Precessing of the Experimental Data——The experimental data were checked by Student's t-test to evaluate the significance of differences between the control and mushroom-treated group.

Results and Discussion

MM-46 and IMC tumor cells (1×10^7 cells) were subcutaneously implanted into C3H mice and CDF_1 mice, respectively. M-Feed was fed to the mice for 30 d, and then each tumor

TABLE I. Antitumor Effects of M-Feed against Various Syngeneic Tumors

Tumor and mouse	Feed	Tumor weight (g)	TIR (%)
MM-46-C3H	Normal	6.3±3.7	0
	M-10%	2.6±1.9	58.8 [a]
	M-20%	2.1±1.2[a]	66.8 [a]
	M-30%	1.5±0.3[b]	76.2
IMC-CDF$_1$	Normal	19.0±3.2	0
	M-10%	12.8±3.7[a]	32.6 [a]
	M-20%	10.4±1.7[c]	45.3 [a]
	M-30%	8.5±0.7[c]	55.4

Significance of differences: (*t*-test) *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$ (12 mice × 3). Mice were given M-feed for 30 d, then growing tumors were extirpated and weighed.

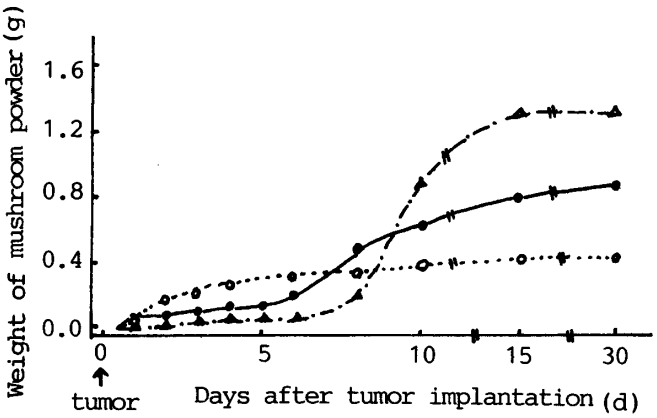

Fig. 1. Amounts of Mushroom Powder Consumed in M-Feed per C3H Mouse

△—△, 30% M-feed; ●—●, 20% M-feed; ○---○, 10% M-feed.

TABLE II. Preventive Effect of 20% M-Feed on Metastasis of MH-134 Carcinoma in C3H Mouse

Feed	No. of metastatic tumor colonies (ave. of 9 mice) (1st)	(2nd)	Prevention (%)
N-feed	17.3±2.3	21.4±3.5	0
M-feed	10.1±2.2[a] (41.6%)	10.6±1.8[a] (50.2%)	45.9

Significance of differences (*t*-test): *a*) $p<0.05$ (10 mice × 2). The number of focal colonies of tumor in the liver were counted at 14 d after tumor implantation.

was extirpated and weighed. The results are compiled in Table I. With increase in the mushroom content in the feed, the tumor inhibition ratio (TIR) increased; the TIR was 66.8% when the feed containing mushroom powder at 20% (20% M-feed) was fed to the MM-46 tumor-bearing mice. This value was larger than that of 53.2% which was obtained when a feed containing powdered shiitake fruit bodies (20% L-feed) at the same concentration was fed to mice implanted with the same tumor cells. When IMC tumor-bearing mice were fed on 20% L-feed, the TIR was only 21.3%. On the other hand, a TIR value of 45.3% was obtained when mice with the same tumor were fed on 20% M-feed. Figure 1 shows daily intake of mushroom powder. During 7—8 d after implantation of the tumor, intake of the M-feed was small but, from the 10th day, the intake returned to a level similar to that seen with the normal feed.

TABLE III. Effect of 20% M-Feed on Phagocytic Activity of ^{3}H-Uridine-Labelled MM-46 Carcinoma Cells by Mϕ from C3H Mouse

Tumor and mouse	Feed	dpm of phagocyted tumor	Ratio
Normal C3H	N-feed	149.1 ±23.2	1.00 [b]
	M-feed	329.7 ±41.1[a]	2.21
MM-46 bearing C3H	N-feed	304.0 ±72.3	2.04 (1.00) [a]
	M-feed	428.9[c] ±41.5[b]	2.88 (1.41)

Significance of differences (*t*-test): *a*) $p<0.05$, *b*) $p<0.01$.
c) Phagocytosed tumor cells/total tumor cells = 13.2%.
(428.9 dpm) (3243 dpm)
Mice were given N- or M-feed for 14 d, then 1×10^4 Mϕ obtained from normal or MM-46 tumor-bearing C3H mice were incubated with 1×10^3 ^{3}H-uridine-labelled tumor cells (3243 dpm) for 60 min. The radioactivity incorporated into Mϕ was counted.

