Antitumor and immunomodulatory activities of medicinal mushroom polysaccharides and polysaccharide-protein complexes in animals and humans (Review)

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Abstract. The number of mushrooms on Earth is estimated at 140 000, yet perhaps only 10 % (approximately 14 000 named species) are known. They make up a vast and yet largely untapped source of powerful new pharmaceutical products. Particularly, and most important for modern medicine, they present an unlimited source for polysaccharides with anticancer and immunostimulating properties. Many, if not all Basidiomycetes mushrooms contain biologically active polysaccharides in fruit bodies, cultured mycelia, and culture broth. The data about mushroom polysaccharides are summarized for 651 species and seven intraspecific taxa from 182 genera of higher Hetero- and Homobasidiomycetes. These polysaccharides are of different chemical composition; the main ones comprise the group of β-glucans. β-(1→3) linkages in the main chain of the glucan and further β-(1→6) branch points are needed for their antitumor action. Numerous bioactive polysaccharides or polysaccharide-protein complexes from medicinal mushrooms are described that appear to enhance innate and cell-mediated immune responses, and exhibit antitumour activities in animals and humans. Stimulation of host immune defense systems by bioactive polymers from medicinal mushrooms has significant effects on the maturation, differentiation, and proliferation of many kinds of immune cells in the host. Many of these mushroom polymers were reported previously to have immunotherapeutic properties by facilitating growth inhibition and destruction of tumour cells. Whilst the mechanism of their antitumor actions is still not completely understood, stimulation and modulation of key host immune responses by these mushroom polymers appears central. Recent evidence suggests that mushroom polymers (β-glucans) may trigger the stimulation of many kinds of immune cells in animals and humans. Several of the mushroom polysaccharide compounds have proceeded through Phases I, II, and III clinical trials, and are used extensively and successfully in Asia to treat various cancers and other diseases. The present review analyzes the pecularities of polysaccharides derived from fruit bodies and cultured mycelia (two main ways of biotechnological production today) in selected examples of medicinal mushrooms.

Key words: active hexose correlated compound (AHCC), beta-glucans, Ehrlich carcinoma, immunomodulator activity, macrophages, polysaccharides, polysaccharide-protein complexes, Sarcoma 180

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Introduction

For millennia, mushrooms have been valued as edible and medical provisions for humankind. 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 & Anke 1998; Borchers *et al.* 1999; Ooi & Liu 1999; Wasser & Weis 1999; Tzianabos 2000; Reshetnikov *et al.* 2001; Wasser 2002; Didukh *et al.* 2003; Rowan *et al.* 2003; Smith *et al.* 2003). 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). Such mushrooms as *Ganoderma lucidum* (W. Curt. : Fr.) P. Karst. (Reishi), *Lentinus edodes* (Berk.) Singer (Shiitake), *Inonotus obliquus* (Ach. ex Pers.) Pilát (Chaga) and many others have been collected and used for centuries 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 area of medicinal mushrooms (Ying *et al.* 1987; Hobbs 1995, 2000; Wasser & Weis 1997a, b, 1999; Stamets 2000; Wasser 2002).

Chihara *et al.* (1969, 1970) published the first scientific report on antitumor activity of polysaccharides from *Lentinus edodes* on mouse Sarcoma 180. Ikekawa *et al.* (1969, 1982) published reports on antitumor activities of essences obtained from fruit bodies of Polyporaceae species (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* (L. : Fr.) Lloyd (Turkey Tail), Lentinan from fruit bodies of *Lentinus edodes,* and Schizophyllan from liquid medium broth product of *Schizophyllum commune* Fr. : Fr. In the 40 years since then 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 to practical use (Mizuno 1996, 1999a; Wasser & Weis 1999; Ikekawa 2001; Wasser 2002; Didukh *et al.* 2003; Rowan *et al.* 2003; Smith *et al.* 2003).

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, proteoglucan Krestin was developed in Japan from the strain of *Trametes 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 & 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 peculiarities. The review summarizes a 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 fruit bodies and pure culture mycelia in selected examples of the studied medicinal mushrooms.

The diversity of mushrooms with antitumor and immunomodulatory polysaccharides

Recent estimates of the number of fungi on Earth range from 500 000 to 9.9 million species, and the generally accepted working figure is 1.5 million (Hawksworth 2001). Meanwhile, the total number of described fungi of all kinds is currently 80 060 species. The figure is based on the total reached by summing the numbers of species in each genus given in the last edition of the *Dictionary of the Fungi* (Kirk *et al.* 2001) and includes all organisms traditionally studied by mycologists: slime molds, chromistan fungi, chytridiaceous fungi, lichen-forming fungi, filamentous fungi, molds, and yeasts. Out of these, mushrooms constitute 14 000 species, calculated from the *Dictionary of the Fungi* (Kirk *et al.* 2001) or go as high as 22 000 (Hawksworth 2001). However, the actual number of such species on Earth is undoubtedly much higher.

By the term 'mushroom' we generally take the definition by Chang & Miles (1992): "a macrofungus with a distinctive fruit body which can be hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand". Two main reasons for the actual 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 (Anderson & Stasovski 1992; Hawksworth 2001) or phylogenetic (Kasuga *et al.* 1999; Taylor *et al.* 1999) species. Studies of compatibility 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) and a varying number of phylogenetic species (Taylor *et al.* 2000).

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). This is also the case for mushrooms, especially those species forming ectomycorrhizas with native trees, although new species continue to be discovered in Europe and North America. In various tropical areas, 22-55 % (in some cases up to 73 %) of mushroom species have proved to be undescribed (Hawksworth 2001).

Considering all this, it is reasonable to estimate the number of mushrooms on Earth at 140 000. Thus, the currently known 14 000 represent perhaps only 10 % of those that exist in nature. Meanwhile, of those approximately 14 000 species that we know today, about 50 % are considered to possess varying degrees of edibility, more than 2000 are safe, and about 700 species are known to possess significant pharmacological properties (Chang 1999; Wasser & Weis 1999; Reshetnikov *et al.* 2001; Wasser 2002). Clearly, mushrooms represent a major and yet largely untapped source of powerful new pharmaceutical products (Wasser 2002).

All the main taxonomic higher Basidiomycetes groups have been investigated for biologically active polysaccharides, and most of them possess such substances. At least 651 species and seven infraspecific taxa representing 182 genera of Hetero- and Homobasidiomycetes mushrooms contain antitumor or immunostimulating polysaccharides, as is evident from Table 1 (adapted from Reshetnikov *et al.* 2001 and Wasser 2002). Naturally collected or artificially grown fruit bodies, pure culture mycelia, and culture filtrate (culture broth) contain biologically active polysaccharides.

Table 1. Higher Basidiomycetes mushrooms containing antitumor or immunostimulating polysaccharides

Taxa (number of species studied)	**Activity against** (% of tumor inhibition)		**Source**
	Sarcoma 180 solid cancer	**Ehrlich solid cancer**	
Heterobasidiomycetes			
Auriculariales – *Auricularia* (3)	70-90	60-80	Ohtsuka *et al.* 1973 Song, C.H. *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)			Ohtsuka *et al.* 1973 Gao, Q.P. *et al.* 1997
Homobasidiomycetes			
Aphyllophoromycetideae			
Cantharellaceae – *Cantharellus* (5), *Craterellus* (2)	60-100	60-90	Ohtsuka *et al.* 1973
Clavariaceae – *Clavaria* (4), *Clavariadelphus* (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 & 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 Mizuno1999b
Corticiaceae – *Aleurodiscus* (1), *Cotylidia* (2), *Laxitextum* (1), *Lopharia* (1), *Merulius* (2), *Phlebia* (2), *Sarcodontia* (1), *Steccherinum* (1), *Stereum* (13)	60-100	60-100	Ohtsuka *et al.* 1973
Coniophoraceae – *Serpula* (1)	70	60	Ohtsuka *et al.* 1973

Table 1. (continued)

Taxa (number of species studied)	Activity against (% of tumor inhibition)		Source
	Sarcoma 180 solid cancer	Ehrlich solid cancer	
Thelephoraceae – *Bankera* (1), *Calodon* (4), *Hydnellum* (2), *Polyozellus* (1), *Sarcodon* (2), *Thelephora* (1)	60-100	70-100	Ohtsuka *et al.* 1973 Song, C.H. *et al.* 1998 Mizuno 2000
Hymenochaetaceae – *Coltricia* (4), *Cryptoderma* (6), *Cyclomyces* (1), *Fuscoporia* (1), *Hymenochaete* (4), *Hymnostilbe* (1), *Inonotus* (6), *Onnia* (1), *Phellinus* (6), *Pyrrhoderma* (1)	60-100	90-100	Ohtsuka *et al.* 1973 Kim, H.M. *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 & Nishijima 1981 Ukai *et al.* 1983 Zhang & 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 & Lin 1979 Min *et al.* 1980 Nakajima, K. *et al.* 1980 Kanayma *et al.* 1986 Mizuno, T. *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
Gasteromycetideae			
Gasteromycetales			
Lycoperdaceae – *Lycoperdon* (2) Phallaceae – *Dictyophora* (1), *Kobayasia* (1)	-	-	Song, C.H. *et al.* 1998 Miyazaki, T. *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

Table 1. (continued)

Taxa (number of species studied)	Activity against (% of tumor inhibition)		Source
	Sarcoma 180 solid cancer	**Ehrlich solid cancer**	
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, C.H. *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.* 1992 Kim, B.K. *et al.* 1982 Ma *et al.* 1991 Ikekawa *et al.* 1982 Kiho *et al.* 1992a, b Mizuno, T. *et al.* 1994 Liu, F. *et al.* 1996 Wang *et al.* 1996 Song, C.H. *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, C.H. *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, C.H. *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* (11), *Cystoderma* (2), *Lepiota* (15), *Leucocoprinus* (3), *Macrolepiota* (2), *Melanophyllum* (1), *Phaeolepiota* (1)	60-100	60-100	Ohtsuka *et al.* 1973 Didukh *et al.* 2003
Coprinaceae – *Coprinus* (16), *Panaeolus* (1), *Psathyrella* (7), *Pseudocoprinus* (1)	60-100	60-100	Ohtsuka *et al.* 1973 Mizuno 2002
Russulales			
Russulaceae – *Lactarius* (18), *Russula* (23)	60-100	70-100	Ohtsuka *et al.* 1973

Structural composition of antitumor and immunomodulatory polysaccharides in mushrooms

Polysaccharides belong to a structurally diverse class of macromolecules, polymers of monosaccharide residues joined to each other by glycosidic linkages. Compared 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 (Kawaguchi 2005). This enormous potential variability in polysaccharide structure gives the necessary flexibility to the precise regulatory mechanisms of various cell-cell interactions in higher organisms.

Mushroom polysaccharides are mostly present 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 & 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, T. *et al.* 1995c).

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* Berk. was demonstrated as having a left-handed, three-fold 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. In heteroglucans, side chains contain glucuronic acid, xylose, galactose, mannose, arabinose, and 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. Heteroglycans' 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 have been investigated; the main types are presented in Table 2.

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

Polysaccharide	Species	References
Glucans		
α-(1→3)-glucan	*Armillariella tabescens* (Scop. : Fr.) Emel	Kiho *et al.* 1992a
Linear α-(1→3)-glucan	*Amanita muscaria* (L.) Hook	Kiho *et al.* 1994
	Agrocybe aegerita (V. Brig.) Singer	Yoshida *et al.* 1996
α-(1→4)-; β-(1→6)-glucan	*Agaricus brasiliensis* S. Wasser *et al.* (= *A. blazei*)	Fujimiya *et al.* 1998b
α-(1→6)-; α-(1→4)- glucan	*Agaricus brasiliensis*	Mizuno, T. *et al.* 1990a
β-(1→6)-glucan	*Lyophyllum decastes* (Fr.) Singer	Ukawa *et al.* 2000
	Armillariella tabescens	Kiho *et al.* 1992a
β-(1→6)-; β-(1→3)-glucan	*Agaricus brasiliensis*	Mizuno, T. *et al.* 1990a
	Grifola frondosa (Dicks. : Fr.) S.F. Gray	Nanba *et al.* 1987
β-(1→6)-; α-(1→ 3)-glucan	*Agaricus brasiliensis*	Mizuno, T. *et al.* 1990a
β-(1→3)-glucuronoglucan	*Ganoderma lucidum*	Wasser & Wies 1997b
Mannoxyloglucan	*Grifola frondosa*	Mizuno, T. *et al.* 1986
Galactoxyloglucan	*Hericium erinaceus* (Bull.) Pers.	Mizuno 1999b
Xyloglucan	*Grifola frondosa*	Mizuno, T. *et al.* 1986
	Albatrellus confluens (Alb. et Schwein.) Kotl. et Pouzar	Mizuno, T. *et al.* 1992
	Pleurotus pulmonarius (Fr.) Quél. (= *P. sajor-caju* (Fr.) Singer)	Zhuang *et al.* 1993

Table 2. (continued)

Polysaccharide	Species	References
Xylogalactoglucan	*Inonotus obliquus*	Mizuno, T. *et al.* 1999a
Mannogalactoglucan	*Pleurotus pulmonarius* *Pleurotus cornucopiae* (Paulet ex Pers.) Roll. *Ganoderma lucidum* *Agaricus brasiliensis*	Kim & Kim 1999 Ebina & Fujimiya 1998
Galactomannoglucan	*Flammulina velutipes* (W. Curt. : Fr.) Singer *Hohenbuehelia serotina* (Pers. : Fr.) Singer *Leucopaxillus giganteus* (Sow. : Fr.) Singer (= *Tricholoma giganteum* Massee)	Ikekawa *et al.* 1982 Mizuno, T. *et al.* 1994 Mizuno, T. *et al.* 1995a
Arabinoglucan	*Ganoderma tsugae* Murrill	Zhuang *et al.* 1994b
Riboglucan	*Agaricus brasiliensis*	Didukh *et al.* 2003
Glycans		
Arabinogalactan	*Pleurotus citrinopileatus* Singer	Zhuang *et al.* 1994a
Glucogalactan	*Ganoderma tsugae*	Wang *et al.* 1993
Fucogalactan	*Sarcodon imbricatus* (L. : Fr.) P. Karst. (= *S. aspratus* (Berk.) S. Ito)	Mizuno, M. *et al.* 2000
α-(1→6)-mannofucogalactan	*Perenniporia fraxinea* (Bull.) Ryvarden (= *Fomitella fraxinea* (Bull.) Imazeki)	Reshetnikov *et al.* 2001
Fucomannogalactan	*Phallus indusiatus* Vent. (= *Dictyophora indusiata* (Vent.) Desv.)	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* *Pleurotus pulmonarius*	Mizuno 1999b Zhuang *et al.* 1993
Mannoglucoxylan	*Hericium erinaceus*	Mizuno 1999b
α-(1→3)-mannan	*Phallus indusiatus*	Ukai *et al.* 1983
Glucomannan	*Agaricus brasiliensis*	Hikichi *et al.* 1999
β-(1→2)-; β-(1→3)-glucomannan	*Agaricus brasiliensis*	Tsuchida *et al.* 2001 Mizuno, T. *et al.* 1999a
Galactoglucomannan	*Lentinus edodes*	Fujii *et al.* 1979

The number of antitumor active fractions in fruit bodies and cultured mycelia of mushrooms is remarkably high. For example, the both water-soluble and water-insoluble polysaccharides obtained from *Inonotus obliquus* sclerotium are two- or threefold higher than those extracted from cultured mycelia (Tab. 4). Another 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).

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

Polysaccharide structure in cultured mycelia may depend on the composition of the nutrient media used for cultivation. Ohno *et al.* (1985, 1986) concluded that antitumor glucan Grifolan extracted from cultured mycelia of *Grifola frondosa* is a β-(1→3)-; β-(l→6)-glucan, the same as in the fruit body of the mushroom. In this experiment, a pure culture was grown in a liquid medium in a stationary culture or with shaking. The mycelium obtained was cultivated for three days more in a buffer

composed of glucose (5 %) and citric acid, pH 4.5. Antitumor active β-(1→3)-, β-(1→6)-glucans were obtained by extraction of mycelium grown on a nutrient medium and by alcohol precipitation of a buffer supernatant (Adachi *et al.* 2002).

The number of polysaccharides extracted from the fruit bodies or the cultured mycelia of the same species is strongly dependent on the methods of fractionation used, but in general the total amount of biologically active polysaccharides in the fruit bodies is higher (Tab. 3). Twenty polysaccharide fractions out of the 29 obtained from *G. frondosa* fruit body demonstrated different values of antitumor activity (Mizuno, T. *et al.* 1986), and 24 polysaccharide fractions out of the 28 obtained from cultured mycelia of this mushroom showed antitumor activity (Zhuang *et al.* 1994a, b).

Table 3. Number of polysaccharide fractions obtained from different Basidiomycetes

Species	Fruit body	Culture mycelium	References
Agaricus brasiliensis	17		Mizuno, T. *et al.* 1990a, b; Didukh *et al.* 2003
Hericium erinaceus	15		Mizuno, T. *et al.* 1990b
Grifola frondosa	29	28	Cun *et al.* 1994; Mizuno, T. *et al.* 1986; Zhuang *et al.* 1994a
Hohenbuehelia serotina	20		Ma *et al.* 1991
Pleurotus pulmonarins	21		Zhuang *et al.* 1993
Pleurotus citrinopileatus	21		Zhuang *et al.* 1994a
Leucopaxillus giganteus	24		Mizuno, T. *et al.* 1995 c, d
Lyophyllum decastes	11		Ukawa *et al.* 2000
Inonotus obliquus	21	8	Mizuno, T. *et al.* 1999b
Ganoderma tsugae	29	16[a]	Wang *et al.* 1993; Zhuang *et al.* 1994b

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

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.

