MINIREVIEW

Mushrooms, Tumors, and Immunity (44412)

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Abstract. **Medicinal properties have been attributed to mushrooms for thousands of years. Mushroom extracts are widely sold as nutritional supplements and touted as beneficial for health. Yet, there has not been a critical review attempting to integrate their nutraceutical potential with basic science. Relatively few studies are available on the biologic effects of mushroom consumption, and those have been performed exclusively in murine models. In this paper, we review existing data on the mechanism of whole mushrooms and isolated mushroom compounds, in particular (1→3)-β-D-glucans, and the means by which they modulate the immune system and potentially exert tumor-inhibitory effects. We believe that the antitumor mechanisms of several species of whole mushrooms as well as of polysaccharides isolated from *Lentinus edodes, Schizophyllum commune, Grifola frondosa*, and *Sclerotinia sclerotiorum* are mediated largely by T cells and macrophages. Despite the structural and functional similarities of these glucans, they differ in their effectiveness against specific tumors and in their ability to elicit various cellular responses, particularly cytokine expression and production. Unfortunately, our data base on the involvement of these important mediators is still rather limited, as are studies concerning the molecular mechanisms of the interactions of glucans with their target cells. As long as it remains unclear what receptors are involved in, and what downstream events are triggered by, the binding of these glucans to their target cells, it will be difficult to make further progress in understanding not only their antitumor mechanisms but also their other biological activities.**

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For millennia, mushrooms have been valued as flavorful foods and as medicinal substances. They are widely sold as nutritional supplements and touted as beneficial for health. Yet, there has not been a critical review attempting to integrate their nutraceutical potential with basic science. The oldest written record of mushrooms as medicinals is an Indian medical treatise from 3000 BC (1). The Chinese "Shen Nong Ben Cao Jing," a compendium of materia medica compiled around 200 BC to 200 AD, includes the beneficial effects of several mushrooms (2); and mushrooms such as *Poria cocos* (Fr.) Wolf and *Polyporus umbellatus* Fries that are still used extensively in Chinese herbal medicine (3). Mushrooms are claimed to exhibit antiviral, antibiotic, anti-inflammatory, hypoglycemic, hypocholesterolemic, and hypotensive activities (4–6). The most thoroughly researched medicinal effect of mushrooms, however, is their antitumor activity in mice as well as in humans, especially in the Shiitake mushroom (*Lentinus edodes*), the Maitake mushroom (*Grifola frondosa*), *Sclerotinia sclerotiorum*, and *Schizophyllum commune*. Classification of these mushrooms is provided in Table I.

A number of bioactive molecules, including antitumor substances, have been identified in numerous mushroom species (7). Polysaccharides have been established to be the most potent antitumor-active compounds. Although many mushrooms contain several polysaccharides that inhibit tumor growth, most are branched (1→3)-β-D-glucans. Details on the physicochemical properties of the glucans, lentinan from *Lentinus edodes*, grifolan (GRN) from *Grifola frondosa*, schizophyllan (SPG) from *Schizophyllum commune*, and SSG from *Sclerotinia sclerotiorum* are provided in Table II. In this review, we focus on studies providing insight into the cellular mechanisms by which polysaccharides modulate the immune system and potentially exert antitumor effects.

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Table I. Classification of the Mushrooms Included in this Review

Common name	Latin name	Family	Class
Shiitake	*Lentinus edodes*	Tricholomataceae	Basidiomycetes
White mushroom	*Agaricus bisporus*	Agaricaceae	Basidiomycetes
Button mushroom	*Schizophyllum commune*	Tricholomataceae	Basidiomycetes
	Sclerotinia sclerotiorum	Pezizaceae	Ascomycetes
Maitake	*Grifola frondosa* (formerly: *Polyporus frondosa*)	Polyporaceae	Basidiomycetes

Table II. Physicochemical Characteristics of Lentinan, Grifolan, SSG, and Schizophyllan

Mushroom/ part used	Isolated compound	Glycosidic linkage back bone	Side chain	Molecular weight[a]	Degree of branching	Higher structure	Ref
Grifola frondosa/ liquid-cultured mycelium	Grifolan (Grifolan LE)	β-(1 → 3)-Glc	β-(1 → 6)-Glc	5×10^6	1/3	Triple helix	(35)
Lentinus edodes/ fruiting body	Lentinan	β-(1 → 3)-Glc	β-(1 → 6)-Glc	$4–8 \times 10^5$	2/5	Triple helix	(109) (81)
Schizophyllum commune/culture medium	Schizophyllan (SPG, Sonifilan, Sizofiran, Sizofilan)	β-(1 → 3)-Glc	β-(1 → 6)-Glc	4.5×10^5	1/3	Triple helix	(110) (111)
Sclerotinia sclerotiorum/ culture filtrate	SSG	β-(1 → 3)-Glc	β-(1 → 6)-Glc		1/2	Triple helix	(112) (113)

[a] The MW of glucans varies with the method used to determine it.
Glucan preparations are never homogeneous in terms of MW but consist of molecules with a wide range of MWs.

General Information on Mushrooms

The word "mushroom" is not a botanically acceptable term but is most commonly defined as the fruiting body of a macrofungus (1, 8, 9). "Fruiting body" (basidiocarp) refers to the spore-producing organ of a fungus, which is formed by the mycelium and consists of stipe (stem or stalk) crowned by the pileus (cap). "Macrofungus" describes a fungus large enough to be seen with the naked eye. Mushrooms belong to only two subdivisions of fungi; the vast majority are basidiomycetes, and a few are ascomycetes. Estimates as to the number of worldwide mushroom species are as high as 10,000 (9) with ≈ 700 edible, 50–200 medicinal, and 50 poisonous species (10). Although many edible mushrooms have alleged medicinal properties, not all medicinal mushrooms are considered edible (e.g., *Ganoderma* and *Coriolus*).

World production of edible cultivated mushrooms was 1.488 million metric tons in 1983/84 (8), 2.176 million metric tons in 1986, and 4.264 million metric tons in 1991 (9). The 1991 crop represented a monetary value of $U.S. 8.5 billion (11), and mushroom products (such as extracts and powders derived from mushrooms) generated $U.S. 1.2 billion. Only six genera of mushrooms account for over 90% of the total world production, namely: *Agaricus*, *Lentinula*, *Pleurotus*, *Auricularia*, *Volvariella*, and *Flammulina* (9). Of these, the white or button mushroom (*Agaricus bisporus*) is available in any American supermarket year-round, and Shiitake (*Lentinus edodes*) and Oyster mushrooms (*Pleurotus* spp.) are now also offered in many stores. Specialty mushrooms, such as Straw mushrooms (*Volvariella volvacea*), Maitake (*Grifola frondosa*), Winter mushrooms (*Flammulina velutipes*), and Woody Ear (*Auricularia* spp.), enjoy increasing popularity. In addition, many American health food stores carry mushroom dietary supplements (e.g., lyophilized whole *Lentinus edodes*, *Grifola frondosa*, and Reishi (*Ganoderma lucidum*)).

