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MINI-REVIEW

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Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides

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Abstract The number of mushrooms on Earth is estimated at 140,000, yet maybe only 10% (approximately 14,000 named species) are known. Mushrooms comprise a vast and yet largely untapped source of powerful new pharmaceutical products. In particular, and most importantly for modern medicine, they represent an unlimited source of polysaccharides with antitumor and immunostimulating properties. Many, if not all, Basidiomycetes mushrooms contain biologically active polysaccharides in fruit bodies, cultured mycelium, culture broth. Data on mushroom polysaccharides have been collected from 651 species and 7 infraspecific taxa from 182 genera of higher Hetero- and Homobasidiomycetes. These polysaccharides are of different chemical composition, with most belonging to the group of β-glucans; these have β-(1→3) linkages in the main chain of the glucan and additional β-(1→6) branch points that are needed for their antitumor action. High molecular weight glucans appear to be more effective than those of low molecular weight. Chemical modification is often carried out to improve the antitumor activity of polysaccharides and their clinical qualities (mostly water solubility). The main procedures used for chemical improvement are: Smith degradation (oxydo-reducto-hydrolysis), formolysis, and carboxymethylation. Most of the clinical evidence for antitumor activity comes from the commercial polysaccharides lentinan, PSK (krestin), and schizophyllan, but polysaccharides of some other promising medicinal mushroom species also show good results. Their activity is especially beneficial in clinics when used in conjunction with chemotherapy. Mushroom polysaccharides prevent oncogenesis, show direct antitumor activity against various allogeneic and syngeneic tumors, and prevent tumor metastasis. Polysaccharides from mushrooms do not attack cancer cells directly, but produce their antitumor effects by activating different immune responses in the host. The antitumor action of polysaccharides requires an intact T-cell component; their activity is mediated through a thymus-dependent immune mechanism. Practical application is dependent not only on biological properties, but also on biotechnological availability. The present review analyzes the pecularities of polysaccharides derived from fruiting bodies and cultured mycelium (the two main methods of biotechnological production today) in selected examples of medicinal mushrooms.

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Introduction

For millennia, mushrooms have been valued by humankind as an edible and medical resource. A number of bioactive molecules, including antitumor substances, have been identified in many mushroom species. Polysaccharides are the best known and most potent mushroom-derived substances with antitumor and immunomodulating properties (Mizuno 1996, 1999a, b, 2002; Lorenzen and Anke 1998; Borchers et al. 1999; Ooi and Liu 1999; Wasser and Weis 1999; Tzianabos 2000; Reshetnikov et al. 2001). Historically, hot-water-soluble fractions (decoctions and essences) from medicinal mushrooms, i.e., mostly polysaccharides, were used as medicine in the Far East, where knowledge and practice of mushroom use primarily originated (Hobbs 1995, 2000). Mushrooms such as *Ganoderma lucidum* (Reishi), *Lentinus edodes* (Shiitake), *Inonotus obliquus* (Chaga) and many others have been collected and used for hundreds of years in Korea, China, Japan, and eastern Russia. Those practices still form the basis of modern scientific studies of fungal medical activities, especially in the field of stomach, prostate, and lung cancers. It is notable and remarkable how reliable the facts collected by traditional eastern medicine are in the study of medicinal mushrooms (Ying et al.

1987; Hobbs 1995, 2000; Wasser and Weis 1997a, b, 1999; Stamets 2000).

Ikekawa et al. (1969) published one of the first scientific reports on antitumor activities of essences obtained from fruiting bodies of mushrooms belonging to the family Polyporaceae (Aphyllophoromycetideae) and a few other families, manifested as host-mediated activity against grafted cancer – such as Sarcoma 180 – in animals (Ikekawa et al. 1982, 1992; Ikekawa 2001). Soon thereafter the first three major drugs were developed from medicinal mushrooms. All three were polysaccharides, specifically β-glucans: krestin from cultured mycelial biomass of *Trametes versicolor* (Turkwey Tail), lentinan from fruiting bodies of *L. edodes*, and schizophyllan from the liquid cultured broth product of *Schizophyllum commune* (Split Gill). For almost 40 years, medicinal mushrooms have been intensively investigated for medicinal effects in in vivo and in vitro model systems, and many new antitumor and immunomodulating polysaccharides have been identified and put into practical use (Mizuno 1996, 1999a; Wasser and Weis 1999; Ikekawa 2001).

Biologically active polysaccharides are widespread among higher Basidiomycetes mushrooms, and most of them have unique structures in different species. Moreover, different strains of one Basidiomycetes species can produce polysaccharides with different properties. For example, the proteoglucan krestin was developed in Japan from the strain *Trametes* (*Coriolus) versicolor* CM-101, whereas polysaccharide-peptide (PSP) in China was developed in submerged culture of the strain Cov-1 of the same species. Both proteoglucans have the same polysaccharide component but with different protein molecules bound to the polysaccharide (Hiroshi and Takeda 1993).

In the present review, antitumor and immunomodulating polysaccharides from higher Basidiomycetes mushrooms are analyzed. More attention is given to their common features than to specific pecularities. The review summarizes the general state of knowledge in the area of biodiversity of mushrooms and their polysaccharides; the chemical structure of polysaccharides and its connection with their antitumor activity, including possible ways of chemical modification; results of experimental testing and clinical use of antitumor or immunostimulating polysaccharides; possible mechanisms of their biological action; and, finally, the difference in polysaccharide fraction composition in fruiting bodies and pure culture mycelia in selected examples of studied medicinal mushrooms.

The vast quantity and diversity of mushrooms with antitumor polysaccharides

The total number of described fungi of all kinds is currently at least 80,060 species; a figure based on the total derived from addition of the numbers of species in each genus given in the latest edition of the *Dictionary of the Fungi* (Kirk et al. 2001). This figure includes all organisms traditionally studied by mycologists: slime molds, chromistan fungi, chytridiaceous fungi, lichen-forming fungi, filamentous fungi, molds, and yeasts.

By the term 'mushrooms', we generally mean the definition of Chang and Miles (1992): 'a macrofungus with a distinctive fruiting body which can be either hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand'. The number of filamentous fungi that are mushrooms in the sense of this definition deduced from the *Dictionary of the Fungi* is at least 14,000 and perhaps as many as 22,000 (Hawksworth 2001). However, the real number of such species on Earth is undoubtedly much higher. Two main reasons for the real number being higher are (1) the great number of as yet undescribed species and (2) the fact that many morphologically defined mushroom 'species' prove to be assemblages of several biological species (Hawksworth 2001).

Most new mushrooms are being discovered in the tropics, especially those species forming ectomycorrhizas with native trees. In various tropical areas, 22–55% (in some cases up to 73%) of mushroom species have proved to be undescribed (Hawksworth 2001). An analysis of the localities from which fungi new to science have been described and catalogued in the *Index of Fungi* in the 10 years from 1990 to 1999 revealed that about 60% of all newly described fungi are from the tropics (Hawksworth 1993, 2001), and this is also the case for mushrooms, although new species continue to be discovered in Europe and North America.

Studies of compatability and molecular sequences between mushrooms previously considered to be the same species on morphological grounds revealed 'cryptic species', i.e., populations functioning as separate biological species but covered by a single scientific name. A single morphologically defined species may consist of 20 or more biological species (Hawksworth 2001).

Taking all this into account, recent estimates of the number of fungi on Earth range from 500,000 to 9.9 million species, of which only 80,060 are named. A working figure of 1.5 million species is generally accepted, and new data suggests that this is not unreasonable. The number of mushrooms on Earth is estimated at 140,000, of which maybe only 10% are known. Meanwhile, of those ~14,000 species that we know today, about 50% are considered to possess varying degrees of edibility, more than 2,000 are safe, and about 700 species are known to possess significant pharmacological properties (Chang 1999; Wasser and Weis 1999; Reshetnikov et al. 2001). Thus, it is clear that mushrooms represent a major and as yet largely untapped source of powerful new pharmaceutical products.