Table 4. Yield of polysaccharide fractions from sclerotia and cultured mycelia of *Inonotus obliquus* (after Mizuno, T. *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

Correlation of structure and antitumor activity 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 & 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.

Obviously, 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 (MW) (Mizuno 1996, 1999a, b). However, distinct variations in antitumor polysaccharides have also been noted. Antitumor polysaccharides may have other chemical structures, such as hetero-β-glucans (Mizuno, T. *et al.* 1995c), heteroglycan (Gao, Q.P. *et al.* 1996b), β-glucan-protein (Kawagishi *et al.* 1990), α-manno-β-glucan, α-glucan-protein (Mizuno, T. *et al.* 1995c), and heteroglycan-protein complexes (Zhuang *et al.* 1993; Mizuno, T. *et al.* 1996).

A triple-helical tertiary conformation of medicinal mushroom β-(1→3)-glucans is known to be important for their immune-stimulating activities, such as macrophage nitrogen oxide synthesis and limulus factor G activation. Denaturation of Lentinan with dimethyl sulfoxide, urea, or sodium hydroxide affected the tertiary structure, while primary structure stayed intact. These conformation changes following progressive denaturation lowered tumor inhibition properties (Maeda *et al.* 1988). The same results, which confirmed the correlation between antitumor activity and triple helix structure, were obtained upon investigation of Schizophyllan (Yanaki *et al.* 1983, 1986), indicating that some of the immuno-stimulating activities are dependent on the triple-helix conformation. However, other activities are independent of it, e.g., synthesis of interferon-γ and colony stimulating factor (Yadomae 2000); this indicates that β-(1→3)-mannan backbone structure is of more importance than the tertiary structure of the molecule.

Unlike β-(1→3)-glucans (ranging from 500 to 2000 Da) with medicinal properties that are heavily dependent on high molecular weight (Mizuno 1996), α-(1→3)-glucuronoxylomannans, which are characteristic of Jelly mushrooms, are not heavily dependent on molecular weight. Gao, Q.P. *et al.* (1996a) reported that acidic hydrolysate fractions of *Tremella fuciformis* fruit bodies' glucuronoxylomannans with molecular weight from 53 to 1 kDa induced human monocytes to produce interleukin-6 as efficiently as non-hydrolyzed heteropolysaccharides. This indicates that the activity may be due to the common structure of the α-(1→3)-mannan backbone (Gao, Q.P. *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 have been developed for *Ganoderma lucidum, Grifola frondosa,* and *Leucopaxillus giganteus.* These schemes include two main procedures: modification of mushroom polysaccharides by the Smith degradation (oxydo-reducto-hydrolysis) and activation by the method of formolysis (Mizuno 1996, 1999a; Mizuno, T. *et al.* 1996). Five polyaldehydes and ten polyalcohols were prepared by the Smith degradation method from five polysaccharide fractions previously obtained from *Grifola frondosa* liquid culture mycelium. For this reason original polysaccharide solutions were first oxidized 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; one-half of the precipitate is lyophilized after dialysis, while the other part is dissolved in hot water and additional fractions are 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 *Grifola 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-glucans or xyloglucans, 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* (Jacq. : Fr.) P. Kumm. 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 chloride 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, and every fourth glucopyranosyl residue was substituted at 0-6 by single D-glucopyranosyl group. 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 & Kuniak 1994). Carboxymethylated glucan from *P. ostreatus* (Pleuran) exhibited immunomodulatory effects especially increased phagocytic activity (Paulik *et al.* 1996).

In a similar manner, water-insoluble, alkali-soluble linear α-(1→3)-glucans obtained from fruit 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 the Smith degradation method 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 Da 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 & 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, W. *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, W. *et al.* 1990; Hobbs 1995). Medical tests indicate that sulfated Schizophyllan with sulfur content of 5 % can be useful as an anti-HIV agent for treatment of HIV-infected hemophiliacs (Hirata *et al.* 1994; Hobbs 1995).

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 BRMs/biologically active substances

We would like to emphasize the principal points of antitumor and immunomodulating effects of mushroom biologically active substances the most significant of which are polysaccharides. Most important among them are: (1) prevention of oncogenesis through 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.

Lentinus edodes

An immense literature treats the anticancer effects of Lentinan on animals and humans, and only the more relevant and recent medical studies will be presented here. Lentinan was first isolated and studied by Chihara *et al.* (1970), who demonstrated that its antitumour effects were greater than those of other mushroom polysaccharides; it was active for some, but not all, types of tumors (Maeda *et al.* 1988). The purified polysaccharide has been shown in numerous xenographs to cause tumor regression and in some cases even a complete response (for extensive review of animal studies, see Hobbs 1995, 2000; Wasser & Weis, 1999; Yap & Ng 2003). The cytostatic effect of Lentinan is due to the activation of the host's immune system. Also, pre-clinical and clinical toxicity with Lentinan is rarely noted. Accumulated information on the antitumor activity, the prevention of metastasis, and the suppression of chemical and viral oncogenesis in animal models by Lentinan is summarized in Table 5 (Wasser & Weis 1999).

While Lentinan is a pure polysaccharide composed only of atoms of carbon, oxygen, and hydrogen, LEM (= glycoprotein from *Lentinus edodes* mycelia) and LAP (= glycoprotein from *L. edodes* culture media) have also demonstrated antitumor activity in xenograph models and clinical trials. Again, both LEM and LAP activate the host immune system (Mizuno 1995). In Japan, Lentinan is presently classified as a medicine whereas LEM and LAP are considered food supplements (nutriceuticals).

There have been numerous clinical trials of Lentinan in Japan, though none has been placebo-controlled and double-blinded. However, Lentinan has been approved for clinical use in Japan for many years, and is manufactured by several pharmaceutical companies. Intraperitoneal Lentinan is widely used as an adjuvant treatment for certain cancers in Japan and China.

Lentinan has proved successful in prolonging the overall survival of cancer patients, especially those with gastric and colorectal carcinoma. In patients with inoperable or recurrent gastric cancer, tumor responses and prolonged median survival were also noted. In a randomized controlled study of patients treated with tegafur, or a combination of Lentinan and tegafur, overall survival was significantly prolonged in the Lentinan plus tegafur group. Of 145 patients, 68 received tegafur alone and 77 received Lentinan plus tegafur. The respective 50 % survival times for the two groups were 92 days (tegafur alone) and 173 days (Lentinan plus tegafur). Sub-group analysis was also carried out by: (1) tumor extension; (2) histology; and (3) Borrman classification. With each prognostic factor the addition of Lentinan significantly prolonged 50 % survival (Furue *et al.* 1981; Taguchi *et al.* 1985).

Table 5. Lentinan – pre-clinical animal models (Wasser & Weis 1999)

Model	Model	Dose of Lentinan (mg/kg × days)	Tumor inhibition ratio (%)	Complete regression of tumor	Decreased tumor occurrence
Allogeneic					
Sarcoma 180	CD-1/ICR	0.2 × 10	78.1	6/10	
		1 × 10	100.0	10/10	
		25 × 10	88.2	0/8	
		80 × 5	-8.5	0/8	
	SWM/Ms	1 × 10	100.0	10/10	
	A/J	4 × 5	96.5	9/10	
	C3H/He	4 × 5	36.2	0/6	
	C57/BI/6	4 × 5	51.8	0/6	
Syngeneic					
A/Ph.MC.S1	A/Ph(A/J)	1 × 10	100.0	18/18	
DBA/2.MC.CS1	DBA/2	1 × 10	76.5	2/7	
P-815	DBA/2	5 × 4	89.0	2/8	
L-5178Y	DB/2	10 × 3	84.0	3/9	
MM-46	C3H/He	5 × 2	100.0	9/9	
Autochthonous					
MC-induced primary	DBA/2	1 × 10	80.5	2/5	
Inhibition of metastasis					
DBA/2.MC.CS-T	DBA.2	1 × 10	94.2		
MH-134	C3H/He	1 × 14	100.0		
Madison-109	BALB/c	25 × 2			
Prevention of oncogenesis					
MC-induced	SWM/Ms	1 × 10			83→31 %
MC-induced	DBA/2	1 × 10			78→37 %
Adenovirus	C3H/He	10 × 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 × 100, where C = average tumor weight of control mice and T = that of Lentinan-treated mice.

Overall, more patients with the combined therapy appeared to survive longer: 19.5 % survived more than one year, 10.4 % more than two years, and 6.5 % more than three years. By the criteria of the Japan Society for Cancer Therapy for Evaluation of Clinical Effects of Cancer Chemotherapy on Solid Tumors, patients treated with Lentinan had a significantly higher response rate (14.9 %) than patients in the control arm (2.0 %).

Lentinan combined with other chemotherapeutic agents appears to have efficacy in a variety of settings (Matsuoka *et al.* 1995). Furthermore, when patients responded well to Lentinan treatment there was a significantly larger response (2.5×) in their killer T cell/suppressor T cell ratio ($CD11^-$ $CD8^+$/ $CD11^+$ $CD8^+$) in peripheral blood. The ratio of NK cells with higher activity to NK with moderate activity ($CD57^-$ $CD16^+$/ $CD57^+$ $CD16^+$) was higher in the responders than in the non-responders and correlated well with survival times. However, these results remain controversial as a later study suggested that lymphocyte subset changes in peripheral blood did not necessarily correlate with the lymphocyte subset changes that were taking place in the tumour (Matsuoka *et al.* 1997).

Few adverse reactions to Lentinan have been noted. In a detailed study of 469 patients, 32 (6.8 %) experienced an adverse reaction – none serious; the total number of episodes was 46 (9.8 %). Only two patients required discontinuation of treatment due to unacceptable tolerance. Perhaps the most intriguing aspect of Lentinan use in conjunction with chemotherapy is its apparent ability to reduce greatly the debilitating effects of the chemotherapy, e.g. nausea, pain, hair loss, and lowered immune status.

Apart from the polysaccharides, *L. edodes* has been shown recently to harbor substances with medicinal properties other than polysaccharides, for instance, the protein Lentin. This protein exerts antifungal activity in tests with *Physalospora pyricola* Nose, *Botrytis cinerea* Pers., and *Mycosphaerella arachidicola* Khohr. (Ngai & Ng 2003). The 27.5 kDa protein has an inhibitory activity on HIV-1 reverse transcriptase and proliferation of leukemia cells.

The antifungal activity was conspicuous at a dose of 10 µg. Inhibition of leukemia cells proliferation was with an IC_{50} of 2 µM, HIV 1 reverse transcriptase with an IC_{50} of 1.5 µM (Ngai & Ng 2003).

Trametes versicolor

Trametes versicolor is not an edible mushroom but from ancient times its extracts have been used in traditional Chinese medicine for therapeutic effects, including the treatment of cancer. Today, two compounds, PSK (polysaccharide-K, commercial name 'Krestin') and PSP (polysaccharide-peptide) are purified from this fungus by deep tank fermentation of the mycelium using a variety of strains. PSK was first isolated in Japan in the late 1960s while PSP was isolated in 1983 in China. Each compound has shown remarkable anticancer properties with few side effects. Remarkably, by 1987 PSK accounted for more than 25 % of total national expenditure for anticancer agents in Japan (Mizuno 1995).

PSK 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 (Hobbs 1995). 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; Smith *et al.* 2003), but seldom has satisfactory results when administered alone.

PSP

While PSK has been almost exclusively developed and tested within Japan, PSP is a product of China and continues to be assessed for efficacy safety by China's scientists and oncologists. There is a close similarity between PSK and PSP polypeptides although PSP lacks fucose and instead contains arabinose and rhamnose. Since the first development of PSP in 1983 there has been rapid progress through human clinical trials. Phase I clinical trials were carried out by Xu (1999) and it was shown that an oral dose of up to 6g/day was well tolerated and devoid of side effects. Patients showed improvement in appetite and general condition, together with a stabilization of haematopoietic parameters.

The Phase II study by the Shanghai PSP Research Group with eight hospitals in Shanghai was carried out on patients with cancers of the stomach, lung, and oesophagus. The dosage was 1 g three times daily for a total of 190 g. Results confirmed the role of PSP as a biological response modifier, improving the immunological status of the patients after surgery, radiotherapy, and/or chemotherapy (Liu & Zhou 1993). Following the success of the Phase II clinical trials, a Phase III trial was conducted in a large cohort of patients (650) in Shanghai hospitals. One hundred eighty-nine patients were randomized to take PSP and placebo; 461 patients were aware of their therapy (Liu, J.X. *et al.* 1999). These trials showed that PSP improved disease-free survival of gastric, oesophageal, and non-small-cell lung cancers substantially reducing the usual unpleasant side effects of conventional treatments (Sun & Zhu 1999; Sun *et al.* 1999). Such protective effect on the immunological functions of conventionally treated patients demonstrates that PSP can be classified as a clinical biological response modifier (BRM). Other BRMs such as LAK cells, IL-2, α IFN or TNF-α are also being used in the treatment of advanced cancer cases (Liu, J.X. *et al.* 1999). However, these drugs in effective doses often produce quite severe side effects such as fevers, chills, rashes, arthralgia, hypotension, pulmonary oedema, congestive heart failure, and CNS toxicities. Combination of PSP with IL-2 has shown dramatic antitumor effects. As side effects of IL-2 are dosage and schedule dependent, it is reasonable to expect that with PSP, a lower dose of IL-2, could be used clinically with subsequent decrease in the severity of the side effects (Liu & Zhou 1993; Liu, J.X. *et al.* 1999). A further observation noted that PSP in combination with radiotherapy induced a significant increase in the percentage of apoptotic cells at 24 h, compared with radiation alone, and it has been surmised that the antitumor mechanism of PSP action may also involve the induction of DNA damage by apoptosis in the target cancer cells (Stephens *et al.* 1991). Another beneficial side of PSP application is a strong amelioration of haematopoietic toxicity (Shiu 1992; Sun *et al.* 1999), a common adverse reaction of radiotherapy and chemotherapy.

In a double blinded Phase II trial in Shanghai hospitals almost 300 patients suffering from gastric, oesophageal or lung cancer were treated with conventional radiotherapy and/or chemotherapy together with PSP or shark liver oil (batyl alcohol). Quality of life was assessed by marked improvement of clinical symptoms along with most symptoms associated with cancer therapy, improvements in blood profiles and/or immune indices, and significant improvement in Karnovsky performance status or body weight. PSP was found to be effective for 82 % of the patients compared with 48 % for batyl alcohol (Liu & Zhou 1993).

Many Phase III clinical trials of PSP combined with conventional therapies have demonstrated significant benefits against cancers of the stomach, oesophagus, and lung (Jong & Yang 1999; Yang 1999). Most studies with PSP have not fully explored the long-term survival benefit, although an open-label, randomized trial in oesophageal cancer showed that PSP did significantly improve one-year and three-year survival (Yao 1999). Liu, L.F. (1999) has commented on the favorable action of PSP in patients receiving bone autologous marrow transplants.

The corpus of laboratory and clinical evidence that PSP offers considerable benefits to patients suffering from cancers of the stomach, oesophagus, and lung led to the Chinese Ministry of Public Health granting it a regulatory license.

Despite the use of PSK and PSP in humans for many years, bioavailability and the pharmacokinetics have received little detailed study. More work in this area, as well as blind RCT's, are required.

PSP produced no teratogenic effects in mice or rats and exerted analgesic action in mice (Jiang *et al.* 1999; Jin 1999). It has been shown that some compounds with proven antitumor and immunomodulatory activities inhibit ovulation and ovarian steroidogenesis, increase the incidence of oocyte degeneration, and demonstrate abortifacient and embryotoxic effects. The lack of deleterious effects of PSP on ovarian follicular development, steroidogenesis, ovulation, quality of ovulated oocytes, pregnancy, and embryo development in mice would suggest that it does not affect female reproduction (Ng & Chang 1997).

Mutagenicity testing can now be viewed against an impressive background of basic scientific knowledge of genetic mechanisms and also in the development of a wide range of experimental procedures that can be used as test systems. Recently, Zhong *et al.* (1999) carried out an extensive series of experiments on possible genetic toxicity of the PSP polysaccharopeptide:

1. Mutagenicity tests to assess genotoxicity of PSP using a special strain of *Salmonella typhimurium* – no evidence of mutagenic activity.

2. Cytotoxicity tests of PSP with V79 Chinese hamster cells *in vitro* – no toxic effects against the V79 cell line.

3. *In vivo* micronucleus tests to the cytogenotoxicity on mammalian somatic cells – PSP showed no evidence of mutagenic potential when administered in this *in vivo* test.

4. Chromosome observation tests, metaphase analysis of bone marrow cells in mice – the results of cytogenic lesions in mice showed that the number of chromosomes had not changed in PSP treated groups even at the high dose rate of 126 mg/kg.

Subchronic toxicity tests were performed with various concentrations of PSP on rats by p.o. administration. PSP was administered at dosage rates of 1.5, 3.0, and 6.0 g/kg body weight every day for up to 62 days. At the time of the final administration of PSP and two weeks after the last administration, the general conditions, i.e., blood indexes, serum biochemistry indexes, and pathohistology indexes of the PSP groups were compared with the control group and no obvious differences were observed (Jiang *et al.* 1999). A further study with mice demonstrated that acute, chronic, genetic, reproductive, and two-generation teratogenic toxicities were very low at 50-100 times the oral clinical dose (Jin 1999 – contains many relevant references to PSP safety tests).