Chemical Composition of Mushrooms. Mushrooms are ≈ 90% water by weight. The remaining 10% consists of 10%–40% protein, 2%–8% fat, 3%–28% carbohydrate, 3%–32% fiber, and 8%–10% ash, with potassium, calcium, phosphorus, magnesium, iron, zinc, and copper accounting for most of the mineral content (5). Most mushrooms contain some vitamins, particularly niacin, thiamin, riboflavin, biotin, and vitamin C. Pro-vitamins A and D have also been detected. Besides actual nutrients, mushrooms contain a wide variety of bioactive molecules including terpenoids, steroids, phenols, nucleotides and their derivatives, glycoproteins, and polysaccharides (6, 7, 9, 12).

Antitumor Effects of Mushrooms. Shiitake has antitumor activity in several experimental murine models. For example, powdered fruit bodies of shiitake (*Lentinus edodes*) were administered orally in the diet (20%) of CDF_1 mice who had received syngeneic IMC carcinoma cells and

C3H/He mice bearing MM-46 carcinoma (13). Shiitake was effective in inhibiting MM-46 carcinoma growth in C3H mice (79% tumor inhibition) but only marginally effective (21%) in inhibiting IMC carcinoma growth in CDF_1 mice. Shiitake intake had a strain-specific effect on macrophage spreading and phagocytosis of latex particles, in both tumor-bearing and nontumor bearing mice (13, 14). For example, shiitake feeding resulted in a decreased spreading rate of macrophages in CDF_1, but an increased spreading rate in C3H mice. Both the spreading rate and phagocytosis of macrophages from tumor-bearing animals of either strain were significantly depressed compared to nontumor-bearing animals. Shiitake feeding improved both parameters in both mouse strains. Only MM-46-bearing C3H mice were restored to normal or even beyond. The use of fluorescent *Listeria* instead of latex particles in the phagocytosis assay in the same two tumor models provided similar results (14). The cytotoxic activity of natural killer (NK) cells and/or lymphokine-activated killer (LAK) cells was established by incubating a spleen lymphocyte suspension from either normal or MM-46 carcinoma-bearing mice with labeled YAC-1 or P-815 tumor cells. Cytotoxic activity was significantly increased in mice on shiitake feed compared to mice given normal feed. Feeding shiitake powder as 20% of the diet to P-815-bearing C3H mice also significantly increased the specific alloreactive cytotoxicity in spleen cells. Pretreatment of the cells with anti-Thy1.2 monoclonal antibodies (mAb) and complement reduced the cytotoxic activity by approximately half in both shiitake-fed mice and mice on normal feed. These data suggest that cytotoxic T cells also participate in the tumor-inhibiting activity of shiitake-feeding. A commendable feature of the preceding studies is that they investigate and compare the response to mushroom feeding in both normal and tumor-bearing mice.

Similar tests have been performed at lower dietary levels (5%) of dried and powdered Shiitake (*Lentinus edodes*), Maitake (*Grifola frondosa*), or Oyster mushroom (*Pleurotus ostreatus*). All decreased the incidence of urinary bladder carcinoma in female 6-week-old ICR mice treated with N-butyl-N"-butanolnitrosoamine (BBN) (15). The chemotactic activity of macrophages and the mitogenic response of lymphocytes to concanavalin A (Con A) were severely suppressed by BBN-treatment. Each of the three mushroom-enriched diets restored these activities to almost normal levels. The cytotoxic activity of LAK and NK cells, also depressed in tumor-bearing mice, was augmented significantly beyond even the levels seen in nontumor-bearing animals not given mushrooms.

The effects of the mushroom with the highest worldwide consumption, the white or button mushroom (*Agaricus bisporus*), have also been studied in mice. The data are contradictory. Toth *et al.* reported that feeding mice with fresh uncooked *Agaricus* resulted in a significantly higher incidence of various tumors compared to controls (16). In this study, mushrooms constituted the only food for 3 days, followed by 4 days during which rodent chow was available. Such a feeding regimen resulted in dramatic weight loss during the days where mushrooms constituted the only available food and equally dramatic weight gain during the days when rodent chow was available (17). However, no increase in mutation frequency was detected with the *lac*I transgenic mouse mutation assay in mice fed either fresh or dried mushrooms for 105 days (17), suggesting that the feeding regimen rather than the consumption of mushrooms contributed to the increased tumor incidence observed by Toth *et al.*

There may be other dietary and procedural factors that contributed to these results. Toth *et al.* when using a different feeding regimen, in which mice had access to rodent chow for 12 hr and to mushrooms only for the remaining 12 hr, found no statistically significant increase in the cancer incidence in Swiss albino mice (18). Unfortunately, it was not specified during which 12 hr (dark or light cycle) the mushrooms were the only food provided. Since mice are night feeders, this would make a difference in the overall mushroom consumption. However, the same researchers recently reported that Swiss mice fed lyophilized *A. bisporus* at 10, 5, and 2.5% of a semisynthetic diet throughout their life had a significantly higher incidence of certain tumors than control mice (19). There was a total absence of a dose-response relationship. This group of researchers is the only one to report an increase in tumor incidence after *A. bisporus* feeding (20). Indeed, in another experiment, diets containing 10, 20, or 30% lyophilized *A. bisporus* dose-dependently inhibited the growth of syngeneic MM-46 and IMC carcinomas in 5–6-week-old male C3H and CDF_1 mice, respectively (21). The peritoneal macrophages from normal C3H mice fed a diet containing 20% *A. bisporus* exhibited significantly increased phagocytic activity when incubated with ^{3}H-uridine-labeled MM-46 carcinoma cells compared to macrophages from mice on normal feed. The phagocytic activity of tumor-bearing mice was higher than that of nontumor bearing animals and was further increased by the *A. bisporus* diet. The cytolytic activity of macrophages from tumor-bearing mice was depressed compared to those of normal mice, but the *A. bisporus* diet increased it to levels higher than in normal mice on normal feed and comparable to the levels seen in normal mice fed *A. bisporus*.

