International Journal of Medicinal Mushrooms, Vol. 1, 31–62 (1999)

Medicinal Properties of Substances Occurring in Higher Basidiomycetes Mushrooms: Current Perspectives (Review)*

Solomon P. Wasser[1,3] and Alexander L. Weis[2]

[1]International Centre for Cryptogamic Plants and Fungi, Institute of Evolution, University of Haifa, Haifa, 31905, Israel; [2]International Myko Biologics, Inc., 14785 Omicron Dr., Suite 104, San Antonio, TX, 78245, USA; [3]M.G.Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Tereshchenkivska Str. 2, Kiev, 252001, Ukraine

ABSTRACT: This review highlights some of the recently isolated and identified substances of higher Basidiomycetes mushroom origin that express promising antitumor, immune modulating, cardiovascular and antihypercholesterolemia, antiviral, antibacterial, antiparasitic, hepatoprotective, and antidiabetic effects. Medicinal mushrooms have a long history of use in folk medicine. Mushrooms useful against cancers of the stomach, esophagus, lungs, etc., are known in China, Russia, Japan, and Korea, as well as the United States and Canada. There are approximately two hundred species of mushrooms that have been found to markedly inhibit the growth of different kinds of tumors. However, most of the mushroom origin antitumor substances have not been clearly defined. Several antitumor polysaccharides, such as hetero-β-glucans and their protein complexes (e.g., xyloglucans, and acidic β-glucan containing uronic acid), as well as dietary fibers, lectins, and terpenoids, have been isolated from medicinal mushrooms. In Japan, Russia, China, and the United States, several different polysaccharide antitumor agents have been developed from the fruiting body, mycelia, and culture medium of various medicinal mushrooms (*Lentinus edodes, Ganoderma lucidum, Schizophyllum commune, Trametes versicolor, Inonotus obliquus,* and *Flammulina velutipes*). Both cellular components and secondary metabolites of a large number of mushrooms have been shown to effect the immune system of the host and therefore could be used to treat a variety of disease states. The information presented in this review is helpful in exploring and understanding the rich traditions of medicinal mushrooms in Eastern and Western cultures and medicine.

KEY WORDS: Dietary fiber, higher Basidiomycetes, immune modulating effect, immunopotentiators, lectins, medicinal mushrooms, polysaccharides, terpenoids, antitumor substances.

INTRODUCTION

Higher Basidiomycetes mushrooms have been used in folk medicine throughout the world since ancient times (Lucas et al., 1957; V. Wasson and R. Wasson, 1957; R. Wasson, 1968; Ying et al., 1987; Yang and Jong, 1989; Mizuno, 1993, 1995a,b, 1996, 1997, 1998a,b; Mizuno, Sakai and Chihara, 1995; Hobbs, 1995; Miles and Chang, 1997; Wasser, 1997; Wasser and Weis, 1997a,b, 1999). Higher Basidiomycetes (mushrooms and toadstools) are not a taxonomic group. They include species from the Basidiomycetes class that have macroscopic fruit bodies (basidioma or ba-

ABBREVIATIONS

BRM: biological response modifer; **BSC:** blood serum cholesterol; **HMGCoA reductase:** 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; **i.p.:** intraperitoneal method; **p.o.:** oral method; **VLDL:** very low density lipoproteins.

*Part of this review has already appeared in *Critical Reviews™ in Immunology*, 1, 1999.

1521-9437/99/$5.00

sidiocarp) which can be either hypogeous or epigeous, large enough to be seen with the naked eye, and usually picked by hand. Higher Basidiomycetes contain approximately 10,000 species from 550 genera and 80 familes (Dudka and Wasser, 1987; Hawksworth et al., 1995). The diagnostic character of Basidiomycetes is the presence of basidium bearing basidiospores. A typical basidium of higher Basidiomycetes is aseptate and has four unicelled hyaline or colored haploid basidiospores (ballisto- or statismospores) dispersed mainly by air. The typical life cycle involves the germination of the basidiospore to give a septate primary (haploid) mycelium. Later during diploidizations, the homo- or heterothallic primary mycelium becomes a secondary (dikariotic) mycelium, which frequently has clamp connections. There is nuclear fusion in the young basidium and meiosis before basidiospore development. The characteristic macroscopic basidioma is generally fleshy and takes a variety of forms. The characteristic features of higher Basidiomycetes could be divided into terresurial or hypogeous, lignicolous or saprobic, mycorrhizal or pathogenic, and edible, medicinal, hallucinogenic, and poisonous mushrooms (Dudka and Wasser, 1987; Hawksworth et al., 1995; Miles and Chang, 1997).

Medicinal mushrooms useful against cancer are known in China, Russia, Japan, and Korea, as well as the United States and Canada. In Russian medicine, an extract from Chaga (*Inonotus obliquus* (Pers.:Fr.) Bond. et Sing.) is used as an antitumor medicine and diuretic. A. Solzhenitsyn, stated in the article entitled "Cancer of White Birch" that a cancerous lesion was cured by application of Chaga, a mushroom that grows on the trunk of the white birch (*Betula alba* L.). In addition, in "Shen Norg Ben Cao Jin" ("Compendium of Materia Medica" of the godly Farmer) of the East Han dynasty in China (100–200 AD) there is reference to medicinal mushrooms such as *Ganoderma lucidum* (Curt.:Fr.) P. Karst. (Reishi), *Dendropolyporus umbellatus* (Pers.:Fr.) Jül., *Tremella fuciformis* Berk. (Ying et al., 1987; Yang and Jong, 1989; Hobbs, 1995; Chang, 1996; Miles and Chang, 1997; Wasser and Weis, 1997a,b, 1999).

Some species of edible higher Basidiomycetes have been found to markedly inhibit the growth of different kinds of tumors. There are approximately two hundred species of higher Basidiomycetes that have been found to possess this activity (Lucas et al., 1957; Gregory et al., 1966; Ying et al., 1987; Yang and Jong, 1989; Mizuno, 1993, 1995a,b, 1996). The search for new antitumor and other medicinal substances from higher Basidiomycetes and the study of the medicinal value of these edible mushrooms have become matters of great interest. Thus, some authors have combined the use of mushrooms both for nutritional (food) and medicinal purposes (Ying et al., 1987; Pai, Jong, and Lo, 1990; Mizuno, 1993; Mizuno, Sakai, and Chihara, 1995; Wasser and Weis, 1997a,b; Miles and Chang, 1997).

Mushrooms are usually used as adaptogens and immunostimulants. First defined by Brekhman (1980), an adaptogen is any substance that meets specific criteria for the category of natural plant-derived[1] "biological response modifier" (BRM) or immunopotentiators. BRMs have been defined as those agents or approaches that modify the host's biological response by a stimulation of the immune system which may result in various therapeutic effects.

The criteria for BRM are:

- It should cause no harm and place no additional stress on the body.
- It should help the body adapt to various environmental and psychological stresses;
- It must have a nonspecific action on the body, supporting all the major systems, including nervous, hormonal, and immune systems, as well as regulating functions.

A large number of mushroom derived compounds, both cellular components and secondary metabolites, have been shown to affect the immune system and could be used to treat a variety of disease states (e.g., Chihara, Maeda, and Hamuro, 1982; Jong, Birmingham, and Pai, 1991; Chihara, 1993; Sakagami and Takeda, 1993; The versatile fungus-food and medicinal properties, T. Mizuno, ed., *Food Rev. Intern.*, 11,1, 1995). Those which appear to enhance or potentiate host resistance are being sought for the treatment

[1]Fungi were generally treated as a part of the kingdom "Plantae." The five-kingdom scheme of Whittaker (1969), adopted rapidly, accepted a separate kingdom "Fungi" (Hawksworth, 1991; Hawksworth et al., 1995) along with bacteria (Monera), plants (Plantae), animals (Animalia), and protists (Protista).

of cancer, immunodeficiency diseases (including AIDS), or generalized immunosuppression after drug treatment.

Edible higher Basidiomycetes are being evaluated for their nutritional value and acceptability, as well as their pharmacological properties. Mushrooms are a nutritionally functional food and a source of physiologically beneficial and noninvective medicines. It was recorded that mushrooms have significant pharmacological effects or physiological properties, such as bioregulation (immunological enhancement), maintenance of homeostasis, regulation of biorhythm, cure of various diseases, and prevention and improvement of life threatening diseases such as cancer, cerebral stroke, and heart diseases. It is also being confirmed that mushrooms have effective substances for decreasing blood cholesterol and could have hypolipidemic, antithrombotic, hypotensive, and other applications (Table 1). Methods for the *in vivo* testing of physiologically active components of mushrooms have been developed using experimental animals (Bobek et al., 1991a,b; Bobek, Ozdin, and Kuniak, 1993; Chihara, 1993; Gunde-Cimerman et al., 1993a,b; Mizuno, 1993, 1996, 1997, 1998a,b; Sakagami and Takeda, 1993; Gunde-Cimerman and Cimerman, 1995; Miles and Chang, 1997; Wasser and Weis, 1997a,b, 1999).

This review highlights some of the most recently isolated and identified substances of mushroom origin which are promising immunomodulators and have demonstrated significant antitumor, cardiovascular, antiviral, antibacterial, antiparasitic, hepatoprotective, and antidiabetic activities.

ANTITUMOR SUBSTANCES OF MUSHROOM ORIGIN

Cancer is the second largest single cause of death in children and adults, claiming more than 6 million lives each year worldwide. Chemoprevention (i.e., the prevention of cancer by ingestion of chemical agents that reduce the risk of carcinogenesis) is one of the most direct ways to reduce morbidity and mortality. Cancer chemopreventive agents include nonsteroidal antiinflammatory drugs (NSAIDs), such as aspirin, ibuprofen, piroxicam, sulindac, and indomethacin. Through the investigation of the mechanism of action of NSAIDs, cyclooxygenase (COS, PGHS, or PGH, prostaglandin-endoperoxide synthase, EC 1.14.99.1) has been established as the key enzyme responsible for prostanoid production (Vane, 1994). Conversion of arachidonic acid to prostaglandin G_2 then to prostaglandin H_2 is catalyzed by two enzymes—COX-1 and COX-2. COX-1 is an isoform of COX, a key enzyme in prostaglandin biosynthesis. A second isoform (COX-2) is induced in inflammatory cells such as monocytes and macrophages upon stimulation by cytokines, mitogens, serum, and endotoxins (Kelloff et al., 1994a,b,c).

Conventional NSAIDs, such as sulindac or indomethacin, inhibit both forms of the enzymes COX-1 and COX-2, but new NSAIDs have been rigorously sought that selective inhibit COX-2. New data shows that COX-2 plays a key role in tumorigenesis and indicates that COX-2-selective inhibitors can be a novel class of therapeutic drugs for suitable chemopreventive agents for many kinds of cancers (Kelloff et al., 1994a,b,c; Elder et al., 1997; Jang et al., 1997; Kalgutkar et al., 1998).

Today, at the beginning of the third millennium, preventives and specific remedies against cancer have not been developed with vaccines and antibiotics. Because cancer cells originate in normal cells which escape growth control and become malignant, it might be feasible to have novel drugs that do not injure normal cells of the host but prevent carcinogenesis and inhibit growth of cancer cells only. Attention has recently focused on the development of immunotherapy to target and eliminate cancer cells, as well as on substances, such as immunopotentiators, immunoinitiators, and BRM, that act to prevent carcinogenesis and induce carcinostasis.

In searching for new cancer chemopreventive agents over the past several years, hundreds of plant extracts have been evaluated for their potential to inhibit COX. For example, an extract derived from *Cassia quinquangulata* Rich. (Leguminosae), collected in Peru, was identified as a potent inhibitor, and on the basis of bioassay divided fraction, resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) was identified as the active substance.