Schizophyllum commune

The polysaccharide derived from this mushroom is a β-(1,3)D-glucan with β-(1,6)D-glucan side-chains and is called Schizophyllan (or Sonifilan, SPG, Sizofilan). As with all glucan preparations, they are never homologous in terms of molecular weight but consist of molecules with a wide range of MWs. In the case of Schizophyllan the molecules are large and are normally administered in the clinical setting via the intramuscular or intraperitoneal route.

Schizophyllan has been shown to be cytostatic in Sarcoma 180 tumor xenographs. The survival of Sarcoma 180 xenographs was not affected by pre-treatment with Schizophyllan, while combined pre- and post-treatment, and post-treatment alone, resulted in increased survival.

Various clinical trials have been carried out in Japan, although many are not blinded. Despite this, Schizophyllan has been approved for clinical use in Japan. Early clinical studies with Schizophyllan in combination with conventional chemotherapy (tegafur or mitomycin C and 5-fluorouracil) in a randomized controlled study of 367 patients with recurrent and inoperable gastric cancer resulted in a significant increase in median survival (Furne 1985). However, a similar study was unable to confirm this apparent success with Schizophyllan. Recently, Schizophyllan has also been shown to increase overall survival of patients with head and neck cancers (Kimura *et al.* 1994).

In a randomized controlled study of Schizophyllan in combination with radiotherapy, Schizophyllan significantly prolonged the overall survival of Stage II cervical cancer patients but not Stage III (Okamura *et al.* 1986, 1989). In a prospective, randomized clinical trial involving 312 patients treated with surgery, radiotherapy, chemotherapy (fluorouracil), and Schizophyllan in various combinations, patients treated with Schizophyllan had a better overall survival than patients who had not received the polysaccharide (Miyazaki, K. *et al.* 1995). The variety of treatment regimes significantly reduced the value of these results. However, separate analyses of patients with 10 % or more activated $CD4^+$ cells out of their total $CD4^+$ population and with more than 25 % activated $CD8^+$ cells before the beginning of treatment showed that in this group the Schizophyllan-induced increase in survival was highly significant. Furthermore, when Schizophyllan was injected intratumorally to cervical cancers there was a significant infiltration of Langerhans cells and T-cells (Nakano *et al.* 1996). Several Japanese pharmaceutical companies currently produce Schizophyllan commercially.

Ganoderma lucidum

Studies indicate that the active constituents of *G. lucidum* possess a variety of therapeutic effects (summarized in Tables 1-2). Numerous polysaccharides demonstrate antitumor and immunostimulating activities. For example, β-D-glucan (GL-1) from the fruit body and as a medium product has a potent effect against Sarcoma 180 in animals. This substance appears to act as a new type of a carcinostatic agent because its effect is based on enhancement of the host's immune system. Unlike general carcinostatic agents (chemotherapy), it appears to be nontoxic. In addition, *G. lucidum* contains other substances

that may enhance various aspects of homeostasis and physis, such as the reduction of blood pressure and blood sugar level, elimination of cholesterol, antithrombotic reaction, hepatitis healing, etc. (Tab. 6). A polysaccharide-enriched fraction stimulates macrophages to increase the production of tumor-necrosis factor (TNF-α) and interleukins.

Table 6. Current biomedical applications of *Ganoderma lucidum* (Hobbs 1995; Chen & Miles 1996; Wasser & Weis 1997b, with additions)

Applications	Source
A. Cosmonaut training in Russia	Wasser & Weis 1997b
1. Improves work capacity	Mizuno, T. *et al.* 1995a, b, c
2. Rapid recovery of normal physiology	Mizuno, T. *et al.* 1995a, b, c
B. Usage with conventional in cancer therapy	
1. Maintains leukocyte counts	Wasser & Weis 1997b
2. Enhances the immune system	Soo 1996; Zhou & Gao 2002
3. Reduces chemotherapy toxicity & elimination of induced leucopenia by chemotherapy & radiation	Hu & But 1987; Chen *et al.* 1995; Mizuno, T. *et al.* 1995a, b, c
4. Accelerates post-surgical recovery	Hseu 1993; Wasser & Weis 1997b
5. Sedation, pain relief & reduction of morphine dependence in terminal cancer patients	Wasser & Weis 1997b; Liu, G.T. 1999
6. Usage during remission to prevent relapses	Wasser & Weis 1997b
C. Cardiovascular disorders	Lee & Rhee 1990; Hobbs 1995; Stamets 2000
1. Coronary dilation & increasing coronary circulation	Soo 1996; Chen & Miles 1996
2. Increases frequency & amplitude of heart contraction	Soo 1996; Chen & Miles 1996
3. Blood pressure regulation together with other medication	Wasser 2005
4. Anti-hyperlipidemic, anti-hypoglycemic & anti-platelet aggregation (blood clots)	Chen & Miles 1996
5. Relief of oxygen deprivation	Yang & Wang 1994; Chen & Miles 1996
D. Immunomodulatory effects	Wasser & Weis 1997b; Smith *et al.* 2003
1. Anti-cancer	Mizuno, T. *et al.* 1980, 1984, 1995a, b, c, 1996; Kino *et al.* 1989; Wasser & Weis 1997b; Smith *et al.* 2003
2. Anti-viral (e.g., anti-HIV)	Gao, Y. *et al.* 2003
3. Anti-bacterial	Yoon *et al.* 1994; Gao, Y. *et al.* 2003
4. Anti-inflammatory	Zhou & Gao 2002; Wasser 2005
5. Therapy of autoimmune disorders	Wasser & Weis 1997b; Zhou & Gao 2002
6. Inhibition of histamine release in allergy & prevention of aphylactic shock	Zhou & Gao 2002; Zhuang & Wasser 2004
E. Usage during remission of cancer & hepatitis B treatment	Lin *et al.* 1994; Mizuno, T. *et al.* 1995a, b, c; Gao, Y. *et al.* 2002; Smith *et al.* 2003
F. Enhancing oxygen utilization	
1. Relief of discomfort of high altitude stress, headaches, dizziness, nausea & insomnia	Zhou & Gao 2002
2. Relief of oxygen deprivation caused by coronary arteries blocked by atheromas, spasms, or clots	Mizuno, T. *et al.* 1995a, b, c
3. Tolerance to hypobaric (low pressure) conditions	Mizuno, T. *et al.* 1995a, b, c
G. Other examples	
1. Usage in combination with other medication	Hobbs 1995; Stamets 2000
2. Anti-aging, anti-oxidant free radical scavenger	Mizuno, T. *et al.* 1995a, b, c; Zhou & Gao 2002; Zhou *et al.* 2002
3. Anti-diabetic	Hobbs 1995; Gao, Y. *et al.* 2004

Additionally, polysaccharides of *G. lucidum* enhance DNA synthesis of spleen cells in mixed lymphocytes culture and stimulate both RNA and DNA synthesis in the bone marrow of mice.

The sterols in *G. lucidum* are reported to act as hormone precursors, while adenosine has been found to inhibit platelet aggregation. Ganoderal A and B glycans of the fruit bodies significantly reduce plasma sugar levels in hyperglycemic mice.

Triterpenes of *G. lucidum* have adaptogenic, anti-hypertensive and anti-allergic potency. Ganoderic acid C appears to be the most active of the anti-allergic constituents followed in activity by ganoderic acids A and D. Ganoderic acid B is the least active of the constituents. Also, ganoderic acids B and D are reported to act as strong anti-hypertensive agents. Ganoderic acids T through Z exhibit antitumor activity against hepatoma cells. Ganoderic acids A through D have been shown to inhibit histamine release (Tab. 7).

Oleic acid (a common element of a balanced diet) and cyclooctasulfur are known to inhibit the release of histamine, thus preventing allergic reactions and inflammation.

Over the last ten years there have been numerous reports of pre-clinical antitumor activity of *G. lucidum* extracts in a variety of tumors (Lee, S.S. *et al.* 1995; Wang *et al.* 1997; Zhou *et al.* 2002). Such extracts effectively inhibited metastasis in animal (mouse) models and increased survival when administered as monotherapy or in combination with conventional chemotherapy (Hwang & Liu 1989; Lee, S.S. *et al.* 1995; Zhou *et al.* 2002). Some pre-clinical studies have suggested that the antitumor action of *G. lucidum* polysaccharides could be the result of its biological response modifying effects (Chang 1996). Ganopoly (an aqueous extract of *G. lucidum*) has been shown in *in vitro* systems and in xenographs to have immunomodulating effects, through the activation of macrophages, T-lymphocytes, and natural killer cells (Gao 2000). An extensive open, non-randomized clinical trial has recently been carried out on patients with advanced cancers using Ganopoly (Gao, Y. *et al.* 2002; Zhou *et al.* 2002). This compound is marketed as an over-the-counter product in Hong Kong, New Zealand, and Australia.

Within the realms of traditional herbal medicine in China and in several Asian countries many cancer patients use *G. lucidum* proprietary extracts as an adjunct to conventional treatment or as the sole therapy. What then can be said of the effectiveness of such products on human cancers? Relatively few clinical studies have so far been published in Chinese while no clinical trials with *G. lucidum* extracts against various human cancers have been published in English peer-reviewed journals (Gao 2000).

Grifola frondosa

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, T. *et al.* 1986; Jong & Birmingham 1990; Wasser & Weis 1999; Stott & Mohammed 2003), 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 sugar component is glucose, while fucose, xylose, mannose, and galactose are minor components (Mizuno, T. *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 in liquid nutrient medium) that was collected before initiation of fruit bodies (Mizuno & Zhuang 1995). In the water-soluble fractions, a fucogalactomannan-protein complex, a glucogalactomannan, a mannogalactofucan, and galactoglucomannofucan-protein complexes were found. In water-insoluble fractions, a mannofucoglucoxylan, a mannoglucofucoxylan-protein complex, a mannofuco-glucoxylan-protein complex, and glucomannofucoxylan-protein complexes were found (Zhuang *et al.* 1994a). Thus, polysaccharides from *G. frondosa* are heteromannans, hetero-fucans, and heteroxylans, or their complexes with protein, i.e., types of polysaccharides that were not found in fruit bodies of this mushroom.

The polysaccharide structure in culture mycelia may depend on the composition of the nutrient medium used for cultivation. Ohno *et al.* (1985, 1986) concluded that antitumor glucan Grifolan extracted from cultured mycelia 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 grown in a liquid medium in stationary culture or with shaking. The mycelium obtained was cultivated for three days more 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 a buffer supernatant (Adachi *et al.* 2002).

Several studies have shown that β-D-glucan and glycoprotein complexes derived from this mushroom have strong antitumor activity in xenographs (Kurashiga *et al.* 1997). More recently, a highly purified extract, β-glucan (β-1,6 glucan branched with a β-1,3-linkage) (Grifron-D©=GD) has become available. GD has considerable immunomodulating and antitumor activities in animal models, and is orally bioavailable (Nishida *et al.* 1988). Maitake D-fraction and crude Maitake powder have demonstrated remarkable inhibition of metastasis in an immuno-competent mouse model, especially in the prevention of hepatic metastases, which in one series of experiments was reduced by 81 % (Maitake powder) to 91 % (D-fraction) (Nanba 1995). GD has been shown to have a cytotoxic effect on human prostate cancer cells (PC9) *in*

Table 7. Major bioactive nutriceutical components of *Ganodema lucidum* and their functions (Chen & Miles 1996; Wasser & Weis 1997b, with additions)

Applications	Source
A. Polysaccharides	
1. Immunomodulatory	
Anti-cancer	Mizuno, T. *et al.* 1992; Zhou *et al.* 2002; Gao, Y. *et al.* 2002
Anti-HIV	Wasser & Weis 1997b
2. Hepato-protective/anti-hepatotoxic	Gao, Y. *et al.* 2003
3. Hypoglycemic	Gao, Y. *et al.* 2004
4. Anti-histamine release. Prevent experimental asthma & contact dermatitis Improving oxygen utilization	Wasser & Weis 1997b
5. Anti-angiogenic	Cheng *et al.* 1986
6. Radiation protective	Chu *et al.* 1988
B. Triterpens and related compounds	
1. Cytotoxic to tumors	Toth *et al.* 1983; Kim & Kim 1999
2. Anti-HIV	Kim & Kim 1999
3. Anti-hyperlipidemic	Liu, K.C. *et al.* 1988; Komoda *et al.* 1989; Kim & Kim 1999
4. Hypotensive	Kim & Kim 1999
5. Anti-platelet aggregation	Gao, Y. *et al.* 2002
6. Hepatoprotective	Lin *et al.* 1991; Chen & Yu 1993
7. Analgesic	Kubota *et al.* 1982
8. Cardioactive	Wasser & Weis 1997b
9. Immunomodulatory	Zhou & Gao 2002
C. LZ-8 (MW 12.420 daltons, 110 amino acid residues)	
1. Anti-hypersensitivity	Kino *et al.* 1989
2. Anti-autoimmune diabetes	Gao, Y. *et al.* 2004
3. Anti-hepatitis B	Zhou & Gao 2002
4. Immunomodulatory	Zhou & Gao 2002
D. Adenosine and derivatives	Kasahara & Hikino 1987
1. 5'-deoxy-5'-methylsulfinyl adenosine Inhibition of platelet aggregation	Wasser & Weis 1997b
E. Organic germanium ($(Ge\text{-}CH_2CH_2CH_2COOH)2O_3$; GE 132 (carboxyethyl germanium sesquioxide)	
1. Antitumor, hepatoma cells, bladder cancer	Gao, Y. *et al.* 2002
2. Anti-Lewis-lung carcinoma	Wasser & Weis 1997b
3. Promoting blood circulation/0_2 utilization	Liu, G.T. 1999
F. Oleic acids & cyclooctasulphur	
1. Inhibition of histamine release	Wasser & Weis 1997b
G. RNA	
1. Anti-viral (encephalitis)	Gao, Y. *et al.* 2003
2. Immunomodulatory	Zhou & Gao 2002

vitro, possibly acting through oxidative stress, and causing 95 % cell death by apoptosis (Fullerton *et al.* 2000; Minato *et al.* 2001). Simultaneous use with various anticancer drugs only slightly enhanced efficacy of most of the drugs tried except for the carmustine/GD combination (90 % reduction in cell viability).

Vitamin C addition reduced the effective level of GD required. This potentiation of GD action by vitamin C and the chemosensitizing effect of GD on carmustine may well have significant clinical implications. Data against using prostate cancer cells *in vitro* have shown that the cytotoxic effects of the anticancer drug was significantly potentiated or enhanced with GD, possibly mediated through the inactivation of glyoxalase I, a vital detoxifying enzyme responsible for detoxification of cytotoxic metabolites / substances. This study suggests that GD may be useful with some anticancer drugs to improve the efficacy of ongoing clinical chemotherapy. The Maitake D-fraction is a relatively new compound and there are a number of clinical trials in breast, prostate, lung, liver, and gastric cancers underway in the US and Japan. Most of these are at an early clinical stage (Phase I / II) (Stott & Mohammed 2003).

Early pilot studies from China published in abstract form involving 63 cancer patients reported a response rate (partial and complete) against solid tumors at 95 % and for leukaemia (type not specified) 90 % (Zhuang & Wasser 2004). A recent Japanese non-randomised clinical study using the D-fraction has been carried out in a variety of advanced cancer patients (n=165). Patients took either oral D-fraction plus crude Maitake powdered tablets, or D-fraction plus placebo tablets in addition to chemotherapy (Nanba 1997a, b). Tumor regression or significant symptomatic improvement was observed in 11 out of 15 advanced hepatocellular carcinomas with D-fraction plus Maitake. When D-fraction plus Maitake was combined with chemotherapy, the overall response rates rose by 12-28 % when results from all cancer types were combined (Fig. 1). Chemotherapy itself could also significantly lower the immune system of patients. They reported that many of the patients recovered from the severe side effects caused by chemotherapy when D-fraction was given. In a similar manner to Lentinan, there are now increasing examples of synergism between Maitake D-fraction and crude Maitake powder and conventional chemotherapy (Nanba 1997a, b).

The US Food and Drug Administration has approved GD for trial under an Investigational New Drug Application (IND) for patients with advanced cancer, and some US-based clinical trials are currently underway at various institutions (Nanba 1997a, b). No details are available yet. In conclusion, GD has few side effects and anecdotal clinical reports appear to suggest that it might alleviate some of the side effects of chemotherapy. The apparent success of crude Maitake powder by oral administration in cancer therapy and immune stimulation would also support its suitability as a nutriceutical.

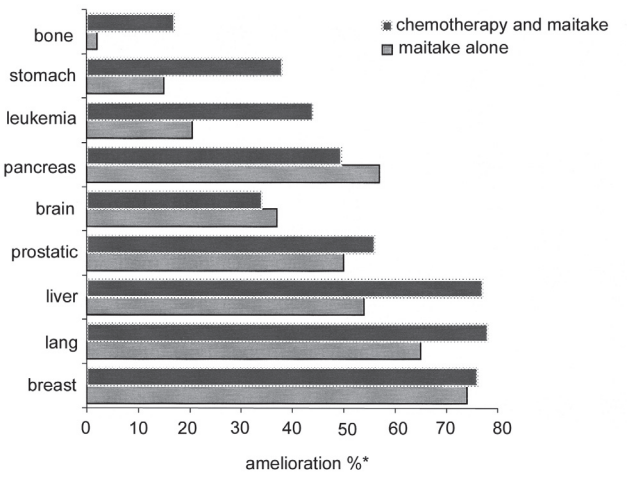

Fig. 1. Effects of Maitake D-fraction on cancer patients (reproduced from Nanba 1997b)

*Definite tumor regression and/or significant symptomatic improvement

Phellinus linteus

Phellinus linteus (Berk. et Curt.) Teng has long been used in traditional Chinese medicine in the form of hot water extracts from the fruit bodies – 'song gen' in Chinese and 'mishimakobsu' in Japanese (Mizuno 2000). In the last decade the effects of these extracts for improving symptoms of digestive system cancers such as oesophageal, duodenal, colorectal, as well as hepatocellular, have been reported by practitioners of TCM. As with most of these mushroom polysaccharide extracts, tumor responses and/or symptomatic improvement (enhanced quality of life) have mainly been reported in combination with conventional chemotherapy in an adjuvant or neo-adjuvant setting (Mizuno 2000). In Korea there has been a major national project involving industry, government, and academic laboratories using fermenter-cultivated mycelium from several *Ph. linteus* strains (Aizawa 1998). The major polysaccharide product has been approved as a medicine and has been manufactured by the Korean New Pharmaceutical Co. since 1997. Similar studies are also taking place in Japan by the Applied Microbiology Laboratory, Obiken Co. Ltd. Meshima, the hot water extracted polysaccharide product now manufactured by the Korean company, has become available in Japan for sale as a functional food (an immunity activation substance).