Antitumor Effects of Isolated Mushroom Constituents. In the late 1960s, Japanese researchers began to investigate systematically whether mushrooms, both wild and cultured, contained substances able to inhibit tumor growth. Since then, a wide variety of mushroom species have been investigated for their antitumor activities (2, 22–25). Although some of the existing literature on antitumor polysaccharides from *Lentinus edodes* and *Grifola frondosa* has been reviewed, only two of these reviews contain references that are less than 10 years old, and only a handful of

those concern the antitumor activities of lentinan and grifolan (26, 27).

For all antitumor compounds from mushrooms, including lentinan, GRN, SPG, and SSG, the initial publications are reports on the fractionation and isolation of these substances and on their ability to inhibit the growth of tumors, most commonly sarcoma 180. These studies do not address the mechanisms by which these compounds exert their antitumor effect; their findings are summarized in Table III.

The Effects of Lentinan, GRN, SPG, and SSG on Different Cell Types

Mouse Macrophages *in vitro*. *In vitro* application of lentinan augmented the cytotoxic response of peritoneal macrophages from eight different mouse strains in a dose-dependent manner (28). The magnitude of the response varied considerably between the strains. Lentinan also induced tumor necrosis factor-α (TNF-α) production by macrophages *in vitro*. Phagocytosis by mouse macrophages was increased by the addition of as little as 0.1 μg/ml lentinan to the culture medium (29). More specifically, lentinan increased the number of fluorescence-labeled microbeads ingested by each cell, either resident or thioglycollate-elicited mouse (A/Ph strain) macrophages, although it did not change the percentage of cells engaged in phagocytosis. Microbeads coated with a combination of C3b and lentinan were ingested more avidly than microbeads coated with C3b only. Preincubation of cells with lentinan also increased their ability to take up C3b-coated microbeads. Mannan had no effect on the lentinan-induced phagocytosis of microbeads but decreased the uptake of C3b-coated beads dose-dependently. Taken together, these results suggest that the stimulation of phagocytosis with lentinan was mediated by both the C3b receptor and a distinct β-glucan receptor.

Lentinan concentrations as low as 0.1 μg/ml increased the *in vitro* pinocytosis of horseradish peroxidase (HRP) and FITC-dextran by resident peritoneal macrophages from female Balb/c mice, but were unable to further increase the high pinocytic activity of thioglycollate-elicited macrophages (30). The HRP-ingestion was inhibited by the addition of mannan to the culture medium. Lentinan was able to counteract this inhibitory effect partially. Interestingly, lentinan opsonized with mouse serum inhibited the uptake of HRP and augmented the inhibition exerted by mannan. These results suggested that the lentinan-induced stimulation of pinocytosis was due to a receptor with specificity for β-glucans.

When applied *in vitro* to human monocytes, lentinan augmented the production of interleukin(IL)-1 as measured by the ability of supernatants to stimulate the proliferation of C3H/HeJ mouse thymocytes (31). A lentinan concentration of 0.01 μg/ml was much more effective than higher concentrations and increased IL-1 production after as little as 5 hr of incubation.

A glucan isolated from *Grifola frondosa* and named grifolan LE (GRN LE) increased the glucose consumption and the activity of a lysosomal enzyme, β-D-glucuronidase, in macrophages from ICR mice *in vitro* (32). It also induced the production of IL-1 by resident macrophages harvested from C3H/HeJ mice. The nomenclature used by these researchers in subsequent publications is extremely confusing. After careful comparison of extraction protocols (32–35), it appears that, though the name "grifolan LE" was changed to grifolan (GRN), it refers to the same purified glucan. GRN was applied at either 100 or 500 μg/ml to proteospeptone-elicited mouse (C3H/HeJ) macrophages (33). Both dosages, but 500 μg more than 100 μg, were effective in stimulating the production of IL-1, TNF-α, and IL-6. IL-6 mRNA, detected by RT-PCR, was also induced by GRN. In the same study, SSG from the culture filtrate of *Sclerotinia sclerotiorum* IFO 9395 did not induce significant TNF-α production. SPG from *Schizophyllum commune* was also ineffective in stimulating the production of IL-6 and TNF-α *in vitro*.

In another study, GRN induced nitrogen oxide (NO) synthesis from peritoneal macrophages *in vitro* (36). The combination of interferon-γ (IFN-γ) and GRN stimulated a highly significant increase in NO production compared to either IFN-γ alone or GRN alone. Similar results were reported when SSG from *Sclerotinia sclerotiorum* was added to the culture medium of peritoneal mouse macrophages (37). In this assay system, NO was not detected after incubation with either SSG or IFN-γ alone, but in combination they induced very high levels of NO. In human monocytes, incubation for 48 hr with as little as 0.1 μl/ml SPG from *Schizophyllum commune* increased acid phosphatase activity although the semiquantitative nature of the assay did not allow to establish statistical significance (38). The cytostatic and cytotoxic activities of human monocytes/macrophages was also augmented by SPG *in vitro*.

Mouse Macrophages *in vivo*. Lentinan significantly increased macrophage cytotoxicity *in vivo* when injected subcutaneously (sc) or intraperitoneally (ip), whereas the intravenous (iv), intradermal and intramuscular (im) routes of administration were slightly less effective (39, 40). Unfortunately, in one of these studies it remained unspecified what treatment, if any, the controls received (39). Lentinan-treated animals also had a higher number of total peritoneal cells and higher percentage of adherent and spreading cells (activated macrophages) than either untreated or saline-injected controls (40). Pretreatment of CBA mice with lentinan at an optimal dose of 2.5 mg/kg significantly enhanced antibody-dependent macrophage-mediated cytotoxicity with IgG1, IgG2a, or IgG3 mAb; no specific lysis was observed with IgG2b, IgA, or IgM (41).