Higher Basidiomycetes mushrooms represent an unlimited source of antitumor or immunostimulating polysaccharides. All main taxonomic mushroom groups have been investigated for biologically active polysaccharides, and most of them possess such substances. At least 651 species and 7 infraspecific taxa representing 182 genera of Hetero- and Homobasidiomycetes mushrooms contain antitumor or immunostimulating polysaccharides, as is evident from Table 1 (adapted from

Table 1 Higher Basidiomycetes mushrooms containing antitumor or immunostimulating polysaccharides

Taxa (number of species studied)	Activity against:		Source
	Sarcoma 180 solid cancer	Ehrlich solid cancer	
Heterobasidiomycetes			
Auriculariales – *Auricularia* (3)	70–90	60–80	Ohtsuka et al. 1973; Ukai et al. 1982; Song et al. 1998
Dacrymycetales – *Calocera* (1) *Dacrymyces* (1)	60–90	60	Ohtsuka et al. 1973
Tremellales – *Exidia* (1) *Guepinia* (1) *Holtermannia* (1) *Phlogiotis* (1) *Protodaedalea* (1) *Pseudohydnum* (1) *Tremella* (2) *Tremellodon* (1)	60–100	70–100	Ohtsuka et al. 1973; Gao et al. 1997
Homobasidiomycetes			
Aphyllophoromycetideae			
Cantharellaceae – *Cantharellus* (5) *Craterellus* (2)	60–100	60–90	Ohtsuka et al. 1973
Clavariaceae – *Clavaria* (4) *Clavariadeiphus* (2) *Clavulinopsis* (4) *Lentaria* (1)	60–90	60–100	Ohtsuka et al. 1973
Clavulinaceae – *Clavulina* (1)	70–90	80	Ohtsuka et al. 1973
Sparassidaceae – *Sparassis* (1)	100	100	Ohtsuka et al. 1973; Ohno et al. 2000; Yadomae and Ohno 2000
Ramariaceae – *Ramaria* (5)	60–80	60–70	Ohtsuka et al. 1973
Hydnaceae – *Hydnum* (1)	70	90	Ohtsuka et al. 1973; Chung et al. 1982
Hericiaceae – *Echinodontium* (2) *Hericium* (2) *Laxitextum* (1)	70–90	60–80	Ohtsuka et al. 1973; Mizuno 1999b
Corticiaceae – *Aleurodiscus* (1) *Cotylidia* (2) *Laxitextum* (1) *Lopharia* (1) *Merulius* (2) *Phlebia* (2) *Sarcodontia* (1) *Sistotrema* (1) *Steccherinum* (1) *Stereum* (13)	60–100	60–100	Ohtsuka et al. 1973
Coniophoraceae – *Serpula* (1)	70	60	Ohtsuka et al. 1973
Thelephoraceae *Bankera* (1) *Calodon* (4) *Hydnellum* (2) *Polyozellus* (1) *Sarcodon* (2) *Thelephora* (1)	60–100	70–100	Ohtsuka et al. 1973; Song et al. 1998; Mizuno 2000
Hymenochaetaceae – *Coltricia* (4) *Cryptoderma* (6) *Cyclomyces* (1) *Fuscoporia* (1) *Hymenochaete* (4) *Hymenostilbe* (1) *Inonotus* (6) *Onnia* (1) *Phellinus* (6) *Pyrrhoderma* (1)	60–100	90–100	Ohtsuka et al. 1973; Kim et al. 1996; Han et al. 1999; Mizuno 2000
Fistulinaceae – *Fistulina* (2)	80	90	Ohtsuka et al. 1973; Ueno et al. 1978
Ganodermataceae – *Ganoderma* (7)	70–100	70–100	Ohtsuka et al. 1973; Nakashima et al. 1979; Miyazaki and Nishijima 1981; Ukai et al. 1983; Zhang and Lin 1999
Polyporaceae – *Amauroderma* (1) *Coriolellus* (1) *Coriolus* (8) *Cymatoderma* (2) *Cystidiophorus* (1) *Daedalea* (1) *Daedaleopsis* (3) *Dendropolyporus* (1) *Favolus* (3) *Fomes* (2) *Fomitella* (1) *Fomitopsis* (5) *Gloeophyllum* (1) *Gloeoporus* (1) *Gloeostereum* (1) *Grifola* (2) *Hirschioporus* (3) *Ischnoderma* (1) *Laetiporus* (2) *Laricifomes* (1) *Lenzites* (1) *Meripilus* (1) *Microporus* (2) *Oxyporus* (1) *Phaeolus* (1) *Piptoporus* (1) *Polyporus* (10) *Poria* (1) *Porodisculus* (1) *Pycnoporus* (1) *Rigidoporus* (2) *Trachyderma* (1) *Trametes* (8) *Trichaptum* (1) *Tyromyces* (5)	70–90	70–100	Ohtsuka et al. 1973; Ito et al. 1976; Ohtsuka et al. 1977; Fujii et al. 1979; Liou and Lin 1979; Min et al. 1980; Nakajima et al. 1980; Kanayma et al. 1986; Mizuno et al. 1992; Gasiorowski et al. 1993; Cho et al. 1996; Nanba 1998; Fullerton et al. 2000
Schizophyllaceae – *Schizophyllum* (1)	70	–	Ohtsuka et al. 1973; Okamura et al. 1986

Table 1 (continued)

Taxa (number of species studied)	Activity against:		Source
	Sarcoma 180 solid cancer	Ehrlich solid cancer	
Gasteromycetideae			
Gasteromycetales			
Lycoperdaceae – *Lycoperdon* (2)	–	–	Song et al. 1998
Phallaceae – *Dictyophora* (1) *Kobayasia* (1)	–	–	Miyazaki et al. 1975; Ukai et al. 1983; Hara et al. 1991; Ishiyama et al. 1996
Boletales			
Boletaceae – *Boletinus* (1) *Boletus* (11) *Filoboletus* (1) *Gyroporus* (1) *Leccinum* (2) *Phylloporus* (1) *Pulveroboletus* (3) *Suillus* (5) *Tylopilus* (3) *Xerocomus* (3)	70–100	90	Ohtsuka et al. 1973
Paxillaceae – *Hygrophoropsis* (1) *Paxillus* (3)	60–90	70–80	Ohtsuka et al. 1973
Strobilomyceteceae – *Boletellus* (2) *Porphyrellus* (1) *Strobilomyces* (1)	60–80	60–70	Ohtsuka et al. 1973
Gomphidiaceae – *Gomphidius* (1) *Chroogomphus* (1)	60–90	60–80	Ohtsuka et al. 1973
Agaricomycetideae			
Agaricales			
Hygrophoraceae – *Camarophyllus* (2) *Hygrocybe* (14) *Hygrophorus* (21)	60–100	70–100	Ohtsuka et al. 1973
Pleurotaceae – *Pleurotus* (4)	–	–	Yoshioka et al. 1972; Chung et al. 1982; Zhuang et al. 1994a; Song et al. 1998
Tricholomataceae – *Armillariella* (3) *Asterophora* (1) *Baeospora* (1) *Cantharellula* (1) *Catathelasma* (2) *Clitocybe* (7) *Collybia* (6) *Dictyopanus* (1) *Flammulina* (1) *Hohenbuehelia* (1) *Hypsizygus* (1) *Laccaria* (6) *Lampteromyces* (1) *Lepista* (3) *Leucopaxillus* (1) *Lyophyllum* (8) *Macrocystidia* (2) *Marasmiellus* (2) *Marasmius* (6) *Melanoleuca* (2) *Mycena* (19) *Omphalina* (1) *Oudemansiella* (3) *Panellus* (1) *Pleurocybella* (1) *Pseudohiatula* (2) *Resupinatus* (1) *Tricholoma* (19 *Tricholomopsis* (4) *Xeromphalina* (3) *Xerula* (2)	60–100	60–100	Ohtsuka et al. 1973; Chung et al. 1982; Ikekawa et al. 1982; Kim et al. 1982; Ma et al. 1991; Ikekawa et al. 1992; Kiho et al. 1992a, b; Mizuno et al. 1994; Liu et al. 1996; Wang et al. 1996; Song et al. 1998; Ukawa et al. 2000
Entolomataceae – *Clitopilus* (2) *Entoloma* (14) *Rhodocybe* (1) *Rhodophyllus* (6)	60–90	60–100	Ohtsuka et al. 1973
Cortinariaceae – *Cortinarius* (25) *Galerina* (6) *Gymnopilus* (3) *Hebeloma* (3) *Inocybe* (19) *Rozites* (1)	60–100	60–100	Ohtsuka et al. 1973
Bolbitiaceae – *Agrocybe* (7) *Bolbitius* (2) *Conocybe* (7)	60–90	70–90	Ohtsuka et al. 1973; Yoshida et al. 1996; Song et al. 1998
Strophariaceae – *Hypholoma* (1) *Kuehneromyces* (1) *Naematoloma* (4) *Pholiota* (8) *Psilocybe* (3) *Stropharia* (2)	60–100	70–100	Ohtsuka et al. 1973; Chung et al. 1982; Song et al. 1998
Crepidotaceae – *Crepidotus* (3) *Tubaria* (1)	60–100	90–100	Nakayoshi et al. 1968; Ohtsuka et al. 1973
Amanitaceae – *Amanita* (21) *Limacella* (1)	60–100	60–90	Ohtsuka et al. 1973; Kiho et al. 1994; Yoshida et al. 1996
Pluteaceae – *Pluteus* (5) *Volvariella* (4)	60–100	70–100	Ohtsuka et al. 1973; Chung et al. 1982; Misaki et al. 1986
Agaricaceae – *Agaricus* (1) *Cystoderma* (2) *Lepiota* (15) *Leucocoprinus* (3) *Macrolepiota* (2) *Melanophyllum* (1) *Phaeolepiota* (1)	60–100	60–100	Ohtsuka et al. 1973; Mizuno 2002
Coprinaceae – *Coprinus* (16) *Panaeolus* (1) *Psathyrella* (7) *Pseudocoprinus* (1)	60–100	60–100	Ohtsuka et al. 1973
Russulales			
Russulaceae – *Lactarius* (18) *Russula* (23)	60–100	70–100	Ohtsuka et al. 1973

