Antitumor 1,3-β-Glucan from Cultured Fruit Body of Sparassis crispa

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Received October 13, 1999; accepted April 10, 2000

Sparassis crispa is an edible mushroom recently cultivable in Japan. Polysaccharide fractions were prepared from the cultured S. crispa by repeated extraction with hot water (SCHWE), cold NaOH (SCCA), and then hot NaOH (SCHA). HWE was further separated by 1 volume (SCHWE1v) or 4 volumes (SCHWE4v) of ethanol-precipitable fractions. By chemical, enzymic, and NMR analyses, the primary structures of SCHWE1v, SCCA, and SCHA were 6-branched 1,3-β-glucan, having one branch in approximately every third mainchain unit. All of these fractions showed antitumor activity to the solid form of Sarcoma 180 in ICR mice with strong vascular dilation and hemorrhage reaction. These fractions also showed enhanced hematopoietic response to cyclophosphamide induced leukopenic mice following intraperitoneal or peroral administration.

Key words: β-glucan; Sparassis crispa; immunomodulation; antitumor activity; hematopoiesis

An immunomodulating substance, a biological response modifier (BRM) or biotherapy is important for treatment of cancer and infectious diseases. β-Glucan is a well-known BRM which is widely distributed in nature and used as a medicine and food.1–5 The effects of “Lentiman” from Lentinus edodes and “Sonifilan” (SPG) from Schizophyllum commune in cancer therapy have been clinically proven.6,7 We have already developed β-glucans, GRN from Grifola frondosa,8–10 SSG and TSG from Sclerotinia sclerotiorum,11–13 OL-2 from Omphalina lapidescens,14 PVS and PVG from Peziza vesiculosa,15 CSBG from Candida spp.,16 and OX-ZYM from a yeast cell preparation zymosan.17,18 We have also prepared several carboxymethyl, hydroxethyl, sulfate, and polyol derivatives of the above β-glucans.18–20 In addition, we have analyzed the mechanism of β-glucan mediated immunopharmacological activity and identified the conformation dependent and independent activity,21–25 i.e. nitrogen oxide production of macrophage by single helical conformer, limulus factor G activation by random coil and single helix, interleukin 8 synthesis of human peripheral mononuclear cell by triple and single helix, and hematopoietic response by triple and single helix. These rather complicated relationships between structure and activity suggest the contribution of multiple receptor-ligand interactions in β-glucan mediated immunopotentiation.

The incidence of cancer is gradually increasing and the spectrum of cancer-prone organs is changing each year. In addition to surgery, irradiation, and chemotherapy, immunotherapy is believed to be an important cancer therapy.26 Immunotherapy and biotherapy include fields, such as BRMs, cytokines, lymphocyte transplantation, gene therapy, and herbal and alternative medicines. Clinical trials of these therapies are being widely carried out, and clinical evidence does suggest their efficacy, although the precise mechanisms are still difficult to understand at the molecular level. Development of other β-glucans is still needed for better biotherapy and to understand the molecular mechanism.

Sparassis crispa is an edible mushroom recently cultivable in Japan. In the present study, polysaccharide fractions were prepared from cultured S. crispa, and the structure and activities of the extracts were examined.

MATERIALS AND METHODS

Materials Fruit bodies of Sparassis crispa were cultured by Minakake Co., Ltd., Tokyo. Sonifilan was generously provided by Kaken Pharmaceutical Co., Ltd., Tokyo. Cyclophosphamide (CY) was from Shionogi & Co., Ltd., Osaka, and Toyopearl HW-40F and HW-65F were from Tosoh Co., Ltd., Tokyo.

Carbohydrate Analyses Carbohydrate content was determined by the phenol–sulfuric acid method. Component sugars were determined by capillary gas-liquid chromatography (Okhura Riken Co., Ltd., Tokyo) of alditol acetate derivatives after complete hydrolysis by 2 M trifluoroacetic acid. A capillary column of fused silica (J & W Scientific, Inc., CA, 30 m×0.262 mm, liquid phase; DB-225, 0.25 mm) was used at 220 °C. The molar ratio was calculated from the peak area of each component (glucose as 100).