In these studies, the effect of lentinan on macrophages was tested in the absence of tumors. Since lentinan is known to have antitumor effects in many different experimental models, the more interesting question to address is whether lentinan alters macrophage activity in tumor-bearing mice. Thus far, that has only been demonstrated after feeding with

Table III. Antitumor Activity of Lentinan, Schizophyllan, Grifolan, and SSG in Different Tumor/Mouse Models

Lentinus edodes: Lentinan mouse strain, age, sex	Tumor	Treatment[a]	Tumor inhibition (%)	Ref
Swiss albino 20 g, no sex	Sarcoma 180 solid tumor	0.2, 1, 5, 25 mg/kg × 10 ip	78.1, 95.1, 97.4, 73.0	(114)
A/Ph, A/Ph, A.BY A.CA, A.SW, DBA/2, Balb/c, C3H/Di, AKR, B10, B10.A, B10.BR, B10.D2, Swiss albino. All adult females	A/Ph.MC.S1 sarcoma Sarcoma 180 solid tumor	1 mg/kg × 10 ip 1 mg/kg × 10 ip	100 12–100 depending on strain	(75)
(A/Ph × B10)F1 8–12 wks, male & female	A/Ph.MC.S1 sarcoma	500 µg/mouse × 1 ip	Strong inhibition (not given in %)	(115)
DBA/2 DBA/2 C3H/He	P815 L5178Y MM46	5 mg/kg × 2, 4, or 6 iv 10 mg/kg × 1, 2, or 3 iv 1, 5, 10 mg/kg × 2	up to 89% up to 89% up to 100% depending on dose and timing	(116)
DBA/2N DBA/2N Balb/c DBA/2N, Balb/cAnN C57BL/6N, C3H/HeN, CBA/JN, A/J, outbred CD-1 ICR, and variousF1 hybrids	DBA/2.MC.CS1 fibrosarcoma 3-methylcholanthrene-induced tumor Sarcoma 180 solid tumor	1, 5, 10 mg/kg × 10 ip 1 mg/kg × 10 1 mg/kg × 10	54, 57.4, 47.3 80.5 no inhibition 36.2–100 depending on the mouse strain or hybrid	(76)
BDF1 = (C57BL/6 × DBA/2)F1 6–10 wks, female	Friends virus-induced FBL-3 erythroleukemia	100 µg/mouse × 5 ip		(51)
Schizophyllum commune: Schizophyllan				
dd albino mice ~20 g, male	Sarcoma 180 solid tumor Sarcoma 37 solid tumor Ehrlich carcinoma solid Sarcoma 180 ascites Sarcoma 37 ascites Ehrlich carcinoma ascites	0.5, 1 5, 10, 50 mg/kg ip 0.625, 1.25, 2.5, 5 mg/kg ip 1, 5 mg/kg ip 5, 10 mg/kg × 10 ip not reported not reported	82, 81, 89, 100, 65 71, 90, 87, 94 96, 98 increased survival[b] no effect no effect	(117)
Donryu rats ~130–140 g, male	Yoshida sarcoma solid	0.7, 0.14, 0.035 mg/kg ip	–15, 36, 74	
Swiss mouse no age, no sex	Yoshida sarcoma ascites spontaneous mammary carcinoma, implanted	dosages not reported 10, 50 mg/kg	no effect no effect	
ICR male; female ddY male; female SWM male; female C57BL/6 male; female C3H/He male; female Balb/c male; female DBA/2 male/female all 6–8 wks	Sarcoma 180 solid tumor	1 mg/kg × 10 on alternate days im	92.5; 97.9 99.4; 93.7 94.3; 89.5 96.2; 89.6 37.1; 48.3 36.1; 24.5 2.6; 14.7	(43)
C3H/He 6–8 wks, male	MM46 mammary carcinoma	0.5, 2, 5, 10 mg/kg × 10 im on alternate days d 1–29 0.5, 2, 5, 10 mg/kg × 10 im on alternate days d 10–28	14.3, 15.6, 49.8, 34.8 0, 34.7, 51.8, 48.2	
C57BL/6	Adenocarcinoma 755	1, 5 mg/kg × 10 im	sign. increase in survival time	
Grifola frondosa: GRM				
ICR no age, no sex	Sarcoma 180 solid	4, 20, 100 200 µg/kg × 5 ip	>38% (20 µg) >99% (100 & 200 µg)	(35)
ICR 2–3 mo, male	Sarcoma 180 solid	4, 7.5, 20, 100 µg/kg × 5 ip	93% (7.5 & 20 µg) >99 (100 µg)	(118)
CDF_1 CDF_1 DBA/2 Balb/c CDF_1 6–8 wks, no sex	P388 leukemia L1210 leukemia L1210 leukemia Meth-A fibrosarcoma IMC carcinoma	75, 150 µg × 5 ip 100 µg × 10 ip 75, 150 µg × 10 ip 75, 150 µg × 5 ip 75, 150 µg × 5 ip	no inhibition no inhibition no inhibition 79.4 & 64.5% 74.5 & 66.9%	(119)
Sclerotinia sclerotiorum: SSG				
BALB/c CDF_1 DBA/2	Meth-A fibrosarcoma IMC carcinoma L1210 Leukemia 6–8 wks, male	100, 250 µg/mouse × 5 (d 1, 3, 5, 7, 9) ip or it, respectively	81, 87% 92, 90% no effect	(120)

[a] Unless otherwise stated, treatment was given once daily starting the day after tumor inoculation.
[b] The significance of the prolonged survival time was not reported.

lyophilized shiitake mushroom, the mushroom from which lentinan is isolated.

The importance of macrophages in the antitumor mechanisms of SPG was examined by blocking macrophage activity *in vivo* with either carrageenan or trypan blue in female 5-week-old JCL/ICR mice inoculated with Sarcoma 180 (42). While trypan blue reduced the tumor growth inhibition seen with SPG from 94% to 68%, carrageenan completely abrogated the antitumor activity of SPG, indicating that macrophages were a crucial cell population for the antitumor effect of SPG. Similar results were obtained by Matsuo *et al.* using trypan blue to inactivate macrophages in 6–8-week-old ICR mice bearing sarcoma 180 tumors and treated with SPG (43). Macrophage spreading and β-glucuronidase activity were also augmented by SPG treatment of normal ICR mice.

Similarly, carrageenan very significantly reduced the tumor-inhibiting effects of lower doses of GRN (20 or 100 μg administered ip on Days 1, 3, 5, 7, and 9 after sarcoma 180 inoculation), but did not affect the GRN-induced tumor inhibition when GRN was given at a dose of 500 μg/mouse (44). GRN administered ip or iv into ICR mice did not increase serum TNF-α levels, as determined with an ELISA (45). However, GRN administered ip 24 hr before an iv LPS injection significantly enhanced the LPS-induced TNF-α production compared to treatment with LPS only. GRN given ip at a dose of 250 μg/mouse (male ICR, 6 weeks old) on Days –13, –8, –5, –3, –2, or –1 before the assay induced significant increases in the NO production by peritoneal macrophages (36). A single injection of various doses of GRN, ranging from 50 μg–2500 μg, increased NO production of ICR peritoneal macrophages dose-dependently, except for a marked decrease at 500 μg compared to both 250 and 1000 μg. GRN was also effective, though to differing degrees, in increasing the NO synthesis of peritoneal macrophages from several other mouse strains, namely AKR, C57BL/6, BALB/c, BALB/c nu/nu (suggesting that NO induction was T-cell independent), C3H/HeN, and C3H/HeJ mice. C3H/HeJ mice have functionally deficient macrophages that are unresponsive to LPS, so that their responsiveness to GRN suggested that GRN induced NO production by a different mechanism than LPS.