Reshetnikov et al. 2001). Naturally collected or artificially growing fruit bodies, pure culture mycelia, and culture filtrate (culture broth) all contain biologically active polysaccharides.

Procedures for polysaccharide purification

After two decades of intensive research on medicinal mushrooms, Mizuno and his co-workers in Japan developed reliable procedures for successful extraction, fractionation and purification of polysaccharides from fruiting bodies or culture mycelia. In general, this scheme involves elimination of low molecular weight substances from mushroom material using 80% ethanol, followed by three successive extractions with water (100°C, 3 h), 2% ammonium oxalate (100°C, 6 h), and 5% sodium hydroxide (80°C, 6 h) (Mizuno 1996, 1999a).

The first extraction results in water-soluble polysaccharides, the other two in water-insoluble polysaccharides. Polysaccharides extracted are further purified using a combination of techniques, such as ethanol concentration, fractional precipitation, acidic precipitation with acetic acid, ion-exchange chromatography, gel filtration, and affinity chromatography. Basically, ion-exchange chromatography through DEAE-cellulose columns separates neutral polysaccharides from acidic ones. Neutral polysaccharides are then separated into α-glucans (adsorbed fraction) and β-glucans (non-absorbed fraction) with the help of gel filtration and affinity chromatography. The same procedure with acidic polysaccharides (after elution with 1 M NaCl) yields purified polysaccharides (Mizuno 1999a).

General schemes for fractional preparations of polysaccharides from mushrooms are shown schematically in Fig. 1. It should be noted that the particular fractionation procedure scheme depends in each case on the polysaccharide composition of the extracted material.

Structural composition of antitumor polysaccharides in mushrooms

Polysaccharides belong to a structurally diverse class of macromolecules, polymers of monosaccharide residues joined to each other by glycosidic linkages. It is noteworthy that, in comparison with other biopolymers such as proteins and nucleic acids, polysaccharides offer the highest capacity for carrying biological information because they have the greatest potential for structural variability. The nucleotides in nucleic acids and the amino acids in proteins can interconnect in only one way whereas the monosaccharide units in polysaccharides can interconnect at several points to form a wide variety of branched or linear structures (Sharon and Lis 1993). This enormous potential variability in polysaccharide structure gives the necessary flexibility to the precise regulatory mechanisms of various cell-cell interactions in higher organisms.

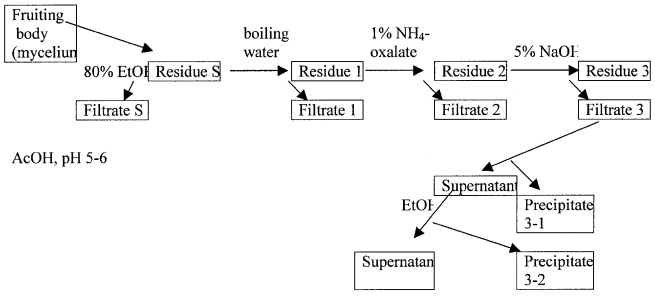

Fig. 1 Fractional preparation of polysaccharides from mushrooms [adapted from Mizuno (1999a) with modification]

Mushroom polysaccharides are present mostly as glucans with different types of glycosidic linkages, such as (1→3), (1→6)-β-glucans and (1→3)-α-glucans, but some are true heteroglycans. The others mostly bind to protein residues as PSP complexes (PSPC; Gorin and Barreto-Berger 1983). The main source of antitumor polysaccharides appears to be fungal cell walls that consist of polysaccharides. However, chitin and chitosan (fungal chitin) have no antitumor activity (Mizuno et al. 1995b).

β-D-glucan is a polysaccharide yielding exclusively D-glucose upon acid hydrolysis (Mizuno 1996, 1999a). As for structure of schizophyllan tertiary conformation, active β-D-glucan has a triple-strand right-winding structure (Marchessault et al. 1977). Acidic glucuronoxylomannan isolated from the fruit body of *Tremella fuciformis* was also demonstrated as having a left-handed, threefold helical backbone conformation (Yui et al. 1995).

Besides the well-known antitumor β-(1→3)-glucans, a wide range of biologically active glucans with other structures have been described. These polysaccharides have linear or branched molecules in a backbone composed of α- or β-linked glucose units, and they contain side chains that are attached in different ways. Heteroglucan side chains contain glucuronic acid, xylose, galactose, mannose, arabinose, or ribose as a main component or in different combinations.

Glycans, in general, are polysaccharides containing units other than glucose in their backbone. They are classified as galactans, fucans, xylans, and mannans by the individual sugar components in the backbone. Heteroglycan side chains contain arabinose, mannose, fucose, galactose, xylose, glucuronic acid, and glucose as a main component or in different combinations.

A wide range of antitumor or immunostimulating polysaccharides of different chemical structure from higher Basidiomycetes mushrooms has been investigated; the main types are presented in Table 2.

The number of antitumor active fractions in the fruit bodies of mushrooms is remarkably high. One example can be seen in an analysis of polysaccharides of fruit bodies of *Pleurotus pulmonarius* (= *P. sajor-caju*): 16 polysaccharide fractions from 21 extractions demonstrated different levels of antitumor activity (Zhuang et al. 1993, Table 3).

Table 2 Chemical structure of antitumor and immunostimulating polysaccharides of higher Basidiomycetes

Polysaccharide	Species	References
Glucans		
α-(1→3)-glucan	*Armillariella tabescens*	Kiho et al. 1992a
Linear α-(1→3)-glucan	*Amanita muscaria*	Kiho et al. 1994
	Agrocybe aegerita	Yoshida et al. 1996
α-(1→4)-; β-(1→6)-glucan	*Agaricus blazei*	Fujimiya et al. 1998b
α-(1→6)-; α-(1→4)- glucan	*Agaricus blazei*	Mizuno et al. 1990a
β-(1→6)-glucan	*Lyophyllum decastes*	Ukawa et al. 2000
	Armillariella tabescens	Kiho et al. 1992a
β-(1→6)-; β-(1→3)-glucan	*Agaricus blazei*	Mizuno et al. 1990a
	Grifola frondosa	Nanba et al. 1987
β-(1→6)-; α-(1→ 3)-glucan	*Agaricus blazei*	Mizuno et al. 1990a
β-(1→3)-glucuronoglucan	*Ganoderma lucidum*	Saito et al. 1989
Mannoxyloglucan	*Grifola frondosa*	Mizuno et al. 1986
Galactoxyloglucan	*Hericium erinaceus*	Mizuno 1999b
Xyloglucan	*Grifola frondosa*	Mizuno et al. 1986
	Polyporus confluens	Mizuno et al. 1992
	Pleurotus pulmonarius	Zhuang et al. 1993
Xylogalactoglucan	*Inonotus obliquus*	Mizuno et al. 1999a
Mannogalactoglucan	*Pleurotus pulmonarius*	Gutiérrez et al. 1996
	Pleurotus cornucopiae	Kim et al. 1994
	Ganoderma lucidum	Cho et al. 1999
	Agaricus blazei	
Galactomannoglucan	*Flammulina velutipes*	Ikekawa et al. 1982
	Hohenbuehelia serotina	Mizuno et al. 1994
	Leucopaxillus giganteus	Mizuno et al. 1995a
Arabinoglucan	*Ganoderma tsugae*	Zhang et al. 1994b
Riboglucan	*Agaricus blazei*	Cho et al. 1999
Glycans		
Arabinogalactan	*Pleurotus citrinopileatus*	Zhang et al. 1994a
Glucogalactan	*Ganoderma tsugae*	Wang et al. 1993
Fucogalactan	*Sarcodon aspratus*	Mizuno 2000
α-(1→6)-mannofucogalactan	*Fomitella fraxinea*	Cho et al. 1998
Fucomannogalactan	*Dictyophora indusiata*	Hara et al. 1991
Mannogalactan	*Pleurotus pulmonarius*	Zhuang et al. 1993
Mannogalactofucan	*Grifola frondosa*	Zhuang et al. 1994a
Xylan	*Hericium erinaceus*	Mizuno 1999b
Glucoxylan	*Hericium erinaceus*	Mizuno 1999b
	Pleurotus pulmonarius	Zhuang et al. 1993
Mannoglucoxylan	*Hericium erinaceus*	Mizuno 1999b
α-(1→3)-mannan	*Dictyophora indusiata*	Ukai et al. 1983
Glucomannan	*Agaricus blazei*	Hikichi et al. 1999
β-(1→2)-; β-(1→3)-glucomannan	*Agaricus blazei*	Tsuchida et al. 2001
		Mizuno et al. 1999b
Galactoglucomannan	*Lentinus edodes*	Fujii et al. 1979