Resveratrol, a phytoalexin found in grapes and other food products, was purified and shown to have cancer chemopreventive activity in assays representative of three major stages of carcino-

TABLE 1
Cross Index of Medically Active Higher Basidiomycetes Mushrooms and Their Medicinal Properties

Taxa	Therapeutic Effects: Antifungal	Antiinflammatory	Antitumor	Antiviral (e.g., anti-HIV)	Antibacterial & Antiparasitic	Blood pressure regulation	Cardiovascular disorders	Hypercholesterolemia, hyperlipidemia	Antidiabetic	Immunomodulating	Kidney tonic	Hepatoprotective	Nerve tonic	Sexual potentiator	Chronic bronchitis
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Auriculariales															
Auricularia auricula-judae (Bull.) Wettst.			✱			✱	✱	✱							✱
Tremellales															
Tremella fuciformis Berk.		✱	✱					✱	✱	✱		✱			✱
Tremella mesenterica Rits.:Fr.						✱									✱
Polyporales															
Schizophyllum commune Fr.:Fr.		✱	✱		✱					✱		✱			
Dendropolyporus umbellatus (Pers.:Fr.) Jül.			✱							✱	✱	✱			✱
Grifola frondosa (Dicks.:Fr.) S.F. Gray	✱		✱	✱	✱	✱			✱	✱		✱			✱
Fomes fomentarius (L.:Fr.) Fr.			✱		✱										
Fomitopsis pimicola (Schw.:Fr.) P. Karst.		✱	✱		✱							✱			
Trametes versicolor (L.:Fr.) Lloyd			✱	✱	✱						✱	✱			
Piptoporus betulinus (Bull.:Fr.) P.Karst.	✱		✱		✱										
Hericium erinaceus (Bull.:Fr.) Pers.			✱							✱			✱		✱
Inonotus obliquus (Pers.:Fr.) Bond. et Sing.		✱	✱							✱		✱			
Lenzites betulina (L.:Fr.) Fr.			✱				✱								
Laetiporus sulphureus (Bull.:Fr.) Murr.	✱		✱												
Ganodermatales															
Ganoderma lucidum (Curt.:Fr.) P.Karst.		✱	✱	✱	✱	✱	✱			✱	✱	✱	✱	✱	✱
Ganoderma applanatum (Pers.) Pat.			✱	✱	✱					✱					
Agaricomycetideae															
Agaricales s.l.															
Pleurotaceae															
Lentinus edodes (Berk.) Sing.		✱	✱	✱	✱	✱		✱	✱	✱	✱	✱		✱	
Pleurotus ostreatus (Jacq.:Fr.) Kumm.			✱	✱	✱			✱					✱		
Pleurotus pulmonarius (Fr.:Fr.) Quél	✱		✱					✱							
Tricholomataceae															
Flammulina velutipes (Curt.:Fr.) P.Karst.	✱	✱	✱	✱						✱					

continued

TABLE 1 (*continued*)

Taxa	Antifungal	Antiinflammatory	Antitumor	Antiviral (e.g., anti-HIV)	Antibacterial & Antiparasitic	Blood pressure regulation	Cardiovascular disorders	Hypercholesterolemia, hyperlipidemia	Antidiabetic	Immunomodulating	Kidney tonic	Hepatoprotective	Nerve tonic	Sexual potentiator	Chronic bronchitis
Therapeutic Effects															
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Oudemansiella mucida (Schrad.:Fr.) v. Höhn.	*														
Armillariella mellea (Vahl.:Fr.) P.Karst.	✖					*	*						*		
Hypsizygus marmoreus (Peck) Bigel.			*												
Marasmius androsaceus (L.:Fr.) Fr.		*											*		
Agaricaceae															
Agaricus blazei Murr.			*												
Agaricus bisporus (J.Lge) Imbach			✖							*	*				
Pluteaceae															
Volvariella volvacea (Bull.:Fr.) Sing.			✖	✖	✖			✖							
Bolbitiaceae															
Agrocybe aegerita (Brit.) Sing.	✖		✖					✖					✖		

* - Commercially developed mushroom product (drug or dietary supplement)
✖ - Non commercially developed mushroom product

genesis. Resveratrol was found to act as an antioxidant and an antimutagen and to induce phase II drug-metabolizing enzymes (antiinitiation activity). It mediated antiinflammatory effects and inhibited COX and hydroperoxidase functions (antipromotion activity), and it induced human promyelocytic leukemia cell differentiation (antiprogression activity). In addition, it inhibited the development of preneoplastic lesions in carcinogen-treated mouse mammary glands in culture and inhibited tumorigenesis in a mouse skin cancer model. These data suggested that resveratrol, a common constituent of the human diet, merits investigation as a potential cancer chemopreventive agent in humans (Jang et al., 1997).

It has been known for many years that selected mushrooms of higher Basidiomycetes origin are effective against cancer of the stomach, esophagus, lungs, etc. (Ying et al., 1987; Yang and Jong, 1989; Hobbs, 1995). However, the components responsible for such activity have not yet been completely identified.

The antitumor activity of the higher Basidiomycetes was first demonstrated by Lucas and his collaborators (1957), who employed extracts of fruiting bodies of *Boletus edulis* Bull.:Fr. and other Homobasidiomycetes in tests against Sarcoma 180 line in mice. In the 1960s calvacin was the most commonly cited natural product isolated from the medicinal mushroom and broadly used in many laboratories as an antitumor agent. Calvacin was isolated from the giant puffball (*Calvatia* (=*Langermannia*) *gigantea* (Batsch:Pers.) Lloyd) by Lucas and his coworkers (1957, 1959). It is interesting to note that calvacin emerged indirectly from the recorded ancient application

and verfication of folk medicine (Lucas et al., 1957). Calvacin was tested against many experimental tumors, including Sarcoma 180, mammary adenocarcinomce 755, leukemia L-1210, and HeLa cell lines.

In 1962, Yohida and his collaborators isolated from *Lampteromyces japonicus* (Kawamura) Sing., an agent active against Ehrlich carcinoma of the mouse. Gregory and collaborators (1966) surveyed more than 7,000 cultures of higher Basidiomycetes for antitumor activity against three rodent tumor systems. Fifty cultures representing 22 species produced, in fermentation media, materials showing inhibitory effects against Sarcoma 180, mammary adenocarcinoma 755, and leukemia L-1210.

Ikekawa and coworkers (1968, 1969) reported that hot water extracts obtained from the fruiting bodies of seven edible wild-growing higher Basidomycetes (*Auricularia auricula-judae* (Bull.) Wettst., *Flammulina velutipes* (Curt.:Fr.) Sing., *Lentinus edodes* (Berk.) Sing., *Pholiota nameko* (T. Ito) S. Ito et Imai, *Pleurotus ostreatus* (Jacq.:Fr.) Quél., *P. spodoleucus* (Fr.) Quél., *Tricholoma matsutake* (S. Ito et Imai) Sing.), showed (except *A. auricula-judae*) a marked host-mediated antitumor activity against Sarcoma 180 in Swiss albino mice. They also produced data related to a component of *L. edodes* (Shiitake mushroom) water-eluted fraction which demonstrated a 94.8% rate of tumor inhibition at a 200 mg/kg/day dose. Interestingly, the alkaline-eluted component gave only a 62.5% tumor inhibition rate at the same dosage. A white powder was also obtained by acetone precipitation of the water-eluted fraction. The tumor completely regressed in six of the nine mice at the same dosage. The loss of body weight in treated mice was not observed.

Using standard methods of fractionation and purification of polysaccharides (Fig. 1), Chihara and coworkers (1969, 1970a,b) isolated a water-soluble antitumor polysaccharide from the fruiting bodies of *Lentinus edodes*, which was named "*Lentinan*" after the generic name of this mushroom. Chihara reported on the antitumor properties of *L. edodes*, and stated that lentinan "was found to almost completely regress the solid type of tumors in synergic host-tumor system A." The antitumor effect of lentinan was originally confirmed by using Sarcoma 180 transplanted in CD-1/ICS mice (Chihara et al., 1969).

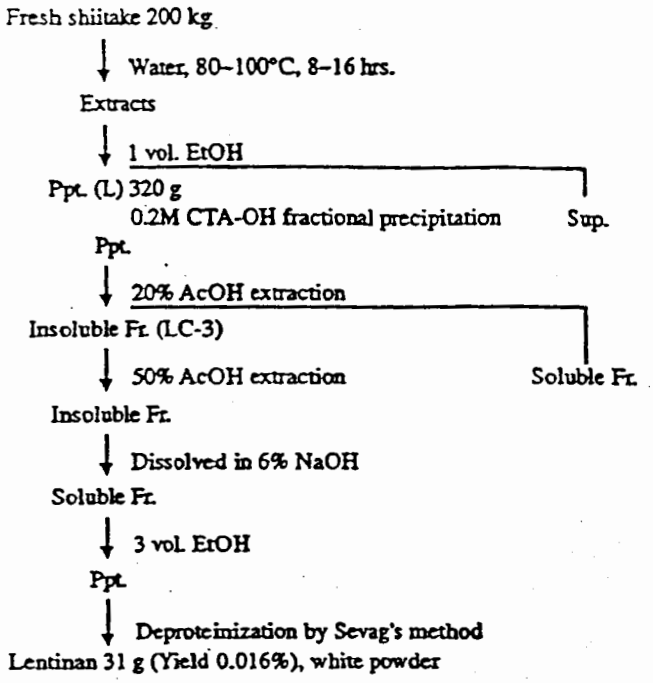

FIGURE 1. Fractionation of antitumor β-D-glucan Lentinan from *Lentinus edodes* (Chihara et al., 1970a,b; Mizuno, 1995a).

Since that time, numerous researchers (e.g., Whister et al., 1976; Zákány, Chihara and Fachet, 1980a,b) have isolated essential polysaccharide substances. Every one of them was a β-D-glucan, a polysaccharide yielding D-glucose by acid hydrolysis. In addition to β-D-glucans, a number of high molecular weight antitumor components were isolated from medicinal mushrooms, including heteroglycans, chitinous substances, peptidoglycans, proteoglycans, lectins, RNA components, dietary fiber, and/or indigestible polysaccharides. Furthermore, as the result of screening for growth inhibition of cultured cancer cells, such as those in carcinoma of the cervical canal (HeLa cells) and the liver (Hepatoma cells), a number of low molecular weight organic substances, such as terpenoids, steroids, novel gamma-pyrones, and novel phenols, were isolated from mushrooms and identified (Jong, Birmingham, and Pai, 1991; Mizuno, 1995a,b,c, 1996, 1997, 1998a,b; Kawagishi, 1995).

Polysaccharides demonstrating a remarkable antitumor activity *in vivo* have been isolated from various species of mushrooms belonging to Auriculariales, Tremellales, Polyporales, Gasteromycetideae and Agaricomycetideae (Table 2), through screening against Sarcoma 180 in mice,

TABLE 2
Antitumor Active Polysaccharides Isolated from Medicinal Higher Basidiomycetes Mushrooms

Taxa	Fruiting body	Submerged cultured mycelial biomass	Liquid cultured broth	Source
1	2	3	4	5
Phragmobasidiomycetes				
Auriculariales				
Auriculariaceae				
Auricularia auricula-judae (Bull.) Wettst.	(1→3)-β-glucan	—	—	Ukai et al., 1983; Misaki & Kakuta, 1995
Tremellales				
Tremellaceae				
Tremella fuciformis Berk.	Glucuronoxylomannan, T-7, T-19 (exopolysaccharides), mannose, xylose, glucuronic acid	Glucuronoxylomannan	Xylose, glucuronic acid, mannose	Ukai et al., 1972, 1978; Misaki & Kakuta, 1995
T. mesenterica Ritz.:Fr.	β-D-glucuronosyl (epitope)	—	—	Misaki & Kakuta, 1995
Homobasidiomycetes				
Aphyllophoromycetideae				
Ganodermatales				
Ganodermataceae				
Ganoderma lucidum (Curt.:Fr.) P. Karst.	FI-1a (β-glucan), FIII-2b (hetero-β-glucan), acidic heteroglucan, chitin xyloglucan	—	β-glucan	Mizuno et al., 1984; Willard, 1990; Wasser & Weis, 1997a
G. applanatum (Pers.) Pat.	FI-1-b-1 (β-glucan)	F-1a-1-b (β-glucan), heteroglucans, peptidoglucans	—	Usui et al., 1981
G. tsugae Murr.	Heteroglucan, hetrogalactan, β-glucan, glucan	Heteroglucan, α-glucan		Wang et al., 1993; Zhang et al., 1994b
Polyporales				
Schizophyllaceae				
Schizophyllum commune Fr.:Fr.	—	—	Sonifilan, SPG or Schizophyllan (β-glucan)	Tabata et al., 1981; Yamamoto et al., 1981
Polyporaceae				
Dendropolyporus umbellatus (Pers.:Fr.) Jül.	GU-2, GU-3, GU-4, AP (β-glucan)	—	(β-glucan)	Ito et al., 1973; Zhu, 1987

continued

TABLE 2 (*continued*)

Taxa	Fruiting body	Submerged cultured mycelial biomass	Liquid cultured broth	Source
1	2	3	4	5
Grifola frondosa (Dick.:Fr.) S.F. Gray	Grifolan (β-glucan), Fa-1a-β (acidic β-glucan), FIII-2c (hetero-β-glucan) xyloglucan, mannoglucan, fucomannoglucan	Heteroglucan protein, mannogalactofucan, heteroxylan, fucoxylan, galactomannoglucan	—	Mizuno, 1997, 1998a; Zhuang et al., 1994a,b
Fomes fomentarius (L.:Fr.) Fr.	β-glucan	β-glucan	—	Ito, Sugiura & Miazaki, 1976
Fomitopsis pinicola (Schw.:Fr.) P.Karst.	F-1a-2-β (β-glucan) α-(1→6)-linked D-galactosyl	α- and β-glucans	—	Mizuno, 1998
Albatrellus confluens (Alb. et Schw.:Fr.) Kotl. et Pouz.	(1→3)-β-D-glucans	(1→3)-β-D-glucans	—	Mizuno et al., 1992a
Trametes versicolor (L.:Fr.) Lloyd	β-glucan	Corielan, PSK, Krestin (β-glucan-protein)	—	Tsukagoshi et al. 1984; Hirose, 1985; Hiroshi & Takeda, 1993; Yang & Jong, 1989
Lenzites betulinus (L.:Fr.) Fr.	β-glucan	—	—	Ikekawa et al., 1968; Fujimoto et al., 1994
Wolfiporia cocos (Schw.) Ryv. et Gilbn.	Pachymaran (β-glucan)	—	—	Narui et al., 1980; Kanayama et al., 1983
Hericium erinaceus (Bull.:Fr.) Pers.	β-glucoxylan, glucoxylan protein, galacoxyloglucan protein	—	—	Kawagishi, Ando & Mizuno, 1990; Mizuno et al., 1992b; Mizuno, 1998b.
Inonotus obliquus (Pers.:Fr.) Boud. et Sing.	Polysaccharide fraction in the Allium-test	—	—	Grzybek, Ochotnicki, & Kohlmunzer, 1983
Gasteromycetideae, Phallaceae				
Dictyophora indusiata Fisch.	T-2 HN (O-acetylated-(1→3)-β-D-mannan), T-3-M[1] (α-(1→3) linked D-mannan), T-3-G, T-4-N, T-5-N (three kinds of β-D-glucans), T-3 Ad (neutral heterogalactan)	—	—	Ukai et al., 1983; Hara & Ukai, 1995
Phallus impudicus L.:Pers.	PI-2 (glucomannan)	PI-2 (glucomannan)	—	Kuznetsovs & Jegina, 1993
Agaricomycetideae				
Agaricales s.l.				
Pleurotaceae				