GRN administered ip at a dose of 1 mg to male 6–8-week-old ICR mice increased H_2O_2 release of polymorphonuclear cells (PMN), but did not stimulate H_2O_2 production of peritoneal cells or PMN incubated with GRN *in vitro* (46). Kupffer cells from 6–10-week-old male ICR mice that had been given an iv injection of 2.5 mg/ml GRN produced significantly higher levels of NO 4 days and particularly 7 days later compared to saline-injected animals (47). Interestingly, the cell surface expression of CD11b, the α-chain of the iC3b receptor, thought to mediate some of the effects of β-glucans, was increased 7 days after GRN-administration. The cytostatic activity against lymphoma EL-4 cells of Kupffer cells from GRN-treated animals was also significantly greater than that of saline-injected mice (47).

Treatment of CDF_1 mice (4–8 weeks old) by iv injection of 10 mg/kg SSG from *Sclerotinia sclerotiorum* gave almost identical results in alveolar macrophages as those reported for peritoneal macrophages following GRN treatment (37). That is, while *in vitro* SSG was unable to induce NO production from alveolar macrophage unless IFN-γ was also present in the culture medium, SSG *in vivo* significantly increased not only the synthesis of NO by alveolar macrophages but also the production of IFN-γ mRNA in the lung. Other *in vivo* effects on macrophages included significant augmentation of spreading ability, phagocytic activity, acid phosphatase activity, H_2O_2 and IL-1 production, candidacidal activity, and cytolytic activity against Lewis lung carcinoma cells.

Human Monocytes/Macrophages *in vivo*. Thirty-three gastric cancer patients, in various stages of the disease, participated in a study on the effects of lentinan at least 6 months after they had undergone gastrectomy (48). Although the most commonly used dosage regime for lentinan is 2 mg once a week, these patients received 2 mg of lentinan iv either at 2- or 4-week intervals. An increase of more than 50% above baseline in IL-1β production by their macrophages was observed in about 70% of the patients. It took longer for this increase to achieve significance in those receiving lentinan only every 4 weeks than in patients treated with lentinan every 2 weeks. The levels of C3b receptor (CR1) and Fcγ receptor were not changed by treatment with lentinan.

T Cells, Natural Killer (NK) Cells, Lymphokine-Activated Killer (LAK) Cells. Early studies examining the cellular mechanism(s) by which lentinan exerts its antitumor effects *in vitro* and *in vivo* established that T cells play an important role (reviewed in Ref. 49). More recent investigations have attempted to identify the effector cells in different tumor/mouse models.

The effect of lentinan on tumor immunity in different strains of congeneic B10 mice with either low or high resistance against Rous sarcoma virus (RSV)-induced tumors was investigated (50). The mice were immunized with syngeneic mitomycin C-treated RSV tumor and were given ip injections of 1 mg/kg lentinan or vehicle for 5 successive days. When subsequently challenged with RSV tumor cells, the percentage of tumor-free high-responder mice was not affected by lentinan treatment. However, in one of the low-responder strains, lentinan significantly increased the number of tumor-free mice. In low-responder mice, lentinan treatment increased both the specific cytotoxicity of their spleen cells against RSV-induced tumors and the nonspecific (i.e., NK or LAK cell-dependent) cytotoxicity against three other types of target cells. In high-responder mice, only the specific, but not the nonspecific, cytotoxicity was slightly increased by lentinan treatment. Pretreatment of the cells with anti-Thy-1.2 (anti-T cell) mAb did not affect the cytotoxic activity in low-responder mice, but abolished it in

high-responder mice, indicating that the effector cells were T cells in the high responders but were non-T cells in the low responders. Passage through a G-10 column removed the cytotoxic activity from the spleen cells of low-responder mice, suggesting that the effector cells were macrophages.

In seven of eight female 6–10-week-old BDF_1 (C57BL/6 × DBA/2 F_1) mice that were inoculated with FBL-3 erythroleukemia cells, 5 mg/kg lentinan administered ip on Days 7–11 resulted in complete tumor regression (51). However, when mAb against both CD4 and CD8 were given ip before the lentinan treatment, the tumor growth inhibition was abrogated. Anti-CD4 or anti-CD8 mAb alone had no effect. Antibody against NK cells (anti NK1.1) slightly reduced the effectiveness of lentinan in inhibiting tumor growth. However, treating mice with anti-CD4 or anti-CD8 had no effect on the cytolytic activity of their spleen cells against ^{51}Cr-labeled FBL-3 cells. In contrast, anti-NK1.1 treatment completely abolished cytolysis. Together, these results indicated that NK activity did not play an important role in this particular mouse/tumor system. The delayed type hypersensitivity (DTH) response against FBL-3, by contrast, was significantly increased in lentinan-treated FBL-3-bearing mice and was abolished by the combination of anti-CD4 and anti-CD8, but not by either one alone and not by anti-NK1.1. Furthermore, the tumor-specific DTH response could be reconstituted in nude mice by a tumor-specific $CD4^+$ T cell clone, and lentinan further increased this DTH response. This combination, however, was only partially effective in inhibiting tumor growth in nude mice. Several other *in vivo* experiments showed similar results (52, 53).

In neonatally thymectomized ICR mice, SPG given intramuscularly did not have a significant effect against the growth of sarcoma 180 solid tumors whereas in nonthymectomized mice, SPG-treatment resulted in ≈ 95% tumor inhibition (43). The same study found that antithymus globulin was equally effective in reducing the tumor inhibition by SPG. Furthermore, intramuscular administration of SPG to B6 mice significantly enhanced their generation of cytotoxic T lymphocytes *in vivo* and augmented the proliferative response of spleen and peripheral lymphocytes to phytohemagglutinin (PHA) and Con A, with maximal stimulation observed 7 days after SPG-administration. The NK activity of spleen cells from B6 mice was significantly greater than that of untreated controls 2 days after intraperitoneal treatment with SPG, but not on Days 5 and 10. Adoptive transfer experiments further established the importance of T cells in the antitumor mechanism of SPG (42).