TABLE IV. Cytolytic Activity of Mϕ from C3H Mice against MM-46 Carcinoma Cells

Tumor and mouse	Feed	Degradation (dpm/10^5 Mϕ)	Degradation	
			B/A (%)	Ratio
Normal C3H	N-feed	754.3± 34.3	23.3	1.00 [a]
	M-feed	1064.6±165.3[a]	32.8	1.41
MM-46 bearing C3H	N-feed	298.3± 82.4	9.2	0.39 (1.00) [c]
	M-feed	1085.0± 97.9[b]	33.5	1.44 (3.69)

Significance of differences: (*t*-test) *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$. Mice were fed for 14 d on N-feed or 20% M-feed, then 1×10^4 peritoneal Mϕ collected from 4 C3H mice were mixed with 1×10^3 ^{3}H-uridine-labelled tumor cells for 60 min. Washing was done with RPMI-1640 medium, then Mϕ which had phagocytosed tumor cells were incubated for 160 min and liberated radioactivity was measured. (*B/A*: liberated dpm from Mϕ/incorporated dpm in Mϕ).

Thus, in the subsequent studies, 20% M-feed was employed. Table II shows the inhibitory effect of the M-feed on the metastasis of MH-134 tumor. MH-134 tumor cells (1×10^7 cells) were subcutaneously implanted into the right abdominal region, the liver was isolated after 14 d of tumor implantation by the abdominal section, and the number of focal colonies of the tumor was counted. A metastasis-inhibiting rate (MIR) of about 46% was obtained with the M-feed. This result indicates that oral administration of *Agaricus bispora* also suppresses metastasis of the tumor. In general, activated Mϕ, Tc cells, NK cells and LAK cells are the cells which play an important role in the immune system of the host in terms of the antitumor activities. In general, antitumor polysaccharides have been reported to exert a cellular immune system-activating activity when they are administered intraperitoneally or intravenously. Thus, the effect of the M-feed on the Mϕ was investigated, and the results are summarized in Table III.

MM-46 tumor cells labelled with ^{3}H-uridine were mixed with Mϕ obtained from normal or MM-46 tumor-bearing C3H mice in an *E/T* ratio of 10 : 1 and incubated at 37 °C for 60 min with MEM medium in an atmosphere of 5% CO_2 in air. The cultured plastic plates were sufficiently washed with MEM medium, and it was confirmed that the washings contained no radioactivity. The adherent Mϕ were detached by pipetting with 0.5% EDTA solution containing fetal calf serum and centrifuged at 1200 rpm for 10 min. The collected Mϕ were adjusted to 1×10^4 cells/ml with PBS and mixed with Univergel II (Nakarai Chemicals Co.) to

lyse the cells, and the radioactivity in the lysate was measured. The outer membrane of Mϕ was inspected under a microscope to confirm the absence of adhering tumor cells. In the same experimental system using fruit bodies of shiitake, no influence was seen on the phagocytic activity of Mϕ from normal C3H mice towards tumor cells.[3] However, in the case of the present mushroom, about 2.2 times higher phagocytic activity was found for Mϕ from mice fed on the M-feed in comparison with those of mice fed on the normal feed. On the other hand, in the case of tumor-bearing mice, administration of the M-feed resulted in a 2.9-fold increase in the phagocytic activity compared to administration of the normal feed. Next, degradation of tumor cells phagocytosed by Mϕ was studied. The results are shown in Table IV.

On the 14th day after implantation of MM-46 tumor, Mϕ were collected from the peritoneal cavity, and they were allowed to phagocytose MM-46 tumor cells labelled with ^{3}H-uridine. The Mϕ which had phagocytosed MM-46 tumor cells were then incubated for 160 min in a humidified atmosphere of 5% CO_2 in air, and radioactivity liberated was measured. In the case of normal feed, the cytotoxicity of Mϕ from tumor-bearing mice against tumor cells was decreased to about 40% of that of Mϕ of normal mice. On the other hand, when tumor bearing mice were raised on the M-feed, the cytotoxicity of Mϕ was increased by 1.44 times in comparison with that of Mϕ from normal mice. This result indicates that administration of the mushroom caused activation of Mϕ. In general, it is well known that SOA is released by Mϕ in phagocytosis; this is one of the criteria of cytolytic activity of

TABLE V. Time Course of SOA Release by Mushroom-Induced Mϕ from Normal C3H Mice

Breeding time (d)	Feed	SOA released (nmol/mg protein)	
		C3H mouse	CDF_1 mouse
0	N-feed	63.2±1.5 (1.00)	52.4±2.1 (1.00)
8	N-feed	61.4±2.1 (0.97)	50.8±1.4 (0.97)
	M-feed	56.3±1.6 (0.89)[a]	47.3±1.2 (0.90)
15	N-feed	60.3±2.2 (0.95)[a]	49.8±1.6 (0.95)
	M-feed	67.2±1.7 (1.06)[b]	50.3±1.3 (0.96)

Significance of differences (*t*-test): *a*) $p<0.05$, *b*) $p<0.01$. Mϕ were obtained from male mice of the C3H strain (5 weeks) and of the CDF_1 strain (6 weeks) which had been maintained on N-feed or 20% M-feed, and SOA production by Mϕ was measured.