An early study by Sasaki *et al.* (1971) explored its tumor-retarding properties. Water-soluble fractions from the fruit bodies and the mycelium of *Ph. linteus* have immune stimulating activity (Lee, J.H. *et al.* 1996) specifically enhancing B-lymphocytes (Song, K.S. *et al.* 1995). Novel types of β-glucan polysaccharides have been identified, which enhance a host-mediated immune response (Han *et al.* 1998). Ikekawa (2001) noted that water fractions of this mushroom did not inhibit the growth of implanted, solid-type sarcoma

tumors in mice. Conversely, Mizuno, M. *et al.* (2000) found that this mushroom had the highest rate of inhibition against implanted Sarcoma 180 tumors in mice, resulting in 96.7 % inhibition. Furthermore, Mizuno (2000) reported clinical studies at Seoul University. In post chemotherapy with 45 stomach cancer patients, *Ph. linteus* significantly enhanced NK activity resulting in recovery of T-3 and T-4 lymphocytes to near-normal conditions. Research by Song, K.S. *et al.* (1995) and Kim, H.M. *et al.* (1996) reported that the water extract from the mycelium induced B-lymphocytes and enhanced cellular immunity. Kim's study noted that the activity of the polysaccharides from the mycelium had a wider range of activity and greater antitumor effects than polysaccharides isolated from other mushroom species. Han *et al.* (1999) found that polysaccharides from Meshima, when combined with the chemotherapeutic agent adriamycin, increased effectiveness against tumor growth and metastasis, while the polysaccharides by themselves did not influence the growth of pulmonary cancers in mice.

A novel β-glycosidase inhibitor, called cyclophellitol, has been isolated from this fungus (Atsumi *et al.* 1990). Shon & Nam (2001) explored the anti-mutagenic properties from the fruit bodies, showing activity in preventing carcinogenesis.

Although there have been only a few Phase II trials, tumor responses to the combination of meshima with conventional chemotherapy have been reported. A considerable number of Korean and Japanese patents are now in place and further trials with meshima polysaccharide product (oral formulation) are ongoing.

Agaricus brasiliensis

Agaricus brasiliensis (= *A. blazei* Murrill ss. Heinem.) was distributed originally in Brazil and was known for several decades under the name of another species – *A. blazei* ss. Murrill (Wasser *et al.* 2002; Didukh *et al.* 2003). 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 the type material of *A. blazei,* shows striking diferences between them. On the basis of existing differences the correct name for the widely cultivated mushroom was proposed as species new for science *Agaricus brasiliensis* (Wasser *et al.* 2002). *A. blazei* ss. Murrill is the North American endemic non-cultivated species known from only three localities: one in Florida and two in South Carolina (Wasser *et al.* 2002).

Agaricus brasiliensis mushroom (the Royal Sun *Agaricus,* ABM, Himematsutake, Cogmelo de Deus), is one of the newly discovered medicinal mushrooms. This delicious edible mushroom is native to a very small area of Brazil, near the city of Sao Paulo. During the 1980s and 1990s, the *A. blazei* mushroom was demonstrated to be an immune system stimulant, promoting the body's natural defense mechanisms to fight a variety of infectious agents including cancers. Immunostimulating activity and antitumor action of *A. blazei* extracts were investigated on different laboratory models, including Sarcoma 180 and Meth-A fibrosarcoma tumor-bearing mice (Kawagishi *et al.* 1989, 1990; Mizuno, T. *et al.* 1990b, 1998; Itoh, H. *et al.* 1994; Ebina & Fujimiya 1998; Fujimiya *et al.* 1998a, 2000; Stamets 2000; Mizuno 2002; Didukh *et al.* 2003).

Antitumor activity

Polysaccharide fractions

Agaricus brasiliensis is used by approximately 300 000-500 000 persons for the prevention of cancer and/or as an adjuvant with cancer chemotherapy drugs after the removal of a malignant tumor (Takaku *et al.* 2001), but the mechanisms of its action need further investigation.

The most intensively studied and thus ubiquitous group of antitumor active substances is comprised of polysaccharides, obtained mostly by extraction from fruit bodies. Now the fruit body is known to contain water-soluble (1→3)-β-D-glucan with (1→6)-β-branch ($F1_o$-a-β) (Mizuno 2002), AB-P (glucan-protein complex with a Glc:protein ratio 34:30) (Reshetnikov *et al.* 2001), three newly reported protein-bond polysaccharide-proteoglucans AB-I, AB-II-b, AB-III-b (Yuexin *et al.* 2002), acidic heteroglucans (1→3)-α-glucan, and (1→6)-β-galactoglucans having Gal, Xyl, Man, Ara, Glc, and uronic acid.

All these fractions, with the exception of AB-I, AB-II-b, AB-III-b, possess pronounced antitumor activity against Sarcoma 180 (Mizuno 2002). By means of 0.9 % sodium chloride and hot water extractions three immunostimulating heteroglucans (AG-2, AG-3, AG-6) were obtained. AG-2 and AG-3 were composed of glucose, galactose, and mannose in the molar ratios of 74.0:15.3:10.7 and 63.6:17.6:12.7, respectively, and AG-6 was composed of glucose and ribose in the molar ratio of 81.4:12.6 (Reshetnikov *et al.* 2001; Didukh *et al.* 2003).

The acid-treated fraction (ATF) induces infiltration of the distant tumor with marked tumoricidal activity, as well as directly inhibits tumor cell growth *in vitro* by inducing apoptotic processing. The ATF has no effect on normal splenic mononuclear mouse cells, indicating that it is selectively cytotoxic for the tumor cells. Cell-cycle analysis demonstrated that ATF induced the loss of S phase in Meth-A tumor cells, but did not affect normal splenic mononuclear cells, which were mainly in the G0G1 phase. The ATF was fractioned into antitumor-active high-molecular (HM) and low-molecular (LM) weight fractions. The most active HM fraction, HM3-G, consists of more than 90 % glucose, the main component being (1→4)-α-D-glucan with (1→6)-β branching, in a ratio of approximately 4:1 (Fujimiya *et al.* 1998b).

The injection of LM extracts (average size 20 kDa) from *A. brasiliensis* into one of the tumors of the double-grafted

tumor system resulted in a marked decrease in tumor cells in both flanks, and a tumor-free rate comparable to that seen with high molecular weight fraction. Like the crude ATF, LM fractions possess a tumor-specific cytocidal activity. The main components of the most active LM3 fraction are α-glucan and β-glucan complex, together with proteoglucan (60 % glucose and 40 % of protein), containing mainly (1→4)-α-D-glucan (Fujimiya *et al.* 1999).

Thus, ATF constituents have a unique mode of action in that they have both a direct cytotoxic action on tumor cells and an indirect immunopotentiating action on tumor-bearing mice. The mechanism of action has not been elucidated yet. It is speculated that the inhibition of the distant tumor might be due to the increased migration of granulocytes, enhanced by the effect of extract injections on the primary tumor side. A decrease of the injected tumor can be, in part, ascribed to a direct action on tumor cells (Fujimiya *et al.* 1999). Another important trait of ATF is that it retained its full tumoricidal activity even when administrated orally (Ebina & Fujimiya 1998; Fujimiya *et al.* 1999; Oshiman *et al.* 2002; Didukh *et al.* 2003).

Besides LM3, other active low molecular weight substances have been isolated from *A. brasiliensis* (Mizuno 2002), namely ABMK-22 and HACCP, which are able to activate the manifestation of the cytokine gene of the macrophages; they are suggested to possess a preventive effect on cancer. ISY-16, obtained from the hot water extract, inhibited the growth of Sarcoma 180 to 80 %. The determination of chemical structure is in progress.

LM weight molecules with biological activity are very important in the design of possible synthetic analogues of natural products (Fujimiya *et al.* 1999).

From the water-insoluble residues of the fruit bodies FIV-2b (xyloglucan containing 9 % of protein and 4 % of uronic acid) and FIII-2b fractions (glucan-protein complex consisting of 43.3 % of protein and 50.2 % of carbohydrate), active against Sarcoma 180, have been obtained (Kawagishi *et al.* 1989). The latter has been shown to be more active. Its polysaccharide part is an almost pure (1→6)-β-D-glucopyranan. This is the first report of the association of antitumor activity with a glucan containing only (1→6)-β linked residues. (1→6)-β-D-glucan was determined as the major carbohydrate component of the fruit bodies (Ohno *et al.* 2001). The data received on partial formolysis of FIII-2-b led to the conclusion that the protein component is essential for antitumor activity (Kawagishi *et al.* 1990).

A homogeneous fraction Ab-2-2N isolated by hot water from the fruit bodies of *A. brasiliensis* has also been elucidated (Dong *et al.* 2002). Ab2-2N is a (1→6), (1→3)-β-D-glucan. It differs from previously reported (1→6), (1→3)-linked-β-D-glucans in that it is water-soluble, contains side (1→3) linkages, and exhibits negative optical rotation. Preliminary *in vitro* trials showed that Ab2-2N exhibits a stimulating effect on T and B cells proliferation. However, it has been shown that a linear (1→6)-β-D-glucan, Islandican, was inactive in terms of antitumor activity in mice (Ohno *et al.* 2001), and that the active center of the antitumor activity is formed not as (1→6)-β-D-glucan, but a highly branched (1→3)-β-glucan segment (Ohno *et al.* 2001).

The liquid culture mycelia is also a source of biologically active substances, mainly polysaccharides. The antitumor active polysaccharide-protein complex, ATOM, inhibits four kinds of established mouse tumors on i.p. or p.o. administration. ATOM is highly effective on subcutaneously implanted Sarcoma 180 mice, against Ehrlich ascites carcinoma, Shionogi carcinoma 42, and Meth-A fibrosarcoma. It has no direct cytotoxic action on tumor cells *in vitro*. Thus, the tumor growth-inhibitory effect of ATOM is apparently due to immunological host-mediated mechanisms. ATOM also induced macrophage activation and alterations of the C3. These effects are probably necessary for the induction of an antitumor effect of ATOM (Ito *et al.* 1997).

The protein-polysaccharide 0041, with glucose and mannose as the main ingredients of the polysaccharide part, is another known active complex (Hikichi *et al.* 1999).

The hot-water soluble fraction of the mycelia contains glucomannan with a main chain of (1→2)-β-D-mannopyranosyl and side chains of (1→3)-β-D-glucopyranosyl. The polysaccharide inhibits Sarcoma 180 and is completely different from the antitumor polysaccharide from fruit bodies of *A. brasiliensis*, (1→6)-β-D-glucan (Mizuno, T. *et al.* 1999a; Tsuchida *et al.* 2001).

The secondary metabolites of the culture mycelia of the species have been investigated only recently. A number of unprecedented skeletal compounds, blazeispirols, previously not reported, have been elucidated (Hirotani *et al.* 2000, 2001, 2002). So far, blazeispirols A, B, C, D, E, F, G, I, U, V, V_1, X, Y, Z, and Z_1 have been revealed (Pl. I, 2). Blazeispirols A-F are the first demonstration of steroids without ring A in a living organism (Hirotani *et al.* 2002). Blazeispirols G and I are des-A-ergostane-type compounds, and U, V, V_1, Z_1 are blazeispirol derivatives.

A liquid medium filtrate separated after submerged cultivation of *A. brasiliensis* also contains an antitumor active substance, namely AB-FP. This is a mannan-protein complex with a small amount of glucose, galactose, and ribose (Reshetnikov *et al.* 2001; Mizuno 2002).

Antitumor polysaccharides from fruit bodies, culture mycelia, or produced extracellularly in a culture medium have different chemical structures. Polysaccharides from fruit bodies represent glucans with different types of glucose unit connections or heteroglucans; culture mycelia contain glucomannans, and mannan-protein complex is produced in a culture medium under submerged cultivation. Polysaccharides or protein-bound polysaccharides (proteoglucans), isolated from *A. brasiliensis,* have shown marked tumoricidal activity in different experimental models (allogeneic, syngeneic mouse tumor models, double grafted tumor system). These models provide screening of anti-metastatic drugs and facilitate examination of the anti-metastatic effects, mechanism of action of these biological response modifiers. Although it has been postulated that the inhibitory action of polysaccharides or proteoglucans results from enhancement of host immunity

against tumor cell growth, no direct evidence has yet been obtained.

Lipid fractions

A considerable effort has been made to reveal and study the antitumor active polysaccharides obtained from the fruit bodies. The antitumor effects of lipid fractions have not been well studied. The lipid fraction isolated from the fruit bodies is highly effective against Sarcoma 180 and highly metastatic, drug-resistant mouse Lewis lung carcinoma (LLC) cells via p.o. or i.p. administration (Takaku *et al.* 2001). The active substance was isolated as ergosterol – a precursor of ergocalciferol. Besides pronounced antitumor activity, administration of ergosterol (both i.p. and p.o.) was devoid of side effects that are usually caused by cancer chemotherapy drugs (e.g., myelotoxicity, immunotoxcity, reduction in body weight). Another interesting fact concerns the antiangiogeneic activity of ergosterol and its derivatives. Ergosterol inhibited the LLC- and Matrigel-induced neovascularization in a dose-dependent way by i.p. administration (Takaku *et al.* 2001), demonstrating ability for direct inhibition of neovascularization. The antitumor activity of ergosterol may be due to the direct inhibition of angiogenesis induced by solid tumors. This is the first report Ergosterol has not been known to exert an antiangiogeneic effect (Takaku *et al.* 2001). Besides ergosterol, six steroids were isolated from acetone extract of *A. brasiliensis* fruit bodies. Three of them effectively inhibited cell proliferation of cervical cancer cells (HeLa cells) (Mizuno 2002).

Lectin

Lectins are ubiquitous carbohydrate-binding non-immunoglobulin proteins. They bind non-covalently to carbohydrates and are readily purified from a wide variety of sources. As a range of carbohydrates occur on all cell surfaces, lectins are used to explore cell membranes and to distinguish different cell types, because cells express distinct carbohydrates that can be detected by specific lectins. Furthermore, lectins' binding to cell surface carbohydrates may affect the behavior of the cell.

A glycoprotein isolated from the fruit bodies was lectin containing 11 % of saccharides and having a molecular weight of 64 000 Da. ABL was composed of four identical subunits with a molecular weight of 16 000 Da. Host-mediated antitumor activity of the lectin was recognized, but it was not significant (Mizuno 2002).

Nucleic acid

An antitumor fraction, FA-2-b-β, was an RNA-protein complex containing an mRNA molecule with a molecular weight of about 10 000 Da. On rare occasions, a host-mediated antitumor nucleic acid complex was obtained from mushrooms.

So, to date, a wide range of biological response modifiers derived from *A. brasiliensis* have been revealed. A clear understanding of their mechanisms of action at different levels is needed in order to apply them successfully to the treatment of human diseases. Besides substances with a relatively known mode of action, *A. brasiliensis* harbors biologically active compounds of unknown or poorly known structure conditioning a number of important properties.

Immunomodulatory activity

The most anticipated pharmacological effect of *A. brasiliensis* is modulation of the immune system against cancer. Neither active substances, nor mechanisms of their action are sufficiently known. A hot water-soluble fraction from *A. brasiliensis* fruit bodies significantly increased positive cell (pan T-cells, helper T-cells, cytotoxic T-cells) populations. The main component in the active polysaccharide is the complex of α-(1→6) and α-(1→4)-glucan, which had already been shown to have anti-tumor activity against Sarcoma 180 (Mizuno, T. *et al.* 1998). Polysaccharide from *A. brasiliensis* may be an effective prophylactic, protecting humans against cancer by stimulating lymphocytes such as cytotoxic T-cells. *A. brasiliensis* boiled water-soluble extract might be an effective stimulator for T-cell and macrophage to IL-1β and IL-1α release, resulting in augmentation of antibody production against sheep red blood cells antigen (Nakajima, A. *et al.* 2002). The ethanol precipitate from an extract of the mycelia-induced tumor necrosis factor alpha (TNF-α), interleukin, and nitric oxide expression from macrophages was found by Sorimachi *et al.* (2001). Fine particles from fruit bodies (ABP-F) and from the mycelium (ABP-M), prepared by mechanical disruption, activated the human complement system in human serum via the alternative pathway. Activation of the alternative pathway was both time- and dose-dependent. When the particles reacted with human serum, the formation of complement-opsonized ABP, iC3b-ABP-F complexes and binding of the complexes to human peripheral blood monocytes were demonstrated *in vitro* by immunofluorescence. Furthermore, the resident human peripheral nucleated cells incubated in the presence of iC3b-ABP-F complexes inhibited the proliferation of the human tumor cell line TPC-1 *in vitro* (Shimizu *et al.* 2002). Another fraction of unknown chemical nature, AB-BDM-2, has also been shown to possess immunomodulating activity. It inhibits cell proliferation by arresting the cell-cycle progression from the G1 transition to the S phase. Interestingly, ATF induced the loss of S-phase in, for instance, Meth-A tumor cells. The fraction has been also shown to mediate through the inhibition of cytokines IL-2, IL-4, IFN-gamma, and cyclin D in a dose-dependent fashion. It

is suggested that AB-BDM-2 contains immunomodulatory agents (Kuo *et al.* 2002).