Lentinus edodes (Berk.) Sing.	Lentinan (β-D-glucans)	KS-2-a-mannan-peptide, LEM, LAP (heteroglucan-protein), EP3	LEM, LAP (heteroglucan-protein), EP3	Chihara et al., 1970a,b; Fujii et al., 1978; Chihara, 1981, 1992; Aoki, 1984a,b; M. Suzuki et al., 1990; Wasser & Wels, 1997b
Pleurotus ostreatus (Jacq.:Fr.) Kumm.	Acidic polysaccharide fraction, HA (β-glucan)	—	β-glucan, heteroglucan	Yoshioka et al. 1972; Solomko, 1992
P. citrinopileatus Sing.	Heteroglucan, β-glucan-protein, glycoprotein (FI, FII, FIII)	—	—	Zhuang et al., 1994a
P. pulmonarius (Fr.:Fr.) Quél. (=*P. sajor-caju* Fr.:Fr.)	Xyloglucan, xylanprotein	—	—	Zhuang et al., 1993
Trichotomataceae				
Panellus serotinus (Pers.:Fr.) Kühn.	Heteroglucan, (1→6)-β-D-glucosyl-branched (1(1→3)-β-D-glucans	—	—	Ma, Mizuno, & Ito, 1991
Omphalina epichysium (Pers.:Fr.) Quel.	OL-2 (β-glucan)	—	—	Mizuno et al., 1995a
Flammulina velutipes (Curt.:Fr.) P. Karst.	EA_6, EA_6-PII (β-glucan-protein)	Proflamin (glycoprotein)	—	Yoshioka et al., 1973; Zeng et al., 1990; Ikekawa, 1995a
Leucopaxillus giganteus (Fr.) Sing.	Mannoxyloglucan, heteroglucan, glucan, xyloglucan, xylogalacetoglucan, galactoxyloglucan	—	—	Zhuang & Mizuno, 1995; Mizuno et al., 1995b
Hypsizygus marmoreus (Peck) Bigel.	β-(1→3)-D-glucan	—	—	Ikekawa, 1995b
Agaricaceae				
Agaricus blazei Murr.	FI_1-a-β (β-glucan), FIII-2-β (β-glucan-protein), FA-1a-β (hetero-β-glucan), FA-2b-β (RNA), FV-1 (insoluble β-glucan)	ATOM (glucomannan-protein)	AB-FP (mannan-protein)	Kawagishi et al., 1990; Mizuno et al., 1990; Mizuno, 1995c
A. bisporus (J.Lge) Imbach	β-glucan	—	—	Mizuno et al., 1995a
Pluteaceae				
Volvariella volvacea (Bull.:Fr.) Sing.	VVG (β-1→3)-D-glucans, α-manno-β-glucan	—	—	Misaki & Kishida, 1995
Strophariaceae				
Pholiota nameko (T.Ito) S.Ito et Imai	Galacto-β-glucan	—	—	Mizuno et al., 1995a
Crepidotaceae				
Crepidotus mollis (Schaeff.:Fr.) Kumm.	CPS (β-glucan)	—	—	Mizuno et al., 1995a
Bolbitiaceae				
Agrocybe aegerita (Brit.) Sing.	α-(1→3)-β-glucans	—	—	Mizuno et al., 1995a

and intraperitoneal (i.p.) or oral (p.o.) methods of administration (Ikekawa et al., 1969; Mori et al., 1986; 1989; Mizuno et al., 1995a; Wasser and Weis, 1997a,b). These antitumor substances are regarded as BRMs that impart their activities based on the activation of host immunological function. The principal component of these substances is (1→3)-β-D-glucans. These components are characterized by the weak antigenicity and the absence of side effects.

In Japan, Russia, China, and the United States, several polysaccharide carcinostatic agents have been developed and commercialized using submerged cultured mycelial biomass of *Trametes versicolor* (PSK, Krestin; Japan), fruiting bodies of *Lentinus edodes* (Lentinan; Japan), *Inonotus obliquus* (Befungin; Russia), *Agaricus blazei* (USA), liquid cultured broth product of *Schizophyllum commune* (Sonifilan, SPG, Schizophyllan; Japan) (Mizuno, 1996; Miles and Chang, 1997).

β-D-GLUCANS

Antitumor polysaccharides[2] isolated from mushrooms (fruit body, submerged cultured mycelial biomass, and liquid cultured broth) are shown in Table 2. As a result of the study (Marchessault et al., 1977), it was clarified that active β-D-glucan show a triple-strand right winding helix structure. β-D-glucans of mushrooms do not always show the antitumor activity. Differences in the activity could be correlated to solubility in water, the size of molecules, branching rate, and form, the β-(1→6)-bonding system in the β-(1→3) major chain. It should be noted that the optimal dose (i.p. or p.o.) has yet to be determined. A fairly large amount of β-glucan is obtainable with dilute alkali which are insoluble in water. Several attempts have been made to enhance activity by chemical modification. β-glucans obtained from *Auricularia auricula-judae* (Misaki and Kakuta, 1995) and *Dendropolyporus umbellatus* (Ito et al., 1973; Zhu, 1987) has been made more water soluble by several modifications:

- carboxymethylation;
- hydroxyethylation;
- polyalcohols formed by BH_4^- reduction after IO_4^- oxidation;
- β-(1→6) branching elimination by a mild Smith degradation;
- glucopyranosyl residue in the principal chain of β-(1→3)-D-glucane being partially converted to 3,6-anhydroglucopyranosyl residue, manopyranosyl residue, and mannosamino pyranosyl residue (Mizuno et al., 1995a,b).

β-D-glucan from *Lentinus edodes* mushroom, lentinan, has been studied more extensively than other similar substances. Lentinan showed prominent antitumor activity not only against allogenic tumors, such as Sarcoma 180, but also against various synergic and autochthonous tumors, and it prevents chemical and viral oncogenesis (Zákány et al., 1980a,b; Suga et al., 1984, 1985, 1986, 1989). The molecular formula of Lentinan is $(C_6H_{10}O_5)n$, the mean molecular weight is about one million—5×10^5 Da, $[\alpha]_D + 20°–22°$ (NaOH). It is a β-D-glucan, as shown by electrophoresis and ultracentrifugation, as well as by other chemical techniques and instrumental analysis (Sasaki and Takatsuka, 1976). Lentinan is not toxic to tumor cells, but inhibits tumor growth by stimulating the immune system (Chihara, 1978). That is, β-D-glucan binds to lymphocyte surfaces or serum specific proteins, which activate macrophage, T-helper, NK, and other effector cells. All of these increase the production of antibodies as well as interleukins (IL-1, IL-2) and interferon (IFN-γ), which are released upon activation of effector cells (Dennert and Tucker, 1973; Hamuro et al., 1976, 1978a,b; Mizuno, 1995a,b). Thus, the carcinostatic effect of lentinan results from the activation of the host's immune system. In animal testing of carcinostatic activity, i.p. administration is used, but p.o. is occasionally effective. Compared to other cancer chemotherapeutic agents, toxicity and adverse reactions of lentinan are rarely noted. With pure β-D-glucan, there is no antigen–antibody reaction nor are there any other disturbances, such as allergy, shock, etc. (Jiang et al., 1986; Cao et al., 1989; Hobbs, 1995).

Lentinan's antitumor activity was significantly stronger than that of polysaccharides from many other fungi (including lichens) or from higher

[2]Comprehensive data about medicinal mushrooms' antitumor polysaccharides are given in the article by T. Mizuno, which is published in this issue.

TABLE 3
Current Antitumor Activity Induced by Lentinan

Tumors	Hosts	Dose of lentinan (mg/kg x days)	Tumor inhibition ratio (%)	Complete regression of tumor	Decreased tumor occurrence
1	2	3	4	5	6
Allogeneic					
Sarcoma 180	CD-1/ICR	0.2 x 10	78.1	6/10	
		1 x 10	100.0	10/10	
		25 x 10	88.2	0/8	
		80 x 5	−8.5	0/8	
	SWM/Ms	1 x 10	100.0	10/10	
	A/J	4 x 5	96.5	9/10	
	C3H/He	4 x 5	36.2	0/6	
	C57Bl/6	4 x 5	51.8	0/6	
Syngeneic					
A/Ph.MC.S1	A/Ph(A/J)	1 x 10	100.0	18/18	
DBA/2.MC.CS1	DBA/2	1 x 10	76.5	2/7	
P-815	DBA/2	5 x 4	89.0	2/8	
L-5178Y	DBA/2	10 x 3	84.0	3/9	
MM-46	C3H/He	5 x 2	100.0	9/9	
Autochthonous					
MC-induced primary	DBA/2	1 x 10	80.5	2/5	
Inhibition of metastasis					
DBA/2.MC.CS-T	DBA/2	1 x 10	94.2		
MH-134	C3H/He	1 x 14	100.0		
Madison-109	BALB/c	25 x 2		10/14	
Prevention of oncogenesis					
MC-induced	SWM/Ms	1 x 10			83→31%
MC-induced	DBA/2	1 x 10			78→37%
Adenovirus 12	C3H/He	10 x 3			79→40%

Note: All tumors were solid, transplanted s.c. Route of lentinan injection was i.p., except i.v. for P-815, L-5178Y, and MM-46. Tumor inhibition ratio = (C-T)C x 100, where C = average tumor weight of control mice and T = that of lentinan-treated mice.
Sources: Chihara et al., 1969, 1970a,b; Ikekawa et al., 1969; Hamuro et al., 1971, 1976; Maeda and Chihara, 1971, 1973; Maeda et al., 1974a,b, 1975, 1984, 1988; Suga et al., 1984, 1985, 1986, 1989; Mori et al., 1987; Mizuno et al., 1995a,b; Mizuno, 1995a,b; Jones, 1995.

plants. It appears to be active in certain animals for some, but not all, types of tumors (Arai et al., 1971; Maeda et al., 1974a,b; Hobbs, 1995). Data on antitumor activity, prevention of metastasis, and suppression of chemical and viral oncogenesis by lentinan are summarized in Table 3.

HETEROPOLYSACCHARIDES AND GLYCOPROTEINS

In addition to water-soluble β-D-glucans, mushrooms also contain β-D-glucans with heterosaccharide chains of xylose, mannose, galactose, and uronic acid extracted by salt and alkali, and β-D-glucan-protein complexes that are present at 10% to 50% in dry matter. Some of them have shown remarkable carcinostatic effects not only by intraperitoneal injection, but also by oral dose (Table 2).

In addition to β-D-glucan, glucuronoglucan, xyloglucan, unannoglucan, xylomannoglucan, and other active heteroglucans and their protein complexes were extracted from *Ganoderma lucidum* for medicinal use and purified using salts, alkali, and DMSO (Mizuno et al., 1984; Willard, 1990; Wasser and Weis, 1997a).

From the extracts of cultured mycelium of *Lentinus edodes*, α-mannan peptide (KS-2) was isolated. Polysaccharide KS-2 (MW 6–9.5 x 10^4 $[\alpha]_D$ = 62°; C = 0.5, water) was obtained by ex-

traction of cultured mycelia of *L. edodes* (strain KSLE 007) with hot water, followed by precipitation with ethanol (Fujii et al., 1978). The product is an α-mannan peptide containing the amino acids serine, threonine, alanine, and proline (as well as residual amounts of the other amino acids). KS-2 was shown to be effective on Sarcoma 180 and Ehrlich's carcinoma, either i.p. or p.o., and to act via an interferon-inducing activity. The acute LD_{50} of KS-2 was found to be extremely high in mice, more than 12,500 mg/kg when administered orally.

The mechanism of action of KS-2 is not clear, although results showed no KS-2 direct cytocidal effect against the tumor cells *in vitro*. Its antitumor activity was observed to be higher at the lower inoculum size of tumor cells, regardless of the routes of KS-2 administration (60% survival rate at 5×10^3 tumor cells/mouse, 10% survival at 1×10^6 tumor cells/mouse). The results also showed that the antitumor activity of KS-2 in mice was always accompanied by the induction of interferon in the sera. Furthermore, preliminary findings indicated that macrophages obtained from KS-2-treated mice exhibited tumoricidal activity (C. Suzuki et al., 1978). Schultz et al. (1977), reported that macrophage became tumoricidal when incubated *in vitro* with interferon. Considering these findings, the antitumor activity of KS-2 may be explained by macrophage activation with or without interferon induction by KS-2.

