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Antitumor Activity of an Immunomodulating Material extracted from a Fungus, *Peziza vesiculosa*

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The antitumor activity of an immunomodulating material, vesiculogen, from a fungus, *Peziza vesiculosa*, on the transplantable sarcoma 180 was examined in mice. Vesiculogen showed antitumor activity against both solid and ascites forms of sarcoma 180.

Keywords——*Peziza vesiculosa*; antitumor activity; sarcoma 180; immunomodulator; fungal product

Many microbial products are well known to possess some immunomodulating activities.[1-3] Most of these materials are of bacterial origin, such as lipopolysaccharide(LPS)[4] and peptidoglycan.[5] Immunomodulators of fungal origin are rare. Therefore, we have examined the mitogenic activity of materials from various species of fruiting body-forming fungi, some of which have been used as foods or herbal medicines. It was found that the water-soluble materials from all species of Pezizaceae (Ascomycetes) and some species of Polyporaceae (Basidiomycetes) tested showed mitogenic activity.[6] Since the mitogenic activity of the material obtained from *P. vesiculosa* which belongs to Pezizaceae was particularly high, the fungus was selected over others as a potential source of fungal immunomodulator. The material (named vesiculogen) was shown to be a B cell mitogen, a polyclonal B cell activator,[7] and an adjuvant (unpublished data). In addition, vesiculogen was capable of enhancing the functions of reticuloendothelial systems, namely phagocytosis and *in vitro* cytostatic action on target tumor cells (unpublished data). Antitumor activities of various kinds of microorganisms and their products have generated much interest during the past few years, and the mechanism of action has been shown to be dependent on their immunopotentiator activities.[8] Thus, we examined whether vesiculogen, which possessed immunomodulating activities as described above, showed antitumor activity *in vivo*.

In this paper, we report the antitumor activity of vesiculogen on transplantable sarcoma 180 in ICR mice.

Materials and Methods

Mice——Male ICR mice, 6 weeks old and weighing 20–25 g, obtained from Tokyo Jikken Dobutsu, Tokyo, were used.

Tumor——Sarcoma 180 tumor cells in ascites form were kindly supplied by Dr. T. Sasaki, The National Cancer Center Research Institute of Japan, and were maintained by weekly passage in ICR mice.

Preparation of Vesiculogen——Vesiculogen was prepared by the method previously described, except for lyophilization.[7] Briefly, the dried fruiting bodies were boiled in water. After filtration, the filtrate was dialyzed against distilled water and the nondialyzable fraction was recovered by lyophilization. This preparation is referred to as vesiculogen in the present paper, and contained 57.6% protein (as bovine serum albumin) and 20.0% carbohydrate (as glucose). Vesiculogen was dissolved in saline and diluted to the desired concentration with saline.

Evaluation of Antitumor Activity——The antitumor activity was evaluated with both the solid and ascites forms of sarcoma 180 tumor cells. For the solid form, tumor cells (2×10^6) were inoculated subcutaneously into the right groin of mice. Vesiculogen was administered several times before and/or after the tumor

inoculation. Thirty-five days after tumor inoculation, the mice were sacrificed. The inhibition ratio was calculated from the tumor weight of the treated group against that of the control group. For the ascites form, groups of mice were inoculated intraperitoneally (*i.p.*) with tumor cells (2×10^6, or 1×10^6). Vesiculogen was administered several times before and/or after the tumor inoculation, and the number of surviving mice in each group was examined to calculate the survival rate and mean survival time.

Results

Antitumor Activity of Vesiculogen on Solid Form of Sarcoma 180

To examine the effect of vesiculogen against solid form of sarcoma 180, mice were administered *i.p.* with various doses of vesiculogen at varying times as indicated in Table I. Control mice received saline instead of vesiculogen solution. The results are shown in Table I. Administration of 200, 2000 or 4000 μg of vesiculogen on days +1, +3, +5, +7 and +9 resulted in significant antitumor activity, and the inhibition ratio was 80.0, 83.2 or 72.9%, respectively. Administration of 2000 μg of vesiculogen on days −9, −7, −5, −3 and −1 was also effective, but the inhibition ratio was below 30.0% ($p<0.05$).