Thus, *A. brasiliensis* fruit bodies, culture mycelia, and culture broth possess compounds exhibiting antitumor, antiviral, antigenotoxic/antimutagenic, and immunomodulatory activities. However, the chemical nature of a number of *A. brasiliensis* biologically active substances remains unknown, as well as the mechanisms of action of both known and unknown active fractions. Moreover, there is one debated aspect concerning consumption and application of this mushroom. It has been shown to contain agaritines. These carcinogenic substances constitute approximately 1 % of dried mass in the fruit bodies and <.02 to .2 % in the mycelia, depending upon the manufacturer and source (Stijve & Amazonas 2001; Stijve *et al.* 2003).

Active hexose correlated compound (AHCC)

A new class of biologically active polysaccharides AHCC was developed in Japan. In contrast to the other anticancer glucans, the glucans of AHCC are low molecular weight, α-1,3 structures (Kidd 2000; Smith *et al.* 2002, 2003).

AHCC is a proprietary compound produced by cultivation and enzymatic modification of mushroom mycelia of several species. The AHCC Research Association was founded in 1986 to promote future study. Over 350 medical doctors and researchers gather in Sapporo (Japan) for the AHCC Research Association symposiums each year since 1994.

Initial studies have evaluated AHCC in a chemoprevention role by assessing its ability to prevent or delay recurrence of hepatocellular carcinoma after surgical resections (Kamiyama 1999). In this non-randomized Phase II trial 44 patients after partial hepatectomies were given oral AHCC at 3 g per day. After one year the AHCC group had a significantly higher 1-year survival and lower recurrence rate than the control group as well as significant lowering of a number of tumor markers (CEA, αFP). This study has only appeared in abstract form, while a second report, again in abstract form (Matsui *et al.* 2002), stated that recurrence was not lower in the AHCC group although the 1-year survival rate was higher.

According to industry analysts in Japan, currently over 700 hospitals and medical clinics recommend AHCC to patients as part of an immune enhancement maintenance regimen. AHCC has been the subject of some 325 clinical studies conducted at prestigious Japanese institutions such as Hokkaido University, Kyorin and Teikyo University. Several studies were slated in the U.S. for 2002 at institutions such as Columbia Presbyterian and Faulkner Hospital. AHCC's most notable observed effects have been in enhancing or boosting the immune system to allow other cancer treatment such as radiation or chemotherapy to work successfully. Researchers have observed an increase in the strength of the body's natural defense mechanisms in patients taking AHCC (Matsushita *et al.* 1998; Kidd 2000; Smith *et al.* 2002).

Mechanisms of antitumor and immunodulating action by mushroom polysaccharides

Mushroom polysaccharides exert their antitumor action mostly via activization of the immune response of the host organism. These substances are regarded as biological response modifiers (Wasser & Weis 1999; Ikekawa 2001; Wasser 2002). 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; (3) they have nonspecific action on the body, supporting some or all of the major systems, including nervous, hormonal, and immune 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, and in the prevention of metastatic tumors, etc., as described above. Polysaccharides 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 & 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. 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 & 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 & 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 the 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 & Weis 1997a; Hobbs 2000).

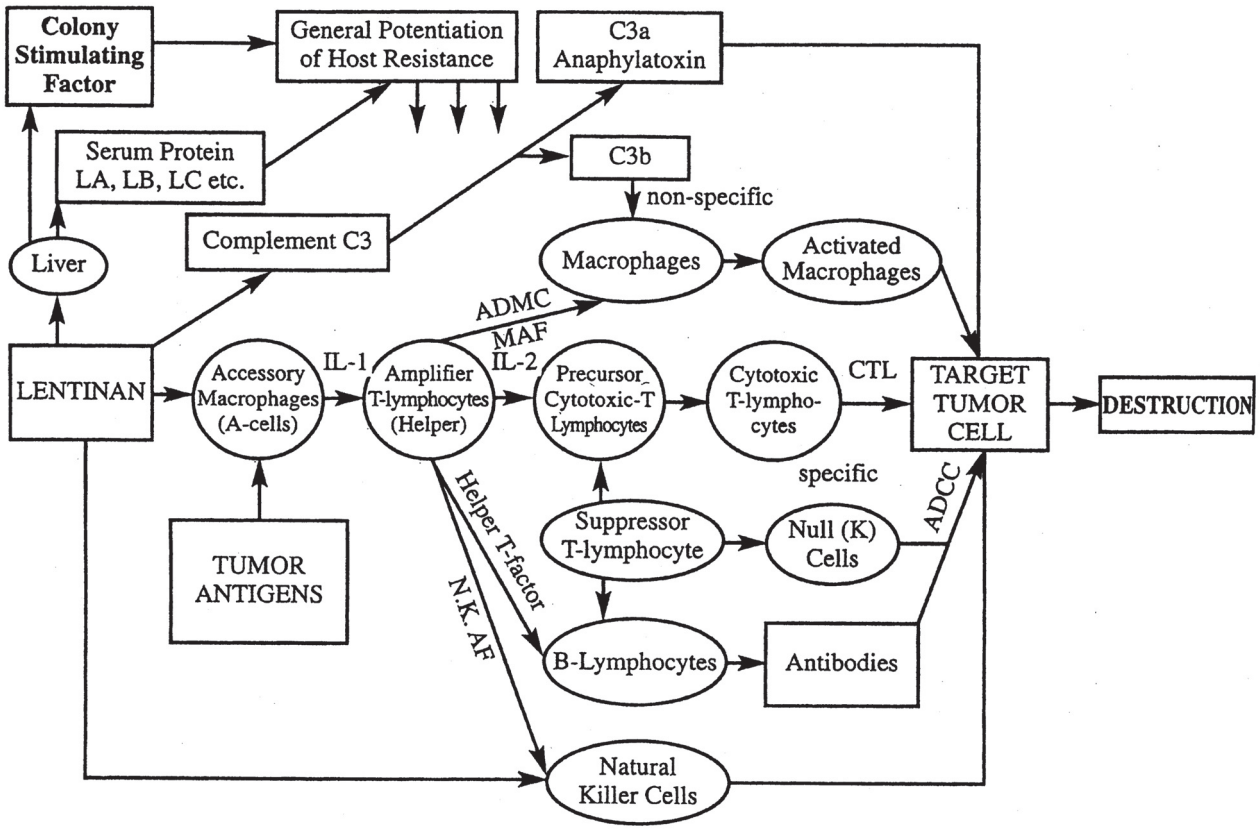

Fig. 2. Possible pathways of Lentinan action (after Chihara *et al.* 1987)

Lentinan's immune-activating ability may be linked to 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 *et al.* (1987) and Hamuro & Chihara (1985), and reviewed by Wasser & Weis (1999) and those for β-D-glucan BRMs (Mizuno 2002) are shown in Figs 2-3.

Conclusions

Higher Basidiomycetes mushrooms are still far from thoroughly studied; even the inventory of their known species is incomplete. The number of mushrooms with known pharmacological qualities is even lower. Nevertheless, the species studied so far represent a vast source of anticancer and immunostimulating polysaccharides. Many, if not all, Basidiomycetes mushrooms contain biologically active polysaccharides. Of the 651 species and seven infraspecific taxa from 182 genera of higher Hetero- and Homobasidiomycetes, the overwhelming majority has been demonstrated to possess pharmacologically active polysaccharides in their fruit bodies, culture mycelia, or culture broth (Reshetnikov *et al.* 2001; Wasser 2002).

Mushroom polysaccharides are different in 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 further 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

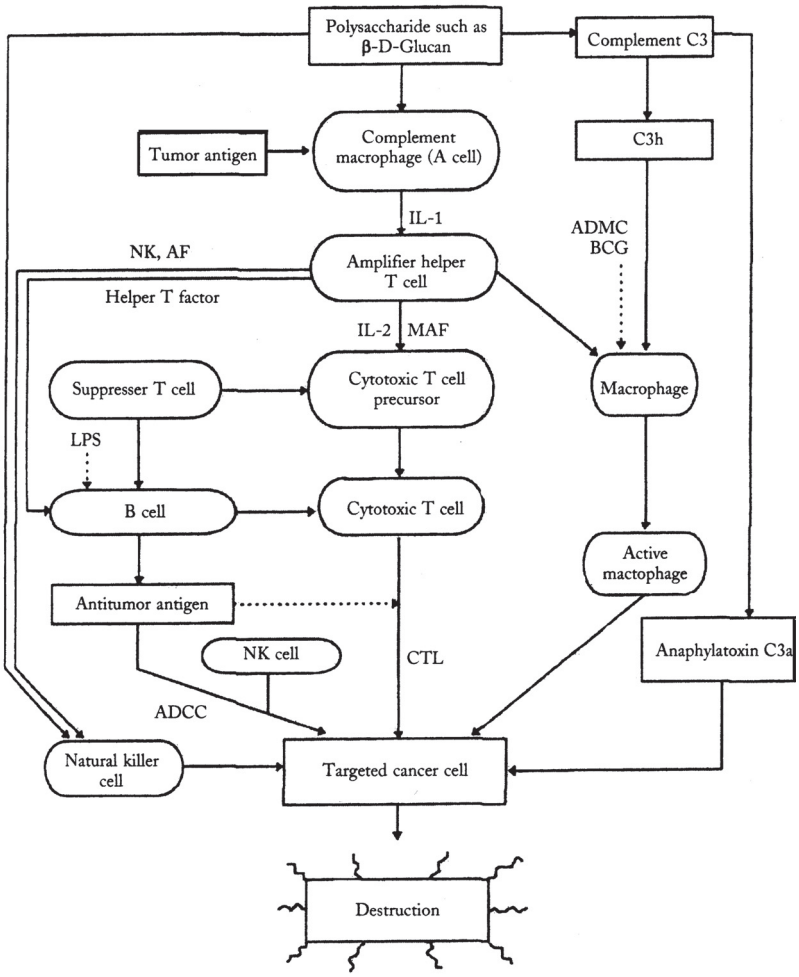

Fig. 3. Possible immune mechanism of biological response modifier (BRM)- β-D-glucan (after Mizuno 2002)

degradation (oxydo-reducto-hydrolysis) and activation by the method of formolysis. The most successful schemes for such methods were developed for *Ganoderma lucidum, Grifola frondosa,* and *Leucopaxillus giganteus.* Carboxymethylation is another chemical method that transforms β-glucans into water-soluble forms.

A large body of experimental and clinical evidence demonstrates the beneficial results of mushroom polysaccharides 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 conjuction 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* (Peck) H. E. Bigelow, *Agaricus brasiliensis,* and others.

The biochemical mechanisms for mediating 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 unique properties but also on biotechnological availability. Isolation and purification of polysaccharides from mushroom material is relatively simple and straightforward, and can be done 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, 2002; Reshetnikov *et al.* 2001; Wasser 2002).

References

Adachi, Y., Suzuki, Y., Jinushi, T., Yadomae, T. & Ohno, N. 2002. TH1-oriented immunomodulating activity of gel-forming fungal (1-3)-Beta-Glucans. – International Journal of Medicinal Mushrooms **4**: 105-120.

Aizawa, K. 1998. Antitumour-effective mushroom meshimakoku *Phellinus linteus.* Gendai-Shorim, Tokyo.

Anderson, J.B. & Stasovski, E. 1992. Molecular phylogeny of northern hemisphere species of *Armillaria.* – Mycologia **84**: 402-414.

Aoki, T. 1984. "Lentinan". – In: R.L. Fenichel & M.A. Chirgis [eds]. Immune Modulation Agents and Their Mechanisms. – Immunology Studies **25**: 62-77.

Atsumi, S., Umezawa, K., Iinuma, H., Naganawa, H., Nakamura, H., Iitaka, Y. & Takeuchi, T. 1990. Production, isolation and structure determination of a novel beta-glucosidase inhibitor, cyclophellitol, from *Phellinus* sp. – Journal of Antibiotics (Tokyo) **43**(1): 49-53.

Borchers, A.T., Stern, T.S., Hackman, R.M., Keen, C.L. & Gershwin, M.E. 1999. Mushrooms, tumours and immunity. – Proceedings of the Society for Experimental Biology and Medicine **221**: 281-293.

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

Chang, R.Y. 1996. Potential application of *Ganoderma* polysaccharides in the immune surveillance and chemoprevention of cancer. – In: D. Royse [ed.]. Mushroom biology and mushroom products, pp. 153-160. Pennsylvania State University, University Park.

Chang, S.T. 1999. Global impact of edible and medicinal mushrooms on human welfare in the 21st century: nongreen revolution. – International Journal of Medicinal Mushrooms **1**: 1-8.

Chang, S.T. & Miles, P.G. 1992. Mushroom biology – a new discipline. – Mycologist **6**: 64-65.

Chen, A.W. & Miles, P.G. 1996. Biomedical research and the application of mushroom nutriceuticals from *Ganoderma lucidum.* – In: D.J. Royse [ed.]. Mushroom biology and mushroom products, pp. 161-176. Pennsylvania State University, University Park.

Chen, R.Y. & Yu, D.Q. 1993. Studies on the triterpenoid constituents of the spores from *Ganoderma lucidum* Karst. – Journal of Chinese Pharmaceutical Sciences **2**: 91-96.

Chen, W.C., Hau, D.M. & Lee, S.S. 1995. Effects of *Ganoderma lucidum* and krestin on cellular immunocompetence in gamma-ray-irradiated mice. – Journal of Chinese Medicine **23**: 71-80.

Cheng, H.H., Tung, C.Y. & Tung, C.T. 1986. The antitumor effect of cultivated *Ganoderma lucidum* extract. III. Effect of *Ganoderma lucidum* extract on human T-cell subsets. – Journal of Chinese Oncological Society **1**: 1-10.

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

Chihara, G., Hamuia, J., Maeda, Y.Y., Arai, Y. & Fukuoka, F. 1970. Fractionation and purification of the polysaccharides with marked antitumour activity especially lentinan from *Lentinus edodes.* – Cancer Research **30**: 2776-2781.

Chihara, G., Hamuro, J., Maeda, Y.Y., Shiio, T., Suga, T., Takasuka, N. & Sasaki, T. 1987. Antitumor and metastasis-inhibitory activities of lentinan as an immuno-modulator: an overview. – Cancer detection and prevention. Supplement: official publication of the International Society for Preventive Oncology, Inc. **1**: 423-443.

Cho, S.M., Yu, S.H. & Shin, G.C. 1996. Biological activities of cultures broth of some wood rotting basidiomycetes. Antimicrobial, plant growth regulatory, antitumor, and enzymatic activities. – Korean Journal of Mycology **24**: 17-24.

Chu, F., Luo, H., Luo, G., Chen, S. & Liu, Z. 1988. Protection of nucleated bone marrow cells of mice against the effect of radiation-induced micronucleus formation with polysaccharides extracted from Ling Zhi (*Ganoderma*). – Fushe Fanghu **8**: 16-20.

Chung, K.S., Choi, E.C., Kim, B.K., Kim, Y.S. & Park, Y.K. 1982. The constituents and culture of Korean Basidiomycetes: Antitumor polysaccharides from the cultured mycelia of some basidiomycetes. – Archives of Pharmacal Research (Seoul) **5**: 17-20.

Cun, Z., Mizuno, T., Ito, H., Shimura, K., Sumiya, T. & Kawade, M. 1994. Antitumor activity and immunological property of polysaccharides from the mycelium of liquid-cultured *Grifola frondosa.* – Journal of Japanese Society for Food Science and Technology **41**: 724-732.

Didukh, M.Ya., Wasser, S.P. & Nevo, E. 2003. Medicinal value of species of the family Agaricaceae Cohn (higher Basidiomycetes) current stage of knowledge and future perspectives. – International Journal of Medicinal Mushrooms **5**: 133-152.

Dong, Q., Yao, J., Yang, X.-T. & Fang, J.-N. 2002. Structural characterization of a water-soluble β-D-glucan from fruiting bodies of *Agaricus blazei* Murr. – Carbohydrate Research **337**: 1417-1421.

Ebina, T. & Fujimiya, Y. 1998. Antitumor effect of a peptide-glucan preparation extracted from *Agaricus blazei* in a double-grafted tumor system in mice. – Biotherapy (Dordrecht) **11**: 259-265.

Fujii, T., Ishida, N., Maeda, H., Mizutani, I. & Suzuki, F. 1979. KS-2-A. US Patent No. 4163780, publ. 08.07.1979.

Fujimiya, Y., Kobori, H., Oshiman, K.I., Soda, R. & Ebina, T. 1998a. Tumoricidal activity of high molecular weight polysaccharides derived from *Agaricus blazei* via oral administration in the mouse tumor model. – Nippon Shokuhin Kagaku Kougaku Kaishi **45**: 246-252.

Fujimiya, Y., Suzuki, Y., Oshiman, K.I., Kobori, H., Moriguchi, K., Nakashima, H., Matumoto, Y., Takahara, S., Ebina, T. & Katakura, R. 1998b. Selective tumoricidal effect of soluble proteoglucan extracted from the basidiomycete, *Agaricus blazei* Murrill, mediated via natural killer cell activation and apoptosis. – Cancer Immunology and Immunotherapy **46**: 147-159.