LEM and LAP extracts from *L. edodes* mushroom mycelium and culture media are glycoproteins containing glucose, galactose, xylose, arabinose, mannose, and fructose (Iizuka et al., 1990). LEM also contains various nucleic acid derivatives, vitamin B compounds, especially B_1 (thiamine), B_2 (riboflavin), and ergosterol (Breene, 1990).

LEM was prepared from an extract of the powdered mycelia of *L. edodes*. After incubation of mycelia on solid medium at 20 to 22°C for 80 to 120 days and before fruiting, the media was powdered and further incubated in the presence of the enzymes naturally present in the mycelia for 50 to 60 hours at 40 to 50°C (which was partially hydrolyzed in the process). When the reaction was completed, the residue was extracted with water (60°C), and the filtrate was freeze dried. The light brown powder obtained was LEM. The yield of LEM is about 6 to 7g/kg of medium. The precipitate obtained from a water solution of LEM by adding 4 volumes of ethanol was named LAP and the yield of LAP is ≈ 0.3g/g of LEM.

LEM and LAP have both demonstrated strong antitumor activity, both orally and by injection, in animals and humans. Both of them were shown to activate the host's immune system (Lin et al., 1987; Mizuno, 1995a,b).

In 1990, an immunoactive substance, EP3 was obtained by fractionation of LEM (M. Suzuki et al., 1990). EP3 is a lignin complex composed of approximately 80% lignin, 10% carbohydrates, and 10% protein. After removal of carbohydrates and protein, biological activity was not affected, but when lignin is removed, activity is reduced. Therefore, the active substance is believed to be a water-soluble lignin containing numerous carboxyl groups (M. Suzuki et al., 1990).

From cultured mycelium of *Agaricus blazei* a glucomannan-protein complex (ATOM) and, from a cultured filtrate, a mannan-protein complex (AB-FP) have been isolated (Kawagishi et al., 1990; Mizuno et al., 1990; Mizuno, 1995c). Both ATOM and AB-FP have shown marked antitumor activity (Mizuno, 1995c). From the fruiting bodies of *A. blazei*, a water-soluble β-(1→6)-D-glucan protein complex (polysaccharides: protein = 50:43 w/w) was isolated, in addition to water soluble β-(1→3)-glucan. It was the first time that the marked anticancer activity was noted on β-(1→6)-D-glucan (Mizuno et al., 1990; Mizuno, 1995c).

From fruiting body of *Flammulina velutipes*, the β-glucan-protein (EA_6) was isolated. EA_6 contains C 41.39%, H 6.92%, N 3.82%, saccharide 70%, protein 30%, $[\alpha]_D$-14.2° (c=0.5, H_2O), and consists of glucose, galactose, mannose, xylose, arabinose, and 16 amino acids (Ikekawa, 1995a). EA_6 exhibited strong antitumor activity against sarcoma 180, Lewis cancer of lung and B-16 melanoma (Zeng et al., 1990; Ikekawa, 1995a).

In addition to the studies on the fruiting body of *F. velutipes*, a new antitumor glycoprotein was found in cultured mycelium. This glycoprotein was named "Proflamin" (Ikekawa et al., 1985). Proflamin is a water-soluble glycoprotein of molecular weight 13,000 ± 4,000 $[\alpha]_D$-52–57° (c = 0.1, 0.1 N NaOH), contains 90% protein and 10% saccharide. Proflamin was isolated by the method illustrated in Fig. 2.

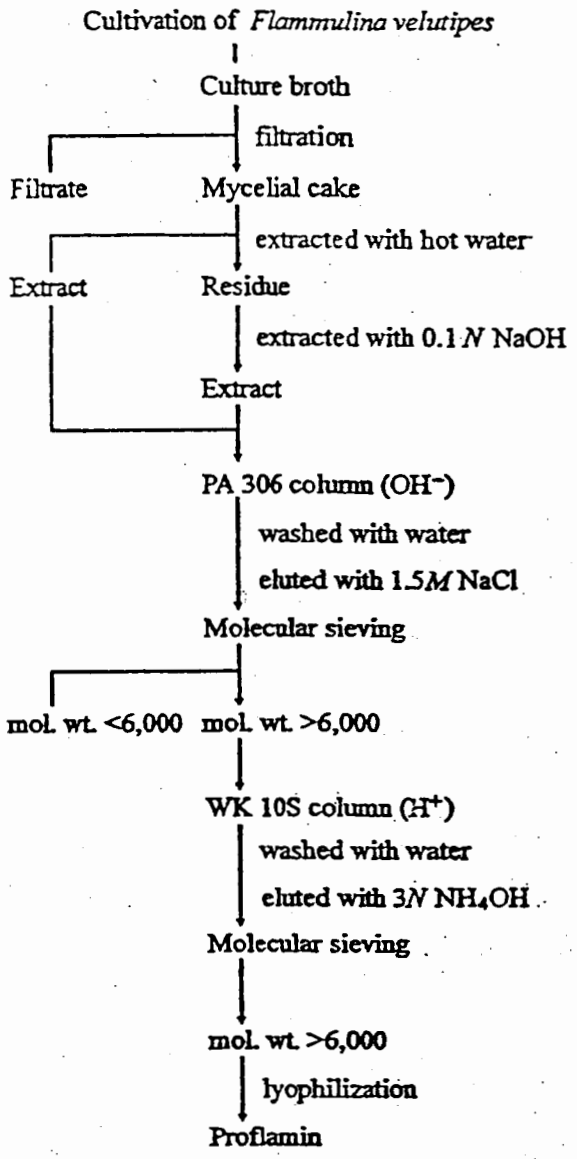

FIGURE 2. Isolation of Proflamin (Ikekawa et al., 1985)

Proflamin is effective against allogeneic and syngeneic tumors by oral administration. Thus it was effective against solid Sarcoma 180, B-16 melanoma, adenocarcinoma 755, and Gardner lymphoma. It was also useful in combination therapy with other antitumor agents. Proflamin augmented antibody formation and activated lymphocyte blastogenesis (Ikekawa, 1995a).

The antitumor activities of *Dictyophora indusiata* (T-2-HN, T-4-N, and T-5-N) against Sarcoma 180 solid tumor were studied (Hara and Ukai, 1995). The potency of antitumor activities of branched (1→3)-β-D-glucans (T-4-N and T-5-N), both of which were extracted with alkaline solutions and soluble in water, was somewhat weaker than those of water-extracted (1→3)-β-D-glucans isolated from other mushrooms (*Lentinus edodes, Ganoderma lucidum, Hericium erinaceus*). In particular, T-4-N has much higher molecular weight (MW: 5.5×10^5 in 0.25 M NaOH), which indicates that the antitumor activity of branched (1→3)-β-D-glucans depends upon their molecular weights. On the other hand, a partially O-acetylated (1→3)-α-D-glucans (T-2-HN) showed significant antitumor activity at only a dose of 25 mg/kg/day × 10, although complete regression of the tumor in mice was not observed.

DIETARY FIBER

High molecular weight materials excreted without digestion and absorption by human beings are called dietary fiber (Vahoumy and Kristchevsky, 1986). Mushrooms contain dietary fibers belonging to β-glucans, chitin, and heteropolysaccharides (pectinous substances, hemicelluloses, polyuronides, etc.)—as much as 10% to 50% in the dried matter. Most of the active polysaccharides, water-soluble or insoluble, isolated from mushrooms can be classified as dietary fibers (i.e. β-glucans, xyloglucan, heteroglucan, chitinous substance and their protein complexes). Because β-glucans and chitinous substances with carcinostatic activity are contained primarily in the dietary fiber of mushrooms and by physicochemical interactions, they absorb such hazardous materials as carcinogenic substances thereby prevent their absorption into the intestine and hastening their excretion (laxative action). Thus they may work effectively to prevent cancer of the colon and rectus (Mizuno, 1996).

LECTINS

The term "lectin" is defined as a carbohydrate–protein of nonimmune origin which agglutinate cells or precipitates polysaccharides or glycoconjugates (Liener, Sharon and Goldstein, 1986; Bog-Hansen and Freed, 1988). Recently several lectins were isolated and purified from higher Basidiomycetes mushrooms (Liener, Sharon and Goldstein, 1986; Mirelman, 1986; Bog-Hansen

and Freed, 1988). Lectins occur as proteins or glycoproteins with highly specific carbohydrate moieties. These identify vital cells, such as erythrocytes, and bind polysaccharides, complex carbohydrates, and proteins. Fungal lectins is useful to study polysaccharides and glycoproteins, as well as enzymatic modifications and cellular membranes. Because of their characteristic sequences, these materials can be used for affinity chromatography, for diagnosis of cancer cells, or as specific binding moieties for targeted cancer therapy. For instance, an N-acetylgalactosamine specific lectin was isolated from the fruiting body of *Grifola frondosa* GFL (Kawagishi, 1995). The isolated lectin agglutinated all types of erythrocytes equally. Molecular weight estimated by gel filtration under various buffers and matrices varied from 30 to 52 kDa. GFL is cytotoxic against HeLa cells. Results obtained by Kawagishi (1995) showed that the cytotoxicity of the lectin of the same Polyporales mushrooms against HeLa cells is the result of the binding of the lectin to the carbohydrate domains of the cells, and independent of aggregation of the cells by the lectin.

TERPENOIDS

Some terpenoids and their derivatives, isolated from Polyporales and Ganodermatales mushrooms, are cytotoxic. These compounds are candidates for antitumor agents. Indeed, about 100 different triterpenoids can be found in fruiting bodies and mycelia of *Ganoderma lucidum* and *G. applanatum*. These include highly oxidized lanostanine-type triterpenoids, such as ganoderic acids A, B, C_1, C_2, D_1, D_2, E_1, E_2, F, G, H, I, J, K_1, K_2, L, Ma, Mb, Mc, Md, Me, Mf, Mg, Mi, Mj, Mk, Mn, N, O, P, Q, S, T, U, V, W, X, Y, and Z, 7-0-methyl ganoderic acid O, trideacetyl ganoderic acid T, ganoderenic acids A, B, C, D, E, F, G, H, I, ganolucidic acids, A, B, C, D and E, lucidenic acids A, B, C, D_1, D_2, E_1, E_2, F, G, H, I, J, K, L, M, ganoderiol type 1 (A, B, F) and type 2 (C, D, E, F, G, H, and I), ganoderal A and B, epoxyganoderiol A, B, C, lucidone A, B, C, furanoganoderic acid and other terpenoid components (all literature cited in Wasser and Weis, 1997a, 1999).

The structures of *Ganoderma* species terpenoids have a lanostane skeleton, and they are classified into several groups based on their carbon numbers and state of oxidation (Komoda et al., 1985). Some triterpenoids (i.e. Ganoderic acids R, T, U-Z) isolated from submerged cultured mycelial biomass have been reported to inhibit growth of hepatoma cells *in vitro* (Toth et al., 1983; Lindequist, 1995).

Omphalotus olearius (DC.:Fr.) Fay. and *Lampteromyces japonicus* (Kawamura) Sing. produce the cytotoxic tricyclic sesquiterpene, illudin S = lamterol (McMorris and Anchel, 1963, 1965; Nakanishi et al., 1965; McMorris et al., 1992; Konno, 1995), which demonstrates anticancer properties and inhibits cancer cell growth by a unique mechanism. It is believed that illudin S undergoes activation by glutathione. The activated form is then capable of covalent binding to DNA. This halts DNA replication and leads to cell death. Illudin S itself is too toxic to be used as a clinical drug. A semisynthetic illudin analog, 6-hydroxymethylacylfulvene (HMAF), demonstrated a superior therapeutic profile and lower toxicity. The compound is obtained from fermentation-derived illudin S by means of a modified Prins reaction. HMAF shows an excellent profile of tumor growth inhibition, evaluated both *in vitro* and in *in vivo* xenograft models. It is particularly promising in inducing tumor regression in refractory tumor cell lines such as MV-522 lung and HT-29 colon cancers. HMAF also inhibits the growth of a number of multidrug-resistant cancer cell-lines. Due to its outstanding therapeutic profile, HMAF is currently undergoing phase I human clinical trials and holds the promise of becoming a valuable new anticancer drug (Weis, 1996).

During the studies of toxic principles, the extract of *L. japonicus* was found to show antitumor activity in rodent tumor models (Yoshida et al., 1962). Illudin S was found to be the active principle. Recently, the anticancer activity of illudin S and M was reevaluated using human tumor cell lines, and it was demonstrated that they showed selective toxicity to certain tumor cells and were effective against those resistant to conventional chemotherapeutic agents; consequently, it was concluded that they may be of potential therapeutic use. McMorris and coworkers (1992) found that illudins behaved as bifunctional alkylating agents, and an analog, dehydroilludin M, showed an improved therapeutic index. Reinvestigation of

the culture extract of *Omphalotus olearius* led to the isolation of several natural congeners, such as illudin A and B.