The most antitumor-active water-soluble fractions from *P. pulmonarius* are Fi_o-a protein-containing xyloglucan with Man:Gal:Xyl:Glc in the polysaccharide at a molar ratio 2:12:42:42, and FA-2 protein-containing mannogalactan consisting of Xyl:Man:Gal (9:35:56 molar ratio). The most antitumor-active water-insoluble polysaccharides are FII-1 protein-containing xylan; FIII-1a protein-containing glucoxylan consisting of Glc:Xyl (40:44 molar ratio), and FIII-2a protein-containing xyloglucan consisting of Xyl:Glc (36:62 molar ratio).

Correlation of structure and antitumor activities of mushroom polysaccharides

Polysaccharides with antitumor action differ greatly in their chemical composition and configuration, as well as their physical properties. Antitumor activity is exhibited by a wide range of glycans extending from homopolymers to highly complex heteropolymers (Ooi and Liu 1999). Differences in activity can be correlated with solubility in water, size of the molecules, branching rate and form. Although it is difficult to correlate the structure and antitumor activity of complex polysaccharides, some relationships can be inferred.

It is obvious that structural features such as β-(1→3) linkages in the main chain of the glucan and additional β-(1→6) branch points are needed for antitumor action. β-glucans containing mainly (1→6) linkages have less activity. High molecular weight glucans appear to be more effective than those of low molecular weight (Mizuno 1996, 1999a, b). However, obvious variations in antitumor polysaccharides have also been noted. Antitumor polysaccharides may have other chemical structures, such as hetero-β-glucans (Mizuno et al. 1995b), heteroglycan (Gao et al. 1996b), β-glucan-protein (Kawagishi

Table 3 Structure and antitumor activity of *Pleurotus pulmonarius* fruit bodies polysaccharides against Sarcoma 180 in mice (after Zhuang et al. 1993). *FI-FA* Water-soluble, *FII-FIII* water-insoluble polysaccharides

Polysaccharid	MW ×10³	Protein (%)	Total sugar (%)	Component sugar (molar %)				Tumor inhibition ratio at 3 weeks (%)
				Glc	Xyl	Man	Gal	
FI_o-a	278	24.1	75.6	43.7	42.3	1.9	11.8	84.8
FI_o-a-α	420	23.5	69.5	24.1	72.5	2.7	0.7	53.1
FI_o-a-β	68	26.3	67.0	53.5	27.2	1.6	17.7	49.8
FI_o-b-α	10	42.1	52.6	56.0	40.7	3.3	–	59.4
FI_o-b-β	24	6.9	84.6	–	16.2	–	83.8	31.7
FA-1	11	27.5	67.7	71.6	5.5	–	22.9	48.7
FA-2	115	16.2	76.1	–	9.4	34.6	56.0	74.6
FA-3	10	75.3	22.5	50.0	14.9	13.1	22.0	34.5
FII-1	19	20.5	62.2	5.2	91.2	–	3.6	90.8
FII-2	17	44.1	50.5	9.2	86.2	–	4.6	8.0
FII-3	13	49.0	50.1	2.9	80.5	–	16.6	8.4
FIII-1a	87	70.5	15.4	39.8	43.7	7.8	8.7	76.9
FIII-1b	24	96.8	3.0	–	97.9	–	2.1	51.6
FIII-2	627	2.8	69.6	33.9	40.3	–	1.9	84.5
FIII-2a	700	2.5	68.8	62.2	35.5	–	2.3	100.0
FIII-2b	190	4.5	74.8	30.9	69.1	–	–	84.6

et al. 1990), α-manno-β-glucan (Mizuno et al. 1995b), α-glucan-protein (Mizuno et al. 1995b) and heteroglycan-protein complexes (Zhuang et al. 1993; Mizuno et al. 1996).

A triple-helical tertiary conformation of medicinal mushroom β-(1→3)-glucans is known to be important for their immune-stimulating activity. When lentinan was denatured with dimethyl sulfoxide, urea, or sodium hydroxide, tertiary structure was lost while primary structure was not affected, but tumor inhibition properties were lowered with progressive denaturation (Maeda et al. 1988). The same results, which confirm the correlation between antitumor activity and triple helix structure, were obtained upon investigation of schizophyllan (Yanaki et al. 1983, 1986).

Mushroom β-(1→3)-glucans exhibit a variety of biological and immuno-pharmacological activities, and many of these activities, such as macrophage nitrogen oxide synthesis, and limulus factor G activation, are dependent on the triple-helix conformation, while others are independent of this conformation, e.g., synthesis of interferon-γ and colony stimulating factor (Yadomae 2000), thus indicating that the α-(1→3)-mannan backbone structure is of more importance than the tertiary structure of the molecule.

Unlike β-(1→3)-glucans with medicinal properties that are strongly dependent on high molecular weight, ranging from 500 to 2,000 kDa (Mizuno 1996), α-(1→3)-glucuronoxylomannans, which are characteristic of Jelly mushrooms, are not strongly dependent on molecular weight. Thus, Gao and co-workers (1996a) reported that acidic hydrolysate fractions of *T. fuciformis* fruit bodies contain glucuronoxylomannans with molecular weights of from 53 to 1 kDa that induce human monocytes to produce interleukin-6 as efficiently as non-hydrolyzed heteropolysaccharide. This indicates that the activity may be due to the common structure of the α-(1→3)-mannan backbone; differences in molecular weight had no obvious influence on the activity of the heteroglycans (Gao et al. 1996b).

Activation of mushroom polysaccharides by chemical modification

Different approaches to improving antitumor activity of mushroom polysaccharides by chemical modification have been described in the literature. The most successful schemes for chemical improvement of mushroom polysaccharides have been developed for *Ganoderma lucidum*, *Grifola frondosa* and *Leucopaxillus giganteus* (= *Tricholoma gigantea*). These schemes include two main procedures: modification of mushroom polysaccharides by Smith degradation (oxydo-reducto-hydrolysis) and activation by the method of formolysis (Mizuno 1996, 1999a; Mizuno et al. 1996). Five polyaldehydes and ten polyalcohols were prepared by the Smith degradation method from five polysaccharide fractions previously obtained from *G. frondosa* liquid culture mycelium. For this reason, original polysaccharide solutions were first oxidised to polyaldehydes by 0.1 M $NaIO_4$ in darkness, then converted into polyalcohols by reduction of $NaBH_4$ in alkaline medium adjusted to pH 8 with 2 M NaOH, and hydrolysed by 1 M H_2SO_4 at room temperature (Zhuang et al. 1994b). Chemical activation of mushroom polysaccharides by the method of formolysis involves degradation of polysaccharides by formic acid in 99% HCOOH solution; the reaction solution is then precipitated with 99% EtOH, and one-half of the precipitate is lyophilized after dialysis, while the other part is dissolved in hot water and additional fractions obtained by alcohol precipitation (Zhuang et al. 1994b). Four formylated polysaccharides and four formolysis products of polysaccharides were prepared by this method from four polysaccharide fractions obtained from *G. frondosa* liquid culture mycelium. Although two of the original

polysaccharides had no activity, their polyaldehyde polyol, formylated, and formolysis derivatives showed significant activity. Polyaldehyde, and polyol-polysaccharides prepared from a polysaccharide with low antitumor activity showed activity higher than the original polysaccharide (Zhuang et al. 1994b). As all original polysaccharide fractions showing elevated activity levels by chemical modification were β-glucan or xyloglucan, it was suggested that the sugar chain was changed or eliminated upon treatment, resulting in improved solubility and activity (Mizuno 1999a).