Fujimiya, Y., Suzuki, Y., Katakura, R. & Ebina, T. 1999. Tumor-specific cytocidal and immunopotentiating effects of relatively low molecular weight products derived from the basidiomycete, *Agaricus blazei* Murrill. – Anticancer Research **19**: 113-118.

Fujimiya, Y., Yamamoto, H., Noji, M. & Suzuki, I. 2000. Peroral effect on tumor progression of soluble β-(1, 6)-glucans prepared by acid treatment from *Agaricus blazei* Murr. (Agaricaceae, Higher Basidiomycetes). – International Journal of Medicinal Mushrooms **2**: 43-49.

Fullerton, S.A., Samadi, A.A., Tortorelis, D.G., Chaudhury, M.S., Mallough, C., Tazaki, H. & Kumo, S. 2000. Induction of apoptosis in human prostate cancer cells with β-glucan (Maitake mushroom polysaccharide). – Molecular Urology **4**: 7-13.

Furne, H. 1985. Clinical evaluation of Schizophyllan (SPG) in gastric cancer – randomised controlled studies. – International Journal of Immunology **7**: 333-336.

Furue, H., Kitoh, I. & Hattori, T. 1981. Phase III study on Lentinan. – Japanese Journal of Cancer Chemotherapy **8**: 944-960.

Gao, Q.P., Jiang, R.Z., Chen, H.Q., Jensen, E. & Seljelid, R. 1996a. Characterization and cytokine stimulating activities of heteroglycans from *Tremella fuciformis.* – Planta Medica **62**: 297-302.

Gao, Q.P., Seljelid, R., Chen, H.Q. & Jiang, R. 1996b. Characterization of acidic heteroglycans from *Tremella fuciformis* Berk. with cytokine stimulating activity. – Carbohydrate Research **288**: 135-142.

Gao, Q.P., Killie, M.K., Chen, H.Q,. Jiang, R.Z. & Seljelid, R. 1997. Characterization and cytokine-stimulating activities of acidic heteroglycans from *Tremella fuciformis.* – Planta Medica **63**: 457-460.

Gao, Y. 2000. The miracle herb scientific reports of *Ganoderma.* Yuangizai Publisher, Taipei.

Gao, Y., Zhou, S., Chen, G., Dai, X. & Ye, J. 2002. A Phase I/II study of a *Ganoderma lucidum* extract (Ganoply) in patients with advanced cancer. – International Journal of Medicinal Mushrooms **4**: 207-214.

Gao, Y., Zhou, Sh., Huang, M. & Xu, A. 2003. Antibacterial and antiviral value of the genus *Ganoderma* P. Karst. species (Aphyllophoromycetideae): a review. – International Journal of Medicinal Mushrooms **5**: 235-246.

Gao, Y., Lan, J., Dai, X., Ye, J. & Zhou, Sh. 2004. A phase I/II study of Ling Zhi mushroom *Ganoderma lucidum* (W. Curt. : Fr.) Lloyd (Aphyllophoromycetideae) extract in patients with type II diabetes mellitus. – International Journal of Medicinal Mushrooms **6**: 96-107.

Gasiorowski, K., Brokos, B., Lamer, Z.E. & Trocha, G.J. 1993. Polysaccharides from *Laetiporus sulphureus* (Basidiomycetes) II. Evaluation of immunostimulative and antitumor activity. – Bulletin of the Polish Academy of Sciences, Biological Sciences **41**: 347-352.

Ghoneum, M., Wimbley, M., Salem, F., McKlain, A., Attallah, N. & Gill, G. 1995. Immunomodulatory and anticancer effects of active hemicellulose compound (AHCC). – International Journal of Immunotherapy **11**: 23-28.

Gorin, P.A.J. & Barreto-Berger, E. 1983. The chemistry of polysaccharides of fungi and lichens. – In: G.O. Aspinall [ed.]. The polysaccharides. Vol. **2**. Pp. 365-409. Academic Press, Orlando, FL.

Hamuro, J. & Chihara, G. 1985. Lentinan, a T-cell oriented immunopotentiator: its experimental and clinical applications and possible mechanism of immune modulation. – In: R.L. Fenichel & M.A. Chirigos [eds]. Immunomodulation agents and their mechanisms, pp. 409-436. Marcel Dekker, New York.

Han, M.D., Lee, E.S. & Kim, Y.K. 1998. Production of nitric oxide in raw 264.7 macrophages treated with ganoderan, the beta glucan of *Ganoderma lucidum.* – Korean Journal of Mycology **26**: 246-255.

Han, S.B., Lee, C.W., Jeon, Y.J., Hong, N.D., Yoo, I.D., Yag, K.H. & Kim, H.M. 1999. The inhibitory effect of polysaccharides isolated from *Phellinus linteus* on tumor growth and metastasis. – Immunopharmacology **41**(2): 157-164.

Hara, C., Kumazawa, Y., Inagaki, K., Kaneko, M., Kiho, T. & Ukai, S. 1991. Mitogenic and colony stimulating factor-inducing activities of polysaccharide fractions from the fruit bodies of *Dictyophora indusiata* Fisch. – Chemical and Pharmaceutical Bulletin **39**: 1615-1616.

Hawksworth, D.L. 1993. The tropical fungal biota: census, pertinence, prophylaxis, and prognosis. – In: S. Isaac, J.C. Frankland, R. Watling & A.J.S. Whalley [eds]. Aspects of Tropical Mycology, pp. 265-293. Cambridge University Press, Cambridge.

Hawksworth, D.L. 2001. Mushrooms: the extent of the unexplored potential. – International Journal of Medicinal Mushrooms **3**: 333-340.

Hikichi, M., Hiroe, E. & Okubo, S. 1999. Protein polysaccharide 0041. EP Patent No. 0939082, publ. 09.01.1999.

Hirata, A., Itoh, W., Tabata, K., Kojima, T., Itoyama, S. & Sugawara, I. 1994. Anticoagulant activity of sulfated schizophyllan. – Bioscience Biotechnology and Biochemistry **58**: 406-407.

Hiroshi, S. & Takeda, M. 1993. Diverse biological activity of PSK (Krestin), a protein-bound polysaccharide from *Coriolus versicolor* (Fr.) Quél. – In: S.T. Chang, J.A. Buswell & S.W. Chiu [ed.]. Mushroom biology and mushroom products, pp. 237-245. Chinese University Press, Hong Kong.

Hirotani, M., Hirotani, S. & Yoshikawa, T. 2000. Blazeispirol D and Z, as the actual intermediates of blazeispirol A biosynthesis from the cultured mycelia of the fungus *Agaricus blazei.* – Tetrahedron Letters **42**: 5261-5264.

Hirotani, M., Hirotani, S. & Yoshikawa, T. 2001. Blazeispirol X and Y, two novel carbon skeletal sterols from the cultured mycelia of the fungus *Agaricus blazei.* – Tetrahedron Letters **41**: 5107-5110.

Hirotani, M., Sai, K., Nagai, R., Hirotani, S., Takayanagi, H. & Yoshikawa, T. 2002. Blazeispirane and protoblazeispirane derivatives from the cultured mycelia of the fungus *Agaricus blazei.* – Phytochemistry **61**: 589-595.

Hobbs, Ch. 1995. Medicinal mushrooms: an exploration of tradition, healing and culture. Botanica Press, Santa Cruz, CA.

Hobbs, Ch.R. 2000. Medicinal value of *Lentinus edodes* (Berk.) Sing. (Agaricomycetideae). A literature review. – International Journal of Medicinal Mushrooms **2**: 287-302.

Hseu, R.Y. 1993. An overview on *Ganoderma* mushrooms. Wann Nian Publishing Co., Taichung, Taiwan.

Hu, B. & But, P. 1987. Chinese materia medica for radiation protection. – Abstracts of Chinese Medicine **1**: 475-490.

Hwang, S.F. & Liu, K.J. 1989. The inhibitory effect on artificial pulmonary metastasis of immune S-180 sarcoma cells by orally administered *Ganoderma lucidum* culture broth. – Journal of Chinese Oncological Society **5**: 10-15.

Ikekawa, T. 2001. Beneficial effects of edible and medicinal mushrooms on health care. –International Journal of Medicinal Mushrooms **3**: 291-298.

Ikekawa, T., Uehara, N., Maeda, Y., Nakanishi, K. & Fukuoka, F. 1969. Antitumor activity of aqueous extracts of edible mushrooms. – Cancer Research **29**: 734-735.

Ikekawa, T., Ikeda, Y., Yoshioka, Y., Nakanishi, K., Yokoyama, E. & Yamazaki, E. 1982. Antitumor polysaccharides of *Flammulina velutipes* 2. The structure of EA-3 and further purification of EA-5. – Journal of Pharmacobiodynamics **5**: 576-581.

Ikekawa, T., Saitoh, H., Feng, W., Zhang, H., Li, L. & Matsuzawa, T. 1992. Antitumor activity of extracts and polysaccharides. – Chemical and Pharmaceutical Bulletin **40**: 1954-1957.

Ishiyama, D., Kawagishi, H., Furukawa, S., Mori, Y., Kojima, F., Okamoto, K. & Sakamoto, H. 1996. Eudesmane derivative and neurocyte factor-producing inducer containing the same as active ingredient. JP Patent No. 8073395, publ. 19.03.1996.

Ito, H., Sugiura, M. & Miyazaki, T. 1976. Antitumor polysaccharide fraction from the culture filtrate of *Fomes fomentatius.* – Chemical and Pharmaceutical Bulletin **24**: 2575.

Ito, H., Shimura, K., Itoh, H. & Kawade, M. 1997. Antitumor effects of a new polysaccharide-protein complex (ATOM) prepared from *Agaricus blazei* (Iwade strain 101) "Himematsutake" and its mechanisms in tumor-bearing mice. – Anticancer Research **17**: 277-284.

Itoh, H., Ito, H., Amano, H. & Noda, H. 1994. Inhibitory action of a (1→6)-beta-D-Glucan-protein complex (FIII-2-b) isolated from *Agaricus blazei* Murrill ("Himematsutake") on Meth A fibrosarcoma-bearing mice and its antitumor mechanism. – Japanese Journal of Pharmacology **66**: 265-271.

Itoh, W., Sugawara, I., Kimura, S., Tabata, K., Hirata, A., Kojima, T., Mori, S. & Shimada, K. 1990. Immunopharmacological study of sulfated schizophyllan (SPG) I: Its action as a mitogen and anti-HIV agent. – International Journal of Immunopharmacology **12**: 225-234.

Jiang, X.-Z., Huang, L.-M., Zhou, Y.-F. & Wang, M.-H. 1999. Subchronic toxicity test of polysaccharide of Yun Zhi (PSP). – In: Q. Yang [ed.]. Advanced Research in PSP, pp. 272-284. Hong Kong Association for Health Care Ltd., Hong Kong.

Jin, T.-Y. 1999. Toxicological research on Yun Zhi polysaccharopeptide (PSP). – In: Q. Yang [ed.]. Advanced Research in PSP, pp. 76-79. Hong Kong Association for Health Care Ltd., Hong Kong.

Jong, S.C. & Birmingham, J.M. 1990. The medicinal value of the mushroom *Grifola.* – World Journal of Microbiology and Biotechnology **6**: 101-127.

Jong, S. & Yang, X. 1999. PSP – a powerful biological response modifier from the mushroom *Coriolus versicolor.* – In: Q. Yang [ed.]. Advanced Research in PSP Advanced Research in PSP, pp. 16-18. Hong Kong Association for Health Care Ltd., Hong Kong.

Kamiyama, Y. 1999. Improving effect of active hexose correlated compound (AHCC) on the prognosis of postoperative hepatocellular cacinoma patients. – European Surgical Research **31**: 216.

Kanayma, H., Togami, M., Adachi, N., Fukai, Y. & Okumoto, T. 1986. Studies on the antitumor active polysaccharides from the mycelia of *Poria cocos:* III. Antitumor activity against mouse tumors. – Yakugaku Zasshi **106**: 307-312.

Karácsonyi, S. & Kuniak, L. 1994. Polysaccharides of *Pleurotus ostreatus:* Isolation and structure of pleuran, an alkali-insoluble β-D-glucan. – Carbohydrate Polymers **24**: 107-111.

Kasahara, Y. & Hikino, H. 1987. Validity of the Oriental medicine. Part 122. Central actions of adenosine, a nucleolide of *Ganoderma lucidum.* – Phytotherapy Research **1**: 173-176.

Kasuga, T., Taylor, J.W. & White, T.J. 1999. Phylogenetic relationships of varieties and geographical groups of the human pathogenic fungus, *Histoplasma capsulatum* Darling. – Journal of Clinical Microbiology **37**: 653-663.

Kawagishi, H., Inagaki, R., Kanao, T., Mizuno, T., Shimura, K., Ito, H., Hagiwara, T. & Hakamura, T. 1989. Fractionation and antitumor activity of the water-insoluble residue of *Agaricus blazei* fruiting bodies. – Carbohydrate Research **186**: 267-273.

Kawagishi, H., Kanao, T., Inagaki, R., Mizuno, T., Shimura, K., Ito, H., Hagiwara, T. & Hakamura, T. 1990. Formulation of a potent antitumor (1→6)-beta-D-glucaan-protein complex from *Agaricus blazei* fruiting bodies and antitumor activity of the resulting products. – Carbohydrate Polymers **12**: 393-404.

Kawaguchi, T. 2005. Cancer metastasis:characterization and identification of the behavior of metastatic tumor cells and the cell adhesion molecules, including carbohydrates. – Current Drug Targets: Cardiovascular and Haematological Disorders **5**: 39-64.

Kidd, P.M. 2000. The use of mushroom glucans and proteoglycans in cancer treatment. – Alternative Medicine Reviews **5**: 4-27.

Kiho, T., Nagai, Y.S., Sakushima, M. & Ukai, S. 1992a. Polysaccharides in fungi: XXIX. Structural features of two antitumor polysaccharides from the fruiting bodies of *Armillariella tabescens.* – Chemical and Pharmaceutical Bulletin **40**: 2212-2214.

Kiho, T., Shiose, Y., Nagai, K. & Ukai, S. 1992b. Polysaccharides in fungi: XXX. Antitumor and immunomodulating activities of two polysaccharides from the fruiting bodies of *Armillariella tabescens.* – Chemical and Pharmaceutical Bulletin **40**: 2110-2114.

Kiho, T., Yoshida, I., Katsuragawa, M., Sakushima, M., Usui, S. & Ukai, S. 1994. Polysaccharides in fungi: XXXIV. A polysaccharide from the fruiting bodies of *Amanita muscaria* and the antitumor activity of its carboxymethylated product. – Biological and Pharmaceutical Bulletin **17**: 1460-1462.

Kim, B.K., Choi, E.C., Chung, K.S., Kang, C.Y., Kim, S.H., Kim, J.S., Kim, Y.J., Lee, K.L. & Lee, J.K. 1982. The constituents of higher fungi of Korea: Antitumor polysaccharides from the carpophores of some Basidiomycetes. – Archives of Pharmacological Research (Seoul) **5**: 21-24.

Kim, H.W. & Kim, B.K. 1999. Biomedical triterpenoids of *Ganoderma lucidum* (Curt. : Fr.) P. Karst. (Aphyllophoromycetideae). – International Journal of Medicinal Mushrooms **1** :121-138.

Kim, H.M., Han, S.B., Oh, G.T., Kim, Y.H., Hong, D.H., Hong, N.D. & Yoo, I.D. 1996. Stimulation of humoral and cell mediated immunity by polysaccharides from mushroom *Phellinus linteus.* – International Journal of Immunopharmacology **18**: 295-303.

Kimura, Y., Tojima, H. & Fukase, S. 1994. Clinical evaluation of sizofiran as assistant immunotherapy in treatment of head and neck cancer. – Acta Oto-laryngologica **511**: 192-195.

Kino, K., Yamashita, K., Yamaoka, J., Watanabe, S., Tanaka, K., Ko, K., Shimizu, K. & Rsunoo, H. 1989. Isolation and characterization of a new immunomodulating protein. Ling Zhi-8 (LZ-8) from *Ganoderma lucidum.* – Journal of Biological Chemistry **264**(1): 472-478.

Kirk, P.M., Cannon, P.F., David, J.C. & Stalpers, J.A. 2001. Ainsworth & Bisby's Dictionary of the Fungi. 9th edn. CAB International, Wallingford.

Komoda, Y., Shimuzu, M., Sonoda, Y. & Sato, Y. 1989. Ganoderic acid and its derivatives as cholesterol synthesis inhibitors. – Chemical and Pharmaceutical Bulletin **37**: 531-533.

Kosuna, K. 1998. Polysaccharides and preparation thereof. US Patent No. 5756318, publ. 26.05.1998.

Kubota, T., Asaka, Y., Miura, I. & Mori, H. 1982. Structures of ganoderic acid A and B. Two new lanostane-type bitter triterpenes from *Ganoderma lucidum.* – Helvetica Chimica Acta **65**: 611-619.

Kuniak, L., Karácsonyi, S., Augusti, J., Ginterova, A., Szecheny, S., Kravarik, D., Dubaj, J. & Varju, J. 1993. A new fungal glucan and its preparation. WO Patent No. 9312243, publ. 24.06.1993.

Kuo, Y.C., Huang, Y.L., Chen, C.C., Lin, Y.S., Chuang, K.A. & Tsai, W.J. 2002. Cell cycle progression and cytokine gene expression of human peripheral blood mononuclear cells modulated by *Agaricus blazei.* – Journal of Laboratory and Clinical Medicine **140**: 176-187.