Leaianafulvene, isolated recently from *Mycena leaiana* (Berk.) Sacc., also belongs to the cyclohumulanoids (Hartting et al., 1990; Konno, 1995). It is probably derived from illudane skeleton by 1,2-migration of either methyl of the gem-dimethyl group. Leaianafulvene is a pigment of the *M. leaiana* mushroom and is cytotoxic. The cytotoxic activities of leaianafulvine are quite pronounced. A 50% lysis of Ehrlich ascetic tumor (ECA) cells were observed at 2.5 ($\mu g\ ml^{-1}$. In ECA cells incorporation of ^{14}C-thymidine and ^{14}C-uridine into trichloroacetic acid perceptible material (DNA, RNA) was inhibited 50% at 10 ($\mu g\ ml^{-1}$, while the incorporation of ^{14}C-leucine into protein was not affected. In the assay for mutagenic activity (spot test without rat liver microsomes) leaianafulvene significantly increased the number of revertants from *Salmonella typhimurium* TA 100 indicating a mutagenic activity for this compound (Hartting et al., 1990).

IMMUNOMODULATING EFFECTS

Polysaccharides from mushrooms do not attack cancer cells directly, but produce their antitumor effects by activating different immune responses in the host. Immunomodulators work mainly by increasing macrophage activity. Macrophages are white blood cells that "eat up" and destroy pathogens, such as bacteria, yeast cells, virus-infected cells, and so on. They reside in great numbers in the mucous membranes of the body—especially throughout the digestive, urinary, and respiratory tracts. They also play a role in the reticuloendothelial system, which is a system of immune cells centered in the spleen, liver, and lymphoid tissues that engulf stored water and toxic chemicals. Macrophages and other phagocytes can be regarded as the body's protective shield. Stimulating this aspect of the immune system helps protect against colds, flu, infections of any kind.

Currently, it is known that many mushroom polysaccharides from *Tremella fuciformis, Schizophyllum commune, Dendropolyporus umbellatus, Grifola frondosa, Hericium erinaceus, Inonotus obliquus, Ganoderma lucidum, G. applanatum, Lentinus edodes,* and *Flammulina velutipes,* etc. (Tables 1 and 2) have shown an ability to stimulate macrophage activity and strengthen immune system.

Of all the mushroom immunomodulators investigated, the most effective is lentinan, from *Lentinus edodes.* Lentinan appears to act as a host defense potentiator (HDP), which is able to restore or augment the responsiveness of host cells to lymphocytokines (interleukins), hormones, and other biologically active substances by stimulating maturation, differentiation, or proliferation of cells involved in host defense mechanisms (Chihara et al., 1987). HDPs are functionally different from biological response modifiers. Thus, e.g., lentinan is able to increase host resistance against various kinds of cancer and infectious diseases, including AIDS.

The initial interactions of lentinan in the human body or animals are not presently known. However, there is a transitory but notable increase in several serum protein components in the α- and β-globulin region, namely complement C3, hemopexin, and ceruloplasmin (Maeda et al., 1974a,b).

Lentinan stimulates various kinds of natural killer cells (NK-cell)-, T-cell-, B-cell-, and macrophage-dependent immune system responses. The antitumor effect of lentinan is abolished by neonatal thymectomy and decreased by the administration of antilymphocyte serum, supporting the concept that lentinan requires immunocompetent T-cell compartments. The effect of lentinan was also inhibited by antimacrophage agents, such as carrageenan. Unlike other well known immunostimulants, lentinan is in a unique class of DT-cell-oriented assistant, in which macrophages play some part (Maeda and Chihara, 1973; Hamuro and Chihara, 1985; Chihara et al., 1987; Maeda et al., 1988).

For example, lentinan can activate NK-cells *in vitro* in the same concentrations that are achieved in the blood plasma of patients treated clinically with lentinan (Sendo et al., 1981; Tani et al., 1992). NK-cell activity is involved in tumor suppression and while these cells do not stimulate T-killer cell activity, or do so only under certain conditions, they are strong T-helper cell stimulants both *in vitro* and *in vivo* (Herberman and

Nunn-Hargrove, 1981; Aoki, 1984a,b; Z. Liu et al., 1988). Using the blood of healthy donors and cancer patients, some authors (Arinaga et al., 1992a; Tani et al., 1993) have shown that lentinan is able to stimulate peripheral blood lymphocytes *in vitro* to increase interleukin 2-mediated LAK-cell (lymphokine-actived killer cell) and NK-cell activity at levels achievable *in vivo* by administration of clinical doses of lentinan. Lentinan has been shown to inhibit suppressor T-cells activity *in vivo* and to increase the ratio of activated T-cells and cytotoxic T-cells in the spleen when administered to gastric cancer patients undergoing chemotherapy (Miyakoshi and Aoki, 1984; Takahashi et al., 1992).

Many interesting biological activities of lentinan have been reported, including (a) an increase in the activation of nonspecific inflammatory responses such as APP (acute phase protein) production (Maeda et al., 1974b), (b), vascular dilation and hemorrhage *in vivo* (Maeda et al., 1984), (c) activation and generation of helper and cytotoxic T-cells (Dresser and Phillips, 1974; Hamuro, Wagner, and Röllinghoff, 1978; Hamuro and Chihara, 1985; Maeda et al., 1988), (d) augmentation of immune mediators like interleukins 1 and 3, colony stimulating factor(s), and migration inhibitory factor (Zákány et al., 1980b; Fruehauf et al., 1982; Izawa et al., 1983), and (e) increasing the capacity of PBM (peripheral blood mononuclear) cells of patients with gastric cancer, and producing IL-1α, IL-1β, and tumor necrosis factor (TNFα) (Arinaga et al., 1992b). Table 4 lists the various biological activities of lentinan as HDPs.

In an *in vivo* study in rats with peritonitis, combined lentinan-gentamicin treatment led to a significantly better survival rate than in the control group. Lentinan activated the peritoneal macrophages, secretory activity of active oxygen and produced cytokines that enhanced the ability of PMNs (=polymorphonuclear leukocytes) to produce active oxygen, which has a bactericidal effect (Shen et al., 1993). Lentinan also increases peritoneal macrophage cytotoxicity against metastatic tumor cells in mice, but was not effective against a highly metastatic tumor type (Ladanyi et al., 1993). Some patients treated with lentinan for carcinomatous pleuritis or carcinomatous peritonitis, have improved with the disappearance of malignancy (Yoshino et al., 1989). Lentinan can activate the normal and alternative pathways of the complement system and can split C3 into C3a and C3b, enhancing macrophage activation (Aoki, 1984b).

Many biological reactions are accelerated and induced by lentinan, including the very important phenomenon of the infiltration of eosinophils, neutrophils, and granulocytes around target tissues. Figure 3 shows early responses initiated by lentinan and possible pathways for inflammatory reactions (Table 4).

Lentinan's immune-activating ability may be linked with its modulation of hormonal factors that are known to play a role in tumor growth. Aoki (1984a) showed that the antitumor activity of lentinan is strongly reduced by administration of thyroxin or hydrocortisone. Lentinan can also restore a tumor-specific antigen-directed delayed-type hypersensitivity reaction (DTHR).

Lentinan is not formally included among the nonspecific immunostimulants (RES stimulants), but it augments the induction of antigen-specific cytotoxic T-lymphocytes, macrophages, and other nonspecific immune responses.

Possible immune system regulating actions of lentinan are summarized by Chihara (1981) in Figure 4.

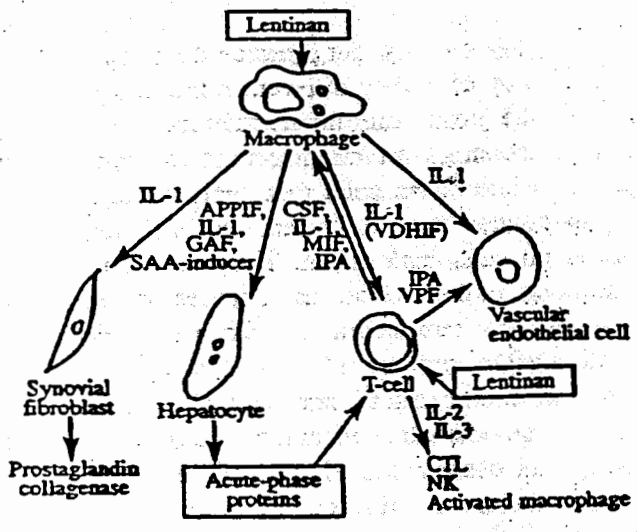

FIGURE 3. Early responses initiated by Lentinan and a possible pathway for inflammatory reactions: APPIF, acute phase protein inducing factor; VDHIF, vascular dilatation and hemorrhage inducing factor; CFS, colony stimulating factor; MIF, migration inhibitory factor; GAF, glucocorticoid antagonizing factor; SAA, serum amyloid A; IPA, plasminogen activator inducer; VPF, vascular permeability factor; CTL, cytotoxic T-lymphocytes; NK, natural killer cells (Mizuno et al., 1995a).

TABLE 4
Biological Activities of Lentinan as Host Defense Potentiators (Mizuno et al., 1995a)

Early reactions peaking 3–24 hrs after treatment	Late reactions peaking 3–7 days after treatment
IL-1 production-inducing factor	IL-1
IL-3	CSF (T-cell)
CSF (macrophage)	Haptoglobin
Acute-phase protein-inducing factor (IL-6)	Ceruloplasmin
Vascular dilatation-inducing factor	Vascular dilatation
Lysozyme activity	Serum amyloid P, C3, C5, factor B
Eosinophil infiltration around cancer tissue	Macrophage infiltration around cancer tissue
	1. T-Cell Participation
Neonatal thymectomy	Abolished antitumor effect
Antilymphocyte serum	Decreased antitumor effect
Helper T-cell *in vitro*	No observed effect
Helper T-cell *in vitro*	Activation or restoration
Cytotoxic T-cell *in vitro*	Augmentation of IL-2 responsivity
Cytotoxic T-cell *in vitro*	Increased responsivity to IL-2
Suppressor T-cell	No induction
Migration inhibitory factor producing T-cell	Activation
IL-3	Increased production
T-cell-derived CSF	Increased production
	2. Natural Killer Cell Participation
NK cell *in vitro*	No effect
NK cell *in vivo*	Activation in C3H/He, but not BALB/c mice
Augmented NK activity by poly I:C or IL-2	More activation when used in Lentinan-treated mice
	3. Macrophage Participation
Antimacrophage agent	Decreased tumor suppressive effect by carrageenan or silica
Macrophage phagocytosis *in vitro*	No effect
Macrophage phagocytosis *in vivo*	Slight activation
Macrophage cytotoxic *in vitro*	No observation
Macrophage cytotoxic *in vivo*	Activation
Macrophage suppressive *in vivo*	Decreased prostaglandin E release from macrophages
IL-1	Increased production *in vitro* and *in vivo*
	4. Antibody Formation
Antibody for SRBC	Increased production with T cells
Antibody-dependent cell mediated cytotoxicity	Activation
	5. Cellular Reactions
Delayed hypersensitivity	Stimulation or restoration
Local cellular reaction	Increased around tumor
Granuloma formation	Increase around schistosoma
	6. Complement Participation
Alternative pathway	Activation
Classical pathway	Activation
C-3 absolute value	Increased production
Total Complement value	Increased production
C-3 splitting activity	Activation

CARDIOVASCULAR AND HYPERCHOLESTEROLEMIA EFFECTS

The major cause of death in the Western countries is coronary artery disease. A primary risk factor is hypercholesterolemia, which contributes to hardening of the arteries. In humans, 50% or more of the total cholesterol is derived from *de novo* synthesis (Rosenfeld, 1989; Steinberg et al., 1989). Clinical intervention studies have demon-

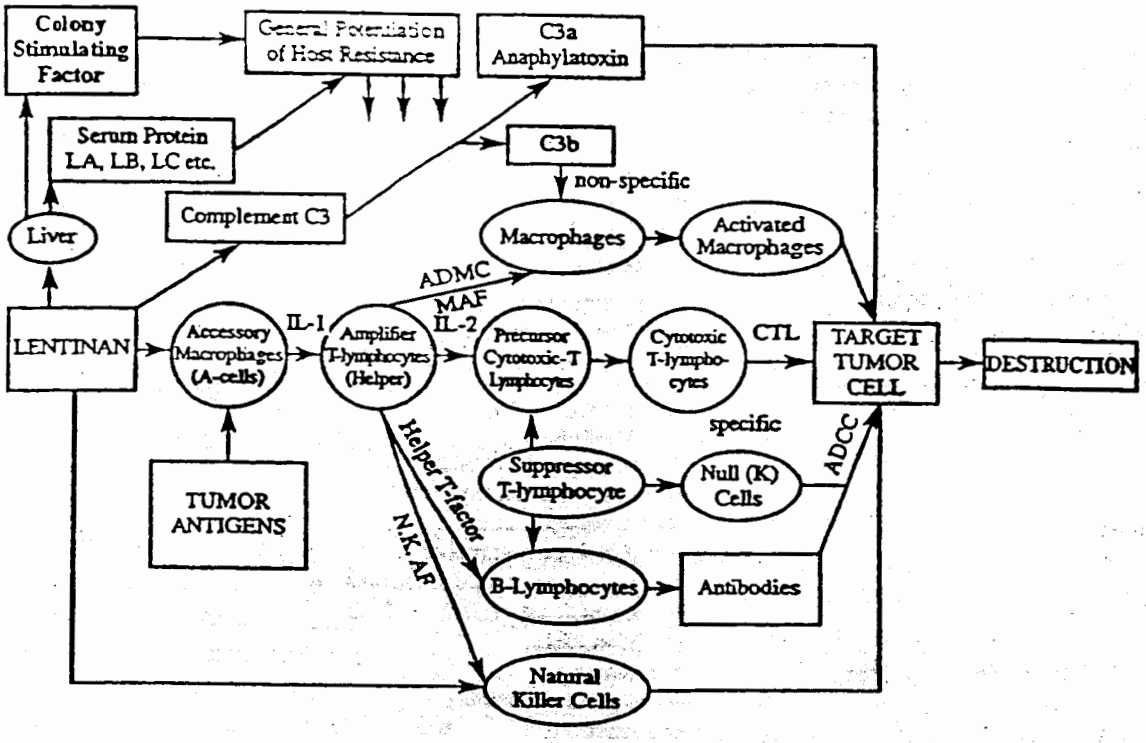

FIGURE 4. Possible mode of action of lentinan as host defense potentiator (Chihara, 1981).

strated the therapeutic importance of correcting the hypercholesterolemia. The initial step in lowering cholesterol is a special diet low in fat and saturated fatty acids and rich in crude fibers.