Zymolase Digestion of Polysaccharide Fractions Each fraction (20 mg) suspended in 10 ml of acetate buffer (50 mM, pH 6.0) was mixed with 1 mg of zymolase 100T (Seikagaku Corp.). After overnight incubation at 45 °C, the reaction mixture was boiled for 3 min to inactivate the enzyme. Four volumes of EtOH was added to precipitate the macromolecular fraction. After centrifugation, the resulting supernatant was concentrated and applied to a Toyopearl HW-40 column. The elution profile of each fraction was monitored by the phenol–H2SO4 method.

NMR Analysis Fractions and authentic materials were dissolved in D2O or Me2SO-d6 and the 1H- and 13C-NMR spectra were determined at 70 °C. Bruker DPX400 and DRX500 instruments equipped with the software “XWIN-NMR” were used.

MALDI-MS Analysis MALDI-TOF-MS analysis was conducted using a Perceptive Biosystems’ Voyager DE-RII equipped with delayed extraction and a nitrogen laser. All analyses were conducted using 2,5-dihydrobenzoic acid (Tokyo Kasei Co.) as matrix.

Antitumor Activity Male ICR mice were obtained from Japan SLC, Inc. (Shizuoka, Japan), they were 5 weeks of age and maintained under specific pathogen-free conditions. Antitumor activity against the solid form of Sarcoma 180 tumor was measured by the method described previously.8
**Sparassis crispa** Fr., air dried, powdered, 25g

- Ext Air dried
- Add H₂O, 500mL, autochaving, 2h
- Ext Add 10% NaOH / 5% urea, 500mL, 4°C, 2days
- Ext Add 10% NaOH / 5% urea, 500mL, 65°C, 1h
- Ext
- Ext Residue suspended in H₂O, dialysis
- Ext Residue dialysis
- Ext Residue
- Ext Sup & turbid parts
- Ext Fiber
- Ext add EtOH, 4L, 4°C, 1day
- Ext 500rpm, 5min
- Ext add H₂O, 200mL, ultrasclerorrser, add EtOH, 200mL
- Ext collect fibrous precipitate
- Ext SCHWE1v (Hot water extracts)
- Ext SCHWE4v
- Ext SSCA2 (Cold alkali extract)
- Ext SCHCA
- Ext SCRC
- Ext Hot alkali extract

**Table 1. Yield and Properties of S. crispa Extracts**

<table>
<thead>
<tr>
<th>Component (Glc : Man : Gal)</th>
<th>% 1,3-β glucan in total carbohydrate (%zymolyase sensitive)</th>
<th>% DEAE-bound in total carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCHWE1v 461 mg</td>
<td>64%</td>
<td>2.5%</td>
</tr>
<tr>
<td>SCHWE4v 415 mg</td>
<td>34%</td>
<td>20 %</td>
</tr>
<tr>
<td>SCCA1 4.97 g</td>
<td>80%</td>
<td>3.9%</td>
</tr>
<tr>
<td>SCCA2 2.1 g</td>
<td>71%</td>
<td>9.8%</td>
</tr>
<tr>
<td>SCHA 1 g</td>
<td>83%</td>
<td>3.8%</td>
</tr>
<tr>
<td>SCRC 4.2 g</td>
<td>—</td>
<td>100 : 3 : 31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 : 4 : 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 : 4 : 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 : 8 : 12</td>
</tr>
</tbody>
</table>

From 25 g of air dried fruit body.

**Monitoring Vasculitis** Vascular reaction of lentian, the vascular dilation and hemorrhage (VDH) reaction, was reported by Maeda et al.27, 29 Vasculitis induced by *S. crispa* extracts was monitored by visual inspection of the ear showing a flare, in accordance with Maeda’s procedure with slight modification. VDH reaction was evaluated as i) strong (+ +) reaction: strong and widespread flare in both ears, ii) partial (+) reaction: partial flare in either ear, and iii) no (+/-/-) reaction: no or only faint flare in one ear.

**Cyclophosphamide Induced Leukopenia** Cyclophosphamide (CY; 200 mg/kg) was administered i.p. to ICR mice on day-0. The number of white blood cells (WBC) was counted microscopically by collecting blood from the tail vein (5 μl) and mixing it with Turk’s solution (45 μl).