***In vivo* Response of Human T, NK, and LAK Cells.** Clinical studies, though none that are placebo-controlled and double-blind, with lentinan and SPG have been conducted in Japan, where both are now approved for clinical use. Lentinan was effective in prolonging the survival of cancer patients, particularly those with gastric and colorectal cancer, (54–57). In contrast to the data on lentinan, SPG was rather ineffective in patients with gastric cancer (stages I-IV) (58). It did, however, prolong the survival time and time to recurrence in stage II cervical cancer patients but not those in stage III (59, 60). It also extended the survival time of patients with head and neck cancer (61).

In seven of ten patients with various cancers, most of whom also received chemotherapy, iv injections of 1 mg of lentinan twice a week significantly increased the NK activity in their peripheral blood mononuclear cells (PBMC) (62). PBMC obtained from nine patients with gastric carcinoma before and 3, 5, and 7 days after they received a single iv dose of 2 mg of lentinan, were incubated with IL-2 to assess their ability to develop LAK activity against the NK-resistant Raji cell line (63). On Day 5 after lentinan administration, LAK activity was significantly increased compared to that observed before the treatment. NK activity (measured in 11 patients), by contrast, was significantly increased only 7 days after the lentinan injection. Changes in subsets of cell populations did not reach significance due to very high variations between the subjects. It is regrettable that the number of participants in this study was so small and that more appropriate methods of statistical analysis were not chosen. In a previous study, the same authors reported that lentinan induced the production of IL-1 and TNF-α by human monocytes/macrophages 3 days after lentinan treatment (64). They hypothesized that these cytokines augmented the ability of T cells to respond to and/or produce IL-2 and that this ability might be related to the enhanced NK and LAK activity observed in this study.

Lentinan, in combination with one of several chemotherapeutic agents, significantly prolonged the survival time in some patients with advanced cancers of various types (65, 66). Those patients who responded to lentinan treatment had a significantly greater increase (about 2.5-fold) in their killer T cell/suppressor T cell ratio ($CD11^-$ $CD8^+$/$CD11^+$ $CD8^+$) in peripheral blood than those patients in whom lentinan was ineffective. The ratio of NK cells with high activity to NK with moderate activity ($CD57^-$ $CD16^+$/$CD57^+$ $CD16^+$) was higher in the responders than in the nonresponders and correlated well with survival time. There are some indications, however, that lymphocyte subset changes in peripheral blood did not necessarily correlate with the lymphocyte subset changes actually occurring in the tumor (67).

In a prospective randomized clinical trial involving 312 patients with cervical carcinomas (FIGO stages IB-IV) treated with surgery, radiotherapy, FU (fluorouracil), and SPG (called sizofiran in this study) in various combinations, 159 patients given im injections of SPG (20–40 mg per week for up to 2 years) survived significantly longer than the 153 patients who did not receive SPG (68). Despite the large sample size, the fact that so many different treatment protocols were used in addition to SPG-injections substantially reduces the meaningfulness of the results obtained in this study. Increases in the percentage of activated $CD8^+$ cells ($HLA\text{-}DR^+$) out of the total $CD8^+$ subset were significantly correlated with disease progression and further aug-

mented by radiotherapy, but decreased by SPG treatment. Separate analysis of patients with 10% or more activated $CD4^+$ cells out of their total $CD4^+$ population and with more than 25% activated $CD8^+$ cells before the beginning of treatment, showed that in this group the SPG-induced increase in survival time was highly significant. By contrast, in patients with less than 10% $CD4^+$ and less than 25% $CD8^+$ cells at the beginning of therapy, SPG treatment resulted in no significant difference in survival time compared to controls. In similar studies, SPG restored $CD4^+$ cell levels more quickly after primary treatment for head and neck cancer (61) and, when given intratumorally (it), significantly increased the infiltration of Langerhans and T cells into cervical tumors (69).

Other Immune Responses Induced by Mushroom Polysaccharides

Acute Phase Proteins (APP). Female ICR mice (20–25 g) received 4 mg/kg lentinan daily for 5 days by ip injection. When an aliquot of their sera was subjected to polyacrylamide gel electrophoresis (PAGE), a marked increase in three serum components was noted (70). These proteins were a haptoglobin-hemoglobin complex, hemopexin, and haptoglobin (71). The same three serum components increased after lentinan treatment were also induced by the administration of 10 mg/kg SPG to ICR mice (43); GRN increased the APP, ceruloplasmin (72).

Vascular Dilatation and Hemorrhage. Vascular dilatation and hemorrhage (VDH, now called hemorrhagic vasculitis) was induced dose-dependently by intraperitoneal injections of lentinan in normal CD-1 mice, but not in carrageenan-treated mice and also not in CD-1 nu/nu mice. The data suggest that this response is mediated by T cells and macrophages (73). Interestingly, bradykinin-induced skin reactions were increased by lentinan treatment when tested in A/J mice, who are sensitive to the induction of the VDH response to lentinan (74). When other strains were tested, it was found that the same strains that showed a VDH response after lentinan treatment also showed high responses to bradykinin. Mice treated with a combination of anti-CD4 and anti-CD8 mAb before receiving lentinan treatment did not show a VDH response, confirming that it was T-cell dependent. Anti-CD4 mAb or anti-CD8 mAb alone, however, had no effect. The bradykinin-induced skin reaction was examined in B10D2 mice bearing S908D2 sarcoma treated with lentinan and FU either alone or in combination. Either of the agents alone had no effect on tumor growth. Their combination resulted in complete regression when given for 10 consecutive days starting 24 hr after, but not 5 weeks after, tumor transplantation. The effective combination treatment augmented the skin reaction whereas none of the other treatments did. These findings suggest that a good correlation exists between the vascular responses and the antitumor activities induced by lentinan.

Responder Analysis to Lentinan

Strain Differences, Genetic Factors, Other Possible Explanations. Three of the studies listed in Table III indicate that striking differences in the response to lentinan and SPG exist between mouse strains (43, 75, 76). Mouse macrophage cytotoxicity *in vitro* (28) and *in vivo* (39), NO synthesis (36) as well as the upregulation of specific (T-cell dependent) or nonspecific cytotoxic (NK cell dependent) responses (50) varied dramatically between mouse strains. Human cancer patients, obviously genetically more diverse than inbred mice, are likely to also show differences in their responses to various biological response modifiers, including mushroom compounds (cf. Refs. 65, 66, 68).