Carboxymethylation is the other chemical method used to transform β-glucans into a water-soluble form. For example, whole fruit bodies of *Pleurotus ostreatus* or their stipes homogenate were treated with 0.15 M sodium hydroxide solution at 95°C for 2 h. The residue collected was washed with water until neutral, then suspended in 0.06% sodium chlorite solution, adjusted to pH 4.5 with acetic acid, and stirred for 6 h at 50°C. The polysaccharide obtained was β-(1→3)-linked glucan, with every fourth glucopyranosyl residue substituted at 0–6 with single D-glucopyranosyl groups. The heterogeneous etherification of the particulate glucan with monochloroacetic acid ($C_2H_3ClO_2$) in alkaline medium gave the sodium salt of the water-soluble *O*-(carboxymethyl) glucan derivative (Kuniak et al. 1993; Karácsonyi and Kuniak 1994). Carboxymethylated glucan from *P. ostreatus* (pleuran) exhibited immunomodulatory effects, especially increased phagocytic activity (Paulik et al. 1996).

In a similar manner, a water-insoluble, alkali-soluble linear α-(1→3)-glucan obtained from fruiting bodies of *Amanita muscaria* and *Agrocybe aegerita* had little or no antitumor effect, while their carboxymethylated products showed potent antitumor activity (Kiho et al. 1994; Yoshida et al. 1996).

Chemical modification of branched mushroom polysaccharides resulting in side-chain reduction can be developed not only by Smith degradation but also by enzymatic reactions. A novel linear polysaccharide comprising α-(1→4)-bonded α-D-glucose units of a molecular weight of 500–10,000 kDa was developed after successive enzymatic treatments of submerged culture broth with amylase, cellulase, and protease (Kosuna 1998).

Linear low molecular weight α-(1→4)-glucans obtained after enzymatic reduction of side chains and protein component (active hexose correlated compounds – AHCC) were demonstrated as having immunomodulatory and anticancer properties (Ghoneum et al. 1995; Matsushita et al. 1998). In 1992, a trial was done in Japan to evaluate the preventive effect of AHCC against recurrence of hepatocellular carcinoma following surgical resection (Kidd 2000).

Sulfated homo- and heteropolysaccharides possessing antiviral activity are widespread in algae, especially in sea algae (Schaeffer and Krylov 2000), but do not naturally occur in higher Basidiomycetes mushrooms. Chemically sulfated schizophyllans with different sulfur content were obtained from β-(1→3)-glucan produced by *Schizophyllum commune* (Itoh et al. 1990; Hirata et al. 1994). It was suggested that the sulfur content in schizophyllan is more important in inhibiting growth of human immunodeficiency virus (HIV) than the molecular weight or the nature of the sugar component (Itoh et al. 1990; Hobbs 1995). The medicinal tests indicate that sulfated schizophyllan with a sulfur content of 5% can be useful as an anti-HIV agent for treatment of HIV-infected hemophiliacs (Hirata et al. 1994; Hobbs 1995).

It is important to realize that chemical modification is necessary in many cases to improve not only the antitumor activity of mushroom polysaccharides, but also their clinical qualities, most importantly water solubility and the ability to permeate stomach walls after oral ingestion.

Testing antitumor and immunomodulating activity of mushroom polysaccharides

Initial data on the antitumor activity of mushroom extracts was circumstantial and in no way solid and reliable. However, many indirect data that were properly collected and processed gave good evidence for the beneficial effects of mushrooms on human health. A good example is an epidemiological study in Nagano Prefecture, Japan, where activity was monitored for several decades. Researchers demonstrated that the cancer death rate of farmers whose main occupation was producing *Flammulina velutipes* (a well known medicinal mushroom in Japan) was remarkably lower than that of the general population in the Prefecture (Ikekawa 1995, 2001). Another similar observation in Brazil brought about extensive studies – and popularity – of *Agaricus blazei* (see below).

I would like to emphasize the principal points of antitumor and immunomodulating effects of mushroom polysaccharides. Most important among them are: (1) prevention of oncogenesis by oral consumption of mushrooms or their preparations; (2) direct antitumor activity against various allogeneic and syngeneic tumors; (3) immunopotentiation activity against tumors in combination with chemotherapy; (4) preventive effect on tumor metastasis.

A good example of preventive effect is given by Japanese research on their popular edible and medicinal mushroom, *Hypsizygus marmoreus* (Ikekawa 2001). Control mice were bred on an ordinary diet and treated mice with a diet containing 5% dried fruit body of *H. marmoreus*. All mice were i.p. injected with a strong carcinogen, methyl-cholanthrene, and carcinogenesis of the mice was investigated. At the end of the 76-week observation, 21 of the 36 control mice developed tumors, but only 3 of 36 mice in the treated group had tumors. The authors concluded that the mechanism of cancer-inhibitory and cancer-preventing activities of edible mushrooms was due to immunopotentiation (Ikekawa 2001).

It is well known from clinical practice that mushroom polysaccharides work best in conjunction with other

forms of 'tough' chemotherapy and surgery, which is, unfortunately, very invasive and has a lot of negative side effects. Lentinan has been studied best in this respect, both in animal models and in human clinical practice. In one study, 275 patients with advanced or recurrent gastric cancer were given one of two kinds of chemotherapy (mitomycin C with 5-fluorouracil or tegafur) either alone or with lentinan injections. The best results were obtained when lentinan was administered prior to chemotherapy and in patients with a primary lesion who had undergone no previous chemotherapy. The results were evaluated on the basis of prolongation of life, regression of tumors or lesions, and the improvement of immune responses (Hamuro and Chihara 1985; Hobbs 1995; Wasser and Weis 1997a).

Metastasis is a very serious and important problem in cancer therapy. A preventive effect of mushroom extracts on cancer metastasis has been studied by many groups, especially at the National Cancer Center Research Institute of Japan. In a successful series of experiments, Lewis lung carcinoma was s.c. transplanted into the foot pads of mice and EA6 or EA6-PII (polysaccharides from *Flammulina velutipes*) were p.o. administered for a period of 10 days. The life span of the group treated with EA6-PII was significantly increased (Ikekawa 2001). Further study was carried out using Meth-A fibrosarcoma: 7 days after the tumor was s.c. transplanted into the abdomen of female BALB/c mice, the solid tumor of each mouse was surgically dissected out, and 7 days after the surgery, a second challenge with the same tumor, Meth-A fibrosarcoma was made s.c. into the other side of the abdomen of the mouse and the re-challenged tumor growth was observed. The results indicated that pre-treatment with EA6 slightly inhibited growth of the rechallenged tumor, but post-treatment was remarkably effective for tumor growth inhibition at a dose of 10 mg/kg (Ikekawa 2001).

Specific preparations from particular medicinal mushrooms have undergone a lot of testing in animal model experiments and in clinics. After isolation of lentinan from *Lentinus edodes* by Chihara in 1969, most of the experimental antitumor testing was performed with this polysaccharide. Its 'father', Chihara himself, was one of the first researchers to report the antitumor properties of lentinan. Originally, its effect was tested by using Sarcoma 180 transplanted in CD-1/ICD mice (Chihara et al. 1969, 1970). Later, lentinan showed prominent antitumor activity not only against allogenic tumors, but also against various synergic and autochtonous tumors (Hamuro and Chihara 1985). Injections of lentinan into mice produced either an 80% reduction in tumor size or complete regression in most of the animals tested (Chihara 1981). A number of clinical tests followed. Among the first of these was a follow-up, randomized control study on Phase 3 patients with advanced and recurrent stomach cancers (Wasser and Weis 1999; Ikekawa 2001). Lentinan therapy showed very good results in prolonging the life span of patients and had no toxic side effects. Similar results were obtained in patients with colorectal and breast cancers. Since then, lentinan became a widely used medicine and dietary supplement in Japan, other Far East countries, and later in the United States and Europe.