**Assessment of Helix Conformation by Congo Red** The change of absorption maximum of Congo Red (Wako Pure Chemical Co., Ltd.) in the presence or absence of polysaccharide fractions was measured by a Hitachi 557 spectrophotometer. An equal volume of polysaccharide fraction (1 mg/ml) and Congo Red solution (2×10⁻⁵ mol) was mixed in sodium hydroxide (final concentration of 0.1 m or 0.35 m) and the absorption maximum of the resulting solution was measured immediately.

**Statistics** Results are expressed as the arithmetic mean±standard deviation (S.D.). Statistical evaluations were performed by the Student's t-test. A value of *p*<0.05 was considered significant.

**RESULTS**

**Preparation of Polysaccharide Fractions** Polysaccharide fractions of *S. crispa* were prepared as shown in Chart 1. Briefly, air-dried and powdered *S. crispa* (25 g) was defatted with CHCl₃:MeOH:H₂O and then extracted with hot water (121°C, 2 h). The polysaccharide fraction was prepared from the extract by precipitation with 1 vol. and then 4 vol. of EtOH (SCHWE1v; 461 mg, SCHWE4v; 415 mg). The form of SCHWE1v was fibrous. The resulting residue was then extracted with cold alkali (10% NaOH/5% urea, 4°C, 2d, twice; SCCA1 and SCCA2), and hot alkali (10% NaOH/5% urea, 65°C, 1 h; SCHA). Polysaccharide fractions of alkali extracts were collected after extensive dialysis (SCCA (SCCA1+SCCA2); 7.1 g, SCHA; 1.0 g). Residue of the alkaline extract was also dialyzed and then lyophilized. Less than 20% of the total component remained after these extraction steps (SCRC; 4.2 g). Protein, carbohydrate and component sugars of these fractions were measured by phenol-sulfuric acid method with glucose as reference, BCA method with bovine serum albumin as reference, and alditol-acetate derivatives, respectively (Table 1).

The major component sugar of all the extracts was glucose (Table 1). Protein content of SCHWE4v and SCCA2 was ca. 20 and 10%, but that of others was less than 4%. The final preparation step of SCCA1 and SCCA2 differed: we used ethanol precipitation for SCCA1 and lyophilisation for SCCA2. Ethanol precipitation is useful to reduce low molec-
ular weight proteins in the extracts. These facts strongly suggested that SCHWE1v, SCCA1 and SCHA were principally “simple polysaccharide” composed of glucan without further purification.

Chemical Characterization of Polysaccharide Fractions
Molecular weight of each extract was analyzed by a column of Toyopearl HW65F equilibrated with 0.3 N NaOH, to rule out, or reduce, interchain interactions such as “gel.” As shown in Fig. 1, all polysaccharide fractions, except for SCHWE4v, showed broad but a symmetrical molecular weight distribution with an average molecular weight of ca. 10^6 Da. Monitored by a BCA protein assay kit, all of the protein constituents in all extracts were eluted from the Bov volume (Fraction no. around 40) of the column (data not shown), thus easily reducing any contaminated proteins.

Carbon-13 NMR spectra of polysaccharide fractions are shown in Fig. 2. Assignment of the spectra was made by comparison with previously published spectra. The anomeric carbon signal presented around 103 ppm was assignable as the β-configuration. Presence of the signal around 86 ppm was assignable as the 1,3-β-linkage. Sharp signals around 70, 73, and 76 ppm were suggested to be the presence of non-reducing terminal glucose. From these assignments, SCHWE1v, SCCA1, SCCA2, and SCHA were strongly suggested to be composed largely of a 6-branched 1,3-β-glucan. In addition, the ratio of branching in SCHWE1v, SCCA1 and SCCA2, estimated from the signal intensities of the triplet signal around 86 ppm, were about one residue in every three main chain units. Other signals appearing between 60 to 80 ppm were also quite similar to that of grifolan and scleroglucan. In contrast, the signal intensity

![Graph](image)

**Fig. 1.** Gel Filtration Chromatography of Polysaccharide Fractions
Aliquot of a polysaccharide fraction (SCHWE1v, SCHWE4v, SCCA1, SCCA2, or SCHA) dissolved in 0.3 N NaOH was applied to a column of Toyopearl HW-65F (1 x 45 cm) equilibrated with 0.3 N NaOH and fractionated. Eluted fractions were collected and monitored by phenol-H2SO4 method. Elution volume of glucose as a reference was Fr. 41.