Drug therapy is the next step. The best known pharmacologic agent that was approved in 1987 is lovastatin (mevinolin) and its analogues (Endo, 1988). This low molecular weight substance is the competitive inhibitor of HMG CoA reductase, the key enzyme in cholesterol metabolism that catalyzes the reduction of HMG CoA into mevalonate.

The best known organisms for potential producers of lovastatin from edible higher Basidiomycetes mushrooms are species of the genus *Pleurotus* (Gunde-Cimerman et al., 1993a,b; Gunde-Cimerman and Cimerman, 1995). The presence of the inhibitor was determined in four species: *P. ostreatus, P. cornucopiae, P. eryngii,* and *P. sapidus*. The highest content of lovastatin was found in the fruiting bodies of the *P. ostreatus*. The appearance of the inhibitor during the development of fruiting bodies was followed and lovastatin was found in the vegetative mycelium, in the primordia, and in different parts of fruiting bodies of different sizes; less lovastatin was found in stipes when compared with pileus (Fig. 5) or in nature stages in the lamellae and basidioiospores (Gunde-Cimerman and Cimerman, 1995).

It was shown that lovastatin at the beginning of mushroom growth is unfortunately distributed in small fruit bodies, and there are no substantial differences between the pileus and the stipe. During fruit body growth, the majority of lovastatin is first transferred to the pileus and later the lamellae. Mature fruiting bodies have a diameter of approximately 15 cm and disperse large amounts of basidiospores. These contain less lovastatin in the lamellae when compared with the smaller, 10 cm diameter, less mature fruiting bodies. These data were the basis of the conclusion that a part of the lovastatin in the completely mature mushroom is probably being transferred to the basidiospores and that in less mature mushrooms this transfer is still incomplete (Gunde-Cimerman and Cimerman, 1995).

In a series of experiments conducted by Bobek et al. (1991a,b, 1993), it has been found that the addition of 2% to 4% of *P. ostreatus* to the hyperlipidemic diet efficiently prevented accumulation of C and triacly-glycerols in both sera and livers of animals with exogenous, endogenous, or genetically induced hyperlipemia. VLDL cholesterol had the dominant role in the reduction of

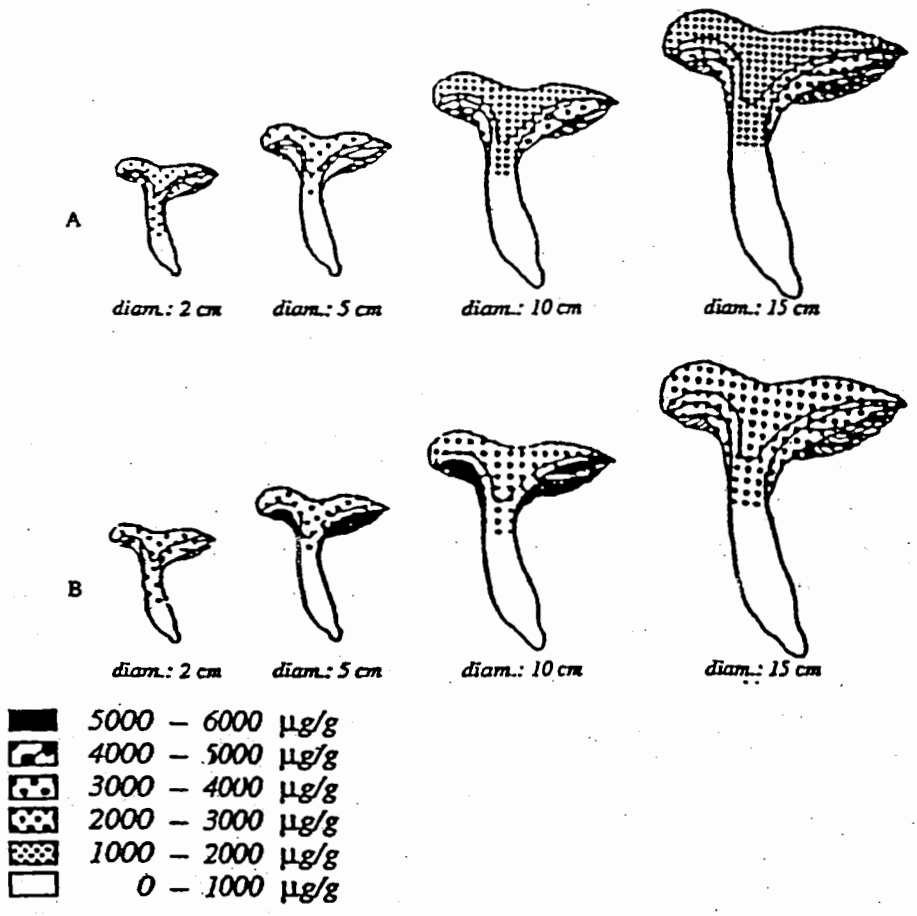

FIGURE 5. Lovastatin content in different parts of *P. ostreatus* sporocarps of different sizes. The extraction was performed with methanol:water (A) and with N_2 + methanol:water (B) (Gunde-Cimerman and Cimerman, 1995).

serum C up to 80% induced by the whole mushroom or its water and 30% ethanol extracts. The authors attributed this effect to the fiber pulp complex of the oyster mushroom, which limits the resorption of C and gastrointestinal tract, and to an undefined substance which influences, as well, metabolism outside the phase of resorption (Bobek et al., 1991a,b, 1993). Ryong et al. (1989) have tested alcohol and water extracts of 20 different edible mushrooms in tissue primary culture of cells isolated from atherosclerotic action. Four mushrooms, including *P. cornucopiae*, had marked effects *in vitro*. Consumption of these mushrooms also decreased atherogenic effects by 20% to 40% in sera collected from coronary heart disease patients. In these experiments, the effect of dietary fibers was excluded and the efficiency was attributed to an unknown active component (Ryong et al., 1989). Authors suggest that this unknown substance is lovastatin which can be found in high quantities in the fruiting bodies of various cultured *Pleurotus* species. Therefore, mature fruiting bodies of *P. ostreatus* could be recommended for consumption as a natural cholesterol lowering agent. Lovastatin appears early in the life cycle of the mushroom, in the mycelia from which primordia are being formed.

It is known that *Lentinus edodes* is able to lower BSC via a factor known as eritadenine (also called "Lentinacin" or "Lentysine"). Eritadenine was isolated from an 80% ethanol extract of Shiitake mushroom fruiting bodies by absorption on an Amberlite IR-120 (H^+) column, followed by elution with 4% NH_4OH (Chibata et al., 1969). A crystalline product had the following properties: mp 261°–263°C, $C_9H_{11}O_4N_5$, MW 253, λ 261.5 nm (ε = 14,508). Na salt mp 266°–268°C (decomposition), $[\alpha]_D$ + 45.5° (C=1, H_2O). Hydrolysis in

6N HCl at 110°C for 72 h yielded glycine and new amino acid. Eritadenine apparently reduces serum cholesterol in mice. Its action is not inhibition of cholesterol biosynthesis, but rather the acceleration of excretion of ingested cholesterol and its metabolic decomposition (Makita et al., 1972, cited in Mizuno, 1995a,b).

Apparently, eritadenine reduces BSC in mice, not by the inhibition of cholesterol biosynthesis, but by the acceleration of the excretion of ingested cholesterol and its metabolic decomposition (S. Suzuki and Oshima, 1974, 1976; Higushi et al., 1978; Yagishita et al., 1977, 1978). Eritadenine has been shown to lower blood levels of cholesterol and lipids in animals (Yamamura and Cochran, 1976). Added to the diet of rats, eritadenine (0.005%) caused a 25% decrease in total cholesterol in as little as one week (Chibata et al., 1969). The cholesterol-lowering activity of this substance is more pronounced in rats fed a high fat diet than in those on a low-fat diet (Rokujo et al., 1969). Although feeding studies with humans have indicated a similar effect, further systematic research is needed. S. Suzuki and Ohshima (1974, 1976) have shown that dietary shiitake mushroom lowered BSC levels. Various studies have confirmed (Kimoto et al., 1976; Kabir and Kimura, 1989) that shiitake mushroom can lower both blood pressure and free cholesterol in plasma, as well as accelerate accumulation of lipids in the liver, by removing them from circulation.

Nucleic acid compounds in *L. edodes* extract have been shown a strong platelet agglutination inhibitive effect (antithrombotic activity). An extract of *L. edodes* with antithrombotic activity was studied by high-performance liquid chromatography (HPLC). ATP, ADP, UDPG, 5'-GMP, 5'-UMP, 5'-CMP, 5'-AMP, uridine, eritadenine, and deoxylentinacin were identified. More antithrombotic activity was shown by 5'-AMP, 5'-GMP, eritadenine, and deoxylentinacin (Hokama and Hokama, 1981; Kabir and Kimura, 1989).

Auricularia auricula-judae has shown the following effects and activities in studies on mice and rats: anticoagulation, lowered total cholesterol, triglyceride, and lipid levels (Chen, 1989; Sheng and Chen, 1990); and antiaggregatory activity on blood platelets, which might make it beneficial for coronary heart disease (Agarwal et al., 1982). This mushroom is traditionally used as an immune tonic.

Tremella fuciformis polysaccharides and spore extracts have demonstrated antilipemic activity. *T. fuciformis* lowered the LDL-cholesterol in rats fed the preparation, which also contained butter, sugar, and egg yolks, by 30% over controls (Nakajima, et al., 1989). *T. fuciformis* polysaccharides prolonged thrombus formation, reduced throumbus size, reduced blood platelet adherence, blood viscosity, and positively influenced other blood coagulation parameters of survival in mice (Sheng and Chen, 1990).

Animal studies on *Armillriella mellea* demonstrated that it decreases heart rate, reduces peripherial and coronary vascular resistance, and increases cerebral blood flow (Chang and But, 1986). AMG-1-a compound isolated from this mushroom exhibits a cerebral-protective effect (Watanabe et al., 1990), and increases coronary oxygen efficiency without altering blood pressure (Y. Zhang et al., 1985).

Grifola frondosa reduced blood pressure in rats without changing plasma HDL levels (Kyoto et al., 1988). Adachi and coworkers (1988) found a blood pressure lowering effect with the powder of *G. frondosa* fed to hypertensive rats in their normal food. The effect was of rapid onset, short-lived, and dose dependent. An aqueous extract of *G. frondosa* also reduced serum cholesterol levels in rats (Mizuno, 1997).

A glycoprotein obtained from submerged cultured mycelial biomass of *Trametes* sp. showed activity (in animal and *in vitro* tests) against experimental hypertension and thrombosis. The protein inhibits blood platelet aggregation and is antihyperlipemic and antiarrhythmic (Ikuzawa, 1985). *Trametes versicolor* has been shown to lower serum cholesterol in animals (Yagishita et al., 1977). PKS (β-glucan-protein) from *T. versicolor* has been used in clinical studies. Tsukagoshi et al. (1984) have reported that PSK causes a significant decrease in LDL cholesterol in hyperlipidemia (stage IIa) patients.

The *Volvariella volvacea* cardioactive proteins are known to lower blood pressure (Cochran, 1978; Yao et al., 1998).