PSK (commercial name krestin) has remarkable immune-enhancing activity and a broad antineoplastic scope. It has been shown to prolong the survival time of radiated mice, stimulate phagocytotic activity of macrophages, and improve the functions of the reticuloendothelial system (Zhu 1987). With regard to its antitumor properties, it acts directly on tumor cells, as well as indirectly in the host to boost cellular immunity (Hobbs 1995; Stamets 2000). It has shown antitumor activity in animals with adenosarcoma, fibrosarcoma, mastocytoma, plasmacytoma, melanoma, sarcoma, carcinoma, and mammary, colon, and lung cancer (Sugimachi et al. 1997). An intriguing feature of this compound is that injection of PSK at one tumor site has been shown to inhibit tumor growth at other sites, thus helping to prevent metastasis. PSK has been used both orally and intravenously in clinical medicine. It has been shown to be effective against many types of cancer (Hobbs 1995; Stamets 2000), but seldom with satisfactory results if administered alone.

The polysaccharide schizophyllan shows antitumor activity against both the solid and ascite forms of Sarcoma 180, as well as against the solid form only of sarcoma 37, Erlich sarcoma, Yoshida sarcoma and Lewis lung carcinoma (Hobbs 1995). Schizophyllan has also increased cellular immunity by restoring suppressed killer-cell activity to normal levels in mice with tumors (Borchers et al. 1999). Best results against radiation damage were found when schizophyllan was administered shortly after or at the same time as radiation, and schizophyllan restored mitosis of bone marrow cells previously suppressed by anticancer drugs (Zhu 1987). Human clinical studies proved the beneficial activity of treatment with schizophyllan for patients with recurrent and inoperable gastric cancer, stage 2 cervical cancer, and advanced cervical carcinoma (Hobbs 1995).

Mechanisms of antitumor and immunomodulating action by mushroom polysaccharides

Mushroom polysaccharides exert their antitumor action mostly via activation of the immune response of the host organism. These substances are regarded as biological response modifiers (BRMs; Wasser and Weis 1999). This basically means that: (1) they cause no harm and place no additional stress on the body; (2) they help the body to adapt to various environmental and biological stresses; and (3) they exert a nonspecific action on the body, supporting some or all of the major systems, including nervous, hormonal, and immune systems, as well as regulatory functions (Brekhman 1980).

The immunomodulating action of mushroom polysaccharides is especially valuable as a prophylactic, a mild and non-invasive form of treatment, and in the prevention of metastatic tumors, etc., as described above. Poly-

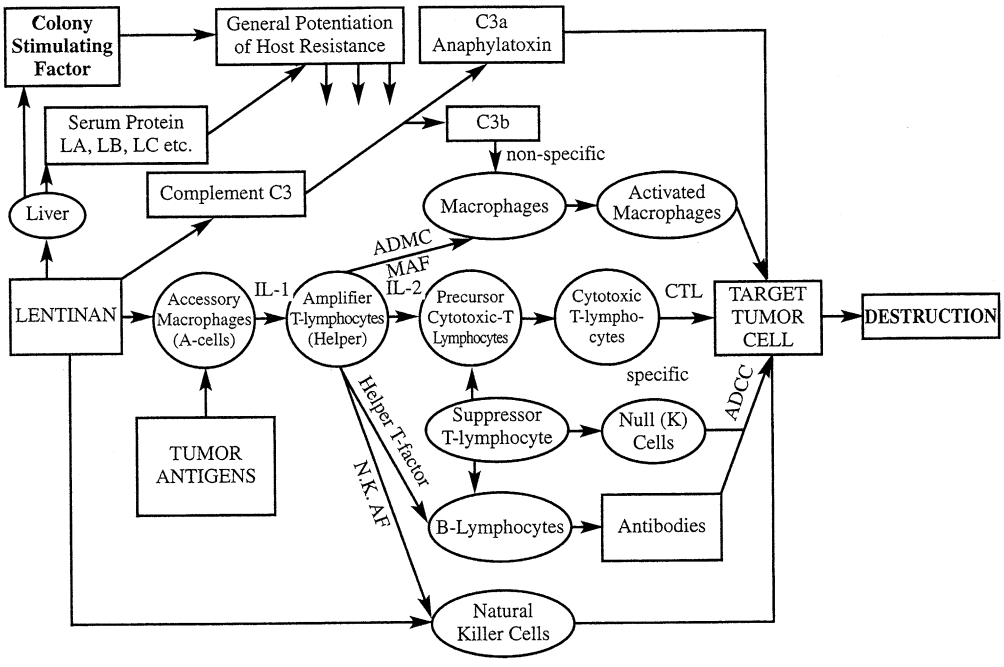

Fig. 2 Possible pathways of lentinan action (after Chihara 1981)

saccharides from mushrooms do not attack cancer cells directly, but produce their antitumor effects by activating different immune responses in the host. This has been verified in many experiments, such as the loss of the antitumor effect of polysaccharides in neonatal thymectomized mice or after administration of anti-lymphocyte serum (Ooi and Liu 1999). Such results suggest that the antitumor action of polysaccharides requires an intact T-cell component and that the activity is mediated through a thymus-dependent immune mechanism. Also, the antitumor activity of lentinan and other polysaccharides is inhibited by pretreatment with antimacrophage agents (such as carrageenan). Thus, the various effects of polysaccharides are thought to be due to potentiation of the response of precursor T cells and macrophages to cytokines produced by lymphocytes after specific recognition of tumor cells (Hamuro and Chihara 1985). In addition, the induction of a marked increase in the amounts of CSF, IL-1, and IL-3 by polysaccharides results in maturation, differentiation, and proliferation of the immunocompetent cells for host defense mechanisms (Hamuro and Chihara 1985). Mushroom polysaccharides are known to stimulate natural killer cells, T-cells, B-cells, and macrophage-dependent immune system responses.

Lentinan is known to be able to restore the suppressed activity of helper T-cells in the tumor-bearing host to their normal state, leading to complete restoration of humoral immune responses (Ooi and Liu 1999). The same effect is true for PSK, while it has no substantial effect on immune responses of the host under normal conditions.

Infiltration of eosinophils, neutrophils, and granulocytes around target tissues is also accelerated by lentinan. It activates secretion of active oxygen and production of cytokines in peritoneal macrophages. Lentinan also increases peritoneal macrophage cytotoxicity against metastatic tumors; it can activate the normal and alternative pathways of the complement system and can split C3 into C3a and C3b, enhancing macrophage activation (Aoki 1984; Wasser and Weis 1997a; Hobbs 2000).

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

Schizophyllan activates macrophages (in vitro and in vivo), which results in augmentation of T-cell activities and increases sensitivity of cytotoxic LAK and NK cells to IL-2 (Mizuno 1996). Although structurally related to lentinan, schizophyllan does not directly activate T-cells (Hobbs 1995). Possible pathways of such actions for lentinan have been summarized in Chihara (1981) and Hamuro and Chihara 1985), and reviewed by Wasser and Weis (1999), and those for β-D-glucan BRMs (Mizuno 2002) are shown in Figs. 2 and 3.

Selected examples of important medicinal mushrooms with antitumor polysaccharides in fruit bodies and cultured mycelium

One of the utmost important edible and medicinal biotechnological species, known as *A. blazei* was revaluated by our group. Analysis of data on cultivated mushroom originating from Brazil, and study of type material of *A. blazei* Murrill shows dramatic differences between