Kurashiga, S., Akuzawa, Y. & Endo, F. 1997. Effect of *Lentinus edodes, Grifola frondosa* and *Pleurotus ostreatus* administration on cancer outbreaks and activities of macrophages and lymphocytes in mice treated with carcinogen. – Immunopharmacology and Immunotoxicology **19**: 175-185.

Lee, J.H., Chuo, S.M., Song, K.S., Hong, N.D. & Yoo, I.D. 1996. Characterization of carbohydratepeptide linkage of acidic heteroglycopeptide with immuno-stimulating activity from mycelium of *Phellinus linteus.* – Chemical and Pharmaceutical Bulletin **44**(5): 1093-1095.

Lee, S.S., Wei, Y.H., Chen, C.H., Wang, S.Y. & Chen, K.Y. 1995. Antitumour effects of *Ganoderma lucidum.* – Journal of Chinese Medicine **6**: 1-12.

Lee, S.Y. & Rhee, H.M. 1990. Cardiovascular effects of mycelium extract of *Ganoderma lucidum:* inhibition of sympathetic outflow as a mechanism of its hypotensive action. – Chemical and Pharmaceutical Bulletin **38**: 1359-1364.

Lin, C.N., Tome, W.P. & Won, S.J. 1991. Novel cytoloxic principles of Formosan *Ganoderma lucidum.* – Journal of Natural Products **54**: 998-1002.

Liou, Y.F. & Lin, K.H. 1979. Preparation of polysaccharides from *Fomes japonicus* (Fr.) Sacc. – screening for antitumor and cytotoxic substances. – Taiwan Yi Xue Hui Za Zhi **78**: 549-557.

Liu, F., Ooi, V.E.C., Liu, W.K. & Chang, S.T. 1996. Immunomodulation and antitumor activity of polysaccharide-peptide complex from the culture filtrates of a local edible mushroom, *Tricholoma labayense.* – General Pharmacology **27**: 621-624.

Liu, G.T. 1999. Recent advances in research of pharmacology and clinical applications of *Ganoderma* P. Karst. species (Aphyllophoromycetideae) in China. – International Journal of Medicinal Mushrooms **1**: 63-68.

Liu, J.X. & Zhou, J.Y. 1993. Phase II clinical trial for PSP capsules. – In: Q. Yong & C. Kwok [eds]. PSP International Symposium, 1993, Hong Kong, Fudan. Pp. 183-208. Fudan University Press, Shanghai, China.

Liu, J.X., Zhou, J. & Liu, T. 1999. Phase III clinical trial for Yun Zhi polysaccharopeptide (PSP) capsules. – In: W. Yang [ed.]. Advanced Research in PSP 1999, pp. 295-303. Hong Kong Association for Health Care Ltd., Fudan.

Liu, K.C., Phounsavan, S.F., Huang, R.L., Liao, C., Hsu, S.Y. & Wang, K.J. 1988. Pharmacological and liver functional studies on the mycelium of *Ganoderma lucidum.* – Chinese Pharmaceutical Journal **40**: 21-29.

Liu, L.F. 1999. PSP in clinical cancer therapy. – In: Q. Yang [ed.]. Advanced Research in PSP, pp. 68-75. Hong Kong Association for Health Care Ltd., Hong Kong.

Lorenzen, K. & Anke, T. 1998. Basidiomycetes as a source for new bioactive natural products. – Current Organic Chemistry **2**: 329-364.

Ma, Y., Mizuno, T. & Ito, H. 1991. Antitumor activity of some polysaccharides isolated from a Chinese mushroom, "Huangmo", the fruiting body of *Hohenbuehelia serotina.* – Agricultural and Biological Chemistry **55**: 2701-2710.

Maeda, Y.Y., Watanabe, S.T., Chihara, C. & Rokutanda, M. 1988. Denaturation and renaturaturation of a β-1,6; 1,3-glucan, lentinan, associated with expression of T-cell-mediated responses. – Cancer Research **48**: 671-675.

Marchessault, R.H., Deslandes, Y., Ogawa, K. & Sundarajan, P.R. 1977. X-ray diffraction data for β-D-glucan. – Canadian Journal of Chemistry **55**: 300-303.

Matsui, Y., Uhara, J., Satoi, S., Kaibori, M., Yamada, H., Kitade, H., Imamura, A., Takai, S., Kawaguchi, Y., Kwon, A.H. & Kamiyama Y. 2002. Improved prognosis of postoperative hepatocellular carcinoma patients when treated with functional foods: a prospective cohort study. – Journal of Hepatology **37**: 78-86.

Matsuoka, H., Seo, Y., Saito, T. & Tanoda, H. 1995. Usefulness of lymphocyte subset change as an indicator for predicting survival time and effectiveness of treatment with the immuno-potentiator Lentinan. – Anticancer Research **15**: 2291-2296.

Matsuoka, H., Seo, Y., Wakasugi, H., Saito, T. & Tanoda, H. 1997. Lentinan potentiates immunity and prolongs the survival time of some patients. – Anticancer Research **17**: 2751-2756.

Matsushita, K., Kuramitsu, Y., Ohiro, Y., Obara, M., Kobayashi, M., Li, Y.Q. & Hosokawa, M. 1998. Combination therapy of active hexose correlated compound plus UFT significantly reduces the metastasis of rat mammary adenocarcinoma. – Anti-Cancer Drugs **9**: 343-350.

Min, H.K., Choi, E.C. & Kim, B.K. 1980. Studies on the constituents of the higher fungi of Korea: 18. Components of *Russula pseudodelica* and *Microporus affinis.* – Korean Journal of Mycology **8**: 13-20.

Minato, K.I., Mizuno, M., Kawakami, S., Tatsuoka, S., Denpo, Y., Tokimoto, K. & Tsuchida, H. 2001. Changes in immunomodulating activities and contents of antitumor polysaccharides during the growth of two medicinal mushrooms, *Lentinus edodes* (Berk.) Sing. and *Grifola frondosa* (Dicks. : Fr.) S.F. Gray. – International Journal of Medicinal Mushrooms **3**: 1-7.

Misaki, A., Nasu, M., Sone, Y., Kishida, E. & Kinoshita, C. 1986. Comparison of structure and antitumor activity of polysaccharides isolated from Fukurotake, the fruiting body of *Volvariella volvacea.* – Agricultural and Biological Chemistry **50**: 2172-2184.

Miyazaki, K., Mizutani, H. & Katakuchi, H. 1995. Activated (HLA-DR+) T-lymphocyte-subset in cervical carcinoma and effects of radiotherapy and immunotherapy with sizofiran on cell-mediated immunity and survival. – Gynecologic Cancer **56**: 412-420.

Miyazaki, T. & Nishijima, M. 1981. Studies on fungal polysaccharides. XXVII. Structural examination of a water-soluble, antitumor polysaccharide of *Ganoderma lucidum.* – Chemical and Pharmaceutical Bulletin **29**: 3611-3616.

Miyazaki, T., Yadomae, T., Terui, T., Yamada, H. & Kikuchi, T. 1975. Studies on fungal polysaccharide. XVII. A new glucuronan "protuberic acid" produced by a fungus *Kobayasia nipponica.* – Biochimecial and Biophysical Acta **385**: 345-353.

Mizuno, M., Shiomi, Y., Minato, K., Kawakami, S., Ashida, H. & Tsuchida, H. 2000. Fucogalactan isolated from *Sarcodon asparatus* elicits release of tumor necrosis factor-alpha and nitric oxide from murine macrophages. – Immunopharmacology **46**: 113-121.

Mizuno, T. 1995. Bioactive biomolecules of mushrooms: food functions and medicinal effects of mushroom fungi. – Food Reviews International **11**: 7-21.

Mizuno, T. 1996. A development of antitumor polysaccharides from mushroom fungi. – Foods and Food Ingredients Journal of Japan **167**: 69-85.

Mizuno, T. 1999a. The extraction and development of antitumor-active polysaccharides from medicinal mushrooms in Japan (Review). – International Journal of Medicinal Mushrooms **1**: 9-29.

Mizuno, T. 1999b. Bioactive substances in *Hericium erinaceus* (Bull. : Fr.) Pers. (Yamabushitake), and its medicinal utilization. – International Journal of Medicinal Mushrooms **1**: 105-119.

Mizuno, T. 2000. Development of an antitumor biological response modifier from *Phellinus linteus* (Berk. et Curt.) Teng (Aphyllophoromycetideae) (Review). – International Journal of Medicinal Mushrooms **2**: 21-33.

Mizuno, T. 2002. Medicinal properties and clinical effects of *Agaricus blazei* Murr. (Review). – International Journal of Medicinal Mushrooms **4**: 299-312.

Mizuno, T. & Zhuang, C. 1995. Maitake, *Grifola frondosa:* pharmacological effects. – Food Reviews International **11**: 135-149.

Mizuno, T., Usui, T., Tomoda, M., Shinkai, K., Shimizu, M., Arakawa, M. & Tanaka, M. 1980. Studies on the host-mediated antitumor polysaccharides. II. Screening test on antitumor activity of various kinds of polysaccharides. – Bulletin of the Faculty of Agriculture Shizuoka University **30**: 41-50.

Mizuno, T., Kato, N., Totsura, A., Takenaka, K., Shinkai, K. & Shimizu, M. 1984. Studies on the host-mediated antitumor polysaccharides. VII. Fractionation, structural features and antitumor activity of water-soluble polysaccharide from "Reishi" the fruit body of *Ganoderma lucidum.* – Journal of Agriculture and Chemistry Society of Japan **58**: 871-880.

Mizuno, T., Ohsawa, K., Hagiwara, N. & Kuboyama, R. 1986. Fractionation and characterization of antitumor polysaccharides from Maitake, *Grifola frondosa.* – Agricultural and Biological Chemistry **50**: 1679-1688.

Mizuno, T., Hagiwara, T., Nakamura, T., Ito, H., Shimura, K., Sumiya, T. & Asakura, A. 1990a. Antitumor activity and some properties of water-soluble

polysaccharides from "Himematsutake", the fruiting body of *Agaricus blazei* Murrill. – Agricultural and Biological Chemistry **54**: 2889-2896.

Mizuno, T., Inagaki, R., Kanao, T., Hagiwara, T., Nakamura, T., Ito, H., Shimura, K., Sumiya, T. & Asakura, A. 1990b. Antitumor activity and some properties of water-insoluble heteroglycans from "Himematsutake", the fruiting body of *Agaricus blazei* Murrill. – Agricultural and Biological Chemistry **54**: 2897-2906.

Mizuno, T., Ando, M., Sugie, R., Ito, H., Shimura, K., Sumiya, T. & Matsuura, A. 1992. Antitumor-activity of some polysaccharides isolated from an edible mushroom, ningyotake, the fruiting body and the cultured mycelium of *Polyporus confluens.* – Bioscience Biotechnology and Biochemistry **56**: 34-41.

Mizuno, T., Ma, Y., Ito, H. & Suzuki, C. 1994. Water insoluble polysaccharide originating in mushroom, its production, and antitumor agent mainly comprising the polysaccharide. JP Patent No. 06-080703, publ. 22.03.1994.

Mizuno, T., Kinoshita, T., Zhuang, C., Ito, H. & Mayuzumi, Y. 1995a. Antitumor-active heteroglycans from Niohshimeji mushroom, *Tricholoma giganteum.* – Bioscience Biotechnology and Biochemistry **59**: 568-571.

Mizuno, T., Saito, H., Nishitoba, T. & Kawagishi, H. 1995b. Antitumor-active substances from mushrooms. – Food Reviews International **11**(1): 23-61.

Mizuno, T., Sakai, T. & Chihara, G. 1995c. Health foods and medicinal usages of mushrooms. – Food Reviews International **11**(1): 69-82.

Mizuno, T., Wang, G., Zhang, J., Kawagishi, H., Nishitoba, T. & Li, J. 1995d. Reishi, *Ganoderma lucidum* and *Ganoderma tusgae:* bioactive substances and medicinal effects. – Food Reviews International **11**(1): 151-166.

Mizuno, T., Yeohlui, P., Kinoshita, T., Zhuang, C., Ito, H. & Mayuzumi, Y. 1996. Antitumor activity and chemical modification of polysaccharides from Niohshimeji mushroom, *Tricholoma giganteum.* – Bioscience Biotechnology and Biochemistry **60**: 30-33.

Mizuno, T., Morimoto, M., Minato, K.I. & Tsuchida, H. 1998. Polysaccharides from *Agaricus blazei* stimulate lymphocyte T-cell subsets in mice. – Bioscience Biotechnology and Biochemistry **62**: 434-437.

Mizuno, T., Minato, K., Ito, H., Kawade, M., Terai, H. & Tsuchida, H. 1999a. Antitumor polysaccharide from the mycelium of liquid-cultured *Agaricus blazei* Murrill. – Biochemistry and Molecular Biology International **47**: 707-714.

Mizuno, T., Zhuang, C., Abe, K., Okamoto, H., Kiho, T., Ukai, S., Leclerc, S. & Meijer, L. 1999b. Antitumor and hypoglycemic activities of polysaccharides from the sclerotia and mycelia of *Inonotus obliquus* (Pers. : Fr.) Pil. (Aphyllophoromycetideae). – International Journal of Medicinal Mushrooms **1**: 301-316.

Nakajima, A., Ishida, T., Koga, M., Takeuchi, T., Mazda, O. & Takeuchi, M. 2002. Effect of hot water extract from *Agaricus blazei* Murrill on antibody-producing cells in mice. – International Immunopharmacology **2**: 12005-1211.

Nakajima, K., Hirata, Y., Uchida, H., Watabe, Y., Taniguchi, T., Obayashi, A. & Tanabe, O. 1980. Polysaccharides having anti-carcinogenic activity and method for producing same. GB Patent No. 2031446, publ. 23.04.1980.

Nakano, T., Oka, K., Hanba, K. & Morita, S. 1996. Intratumoral administration of sizofilan activates Langerhans cells and T-cell infiltration in cervical cancer. – Clinical Immunology and Immunopathology **79**: 79-86.

Nakashima, S., Umeda, Y. & Kanada, T. 1979. Effect of polysaccharides from *Ganoderma applanatum* on immune response. I. Enhancing effect on the induction of delayed hypersensitivity in mice. – Microbiology and Immunology **23**: 501-513.

Nakayoshi, H., Watanebe, T., Yamamura, Y. & Ono, M. 1968. Suppression of Sarcoma 37 in mice by the treatment with extracellular polysaccharide produced by a strain of *Crepidotus* sp. – Japanese Journal of Experimental Medicine **38**: 437-442.

Nanba, H. 1995. Activity of Maitake D-fraction to inhibit carcinogenesis and metastasis. – Annals of the New York Academy of Sciences **768**: 243-245.

Nanba, H. 1997a. Maitake D-fraction: healing and preventive potential for cancer. – Journal of Orthomolecular Medicine **12**: 43-49.

Nanba, H. 1997b. Effect of Maitake D-fraction on cancer prevention. – Annals of the New York Academy of Sciences **833**: 204-207.

Nanba, H. 1998. Proteoglycan and antidiabetic drug. JP Patent No. 182702, publ. 07.07.1998.

Nanba, H., Hamaguchi, A. & Kuroda, H. 1987. The chemical structure of an antitumor polysaccharide in fruit bodies of *Grifola frondosa* (Maitake). – Chemical and Pharmaceutical Bulletin **35**: 1162-1168.

Ng, T.B. & Chang, W.Y. 1997. Polysaccharopeptide from the mushroom *Coriolus versicolor* possesses analgesic activity but does not produce adverse effects on female reproduction or embryonic development in mice. – General Pharmacology **29**: 269-273.

Ngai, P.H.K. & Ng, T.B. 2003. Lentin, a novel and potent antifungal protein from shitake mushroom with inhibitory effects on activity of human immunodeficiency virus-1 reverse transcriptase and proliferation of leukaemia cells. – Life Sciences **73**: 3363-3374.

Nishida, I., Nanba, H. & Kuroda, H. 1988. Antitumour activity exhibited by orally administered extracts from fruit-body of *Grifola frondosa* (Maitake). – Chemical and Pharmaceutical Bulletin **36**: 1819-1827.

Ohno, N., Iino, K., Takeyama, T., Suzuki, I., Sato, K., Oikawa, S., Miyazaki, T. & Yadomae, T. 1985. Structural characterization and antitumor activity of the extracts from matted mycelium of cultured *Grifola frondosa.* – Chemical and Pharmaceutical Bulletin **33**: 3395-3401.

Ohno, N., Adachi, Y., Suzuki, I., Sato, K., Oikawa, S. & Yadomae, T. 1986. Characterization of the antitumor glucan obtained from liquid-cultured *Grifola frondosa.* – Chemical and Pharmaceutical Bulletin **34**: 1709-1715.

Ohno, N., Miura, N.N., Nakajima, M. & Yadomae, T. 2000. Antitumor 1,3-beta-glucan from cultured fruit body of *Sparassis crispa.* – Biological and Pharmaceutical Bulletin **23**: 866-872.

Ohno, N., Furukawa, M., Miura, N.N., Adachi, Y., Motoi, M. & Yadomae, T. 2001. Antitumor β-glucan from teh cultured fruit body of *Agaricus blazei.* – Biological and Pharmaceutical Bulletin **24**: 820-828.

Ohtsuka, S., Ueno, S., Yoshikumi, C., Hirose, F., Ohmura, Y., Wada, T., Fujii, T. & Takahashi, E. 1973. Polysaccharides having an anticarcinogenic effect and a method of producing them from species of Basidiomycetes. GB Patent No. 1331513, publ. 26.09.1973.