ANTIVIRAL, ANTIBACTERIAL, AND ANTIPARASITIC EFFECTS

Different substances occurring in higher Basidiomycetes are effective against various kinds

TABLE 5
Spectrum of Mycoses and Mycetes Related to AIDS

Mycoses	Causative organisms/saprophytes	Main target issues	Incidence %
Dermatophytoses	Anthropophillic dermatophytes: *Trichophyton rubum, Epidermophyton floccosum*, and others	Skin and appendages	80–90
Candidoses	*Candida albicans, C. tropicalis, C. parapsilosis, C. guilliermondii, C. krusci*, and other species	Oral cavity; skin; vagina; oesophagus	70–90 25–30 20–25 10–15
Torulopsidoses	*Torulopsis glabrata, T. candida*	Intestinal tract; Parasitic; Saprobic	 1–2 70–90
Trichosporosis	*Trichosporon cutaneum*	Systemic, mainly brain	<1
Cryptococcosis	*Cryptococcus neoformans*	Brain (lungs, skin)	5–7
Histoplasmosis "American"	*Histoplasma capsulatum*	Lungs; lymphatic system	1(–2)
"American"	*Histoplasma duborsii*	Skin; lungs; lymphatic system	1(–5)
Coccidioidomycosis	*Coccidioides immitis*	Lungs; brain	Sporadic
Aspergillosis	*Aspergillus fumigatus, A. flavus, A. nidulans, A. glaucus, A. terreus* and other species	Respiratory tract; sinuses; intestinal tract; brain; liver; kidneys	Sporadic
Blastomycosis ("N. American Blastomycosis")	*Blastomyces dermatitidis*	Lungs; skin; bones	Sporadic
Paracoccidioidomycosis ("S. American Blastomycosis")	*Paracoccidioides brasiliensis*	Lungs; oral/nasal cavity; gastrointestinal mucosa; lymph vessels/nodes; skin	Sporadic
Sporotrichosis	*Sporothrix brasiliensis*	Skin; lymph vessels; brain	Sporadic
Mycoses caused by opportunistic molds	Various species of *Fusarium, Paecilomyces, Alternaria, Drechslera, Mucor, Phizopus, Absidia, Pseudollescheria, Penicillium* species and other molds	Various: lungs; brain; bones; sinuses; skin and other tissues/ organs	Sporadic

Source: Male, 1991.

of viral, bacterial,[3] and parasitic infections, including AIDS (Bose, 1946, 1955; Hervey, 1947; Brain, 1950; Benz, 1959; Broadbent, 1964; Benedict and Brady, 1972; Cochran, 1978; K. Chang, 1981; Kanai and Kondo, 1981; Akiyama et al., 1981; Aoki, 1984a; Tochikura et al., 1987a,b, 1988; H. Suzuki et al., 1986, 1989, 1990; Dudka and Wasser, 1987; Ying et al., 1987; Sorimachi et al., 1990; Jong, Birmingham and Pai, 1991; Koga et al., 1991; Irinoda et al., 1992; Sarkar et al., 1993; Hobbs, 1995; Wasser and Weis, 1997a,b, 1999). An important area of research of different substances occurring in higher Basidiomycetes mushrooms deals with their ability to mobilize the body's humoral immunity to ward off bacterial, viral, or parasitic (including microfungi) infections resistant to antibiotics. Many cancer and AIDS patients die of opportunistic infections because of immunodysfunction. The spectrum of mycoses and mycetes seen in AIDS are summarized in Table 5. It is extremely important to pro-

[3]Complete data till 1978 about antibacterial effects of medicinal mushrooms are published in an article by Cochran (1978).

tect AIDS patients from these opportunist infections. According to Tochikura et al. (1987a,b) lentinan, from *Lentinus edodes,* when used in combination with azidothymidine (AZT), suppressed the surface expression of human immunodeficiency virus (HIV) on T-cells more than AZT did alone. Lentinan and sulfated lentinan exhibited a potent anti-HIV activity, resulting in inhibition of viral replication and cell fusion. AIDS therapy must include a strategy to enhance the immune system. Among the various therapeutic approaches used in HIV patients, prevention of the development of AIDS symptoms in carriers should be stressed. This can be realized by use of HDPs such as lentinan, or its related substances. For example, LEM is also useful in the treatment of AIDS. It has been shown to inhibit HIV infections of cultured human T-cells (Iizuka, 1990), and it potentates the effects of AZT against viral replication *in vitro.* The mechanism of its action is not known for certain, but the extract was found to activate macrophages and stimulate the production of interleukin 1 (Tochikura et al., 1988).

Water-soluble lignans from EP3 and EPS4 from *L. edodes* mushroom mycelium has shown antiviral and immunomodulating effects (Hanafusa et al., 1990). A water-soluble extract of mycelium known as JLS and JLS-18 has the ability to block the release of *herpes simplex* virus type 1 in animals (Sarkar et al., 1993). JLS-18, consisting of 65% to 75% lignin, 15% to 30% polysaccharides, and 10% to 20% protein, has inhibited the herpes virus both *in vitro* and *in vivo* (Koga et al., 1991).

In addition, lentinan has shown (a) antiviral activity in mice against VSV (vesicular stomatitis virus), encephalitis virus, Abelson virus, and adenovirus type 12; (b) stimulated nonspecific resistance against respiratory viral infections in mice; (c) conferred complete protection against an LD75 challenge dose of virulent mouse influenza A/SW15; (d) enhanced bronchoalveolar macrophage activity; (e) increased resistance to the parasites *Schistosoma japonicum* and *Sch. mansoni;* (f) exhibited activity against *Mycobacterium tuberculosis* bacilli resistant to antituberculosis drugs, *Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus, Candida albicans, and Saccharomyces cerevisiae;* and (g) increased host resistance to infections with the potentially lethal *Listeria monocytogenes.* Antibacterial polyacetylene compounds, centinamycin A and B, have also been identified in shiitake mushroom. Table 6 summarizes the antiinfective activity of lentinan and its derivatives.

Eritadenine, a compound that effects cholesterol metabolism, also processes antiviral properties (Cochran, 1978).

It should be noted, that a protein fraction of shiitake mushroom fruiting bodies, labeled FBP (fruiting body protein), prevented infection of plants with tobacco mosaic virus (TMV). The binding of the virus to the plant cells was inhibited by FBP (Wasser and Weis, 1997b).

Armillariella mellea shows antibiotic action *(in vitro)* against the pathogenic bacteria *Staphylococcus aureaus, Bacillus cereus,* and *B. subtilis.* Armillaric acid, recently isolated from *A. mellea,* inhibits Gram-positive bacteria and yeasts (Obuchi et al., 1990). *In vitro* studies with the mycelial extract of *A. mellea* also showed significant antibacterial activity against Gram-positive bacteria (Donnelly et al., 1985, 1986; Donnelly and Hutchinson, 1990).

The polysaccharide schizophyllan from *Schizophyllum commune* demonstrated protective effects against *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli,* and *Klebsiella pneumoniae* infections in mice (Komatsu et al., 1973; Cochran, 1978).

An alcoholic extract of *Dendropolyporus umbellatus* has demonstrated antibiotic actions *in vitro* against *Staphyllococcus aureus* and *Escherichia coli* (Chang and But, 1986).

Species of the genus *Trametes* contain coriolin, an antibiotic that has been shown to inhibit Gram-positive bacteria and *Trichomonas vaginalis* (Takeuchi et al., 1969; Ying et al., 1987).

Sesquiterpenes velleral and isovelleral isolated from *Lactarius vellereus* have strong antibacterial (against *Escherichia coli*) and anifungal (*Candida utilis*) activity (Sterner et al., 1985).

Ganoderma species (*G. lucidum G. applanatum, G. oregonense*) has shown a high degree of activity against *Staphyllococcus, Streptococcus* species, and *Bacillus pneumoniae,* perhaps because of increased immune system activity and its antiviral effect, induced by interferon production (Yoon et al., 1994; Wasser and Weis, 1997a).

Agaricus campestis produces campestrin

TABLE 6
Antiviral, Antibacterial, and Antiparasitic Effects of Lentinan and Its Derivatives

Infections	Polysaccharide	Effects
Viruses		
Andenovirus Type 12	Lentinan	Antitumorigenic
Abelson virus	Lentinan	100% cure *in vivo*
VSV-*encephalitis virus*	Lentinan	100% cure *in vivo*
Herpes simplex I & II	Lentinan	Increased resistance
Herpes simplex I & II	JLS-18	Increased resistance
Human immunodeficiency virus	Lentinan	Combined with AZT
(HIV-I)	Lentinan sulfate	Inhibited infectivity
HIV	LEM	Combined with AZT
HIV	EP3	Inhibited infectivity
HIV	EPS4	Inhibited infectivity
Bacteria	Lentinan	
Bacillus subtilis	Lentinan	100% cure *in vivo*
Mycobacterium tuberculosis	Lentinan	Relapse prevention
Listeria monocytogenes	Lentinan	100% cure *in vivo*
Staphyllococcus ayrsus	Lentinan	100% cure *in vivo*
Parasites		
Schistosoma mansoni	Lentinan	Inhibition *in vivo*
Schistosoma japonicum	Lentinan	Inhibition *in vivo*
Mesocestoides corti	Lentinan	Granuloma formation
Fungi		
Candida albicans	Lentinan sulfate	100% cure *in vivo*
Trichophyton sp.	Lentinan sulfate	100% cure *in vivo*

Source: Kanai and Kondo, 1981; Hamada, 1981; K. Chang, 1981; Aoki, 1984b; Irinoda et al., 1992; Mizuno, 1995a,b; Hobbs, 1995, with our additions.

which is effective against Gram-positive and Gram-negative bacteria (Bose, 1955). *A. bisporus* and *A. arvensis* are resistant to Gram-positive and Gram-negative bacteria (Ying et al., 1987). *A. xanthoderma* contains antibiotics psalliotin (4-hydroxybenzenediazonium), which was separated from culture fluid. It is inhibitors against Gram-positive bacteria (Dornberger et al., 1986).

Hepatoprotective Effects

In the last 15 to 20 years, medicinal mushrooms have been subject to various laboratory studies with animals as well as clinical studies with humans. They are thought to be beneficial for a wide variety of hepato disorders, including hepatitis. Hepatitis B, for example, is spreading quietly through blood contact, sex, and birth. The virus currently infects 350 million people worldwide, according to the World Health Organization (WHO). Most do not have symptoms. But in a fraction of cases, those infections lead to liver failure or liver cancer, deadly complications that each year kill more than 1 million people around the world (Marshall, 1998).

Sugano and coworkers (1982) showed that injection of LEM from *Lentinus edodes* slowed the growth of cancerous liver tumor in rats. A polysaccharide fraction from the Shiitake mushroom demonstrated liver protective action in animals, as well as the ability to improve liver function and enhance the production of antibodies to hepatitis B (Lin and Huang, 1987; Miroguchi et al., 1987; Amagase, 1987; Mizuno, 1995a,b).

Lentinan has shown favorable results in treating chronic persistent hepatitis and viral hepatitis B. Forty patients with chronic viral hepatitis B and seropositive for HBe antigenemia were given 6 grams of LEM daily (orally) for four months. The study focused on the number of patients seroconverting from HBe antigen positive to anti-HBe positive, which was 25% after LEM therapy and was higher in patients with chronic active hepati-

tis (36.8%). In addition, 17 patients (43%) became seronegative for HBe antigen. Liver function tests improved even in patients who remained serapositive, had raised plasma albumin, and adjust protein metabolism (Zhu et al., 1985; Lin et al., 1987; Amagase, 1987).

In combination with polysaccharides from Reishi mushroom (*Ganoderma lucidum*) and Turkey tails (*Trametes versicolor*), lentinan has improved SGPT and completely GPT levels in the livers of mice with toxic hepatitis (Zhang and Luan, 1986; Wasser and Weis, 1997a). Crude extracts of Shiitake mushroom cultures have demonstrated liver-protecting actions (Y. Lin, 1987; Hobbs, 1995; Wasser and Weis, 1997b).

Reishi mushroom has shown favorable results in treating hepatitis, especially in cases without severe impairment of liver functions. In a study of 355 cases of hepatitis B treated with the Wulingdan pill, which includes the fruiting body of Reishi mushroom, 92.4% of the patients had positive results (Chang and But, 1986; Yan et al., 1987). In a clinical report from the MARA Institute of Technology (Malaysia), a lyophilized extract of Reishi mushroom was beneficial in alleviating the symptoms of patients suffering from hepatitis B by significantly reducing the SGOT and SGPT levels and leading to seroconversion after three months of administration (Soo, 1994).

A hepatoprotective effect was found in the extract of the Maitake mushroom (*Grifola frondosa*) when given to rats (300mg/kg) in a hepatitis model (paracetamol-induced) (Lee et al., 1992; Ooi et al., 1993).

A polysaccharide derivative from the alcoholic extract of *Dendropolyporus umbellatus* had demonstrated hepatoprotective effects in mice (Lin and Wu, 1988). In 39 patients with cirrhosis of the liver with ascites who failed to respond to other treatment, a modified traditional herbal formula "Wu Ling San" with 2.3 g of fruit body of *D. umbellatus*, was reported to bring about a clinical cure in 17 cases, marked improvement in 7, and significant improvement in 12 (Bensky and Barolet, 1990).

Schizophyllan (SPG) from Split Gill mushroom (*Schizophyllum commune*) indicated (*in vitro*) that chronic hepatitis B patients could benefit from SPG, because SPG can enhance immunological responsiveness to the virus, particularly in interferon-g production (Kakumu et al., 1991).

Pharmacological activities that may be the result of the protein bound polysaccharide (PSK) from turkey tail mushroom (*Trametes versicolor*) support hepatic function, and indicate on possible prevention of liver cancer (Wang, 1989). In China, *T. versicolor* is considered useful for hepatitis B and chronic active hepatitis (Ying et al., 1987). *Tremella fuciforms* polysaccharides and spore extracts have demonstrated liver-protective (p.o.) activities (Zhou et al., 1989).