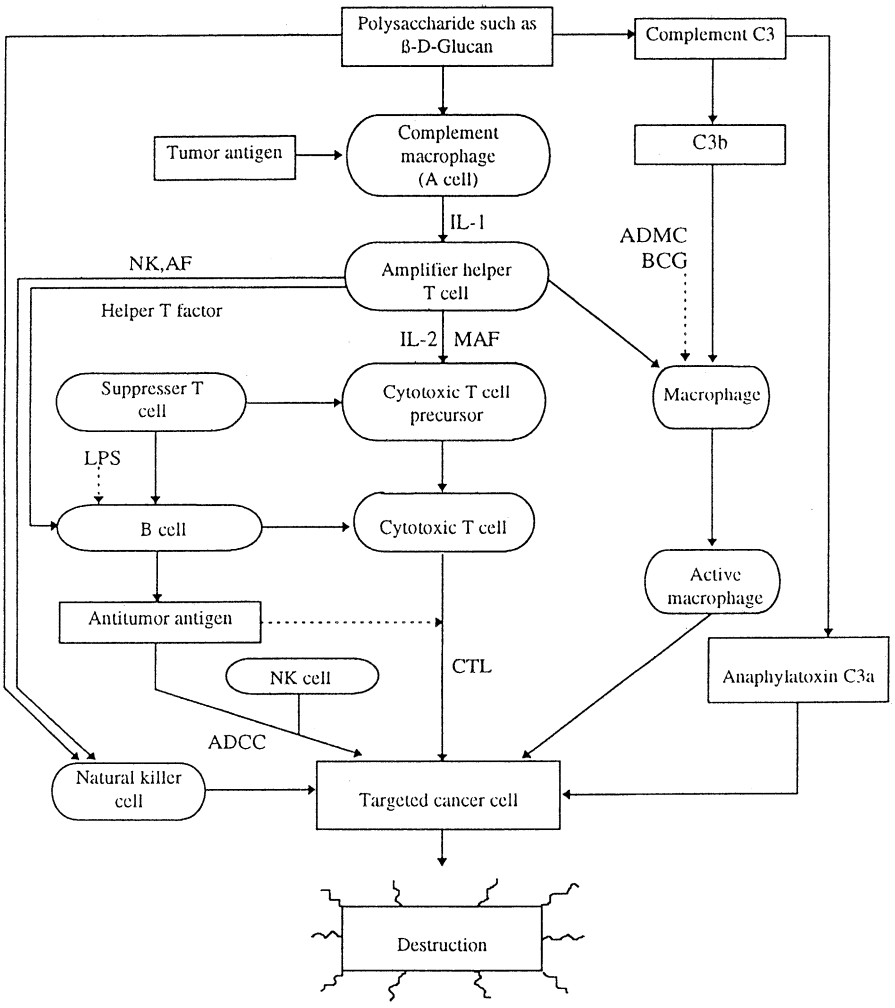

Fig. 3 Possible immune mechanism: β-D-glucan biological response modifier (BRM) (after Mizuno 2002)

them. On the basis of existing differences the correct name for widely cultivated mushroom was proposed as new for science species *Agaricus brasiliensis* S. Wasser et al. *A. blazei* is the North American endemic not cultivated species known from only three localities – one in Florida and two in South Carolina (Wasser et al. 2002).

Agaricus blazei mushroom (the Royal Sun *Agaricus*, ABM, Himematsutake, Cogmelo de Dues) is one of the more newly discovered medicinal mushrooms. This delicious edible mushroom is native to a very small area of the mountains of Brazil, near the town of Sao Paulo. Epidemiologists studying the native population in this area found that they had a very low incidence of a variety of illnesses, including cancers as well as viral and bacteria-induced diseases, and a disproportionately high number enjoyed longevity. Eventually this was correlated with constant consumption of *A. blazei* mushroom in their normal diet. During the 1980s and 1990s, *A. blazei* was demonstrated to be an immune system stimulant, promoting the body's natural defense mechanisms to fight a variety of infectious agents and conditions, including cancer. The immunostimulating activity and antitumor action of *A. blazei* extracts were investigated in different laboratory models, including Sarcoma 180 and (Meth-A) fibrosarcoma tumor-bearing mice (Kawagishi et al. 1989, 1990; Mizuno et al. 1990b, 1998; Mizuno 2002; Itoh et al. 1994; Ebina and Fujimiya 1998; Fujimiya et al. 1998a, 2000; Stamets 2000). Of the 17 polysaccharide fractions obtained from *A. blazei* fruit bodies (Mizuno et al. 1990a; Mizuno 2002), 7 were demonstrated to have antitumor activity. Analyses of physico-chemical properties of water-soluble polysaccharide fractions having high antitumor activity showed that their main components were β-(1→6)-; β-(1→3)-glucan, acidic β-(1→6)-; α-(1→4)-glucan, and acidic β-(1→6)-; α-(1→3)-glucan (Mizuno et al. 1990a). *A. blazei* was the first mushroom described to contain antitumor glucan with a β-(1→6)-linked backbone, unlike the well-known β-(1→3)-glucans. The antitumor proteoglucan HM3-G from *A. blazei* fruit bodies, which mediated natural killer cell activation and apoptosis, has a molecular mass of 380 kDa and consists of more than 90% glucose, the main component being α-(1→4)-glucan with β-(1→6)-branching, at a ratio of approximately 4:1 (Fujimiya et al. 1998b). It is interesting to note that a low molecular weight fraction, LM-3, with an average weight of 20 kDa, composed of α-(1→4)-glucan with β-(1→6)-branching, also demonstrated tumor-specific cytocidal and immunopotentiating

effects (Fujimiya et al. 1999), whereas pure glucan obtained from antitumor β-(1→6)-glucan-protein complex, isolated from water-insoluble residue of *A. blazei* fruiting bodies, did not exhibit strong activity (Kawagishi et al. 1990). Three immunostimulating heteroglucans (AG-2, -3, and -6) were extracted with 0.9% sodium chloride and hot water from fruiting bodies of *A. blazei* from among the six polysaccharides obtained (Cho et al. 1999). AG-2 and AG-3 were composed of glucose, galactose and mannose in the molar ratios 74.0:15.3:10.7 and 63.6:17.6:12.7, respectively; and AG-6 was composed of glucose and ribose at a molar ratio of 81.4:12.6.

A xyloglucan (Xyl:Glc, molar ratio =2:10) containing 9% protein obtained by fractionation and purification of *A. blazei* extract showed significant activity against Sarcoma 180 in mice (Mizuno 2002).

Not only fruit bodies but also cultured mycelia of *A. blazei* are a source of antitumor polysaccharides. An antitumor organic substance called ATOM was developed from *A. blazei* (Iwade strain 101), which is a PSPC (Ito et al. 1997). Another PSPC, 0041, was obtained from submerged culture mycelium; the main components of this polysaccharide are glucose and mannose (Hikichi et al. 1999). A new antitumor polysaccharide active against Sarcoma 180 was recently separated from liquid cultured mycelium of *A. blazei*: β-(1→2)-; β-(1→3)-glucomannan (Tsuchida et al. 2001). This polysaccharide appears to be completely different from the antitumor polysaccharides from fruiting bodies of *A. blazei* (Mizuno et al. 1999b).

A liquid medium filtrate separated from mycelium after submerged cultivation of *A. blazei* contained mannan-protein complex (AB-FP) with a molecular weight of 10^5–10^7 Da and a small amount of glucose, galactose, and ribose. The yield of AB-FP was 575 mg/1 liquid medium filtrate, and it possesses significant antitumor activity (Mizuno 2002).

Thus, antitumor polysaccharides investigated in *A. blazei* fruit body, culture mycelia, or produced extracellularly in a culture medium have different chemical structures. Polysaccharides from fruit bodies represented glucans with different types of glucose unit connections or heteroglucans; culture mycelia contained glucomannans, and mannan-protein complex was produced in a culture medium under submerged cultivation.

Ganoderma tsugae is the other medicinal mushroom in which polysaccharides have been well investigated in both the fruit body and mycelia. Seven glycans with strong antitumor activities were obtained from 14 water-soluble and 15 water-insoluble fractions extracted from *G. tsugae* fruit bodies (Wang et al. 1993). Water-soluble fractions were protein-containing glucogalactans associated with mannose and fucose, and water-insoluble fractions represented protein-containing β-(1→3)-glucans with different protein content.

Sixteen water-soluble polysaccharides were extracted from *G. tsugae* mycelium and examined for antitumor effects on Sarcoma 180 in mice (Zhang et al. 1994b). The three active polysaccharides obtained were: a glycan-protein complex containing 9.3% protein, with a heteropolysaccharide composed of mannose and xylose; a glucan-protein complex containing 25.8% protein; and a glycan-protein with glucose as the main component, and associated with arabinose, mannose, xylose, and galactose. Comparison of active water-soluble polysaccharides obtained from fruit body and mycelium showed that those from the fruiting body were glucogalactan-protein complexes, but those of the mycelium were homoglucan-protein complexes or a heteroglycan composed of mannose and xylose.