![Graph](image)

**Fig. 2.** 13C-NMR Spectra of Polysaccharide Fractions
Twenty milligrams of a polysaccharide fraction was dissolved in 0.6 ml of DMSO-d6. 13C-NMR spectrum was scanned at 70 °C overnight as described in Materials and Methods. A) SCHWE1v, B) SCCA1, C) SCCA2, D) SCHA.

![Graph](image)

**Fig. 3.** Metachromasy of Congo Red by Polysaccharide Fractions
Absorption maximum of Congo Red in the presence of polysaccharide fractions at 0.1 x and 0.35 x NaOH were measured as described in Materials and Methods.
appearing around 86 ppm in SCHA was not equal intensity, thus the ratio of branching would be lower. The signal intensity of SCHA was similar to HA-β-glucan prepared from Pleurotus ostreatus and suggested to be about one residue in every four main chain unit. Lower ratio of branching in SCHA was also suggested by the zymolyase digestion shown below (Fig. 5).

The physicochemical property of the polysaccharide fractions was also examined by Congo Red induced metachromasy, which is a well-known property of a high molecular weight and gel forming 1,3-β-glucan. All the fractions, except for SCHW4v, showed metachromasy with Congo Red, and the absorption maximum was returned to a shorter wavelength in 0.3 N NaOH (Fig. 3). Content of 1,3-β-glucan in each fraction was also examined by digestion by an endo-1,3-β-glucanase, zymolyase, digestion (Table 1). The majority (50—75%) of SCHWE1v, SCCA, and SCHA were degraded and resulted in the production of oligosaccharides. The oligosaccharide fractions were analyzed by a column of Toyopearl HW40 (Fig. 4) and by MALDI-TOF-MS (data not shown). Major oligosaccharides were suggested to be glucotri- and glucotetroside. Carbon-13 NMR analysis of the zymolyase-resistant part, prepared by ethanol precipitation, suggested the presence of linkages other than 6-branched 1,3-β-glucan: 1,6-β-glucan and α-glucan (Fig. 5). Each fraction was also applied to DEAE-Sephadex A-25 equilibrated with 8 M urea. After collecting the passed through fraction, the adsorbed fraction was eluted with 8 M urea containing 1 M NaCl. As shown in Table 1, more than 80% of the polysaccharide fraction was neutral.

**Biological Activity of Polysaccharide Fractions** Chemical analysis strongly suggested that the structural feature of

![Fraction no.](image)

**Fig. 4. Elution Profile of Zymolyase Digested Polysaccharide Fractions from Toyopearl HW40**

Sonifilan, SCCA1 and SCHA were digested with zymolyase as described in Materials and Methods. After ethanol precipitation, the ethanol layer was concentrated and applied to a column of Toyopearl HW40F (2×90 cm) equilibrated with distilled water. The elution profile was monitored by phenol-H₂SO₄ method. DP1, 2, 3, and 4 represent elution volume of glucose, laminara-biose, laminara-triose, and laminara-tetrose, respectively.

![PPM](image)

**Fig. 5. ¹³C-NMR Spectra of Zymolyase Resistant Moiety in DMSO-d₆**

Macromolecular fractions of the zymolyase digested polysaccharide fractions were collected by ethanol precipitation. A) SCHWE1v; B) SCCA2; C) SCHA.
the polysaccharide fractions was characteristic of so-called "fungal antitumor β-glucan," and the biological activity of these fractions was screened. The extracts showed strong antitumor activity to the solid form of Sarcoma 180 in mice even by three intraperitoneal administrations of 20 μg/mouse (Table 2). The activity of SCHWE4v was weaker, probably because of the lower content of 1,3-β-glucan. Polysaccharide fractions also induced temporary but strong vasculitis, which gradually disappeared after administration was terminated. The vasculitis was similar to the syndrome known as VDH (vascular dilation and hemorrhage) reaction, a characteristic phenomenon of a neutral polysaccharide showing antitumor activity, such as lentian. The VDH reaction for the vasculitis induced by S. crispae extracts. As shown in Table 3, SCHWE1v, SCA1, and SCAH showed significant reduction on day 5. In Fig. 6(A), SCA1 was administered i.p. simultaneously with CY. The number of WBC in the SCA1 administered group was significantly higher than in the CY-administered group from days 7 to 11, as true of other antitumor β-glucans. The hematopoietic response was also examined by peroral administration (Fig. 6(B)). SCA1 was perorally administered once a day during the experiment, and it enhanced recovery of the WBC count compared with the saline administered control group. The number of WBC was higher than in the CY group from day 7 to 9 and returned to normal level on day 11.