TABLE VI. Time Course of SOA Release by Mushroom-Induced Macrophages from MM-46 Carcinoma-Bearing Mice

Days after tumor implantation	Feed	SOA released (nmol/mg protein)	
		C3H-MM-46 tumor	CDF_1-IMC tumor
0	N-feed	60.2±1.2 (1.00)	48.0±1.9 (1.00)
8	N-feed	49.7±1.1 (0.83)	38.2±2.3 (0.80)
	M-feed	63.4±1.7[a] (1.05)	41.4±1.5[a] (0.86)
15	N-feed	31.3±1.7[a] (0.52) [b]	21.2±0.9 (0.44) [a]
	M-feed	79.2±1.2[b] (1.31)	42.2±1.7[a] (0.88)

Significance of differences (*t*-test): *a*) $p<0.01$, *b*) $p<0.001$. SOA production of Mϕ obtained from MM-46-bearing C3H mice given 20% M-feed was measured.

activated Mϕ. As shown in Table V, the amounts of SOA released by Mϕ obtained from normal C3H mice and CDF_1 mice were determined. Administration of the M-feed did not increase SOA production by Mϕ from C3H mice or CDF_1 mice.

However, as shown in Table VI, in the case of Mϕ obtained from MM-46 tumor-bearing C3H mice, the amount of released SOA decreased to 0.83 on the 8th day and 0.52 on the 15th day. In CDF_1 mice, the SOA production decreased to 0.80 on the 8th day and 0.44 on the 15th day after implantation of IMC tumor. On the other hand, the production of SOA by Mϕ obtained from MM-46 tumor-bearing C3H mice which were bred on M-feed returned to the level recorded for normal mouse Mϕ on the 8th day and showed a 1.3-fold increase on the 15th day. In the case of IMC tumor-bearing CDF_1 mice, the SOA production from Mϕ was only 80% of that of normal mice given M-feed. These results indicate that the tumor growth inhibition by M-feed is at least partly due to activation of the cytotoxicity of Mϕ or prevention of deterioration of the immune functions caused by the tumor-bearing state. Next, the effects of mushroom powder on NK cells, nonspecifically injuring tumor cells, and Tc cells, specifically injuring only the antigen-presenting cells, were studied. First of all, NK cells, which are induced from pre-NK cells by interferon, were studied. Spleen cells were used as a cell group containing NK cells, and YAC-1 tumor cells were labelled with ^{3}H-uridine as target cells. The results are shown in Table VII.

The M-feed was given to C3H mice, the spleen was extirpated after predetermined time intervals, non-adherent spleen cell suspension was prepared, and the cytotoxicity of this cell fraction against YAC-1 tumor cells (believed to be most susceptible to NK cells) was measured. As a result, in both the whole spleen cell fraction and non-adherent spleen cell fraction, the strongest NK cells activity was obtained when the mice were bred on M-feed for 8 d. The NK cells activity of whole spleen cells was potentiated about 1.5 times by mushroom, compared with that for the same cell fraction from the mice given the normal feed, while the cytotoxicity of NK cells was increased by 2.4 times in non-adherent spleen cells obtained from C3H mice maintained on M-feed. MM-46 tumor-bearing C3H mice were bred on M-feed, and spleen cells containing NK cells were obtained from the extirpated spleen. The cytotoxic activity against P-815 tumor cells was assessed. The cytotoxicity in tumor-bearing mice may be due in part to LAK cells activated by lymphokine secreted by T cells. Thus, in this experiment, the cytotoxicity was tentatively expressed as LAK cells activity. As shown in Table VIII, the whole cell suspension prepared from the spleen of MM-46 tumor-bearing mice administered the normal feed on the 18th day showed only 0.46 times the cytotoxic activity of

TABLE VII. Effect of M-Feed on NK Cells Activity of Whole Spleen Cells and Non-adherent Spleen Lymphocytes in Relation to the Period of Administration (Normal C3H Mice)

Days of M-feed	NK cells activity (%) (relative cytotoxicity)	
	Whole spleen cells	Non-adherent spleen lymphocytes
Control feed	14.1±0.3 (1.00)	7.7±1.3 (1.00)
4	19.4±0.2[c] (1.38)	12.8±0.8[b] (1.66) [b] [c]
8	21.2±1.1[b] (1.50)	18.3±1.4[a] (2.38)
12	17.8±1.7[a] (1.26)	16.2±2.4 (2.10)

Significance of differences (*t*-test): *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$. After predetermined time intervals, the spleens were extirpated from five C3H mice maintained on 20% M-feed and the cytotoxicity of NK cells prepared from the spleens was measured.