Grifola frondosa is one of the most popular medicinal mushrooms. Fruit bodies of this mushroom contain β-(1→3)-; β-(1→6)-glucan, acidic β-D-glucan (Mizuno et al. 1986; Jong and Birmingham 1990; Wasser and Weis 1999), and β-(1→6)-; β-(1→3)-glucan (Nanba et al. 1987) in the water-soluble polysaccharide fraction. Water-insoluble fractions include an acidic xyloglucan with a Glc:Xyl molar ratio of 100:82 and 16.5% glucuronic acid; an acidic heteroglycan containing 3.8% protein, component sugars Glc:Xyl:Man:Fuc (100:58:34:14); and three acidic glycoproteins with molecular masses of 20–100 kDa. The major component sugar is glucose, while fucose, xylose, mannose and galactose are minor components (Mizuno et al. 1986). Thus, all polysaccharides detected in *G. frondosa* fruit bodies are β-glucans with different chain conformation, heteroglucans, or glucoproteins.

In contrast to fruit body polysaccharide composition, no β-glucan has been detected among antitumor active fractions obtained from culture mycelium (grown on Whatman filter paper soaked with liquid nutrient medium) that was collected before initiation of fruit bodies (Mizuno and Zhuang 1995). In the water-soluble fractions, a fucogalactomannan-protein complex, a glucogalactomannan, a mannogalactofucan, and a galactoglucomannofucan-protein complex were found. In water-insoluble fractions, a mannofucoglucoxylan, a mannoglucofucoxylan-protein complex, a mannofucoglucoxylan-protein complex, and a glucomannofucoxylan-protein complex were found (Zhuang et al. 1994a). Thus, polysaccharides from *G. frondosa* are heteromannans, heterofucans, and heteroxylans, or their complexes with protein, i.e., types of polysaccharide that were not found in fruit bodies of this mushroom.

It must be stated that the polysaccharide structure in cultured mycelia may depend on the composition of the nutrient medium used for cultivation. Thus, Ohno and coworkers (1985, 1986) concluded that the antitumor glucan grifolan extracted from cultured mycelium of *G. frondosa* is a β-(1→3)-, β-(1→6)-glucan, the same as in the fruit body of the mushroom. In this experiment, a pure culture was growing in liquid medium in stationary culture or with shaking. The mycelium obtained was additionally cultivated for 3 days in a buffer composed of glucose (5%) and citric acid, pH 4.5. Antitumor active β-(1→3)-, β-(1→6)-glucans were obtained both by extraction of mycelium grown on a nutrient medium and by alcohol precipitation of buffer supernatant (Adachi et al. 2002).

Table 4 Number of polysaccharide fractions obtained from different Basidiomycetes

Species	Fruit body	Culture mycelium	References
Agaricus blazei	17		Mizuno et al. 1990a
Hericium erinaceus	15		Mizuno 1999b
Grifola frondosa	29	28	Mizuno et al. 1986; Cun et al. 1994; Zhuang et al. 1994a
Hohenbuehelia serotina	20		Ma et al. 1991
Pleurotus pulmonarius	21		Zhuang et al. 1993
Pleurotus citrinopileatus	21		Zhang et al. 1994a
Leucopaxillus giganteus	24		Mizuno et al. 1995a
Lyophyllum decastes	11		Ukawa et al. 2000
Inonotus obliquus	21	8	Mizuno et al. 1999a
Ganoderma tsugae	29	16[a]	Wang et al. 1993; Zhang et al. 1994b

[a] Number of fractions of water-soluble polysaccharides only

The number of polysaccharides extracted from fruiting body or cultured mycelium of the same species is strongly dependent on the method of fractionation used but, in general, the total amount of polysaccharides in fruiting bodies is higher (Table 4).

The number of fractions indicated in Table 4 includes, in some cases, not only finally purified polysaccharides but also some intermediate fractions that were tested for antitumor activity.

The proportion of biologically active polysaccharide fractions in fruit body and culture mycelium is very high. Thus, 20 of 29 polysaccharide fractions obtained from *G. frondosa* fruit body exhibited different levels of antitumor activity (Mizuno et al. 1986), and 24 of 28 polysaccharide fractions obtained from culture mycelium of this mushroom showed antitumor activity (Zhuang et al. 1994a).

The total number of polysaccharides extracted from the fruit body is higher, in general, than that obtained from culture mycelium. For example, the total of both water-soluble and water-insoluble polysaccharides obtained from *I. obliquus* sclerotium is 2–3 times higher than that extracted from cultured mycelium (Table 5).

Table 5 Yield of polysaccharide fractions from sclerotia and culture mycelium of *Inonotus obliquus* (after Mizuno et al. 1999b)

Water-soluble polysaccharides, g/kg dry weight		Water-insoluble polysaccharides, g/kg dry weight	
Sclerotium			
FIS-I	164.5	FII	2.64
FIS-II	12.0	FIII-1	42.48
		FIII-2	87.84
Mycelium			
FI	53.9	FII	43.15
		FIII-1	4.6
		FIII-2	21.1

Conclusions

Higher Basidiomycetes mushrooms are still far from being thoroughly studied; even the inventory of known species is incomplete, comprising maybe only 10% of the true number of species existing (Hawksworth 2001; Kirk et al. 2001). The number of mushrooms with known pharmacological qualities is much lower still. Nevertheless, the species studied so far represeent a vast source of anticancer and immunostimulating polysaccharides. Many, if not all, Basidiomycetes mushrooms contain biologically active polysaccharides. Of the 651 species and 7 infraspecific taxa from 182 genera of higher Hetero- and Homobasidiomycetes, the overwhelming majority have been demonstrated to possess pharmacologically active polysaccharides in their fruit bodies, culture mycelia, or culture broth (Reshetnikov et al. 2001).

Mushroom polysaccharides are of different chemical composition, mainly belonging to the group of β-glucans (Mizuno 1999a, 2000). The antitumor polysaccharides from various mushrooms are characterized by their molecular weight, degree of branching, and higher (tertiary) structure. It is evident that such structural features as β-(1→3) linkages in the main chain of the glucan and additional β-(1→6) branch points are needed for antitumor action. The β-glucans containing mainly (1→6) linkages have less activity. High molecular weight glucans appear to be more effective than those of low molecular weight (Mizuno 1996, 1999a, b). Unlike β-(1→3)-glucans, α-(1→3)-glucuronoxylomannans, which are characteristic of jelly mushrooms, are not strongly dependent on molecular weight.

Different approaches exist to improve the antitumor activity of mushroom polysaccharides by chemical modification, which is also necessary to improve their clinical qualities, water solubility and ability to permeate stomach walls after oral ingestion. Two main procedures for chemical improvement are: modification of mushroom polysaccharides by Smith degradation (oxydo-reductohydrolysis) and activation by the method of formolysis. The most successful schemes for such methods have been developed for *Ganoderma lucidum*, *Grifola frondosa* and *Leucopaxillus giganteus* (= *Tricholoma gigantea*). Carboxymethylation is another chemical method that transforms β-glucans into a water-soluble form.

A large body of experimental and clinical evidence demonstrates the beneficial results of mushroom poly-

saccharides for the following purposes: (1) prevention of oncogenesis by oral consumption of mushrooms or their preparations; (2) direct antitumor activity against various allogeneic and syngeneic tumors; (3) immunopotentiation activity against tumors in conjunction with chemotherapy; (4) preventive effects on tumor metastasis. Most of the clinical evidence comes from the commercial polysaccharides lentinan, PSK (krestin), and schizophyllan, but there are also impressive new data for polysaccharides from *Phellinus linteus*, *Flammulina velutipes*, *Hypsizygus marmoreus*, *A. blazei* and others.

The biochemical mechanisms that mediate the biological activity of polysaccharides are still not clearly understood. Polysaccharides from mushrooms do not attack cancer cells directly, but produce their antitumor effects by activating different immune responses in the host. The antitumor action of polysaccharides requires an intact T-cell component; their activity is mediated through a thymus-dependent immune mechanism (Borchers et al. 1999). Mushroom polysaccharides are known to stimulate natural killer cells, T-cells, B-cells, and macrophage-dependent immune system responses. The immunomodulating action of mushroom polysaccharides is especially valuable as a means of prophylaxis, a mild and non-invasive form of treatment, prevention of metastatic tumors, and as a co-treatment with chemotherapy.

A wide range of biologically active polysaccharides is found among higher Basidiomycetes mushrooms, and their practical application is dependent not only on their unique properties but also on biotechnological availability. Isolation and purification of polysaccharides from mushroom material is relatively simple and straightforward, and can be carried out with minimal effort (Mizuno 1996, 1999a). Mycelia formed by growing pure cultures in submerged conditions are of constant composition, and submerged culture is the best technique for obtaining consistent and safe mushroom products (Wasser et al. 2000; Reshetnikov et al. 2001).

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