DISCUSSION

We have long been working on the relationship between structure and immunomodulating activity of β-glucans, using G. frondosa, P. vesiculosa, S. sclerotiorum, S. commune, O. lapidescens, G. lucidum, S. cerevisiae, C. albicans, and M. furfur. During this period, we found the activity was significantly dependent on

Table 2. Antitumor Activity of Polysaccharide Fractions of S. crispae

<table>
<thead>
<tr>
<th>Name</th>
<th>Dose (μg/mouse)</th>
<th>CR/total</th>
<th>Mean±S.D.</th>
<th>% inhibition</th>
<th>r-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0/9</td>
<td>16.19±8.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCHWE4v</td>
<td>500</td>
<td>4/10</td>
<td>1.43±2.56</td>
<td>91.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SCHWE1v</td>
<td>500</td>
<td>8/10</td>
<td>0.08±0.25</td>
<td>99.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>6/10</td>
<td>0.91±2.27</td>
<td>94.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>2/10</td>
<td>2.65±4.02</td>
<td>83.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SCA1</td>
<td>500</td>
<td>5/10</td>
<td>1.61±4.57</td>
<td>90.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>2/10</td>
<td>0.70±1.39</td>
<td>95.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>3/10</td>
<td>6.14±6.59</td>
<td>62.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SCAH</td>
<td>500</td>
<td>6/10</td>
<td>0.16±0.25</td>
<td>99.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>10/10</td>
<td>0.00±0.00</td>
<td>100.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>2/10</td>
<td>7.31±8.11</td>
<td>54.9</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

S180 (5×10⁶/mouse) was s.c. administered (on day 0) into right groin of ICR mice (male), 6 weeks old. Each fraction was i.p. administered on days 7, 9, and 11. Whole tumor was collected on day 35 and weighed. Lower doses of SCHWE4v (100, 20) did not show antitumor activity in this experimental schedule.

![Graph A](image1.png)

![Graph B](image2.png)

Fig. 6. Effect of Intraperitoneally (A) or Perorally (B) Administered CA on WBC Count of CY-Induced Leukopenic Mice

Leukopenia was induced by the intraperitoneal administration of cyclophosphamide (200 mg/kg) on day 0. (A) SCA1 (250 μg/mouse in 0.2 ml saline) was intraperitoneally administered simultaneously. (B) SCA1 (50, 100, or 200 μg/mouse in 0.2 ml saline) was perorally administered once a day from day 1 during the experiment. WBC were monitored daily.
the molecular weight, degree of branching, and conformation, in a preclinical animal study. Cancer immunotherapy has been applied for many patients, and enhancement of the quality of life of patients is also an important goal of cancer treatment. Establishment of a molecular mechanism for use in biotherapy is still needed. S. crispa is a mushroom recently cultivable in Japan. Following preliminary investigation it was found that the β-glucan content of S. crispa was 43.6%, as measured by so-called "enzyme method" by the Japan Food Research Laboratories. Thus, we thought it possible that S. crispa might be a good source as a healthy food as well as a candidate for immunomodulating medicine. As shown above, S. crispa was found to be a good source material to prepare the antitumor β-glucan with very high yield. The structure of the main component was 6-branched 1,3-β-glucan, and the ratio of branches was approximately one in every third main chain unit. We previously reported that the optimum dose of GF-1, a hot-water extracted glucan fraction of G. frondosa (fruit body), to show antitumor activity was 2000 μg/mouse×10 times, using an assay protocol of solid form Sarcoma 180 in ICR mice. In contrast, the optimum dose of SCHWE1v was only 20 μg/mouse×3 times. These facts strongly suggested that content as well as extractability of 1,3-β-glucan of S. crispa was significantly higher than G. frondosa.