Maeda *et al.* investigated whether the responsiveness of mice to two of the biological activities induced by lentinan, the APP and VDH responses, were under genetic control (77). They divided 20 inbred mouse strains into sensitive and resistant strains according to the levels of APP they produced when treated with lentinan. F_1 hybrids generated from two sensitive parent strains were sensitive; offspring from two resistant parent strains were resistant; and the progeny of all crosses between a sensitive and a resistant parent were resistant, indicating that the resistant phenotype is dominant. Analysis of backcrosses suggested that responsiveness to lentinan was under the control of a single major gene, which they designated as *ltn*-1. Similar experiments were conducted for the VDH response, which was found to be controlled also by a single major and dominant gene, named *ltn*-2. However, the strain distribution for the APP response to lentinan was different from that for the VDH response. Although the data were not shown, the responsiveness to lentinan in terms of APP production was reported to be distributed to different strains than sensitivity to induction of APP by LPS.

IL-6 plays a major role in the induction of APPs. Maeda and colleagues also examined the levels of IL-6 mRNA in the spleens of SWR/J mice, a strain sensitive to APP-induction by lentinan, and in MA/MyJ and AKR/J mice, both resistant strains (78). While two peaks in IL-6 mRNA induction, one at 4 hr and one at Day 7 after an ip injection of 10 mg/kg of lentinan, were observed in the high APP-responders, only one peak at 4 hr occurred in nonresponders. This agreed well with their findings that the highest levels of several APPs were found on Day 7 and suggested that *ltn*-1 participated in IL-6 mRNA expression (78).

In further studies on the genetic control of APP responses induced by lentinan, Maeda and co-workers used PCR-simple sequence length polymorphism, followed by linkage analysis between each microsatellite locus and the *ltn*-1 phenotype, to determine two possible loci for *ltn*-1 on chromosomes 3 and 11, respectively, designated as *ltn*1.1 and *ltn*1.2 (79). The *ltn*1.1 locus was near the loci of *Csfm*

(whose product is macrophage colony stimulating factor M-CSF) and of the *Il6r* (encoding the IL-6 receptor). The *ltn*1.2 locus was located near a cluster of cytokine genes, such as *Csfgm*, *Il*3, *Il*13, *Il*4, and *Il*5. In a subsequent paper, Maeda *et al.* reported the same findings but called the two recessive genes *ltnr*1 and *ltnr*2 (80). Using the same procedure as reported in the previous paper, they concluded that VDH is controlled by one dominant major gene, *Ltnr*3, and three dominant minor genes, *Ltnr*4, *Ltnr*5, and *Ltnr*6 (capitalization by the authors) mapped to chromosomes 6, 9, 15, and 16, respectively (80).

Structure-Function Interactions. The antitumor polysaccharides from various mushrooms are characterized by three physicochemical properties: 1) molecular weight (MW); 2) degree of branching; and 3) higher (tertiary) structure (see also Table II). Generally, these glucans are large molecules, yet their molecular weights range from 200,000 to over 1 million Da. The degree of branching ranges from 2:3 to 1:5 for the polysaccharides with the strongest antitumor activity. All three structural features appear to play a role in many of the antitumor activities of the β(1→3)-glucans.

The tertiary (i.e., higher) structure of mushroom polysaccharides is difficult to determine. Many of them are water-soluble, gel-forming substances that do not easily crystallize. Nonetheless, x-ray diffraction of lentinan combined with theoretical conformational analysis revealed it to have a triple helical structure (81). When lentinan was denatured with either dimethyl sulfoxide (DMSO), urea, or NaOH, tertiary structure (measured by changes in optical rotation) was lost, but primary structure was not affected since renaturation restored the original optical rotation (82). Tumor inhibition in a P815 mastocytoma/DBA/2 system was lowered with progressive denaturation. DMSO- or urea-treatment of lentinan also lowered the VDH response, but it was restored when renatured lentinan samples were used. APP production, a T-cell independent response, was unaffected by denaturation. Taken together, these results suggested that tertiary structure plays an important role in some of the antitumor mechanisms of lentinan. Similar results were obtained in structure-function studies with SPG (83), GRN (32, 84), and SSG (84). It is noteworthy that different biological activities of a specific polysaccharide were affected by changes in MW to varying degrees but that only the native substance had the full range of immunomodulatory activities.

Kinetics. Numerous studies reviewed above indicated that the biological activities of various mushroom antitumor compounds follow distinct kinetics. One group of researchers determined the kinetics, particularly of early biochemical events, *in vivo,* by injecting (1→3)-β-D-glucanase at various time points after the injection of GRN, thereby inactivating it (72, 85). After determining the optimal dose for (1→3)-β-D-glucanase *in vitro* and *in vivo*, GRN was injected ip at a dose of 1 mg/mouse 4 days before transplantation of sarcoma 180, then glucanase was administered ip 0, 6, 12, 24, 48, or 72 hr later (85). Simultaneous administration of GRN and glucanase completely abrogated the tumor inhibition seen with GRN alone. This effect gradually decreased the more the time increased between the GRN-injection and the treatment with glucanase, but had not fully subsided after 48 hr. Injection of thyoglycollate, a known inducer of peritoneal exudate cells, shortened the period during which glucanase treatment affected the GRN-induced antitumor effects to 24 hr. From the length of time that could elapse between the GRN and the glucanase injection, the authors concluded that induction of peritoneal exudate cells, carbon clearance, and acid phosphatase required less than a day of exposure to GRN; 6 hr of exposure to GRN were enough to see significant stimulation of ceruloplasmin activity, but IL-1 production and mitogenic response required the presence of GRN for 3 days (72). We should also note that most glucans are retained in the body for long periods, usually at least a month (86–88), but oxidative degradation of β-1,3-glucans occurs (89). Kuppfer cells play an important role in the metabolism of SSG, and possibly do so for other glucans as well. Metabolism (i.e., oxidative degradation) does not, however, appear to be the only mechanism for inactivating glucans. Although tissue distribution studies indicated that some glucans were retained in the liver for over a month, they did not retain their full biological activity for that long (90).