Table I. Antitumor Activity of Vesiculogen on Solid Form of Sarcoma 180[a)]

Dose (μg/mouse)	Dosage schedule (day)	No. of mice	Mean weight of tumors ± SD (g)[b)]	Inhibition ratio(%)	Significance[c)]
200	+1, +3, +5, +7, +9	10	0.76±0.45	80.0	$p<0.001$
2000	+1, +3, +5, +7, +9	10	0.64±0.48	83.2	$p<0.001$
4000	+1, +3, +5, +7, +9	8[d)]	1.03±0.43	72.9	$p<0.001$
2000	−9, −5	10	2.80±0.86	26.3	n.s.
2000	−9, −7, −5, −3, −1	10	2.76±0.82	27.4	$p<0.05$
Control	+1, +3, +5, +7, +9	10	3.80±1.24	—	

a) Sarcoma 180 cells (2×10^6) were inoculated on day 0.
b) Arithmetic mean weight of tumors ± standard deviation.
c) The significance (p value) of differences between the control and experimental group was evaluated according to Student's t test, $p<0.05$ being taken as a significant difference. n.s. = not significant.
d) Two mice died within 5 days after the administration of vesiculogen.

Antitumor Activity of Vesiculogen on Ascites Form of Sarcoma 180

The effect of vesiculogen against ascites form of sarcoma 180 was examined in relation to the dosage and time schedule in comparison with solid form. However, there was no group that showed significant activity. In addition, a high dose (2000 or 4000 μg/mouse) of vesiculogen was toxic to mice, particularly when vesiculogen was administered *i.p.* to mice that had been given tumor cells *i.p.* (data not shown). Then the effect of low doses (50 to 1000 μg/mouse) of vesiculogen in mice inoculated with 1×10^6 of tumor cells was examined. The results are shown in Table II. Administration of 50 μg of vesiculogen exhibited the

Table II. Antitumor Activity of Vesiculogen on Ascites Form of Sarcoma 180[a)]

Dose (μg/mouse)	35-day survivors/ No. of tested mice	Mean survival days ± SD[b)]	Significance[c)]
50	6/10	28.1± 9.5	$p<0.01$
100	4/10	25.5±10.5	$p<0.02$
200	4/10	24.1±10.6	$p<0.05$
500	5/10	25.5±10.8	$p<0.02$
1000	2/10	18.5± 9.1	n.s.
Control	0/10	14.5± 7.0	

a) Sarcoma 180 cells (1×10^6) were inoculated on day 0. Vesiculogen was administered *i. p.* to mice on days −5, −2, +2, +5, +8 and +11. Results on day +35 are shown.
b) Arithmetic mean survival days ± standard deviation.
c) See Table I.

most effective activity, giving a 60% survival rate. The survival rate in the group administered with 100, 200 or 500 μg of vesiculogen was 40, 40 or 50%, respectively. These survival rates are significant ($p<0.05$). However, 1000 μg of vesiculogen was less effective than other doses (not significant).

Discussion

The present data demonstrate that vesiculogen extracted from fruiting bodies of a fungus, *P. vesiculosa*, which belongs to Ascomycetes showed antitumor effect against both solid and ascites forms of sarcoma 180, although the effect was not remarkable. Various kinds of fungi, such as *Coriolus versicolor*,[9,10] *Lentinus edodes*,[11] and *Grifola umbellata*,[12] have been reported to produce antitumor material, but almost all of these fungi belong to Basidiomycetes. Therefore, it is interesting that *P. vesiculosa* which belongs to Ascomycetes, also produces an antitumor material.

The antitumor effect of vesiculogen is considered to be mediated by the immune system. The reasons are that: 1) vesiculogen does not seem to have a direct cytotoxic effect on cultured tumor cells *in vitro* (data not shown); 2) although the inhibitory effect was not remarkable, the administration of vesiculogen before the tumor inoculation did result in antitumor activity (Table I); 3) vesiculogen acts as an immunoadjuvant on the antibody response; and further 4) vesiculogen is capable of enhancing the functions of reticuloendothelial systems, namely the *i.p.* injection of vesiculogen into mice increased the clearance rate of carbon in the circulating blood, and peritoneal macrophages collected from the mice showed a cytostatic effect on target syngeneic tumor cells (unpublished data). We are now examining whether the macrophages activated with vesiculogen exhibit antitumor activity *in vivo*. Since vesiculogen is a crude extract, the relation between the molecule possessing antitumor activity and the immunological activator is not clear. The purification of these materials is in progress.

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