Primary structure of the antitumor β-glucan from fungi is principally 6-branched 1,3-β-glucan. The degree of branching is varied: grifolan from G. frondosa has one branch in every third main chain unit, SSG from S. sclerotiorum has one in every other main chain unit, OL-2 from O. lapidescens has two in every third main chain unit, and PVG from P. vesiculosa has one in every fifth main chain unit. Zymolysis is a commercially available endo 1,3-β-glucanase. We previously showed that SSG, a highly branched β-glucan, was not digestible by zymolysis. In this study we used zymolysis to elucidate the architecture of the structure; all of the 1,3-β-glucan portion was digested by zymolysis treatment, thus suggesting the absence of a highly branching region in the polysaccharide fractions. This was also supported by the fact that the structure of the macromolecular fractions remaining after zymolysis digestion did not contain 1,3-β-glucans as assessed by carbon-13 NMR spectroscopy, especially absence of the signals around 86 ppm which is assignable as 1,3-β-linked glucose. Structure of the macromolecular fractions was not examined in detail, but the carbon 13-NMR spectra suggested the presence of α- and β-glucans having various linkages. Oligosaccharide fractions of the zymolysis digests were analyzed by gel filtration chromatography (Fig. 4). The pattern of SCCA was very similar to that of SPG, strongly suggesting a similarity in the degree of branching. In contrast, the digest of SCA contained a larger proportion of lower MW fractions (DP2 and DP1), suggesting less branching and consistent with the result of carbon-13 NMR (Fig. 2).

The VDH reaction reported by Maeda et al. was a T-cell-mediated vascular reaction appearing following administration of a neutral polysaccharide possessing antitumor activity, such as lentian. Dominant genes for VDH induction were recently analyzed by the polymerase chain reaction-simple sequence length polymorphism (PCR-SSCP) technique and one major gene (lentinant responsive gene, Ltnr3) and three minor genes were identified by Maeda et al. It was also reported that Ltnr3 was closely linked to a microsatellite marker D6Mit135 on chromosome 6. As shown in Table 3, extracts of S. crispa also showed vasculitis, suggesting an immunomodulating property similar to lentinant. Enhancement of the vascular permeability, especially around tumor tissue, is an important property in establishing and rejecting tumor cells, because of increasing leukocyte traffic to the tissue. VDH reaction was induced not only in the tumor tissue but also in a variety of blood vessels. Although metastasis is one big problem in cancer therapy, it is usually difficult to identify the site of micrometastasis. VDH reaction might help to transport leukocytes to the tumor tissue for micrometastasis even in an unidentified location.

The surface barrier, including mucosal surface, acts as a specialized immune system, a mucosa-associated lymphoreticular tissue (MALT). Leukocytes are also specifically localized and differentiated in MALT. Many of the stimuli as well as invading microbes enter through such a surface barrier, even though the molecular weight of the material is significantly large. Many of the clinically important allergic reactions are mediated by pollen, fungi, and mites, and are infiltrated through such surface barrier. These facts strongly suggest that the surface barrier is not just a physical barrier, but has receptors and/or machinery sensitive to substances on the surrounding surface, and establishes a specific acquired immune response. During the study of immunopotentiating action of fungal β-glucans, we have shown that a highly branched 1,3-β-glucan, SSG, displayed various activities by peroral administrations: i) enhancement of lymphocytes to mitogenic stimuli, ii) activation of NK cell and macrophage, iii) inhibition of tumor growth, iv) cytokine production, v) enhancement of function of Peyer's patch cells, vi) inhibition of metastasis of Lewis lung carcinoma, vii) enhancement of alveolar macrophage function, and so on. Interestingly, we found no detectable concentration of SSG adsorbed from the gut and circulating in the body fluid. SSG thus might activate gut-associated lymphoreticular system directly. Yoshikai and collaborators have shown that peroral administration of a branched 1,6-α-glucan (RBS) potentiates a host defense system by: i) antitumor activity, ii) augmentation of mitogen-induced IL-2 production, and iii) protection from 5-FU induced leukopenia. In this study we have shown that polysaccharide fractions of S. crispa had immunopotentiating action by both systemic and peroral administration. We have shown only a limited number of the activities tested, however, the extract may also activate various functions of MALT by peroral administration.

Acknowledgments The authors wish to thank Mr. M. Uchiyama and Mr. S. Masuzawa for excellent technical assistance.

REFERENCES