Molecular Interactions of Mushroom Compounds. Some of the activities of macrophages induced by mushroom β-1,3-glucans appear to be mediated by a receptor for β-glucans (29, 30, 33, 47, 85). A receptor for particulate activators of the alternative pathway, such as unopsonized zymosan, a particulate yeast (i.e., fungal) cell wall component consisting of an α-mannan and a β-glucan, is present on human monocytes. Several biological events triggered by the activation of this receptor on human monocytes by unopsonized zymosan were inhibitable by β-glucans, but not by an α-mannan, identifying it as a β-glucan receptor that was distinct from the α-mannan receptor known to exist on human monocytes (91). It was also distinct from the Fc, and fibronection receptors, and the receptor for C3b (CR1). On the other hand, the CR3 molecule (the receptor for iC3b, now designated as CD11b/CD18) contains two separate binding sites for β-glucans and fixed iC3b (92). In this context, it is noteworthy that GRN was reported to upregulate the cell surface expression of CD11b on Kupffer cells (47). Nonetheless, whether a receptor is responsible for any of the actions stimulated by mushroom-derived β-glucans is still unclear. One of the effects of the binding of zymosan or its β-glucan moiety to the β-glucan receptor(s) is the mediation of the release of lysosomal enzymes (93). As reported above, SPG, GRN, and SSG increased the release of lysosomal enzymes from mouse macrophages (32, 37, 43). Both the β-glucan receptor and CR3 are thought to play a role in activating the phagocytic re-

sponse of macrophages (91, 92). Increased phagocytic activity was reported after incubating mouse macrophages with lentinan (29, 30) or treating mice with SSG (37, 94). Lentinan may stimulate pinocytosis through a β-glucan receptor, and phagocytosis through both a β-glucan receptor and the C3b receptor (complement receptor type 1, CR1) (29, 30). This agrees with the finding that binding of lentinan to human monocytes is strongly inhibited by anti-CR1 mAb (95). From the data, it was impossible to determine whether the other receptor involved was CR3 or the distinct β-glucan receptor. The production of cytokines, such as TNF-α and IL-1β, is another macrophage response to zymosan binding to the β-glucan receptor (96). The induction of these cytokines from mouse and human macrophages has been reported for lentinan (28, 31, 48), GRN (32, 33, 47), and SSG, though only *in vivo* (37, 94). Furthermore, zymosan-binding to the β-glucan receptor induces the release of reactive oxygen species such as superoxide anion and H_2O_2. Such responses have also been observed, if not *in vitro*, but after treatment of mice with GRN (46) and SSG (37).

Two findings suggest that the specific β-glucan receptor is not, or only partially, responsible for the macrophage activities induced by lentinan, GRN, SSG, and SPG. All four glucans, though to differing extents, inhibit the zymosan-induced H_2O_2 production and phagocytosis by mouse peritoneal macrophages (97), although in other studies at least some of them could themselves induce these activities. This suggests, on the one hand, that they are able to bind to the β-glucan receptor but that, on the other hand, they do not activate H_2O_2 production and phagocytosis through the same pathway as does zymozan. Furthermore, the kinetics of the induction of various macrophage activities by mushroom glucans are considerably different from those seen with zymosan, further indicating that mushroom polysaccharides might act through different pathways and/or receptors (33, 85).

Conclusion

Despite the fact that dietary supplements consisting of whole mushroom extracts enjoy increasing popularity, there is a paucity of data regarding a beneficial effect of their consumption. Here, we review murine studies indicating that feeding whole mushrooms inhibits growth of existing tumors. However, no research is available on the prophylactic effects of mushroom intake on, for example, the development of spontaneous tumors. Epidemiological data to that effect are almost equally nonexistent although one report in the literature of a survey conducted among Japanese mushroom growers indicated that eating mushrooms was associated with a lower death rate from cancer than the national average (see reference 9 listed in Ref. 98). Instead, much research has been conducted on isolated purified mushroom constituents, particularly on β-1,3 glucans. Since mammals lack the necessary enzyme, β-1,3-glucanase, to digest these polysaccharides, they are most commonly administered by parenteral routes. Of the four mushroom (1→3)-β-glucans reviewed herein, lentinan and GRN had no antitumor activity when administered orally (5, 99), SPG was only marginally effective (43), but SSG significantly inhibited pulmonary metastasis when given orally, though at a dose 10-fold higher than when injected ip (100).

Similarly, various other mushroom polysaccharides and related compounds have been reported to exert tumor-inhibitory effects after oral administration, including two glucans from *Lentinus edodes*, LEM (101) and KS-2 (102) as well as several glycoproteins isolated from various other mushrooms (98, 103, 104). By contrast, oral administration of PSP, another glycoprotein from *Coriolus versicolor* was recently reported to have no enhancing effect on various immune parameters in young and old mice, although it did significantly increase the DTH response of old mice (105).

It appears surprising that indigestible polysaccharides would have any biological activity after oral ingestion. Until recently, it had been assumed that such polysaccharides were either a) absorbed only after they have been digested into monosaccharides or at most small oligosaccharides or b) not digested, and therefore not absorbed, at all. However, it has now been established by several research groups that at least some indigestible polysaccharides are partially absorbed after oral ingestion, as evidenced by the fact that remnants with molecular weights as high 20,000 Da are detected in plasma or serum of experimental animals as well as humans (106–108). However, mushroom-derived (1→3)-β-glucans lose much of their antitumor-activity with decreasing molecular weight. If therefore their absorption followed a similar course, such small remnants would be unlikely to account for the tumor inhibition seen after oral consumption of those polysaccharides. Unfortunately, no data exist on the metabolism and tissue distribution of mushroom (1→3)-β-glucans or any other biologically active mushroom compounds from the whole extract after oral administration despite the relative ease with which metabolically labeled mushroom extracts could be obtained.

An alternate route for biological activity of orally ingested mushroom polysaccharides could involve contact with and activation of circulating cellular components of the gut-associated lymphoid tissue (GALT). These activated cells could subsequently migrate to other tissues and then exert immunomodulatory effects.

However, it cannot be overlooked that oral ingestion of whole mushroom extracts of *Lentinus edodes* and *Grifola frondosa* can modulate certain immune functions even though the oral administration of their polysaccharides, lentinan and GRN, is ineffective. This suggests that constituents other than polysaccharides have biological activity. In this context, it is noteworthy that consumption of *Lentinus edodes* as part of a regular diet had immunostimulatory as well as immunosuppressive effects in mice depending on the strain and the specific immune function investigated (13, 14). Humans are unlikely to make mushrooms part of their daily diet, but might regularly ingest mushrooms in the

form of dietary supplements. Whether the amount consumed by this route is sufficient to affect immune function in sick or healthy humans will be the subject of future research. More importantly, research is needed to establish whether orally ingested mushrooms or mushroom supplements enhance or downregulate immune functions in humans and what the long-term effects of such immune modulation might be.

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