The chemically modified form of pachyman (kind of polysaccharides), carboxymethylpachymaran from *Wolfiporia cocos* was reported to produce an "immediate cure" of chronic viral hepatitis in human clinical studies. Following two courses of the carboxymethylpachymaran (60 mg, i.m.), no side effects were found and a normalization was seen in immune functions, it has also been useful to treat arrhythmia (Guo et al., 1984; Ding, 1987).

Antidiabetic Effect

Diabetes is one of the world's oldest known diseases. At present there are approximately 250 million people with diabetes worldwide. In recent years, diabetes has become the fourth leading cause of death by disease in the United States, for example, and the leading cause for the development of related disorders such as kidney disease, vessel disease, blindness, impotence, and gangrene (Campbell, 1997).

Depending on the nature of the disease, insulin and certain synthetic drugs like sulphonylureas, biguanidines, and acarbose, are widely used in its treatment. However, in recent years evidence of cases of "insulin resistance" and the occurance of side effects when some of the conventional drugs are administered for prolonged periods of time, have triggered the search for safe and effective alternatives. Several plant and mushroom extracts and isolated substances have been examined for antidiabetic activity, with a view to identify alternative treatment strategies for diabetes (Hobbs, 1995; Hackman, 1996; Majeed, 1998).

Maitake or Hen-of-the-Woods mushroom (*Grifola frondosa*) has a antidiabetic effect. Glu-

cose tolerance tests were conducted on mice, model animals of NIDDM (noninsulin dependent diabetes mellitus). The elevated blood glucose levels after 15 min. and 30 min. of maitake fed group were 0.64 times and 0.76 times those of the control group respectively, indicating inhibition of a significant blood glucose increase. Insulin receptor capability of liver cells then was examined using the liver perfusion method. Downward regulation was observed among the maitake-fed group, while a state of tolerance was seen among the control group. Next, glucose absorption activity at the enteron and sucrase activity at the mucosa of the small intestine were examined. Neither inhibition of glucose at the small intestine nor inhibition of sucrase activation was observed with Maitake powder or X-fraction was administered. These results suggested that Maitake anti-diabetic activity is not related to the inhibition of glucose absorption at the entereon, but with the process of metabolism of absorbed glucose (Kubo and Namba, 1996). Feeding maitake fruiting body powder (20% of the diet for 21 days at 1 g/day) to genetically diabetic rats has also lowered blood glucose levels in a noninsulin dependent diabetes mellitus model (Kubo et al., 1994). The blood glucose lowering effect was said to be a result of a high (150,000) molecular-weight glycoprotein from the hot water decoction and from compounds of an ether-ethanol extract.

Gandoderan A and B, glucans of the fruit body of *Ganoderma lucidum*, significantly reduced plasma sugar levels in mice (Hikino et al., 1985). A coreolan (β-glucan-protein) obtained from submerged cultured mycelial biomass of *Trametes versicolor* showed activity (in animal and *in vitro* tests) against experimental diabetes (Ikuzawa et al., 1985).

ACKNOWLEDGMENTS

The authors are grateful to Professor Takashi Mizuno (Shizuka University, Japan) and Dr. N. Gunde-Cimerman (National Institute of Chemistry, Slovenia) who kindly provided us with reprints and information on medicinal mushrooms. We are grateful to Dr. P.A. Volz (US) and Dr. C. Levensohn (US) for skillful comments on the contents and style of the manuscript. We also thank Ms. Sabrina Rodriguez for help in preparing the text for publication.

REFERENCES

Adachi K. et al. 1988. Blood pressure-lowering activity present in the fruit body of *Grifola frondosa* (Maitake). *Chem Pharm Bull*, 36, 1000–1006.

Agarwal K. C. et al. 1982. Inhibition of human and rat platelet aggregation by extracts of Mo-er (*Auricularia auricula*). *Thromb Haemostas (Stuttgart)*, 48, 162–165.

Akiyama Y. et al. 1981. Immunological characteristics of anti-tumor polysaccharides lentinan and its analogues, an immune adjuvants. In: Manipulation of host defense mechanisms, Aoki T. et al., ed., Excerpta Medica (International Congress Series 576). Amsterdam. 227–243.

Aoki T. 1984a. Antibodies to HTLV I and HTLV III in sera from two Japanese patients, one with possible pre-AIDS. *Lancet*, Oct. 20, 936–937.

Aoki T. 1984b. "Lentinan." In: Immune modulation agents and their mechanisms, Fenichel R. L. and Chirgis M. A., eds., *Immun Stud*, 25, 62–77.

Amagase H. 1987. Treatment of hepatitis B patients with *Lentinus edodes* mycelium. Proceedings of the XII International Congress of Gastroenterology, Lisbon, p. 197.

Arai Y. et al. 1971. Effect of immunosuppressive agents on antitumor action of *Lentinan*. *Japan J Cancer Res (Gann)*, 62, 131–134.

Arinaga S. et al. 1992a. Enhanced induction of lymphokine-activated killer activity after Lentinan administration in patients with gastric carcinoma. *Int J Immunopharmacol*, 14, 535–539.

Arinaga S. et al. 1992b. Enhanced production of interleukin 1 and tumor necrosis factor by peripheral monocytes after Lentinan administration in patients with gastric carcinoma. *Int J Immunopharmacol*, 14, 43–47.

Benedict R. C. and Brady L. R. 1972. Antimicrobial activity of mushroom metabolites. *J Pharm Sci*, 61, 180–182.

Bensky D. and Barolet R. 1990. Chinese herbal medicine: Formulas and strategies. Easte and Press, Seattle, WA.

Benz G. 1959. Marasin, an antibiotic polycetylene isolated from culture medium of *Marasmius ramealis*. *Arkiv Kemi*, 14, 305–321.

Bobek P. et al. 1991a. Cholesterol-lowering effect of the mushroom *Pleurotus ostreatus* in hereditary hypercholesterolomic rats. *Ann Nutr Metab*, 35, 191–195.

Bobek P. et al. 1991b. Effect of mushroom *Pleurotus ostreatus* and isolated fungal polysaccharide on serum and liver lipids in Syrian hamsters with hyperlipoproteinemia. *Nutrition*, 7, 105–108.

Bobek P., Ozdin L., and Kuniak L. 1993. Influence of water and ethanol extracts of the oyster mushroom (*Pleurotus ostreatus*) on serum and liver lipids of the Syrian hamster. *Nährung*, 37(6), 571–575.

Bog-Hansen C. T. and Freed J. L. D. 1988. Lectins: Biology, biochemistry, clinical biochemistry. Sigma, St. Louis.

Bose S. R. 1946. Antibiotics in a polypours (*Polystictus sanguineus*). *Nature*, 158, 292–296.

Bose S. R. 1955. Campestrin, the antibiotic of *Psalliota campestris*. *Nature*, 175, 468.

Brain P. W. 1950. Antibiotics produced by fungi. *Bot Rev*, 17, 357–430.

Breene W. 1990. Nutritional and medicinal value of special mushrooms. *J Food Prod*, 53, 883–894.

Brekhman I. I. 1980. Man and biologically active substances. Pergamon Press, New York.

Broadbent D. 1964. Antibiotics produced by fungi. *Bot Rev*, 32, 219–242.

Campbell R. K. 1997. Type II Diabetes mellitus: Disease state management. *Retail Pharmacy News*, October, 15–18.

Cao X. et al. 1989. Immunostimulation by polysaccharide of *Lentinus edodes* in mice. *Zhongguo Zhongyao Zazhi*, 14, 110–111.

Chang H. M. and But H. P. P. 1986. Pharmacology and applications of Chinese Materia Medica Vol. 1, World Scientific, Singapore.

Chang K. S. S. 1981. Lentinan-mediated resistance against VSV-encephalitis, Abelson virus-induced tumor, ad trophoblastic tumor in mice. In Manipulation of host defense mechanisms, Aoki T. et al., eds. Excerpta Medica (International Congress Series 576), Amsterdam.

Chang S. T. 1993. Mushroom biology: The impact on mushroom production and mushroom products. In Mushroom biology and mushroom products, Chang S. T., Buswell J. A., and Chiu S. W. et al., eds. Chinese Univ. Press, Hong Kong, 3–20.

Chang S. T. 1996. Mushroom research and development-equality and mutual benefit. In Mushroom biology and mushroom products, Royse, ed., Pennsylvania State University Press, 1–10.

Chen Q. 1989. Antilipemic effect of polysaccharides from *Auricularia auricula*, *Tremella fuciformis*, and *Tremella fuciformis* spores. *Zhongguo Yaoke Daxue Xuebae*, 20, 344–347.

Chibata I. et al. 1969. Lentinacin: A new hypocholesterolemic substance in *Lentinus edodes*. *Experientia*, 25, 1237–1238.

Chihara G. 1978. Antitumor and immunological properties of polysaccharides from fungal origin. *Mushr Sci*, 9(2), 797–814.

Chihara G. 1981. The antitumor polysaccharide Lentinan: An overview. In Manipulation of host defense mechanisms, Aoki T. et al., ed., Excerpta Medica (International Congress Series 576), Amsterdam.

Chihara G. 1992. Immunopharmacology of Lentinan, a polysaccharide isolated from *Lentinus edodes*: Its application as a host defence potentiator. *Int J Oriental Med*, 17, 57–77.

Chihara G. 1993. Medical aspects of lentinan isolated from *Lentinus edodes* (Berk.) Sing. In Mushroom biology and mushroom products, Chang S. T., Buswell J. A., and Chiu S. W., eds., Chinese University Press, Hong Kong. 261–266.

Chihara G., Maeda Y. Y., Hamuro J., Sasaki T., and Fukuoka F. 1969. Inhibition of mouse sarcoma 180 by polysaccharides from *Lentinus edodes* (Berk.) Sing. *Nature*, 222, 687–688.

Chihara G., Hamuro J., Maeda Y. Y., Arai Y., and Fukuoka F. 1970a. Antitumor polysaccharide derived chemically from natural glucan (pachyman). *Nature*, 225, 943–944.

Chihara G., Hamuro J., Maeda Y. Y., Arai Y., and Fukuoka F. 1970b. Fractionation and purification of the polysaccharides with marked antitumor activity, especially Lentinan, from *Lentinus edodes* (Berk.) Sing. (an edible mushroom). *Cancer Res*, 30, 2776–2781.

Chihara G., Maeda Y. Y., and Hamuro J. 1982. Current status and perspectives of immunomodulators of microbial origin. *Int J Tissue React*, 4, 207–225.

Chihara G. et al. 1987. Antitumor and metastasis-inhibitory activites of Lentinan as an immunomodulator: An overview. *Cancer Detect Prev* (Suppl), 1, 423–443.

Cochran K. W. 1978. Medical effects. In The biology and cultivation of edible mushrooms, Chang S. T. and Hayes W. A., eds., Academic Press, New York, San Francisco, London. 160–187.

Dennert G. and Tucker D. 1973. Antitumor polysaccharide Lentinan-a T-cell adjuvant. *J Natl Cancer Inst*, 51, 1727–1729.

Ding G. 1987. Anti-arrhythmia agents in traditional Chinese medicines. Abstracts of Chinese Medicines 1, 287–308.

Donnelly D. M. X. and Hutchinson R. M. 1990. Armillane, a saturated sesquiterpene ester from *Armillaria mellea*. *Phytochemistry*, 29, 179–182.

Donnelly D. M. X. et al. 1985. Antibacterial sesquiterpene aryl esters from *Armillaria mellea*. *J Nat Prod*, 48, 10–16.

Donnelly D. M. X. et al. 1986. New sesquiterpene aryl esters from *Armillaria mellea*. *J Nat Prod*, 49, 111–116.

Dornberger K. et al. 1986. Evidence for the occurrence of the 4-hydroxybenzenediazonium in the extract of *Agaricus xanthodermus*. *Tetrahedron Lett*, 27, 559–560.

Dresser D. W. and Phillips J. M. 1974. The orientation of the adjuvant activities of *Salmonella typhosa* lipopolysaccharide and Lentinan. *Immunology*, 27, 895–902.

Dudka I. A. and Wasser S. P. 1987. Reference book of a mycologist and mushroomers. Kiev, Nauk. Dumka. 536p.

Elder D. S. E., Halton D. E., Hague A., and Paraskeva C. 1997. Induction of apoptotic cell death in human colorectal carcinoma cell lines by a cyclooxygenase - (COX-2) - selective nonsteroidal anti-inflammatory drug: Independence from COX-2 protein expression. *Clin Cancer Res*, 3, 1679–1683.

Endo A. 1988. Chemistry, biochemistry, and pharmacology of HMG CoA reductase inhibitors. *Klin Wochenschr*, 66, 421–427.

Fruehauf J. P., Bonnard G. D. and Herberman R. B. 1982. The effect of Lentinan on production of interleukin-1 by human monocytes. *Immunopharmacology*, 5, 65–74.

Fujii T. et al. 1978. Isolation and characterization of a new antitumor polysaccharide, KS-2, extracted from culture mycelia of *Lentinus edodes*. *J Antibiot*, 31, 1079–1090.

Fujimoto H. et al. 1994. Isolation and characterization of immunosuppressive components of three mushrooms, *Piso-*