TABLE VIII. Effect of 20% M-Feed on LAK Cells Activity of Whole Spleen Cells and Non-adherent Spleen Lymphocytes in Relation to the Period of Tumor-Bearing

Days of tumor-bearing		LAK cells activity (%) (relative cytotoxicity)			
		Whole spleen cells		Non-adherent spleen lymphocytes	
Control mouse	N-feed	11.3 ± 1.8	(1.00)	5.9 ± 2.3	(1.00)
6	N-feed	11.8 ± 1.2	(0.96)	6.7 ± 1.4	(1.14)[a]
	M-feed	8.7 ± 1.5	(0.73)	4.3 ± 0.8	(0.73)
12	N-feed	8.5 ± 0.9[a]	(0.75)[a]	5.1 ± 1.2	(0.86)[a]
	M-feed	11.4 ± 1.7[a]	(1.01)	6.4 ± 0.7[b]	(1.08)
18	N-feed	5.3 ± 1.1	(0.46)[c]	2.1 ± 1.2	(0.36)[c]
	M-feed	15.5 ± 1.2[c]	(1.37)	7.7 ± 1.3[a]	(1.30)

Significance of differences (*t*-test): *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$. TIR: 12 d 62.9%; 18 d 66.7%. Spleen cells containing LAK cells were obtained from MM-46 tumor-bearing C3H mice maintained on M-feed, and the cytotoxic activity against P-815 tumor cells was assessed. The cytotoxicity in tumor-bearing mice may be due in part to LAK cells activated by lymphokine secreted by T cells. Thus, in this experiment, the cytotoxic activity was taken as activity of LAK cells.

TABLE IX. Effect of 20% M-Feed on the Development of Alloreactive Cytotoxic T-Lymphocytes

Feed	Treatment	Cytolysis	Ratio	
N-feed	Non	18.21 ± 0.74	1.00	1.00
	Anti-Thy 1.2+C′	10.37 ± 1.18	0.57	
M-feed	Non	27.43 ± 1.07[b]	1.00	1.51
	Anti-Thy 1.2+C′	13.77 ± 0.96[a]	0.50	

Significance of differences (*t*-test): *a*) $p<0.05$, *b*) $p<0.01$. After sensitization by grafting of P-815 tumor cells as antigen cells into C3H mice, mice were given M-feed for 11 d. Then the cytotoxicity of spleen cells containing no T cells was measured.

normal mice and that of the non-adherent spleen lymphocyte suspension showed only 0.36 times. However, when the tumor-bearing mice were given M-feed for the same time, the cytotoxic activity of LAK cells of both cell suspensions was increased to 1.37 and 1.30 times of the level of normal mice, respectively. These results indicate that mushroom powder directly potentiates the cytotoxicity of LAK cells and NK cells or accelerates the differentiation of inactive pre-NK cells to active NK cells, even when administered orally. Moreover, the increase in the LAK cells activity was higher than that when shiitake powder was administered. Generally, T cells (Tc) show specific cytotoxicity against allogeneic cells used for immunization. Hamuro *et al.*[9,10] reported that such induction of Tc cells enhanced by i.p. or i.v. administration of a β-glucan. Thus, whether mushroom fruit bodies show such an enhancing effect or not was studied, and the results are summarized in Table IX.

After sensitization by grafting, P-815 tumor cells as the antigen cells were inoculated intraperitoneally into C3H mice, then the mice were given M-feed for 11 d, the spleen was extirpated and whole spleen cells and spleen cell suspension in which T cells had been treated with anti-Thy 1.2 and complements (C′) were prepared. The cytotoxicity of the whole spleen cell preparation was increased 1.5 times by mushroom administration, while the fraction lacking T cells showed a marked decrease. These results suggest that the increased cytotoxicity

is due to T cells. That is, the mushroom fruit bodies enhance the induction of Tc cells. Recently, Toth and Erickson[11] reported that when mice were given uncooked fresh mushroom for 60—70 weeks, tumors were induced in the bone, liver and other organs. However, we found no mushroom-induced tumors in mice fed for 13 months on dried mushroom powder. On the basis of the above results, we consider that oral administration of mushroom fruit bodies not only activates effector cells such as Mϕ, LAK cells and Tc cells, but also potentiates host immune function, which is suppressed in the tumor-bearing state.

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