Ohtsuka, S., Ueno, S., Yoshikumi, C., Hirose, F., Ohmura, Y., Wada, T., Fujii, T. & Takahashi, E. 1977. Polysaccharides and a method for producing same. US Patent No. 4051314, publ. 27.09.1977.

Okamura, K., Suzuki, M. & Chihara, T. 1986. Clinical evaluation of Schizophyllan combined with irradiation in patients with cervical cancer. A randomised controlled study. – Cancer **58**: 865-872.

Okamura, K., Suzuki, M. & Chihara, T. 1989. Adjuvant immunochemotherapy: two randomised controlled studies of patients with cervical cancer. – Biomedicine and Pharmacotherapy **43**: 17-181.

Ooi, V.E.C. & Liu, F. 1999. A review of pharmacological activities of mushroom polysaccharides. – International Journal of Medicinal Mushrooms **1**: 195-206.

Oshiman, K., Fujimiya, Y., Ebina, T., Suzuki, I. & Noji, M. 2002. Orally administered beta-1,6-D-polyglucose extracted from *Agaricus blazei* results in tumor regression in tumor-bearing mice. – Planta Medica **67**: 610-614.

Paulik, Š., Švrčec, K., Mojžišová, J., Ďurove, A., Benišek, Z. & Húska, M. 1996. The immunomodulatory effect of the soluble fungal glucan (*Pleurotus ostreatus*) on delayed hypersensitivity and phagocytic ability of blood leucocytes in mice. – Journal of Medical and Veterinary Biology **43**: 129-135.

Reshetnikov, S.V., Wasser, S.P. & Tan, K.K. 2001. Higher Basidiomycota as source of antitumor and immunostimulating polysaccharides. – International Journal of Medicinal Mushrooms **3**: 361-394.

Rowan, N.J., Smith, J.E. & Sullivan, R. 2003. Immunomodulatory activities of mushroom glucans and polysaccharide-protein complexes in animals and humans (a review). – International Journal of Medicinal Mushrooms **5**: 95-110.

Sasaki, T., Arai, Y., Ikekawa, T., Chihara, G. & Fukuoka, F. 1971. Antitumor polysaccharides from some Polyporaceae, *Ganoderma applanatum* (Pers.) Pat. and *Phellinus linteus* (Berk. et Curt.) Aoshima. – Chemical and Pharmaceutical Bulletin **19**(4): 821-826.

Schaeffer, D.J. & Krylov, V.S. 2000. Anti-HIV activity of extracts and compounds from algae and Cyanobacteria. – Ecotoxicology and Environmental Safety **45**: 208-227.

Shimizu, S., Kitadam, H., Yokota, H., Yamakawa, J., Muryama, T., Sugiyama, K., Izumi, H. & Yamaguchi, N. 2002. Activation of the alternative complement pathway by *Agaricus blazei* Murrill. – Phytomedicine **9**: 536-545.

Shiu, W.C.T. 1992. A clinical study of PSP on peripheral blood counts during chemotherapy. – Physiological Research **6**: 217-218.

Shon, Y.H. & Nam, K.S. 2001. Antimutagenicity and induction of anticarcinogenic phase II enzymes by basidiomycetes. – Journal of Ethnopharmacology **77**(1): 103-109.

Smith, J.E., Rowan, N. & Sullivan, R. 2002. Medicinal mushrooms. Their therapeutic properties and current medicinal usage with special emphasis on cancer treatments. A special report Commissioned by Cancer Research UK. The University of Strathclyde in Glasgow, Glasgow.

Smith, J.E., Sullivan, R. & Rowan, N.J. 2003.The role of polysaccharides derived from medicinal mushrooms in cancer treatment programs: Current perspectives (Review). – International Journal of Medicinal Mushrooms **5**: 217-234.

Song, C.H., Jeon, Y.J., Yang, B.K., Ra, K.S. & Kim, H.I. 1998. Anti-complementary activity of endopolymers produced from submerged mycelial culture of higher fungi with particular reference to *Lentinus edodes.* – Biotechnology Letters **20**: 741-744.

Song, K.S., Cho, S.M., Lee, J.H., Kim, H.M., Han, S.B., Ko, K.S. & Yoo, I,D. 1995. B-lymphocyte stimulating polysaccharide from mushroom *Phellinus linteus.* – Chemical and Pharmaceutical Bulletin **43**(12): 2105-2108.

Soo, T.S. 1996. Effective dosage of the extract of *Ganoderma lucidum* in the treatment of various ailments. – In: D. Royse [ed.]. Mushroom biology and mushroom products, p. 177-186. Penn State University, University Park.

Sorimachi, K., Akimoto, K., Ikehara, Y., Inafuku, K., Okubo, A. & Yamazaki, S. 2001. Secretion of TNF-alpha, IL-8 and nitric oxide by macrophages activated with *Agaricus blazei* Murrill fractions *in vitro.* – Cell Structure and Function **26**: 103-108.

Stamets, P. 2000. Growing gourmet and medicinal mushrooms. 3rd edn. Ten Speed Press, CA, Berkeley.

Stephens, L.C., Ang, K.K. & Schulthesis, T.E. 1991. Apoptosis in irradiated murine tumours. – Radiation Research **127**: 308.

Stijve, T. & Amazonas, M.A.L. de A. 2001. *Agaricus blazei* Murrill, un noveau champignon gourmet et medicament qui nous vient de Bresil. – Miscellanea Mycologica **69**: 41-47.

Stijve, T., Pittel, A., Andrey, D., Amazonas, M.A.L. & Goessler, W. 2003. Potential toxic constituents of *Agaricus brasiliensis* (*A. blazei* ss. Heinem), as compared to other cultivated and wild-growing edible mushrooms. – Deutsche Lebensmittel-Rundschau **99**: 475-481.

Stott, K. & Mohammed, C. 2003. Cultivation of the edible and medicinal mushroom *Grifola frondosa* (Dicks. : Fr.) S.F. Gray (Maitake)-relevance of literature to production in Australia (review). – International Journal of Medicinal Mushrooms **5**: 199-216.

Sun, T. & Zhu, Y. 1999. The effect of PSP on immune function and living quality in patients receiving chemotherapy for gynaecological malignancies. – In: Q. Yang [ed.]. Advanced Research in PSP, pp. 308-309. Hong Kong Association for Health Care Ltd., Hong Kong.

Sun, Z., Yang, Q-Y. & Fei, H.-L. 1999. The ameliorative effect of PSP on the toxic and side reactions of chemo- and radiotherapy of cancers. – In: Q. Yang [ed.]. Advanced Research in PSP, pp. 304-307. Hong Kong Association for Health Care Ltd., Hong Kong.

Taguchi, T., Furue, H., Kimura, T., Kondo, T., Hattori, T., Ito, I. & Ogawa, N. 1985. End-point results of Phase III study of Lentinan. – Japanese Journal of Cancer Chemotherapy **12**: 366-380.

Takaku, T., Kimura, Y. & Okuda, H. 2001. Isolation of an antitumor compound from *Agaricus blazei* Murrill and its mechanism of action. – Journal of Nutrition **5**: 1409-1413.

Taylor, J.W., Geiser, D.M., Burt, A. & Koufopanou, V. 1999. The evolutionary biology and population genetics underlying strain-typing. – Clinical Microbiology Reviews **12**: 126-146.

Taylor, J.W., Jacobson, D.J., Kroken, S., Kasuga, T., Geiser, D.M., Hibbett, D.S. & Fisher, M.C. 2000. Phylogenetic species recognition and species concepts in fungi. – Fungal Genetics and Biology **31**: 21-32.

Toth, J.O., Luu, B. & Ourisson, G. 1983. Ganodermic acids T-Z, cylotoxic triterpenes from *Ganoderma lucidum* (Polyporaceae). – Tetrahedron Letters **24**: 1081-1084.

Tsuchida, H., Mizuno, M., Taniguchi, Y., Ito, H., Kawade, M. & Akasaka, K. 2001. Glucomannan separated from *Agaricus blazei* mushroom culture and antitumor agent containing as active ingredient. JP Patent No. 11-080206, publ. 26.03.2001.

Tzianabos, A.O. 2000. Polysaccharide immunomodulators as theurapeutic agents: structural aspects and biological function. – Clinical Microbiology Reviews **13**: 523-533.

Ueno, S., Yoshikumi, C., Hirose, F., Omura, Y., Fujii, T., Ohara, M. & Matsunaga, K. 1978. Preparation of anti-tumor polysaccharides. JP Patent No. 53109915, publ. 26.09.1978.

Ukai, S., Kiho, T., Hara, C., Morita, M., Goto, A., Imaizumi, N. & Hasegawa, Y. 1983. Polysaccharides in fungi: XIII. Antitumor activity of various polysaccharides isolated from *Dictyophora indusiata, Ganoderma japonicum, Cordyceps cicadae, Auricularia auricula-judae* and *Auricula* sp. – Chemical and Pharmaceutical Bulletin **31**: 741-744.

Ukawa, Y., Ito, H. & Hisamatsu, M. 2000. Antitumor effect of (1→3)-beta-D-glucan and (1→6)-beta-D-glucan purified from newly cultivated mushroom, Hatakeshimeji (*Lyophyllum decastes* Sing.). – Journal of Bioscience and Bioengineering **90**: 98-104.

Wang, G., Zhang, J., Mizuno, T., Zhuang, C., Ito, H., Mayuzumi, H., Okamoto, H. & Li, J. 1993. Antitumor active polysaccharides from the Chinese mushroom Songshan Lingzhi, the fruiting body of *Ganoderma tsugae.* – Bioscience Biotechnology and Biochemistry **57**: 894-900.

Wang, H.X., Ng, T.B., Ooi, V.E.C., Liu, W.K. & Chanf, S.T. 1996. A polysaccharide-peptide complex from culture mycelia of the mushroom *Tricholoma mongolicum* with immunoenhancing and antitumor activities. – Biochemistry and Cell Biology **74**: 95-100.

Wang, S.Y., Hsu, M.L. & Hsu, H.C. 1997. The antitumour effect of *Ganoderma lucidum* is mediated by cytokines released from activated macrophages and T lymphocytes. – International Journal of Cancer **70**: 699-705.

Wasser, S.P. 2002. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. – Applied Microbiology and Biotechnology **60**: 258-274.

Wasser S.P. 2005. Reishi or Ling Zhi (*Ganoderma lucidum*). – In: P.M. Coates, M.R. Blackman, G.M. Gragg., M. Levine, J. Moss & J.D. White [eds]. Encyclopedia of dietary supplements. Pp. 603-622. Marcel Dekker, New York.

Wasser, S.P. & Weis, A.L. 1997a. Shiitake mushrooms [*Lentinus edodes* (Berk.) Sing.]. – In: E. Nevo [ed.]. Medicinal mushrooms. Peledfus, Haifa, Israel.

Wasser, S.P. & Weis, A.L. 1997b. Reishi mushroom [*Ganoderma lucidum* (Curt. : Fr.) P. Karst.]. – In: E. Nevo [ed.]. Medicinal mushrooms. Peledfus, Haifa, Israel.

Wasser, S.P. & Weis, A.L. 1999. Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives (Review). – International Journal of Medicinal Mushrooms **1**: 31-62.

Wasser, S.P., Nevo, E., Sokolov, D., Reshetnikov, S.V. & Timor-Tismenetsky, M. 2000. Dietary supplements from medicinal mushrooms: diversity of types and variety of regulations. – International Journal of Medicinal Mushrooms **2**: 1-19.

Wasser, S.P., Didukh, M.Ya., Amazonas, M.A.L.A., Nevo, E., Stamets, P. & Eira, A.F. 2002. Is widely cultivated culinary-medicinal Royal Sun Agaricus (the Himematsutake mushroom) indeed *Agaricus blazei* Murrill? – International Journal of Medicinal Mushrooms **4**: 267-290.

Xu, L.Z. 1999. The antitumor and antivirus activity of PSP. – In: Q. Yang [ed.]. Advanced Research in PSP, pp. 62-67. Hong Kong Association for Health Care Ltd., Hong Kong.

Yadomae, T. 2000. Structure and biological activities of fungal β-1,3-glucans. – Yakugaku Zasshi **120**: 413-431.

Yadomae, T. & Ohno, N. 2000. *Sparassis crispa* Fr. extract. JP Patent No. 2000-217543, publ. 08.08.2000.

Yanaki, T., Ito, W., Tabata, K., Kojima, T., Norizuye, T., Takano, N. & Fujita, H. 1983. Correlation between the antitumor activity of a polysaccharide schizophyllan and its triple-helical conformation in dilute aqueous solution. – Biophysical Chemistry **17**: 337-342.

Yanaki, T., Ito, W. & Tabata, K. 1986. Correlation between antitumor activity of schizophyllan and its triple helix. – Agricultural and Biological Chemistry **509**: 2415-2416.

Yang, Q.Y. 1999. History present status and perspectives of the study of yun zhi polysaccharides. – In: Q. Yang [ed.]. Advanced Research in PSP, pp. 5-15. Hong Kong Association for Health Care Ltd., Hong Kong.

Yang, Q.Y. & Wang, M.M. 1994. The effect of *Ganoderma lucidum* extract against fatigue and endurance in the absence of oxygen. – In: P.K. Buchanan, R.S. Hseu & J.M. Moncalvo [eds]. 5th International Mycological Congress (IMC5) Abstracts, Vancouver, BC, Canada, 14-21 August 1994. P. 249. Vancouver, BC.

Yao, W. 1999. Prospective randomised trial of radiotherapy plus PSP in the treatment of oesophageal carcinoma. – In: Q. Yang [ed.]. Advanced Research in PSP, pp. 310-313. Hong Kong Association for Health Care Ltd., Hong Kong.

Yap, A.-T. & Ng, M.-L. 2003. Immuno-potentiating properties of lentinan (1→3)-β-D-glucan extracted from culinary-medicinal shiitake mushroom *Lentinus edodes* (Berk.) Singer (Agaricomycetideae). – International Journal of Medicinal Mushrooms **5**: 339-358.

Ying, J., Mao, X., Ma, Q., Zong, Y. & Wen, H. 1987. [Icones of medicinal fungi from China]. Science Press, Beijing. (In Chinese)

Yoon, S.Y., Eo, S.K., Kim, Y.S., Lee, C.K. & Han, S.S. 1994. Antimicrobial activity of *Ganoderma lucidum* extract alone and in combination with some antibiotics. – Archives of Pharmacal Research (Seoul) **17**(6): 438-442.

Yoshida, I., Kiho, T., Usui, S., Sakushima, M. & Ukai, S. 1996. Polysaccharides in fungi. XXXVII. Immunomodulating activities of carboxymethylated derivatives of linear (1→3)-alpha-D-glucans extracted from the fruiting bodies of *Agrocybe cylindracea* and *Amanita muscaria.* – Biological and Pharmaceutical Bulletin **19**: 114-121.

Yoshioka, Y., Ikekawa, T., Nida, M. & Fukuoka, F. 1972. Studies on antitumor activity of some fractions from basidiomycetes. I. An antitumor acidic polysaccharide fraction of *P. ostreatus* (Fr.) Quél. – Chemical and Pharmaceutical Bulletin **20**: 1175-1180.

Yuexin, L., Zhuqiu, Ye., Yanan, H. & Hualing, X. 2002. Fractionation and characterization of water-soluble polysaccharides from culinary-medicinal mushroom, *Agaricus blazei* Murrill (Agaricomycetideae) fruit body. – International Journal of Medicinal Mushrooms **4**: 131-321.

Yui, T., Ogawa, K., Kakuta, M. & Misaki, A. 1995. Chain conformation of a glucurono-xylo-mannan isolated from fruit body of *Tremella fuciformis* Berk. – Journal of Carbohydrate Chemistry **14**: 255-263.

Zhang, Q.H. & Lin, Z.B. 1999. The antitumor activity of *Ganoderma lucidum* (Curt. : Fr.) P. Karst. (Ling Zhi) (Aphyllophoromycetideae) polysaccharides. – International Journal of Medicinal Mushrooms **1**: 207-215.

Zhong, B.-Z., Zhon, Y.G., Zhou, L.-F. & Yang, Q.-Y. 1999. Genetic toxicity test of Yun Zhi polysaccharide (PSP). – In: Q. Yang [ed.]. Advanced Research in PSP, pp. 285-294. Hong Kong Association for Health Care Ltd., Hong Kong.

Zhou, Sh. & Gao, Y. 2002. The immunomodulating effects of *Ganoderma lucidum* (Curt. : Fr.) P. Karst. (Ling Zhi, reishi mushroom) (Aphyllophoro mycetideae). – International Journal of Medicinal Mushrooms **4**: 1-12.

Zhou, Sh., Gao, Y., Chen, G., Dai, X., Ye, X. & Gao, H. 2002. A phase I/II study of a *Ganoderma lucidum* (Curt. : Fr.) P. Karst. (Ling Zhi, Reishi mushroom) extract in patients with chronic hepatitis B. – International Journal of Medicinal Mushrooms **4**: 321-328.

Zhu, D. 1987. Recent advances on the active components in Chinese medicines. – Abstracts of Chinese Medicine **1**: 251-286.

Zhuang, C. & Wasser, S.P. 2004. Medicinal value of culinary-medicinal Maitake mushroom *Grifola frondosa* (Dicks. : Fr.) S.F. Gray (Aphyllophoromycetideae). Review. – International Journal of Medicinal Mushrooms **6**: 287-313.

Zhuang, C., Mizuno, T., Shimada, A., Ito, H., Suzuki, C., Mayuzumi, Y., Okamoto, H., Ma, Y. & Li, J. 1993. Antitumor protein-containing polysaccharides from a Chinese mushroom Fengweigu or Houbitake, *Pleurotus sajor-caju* (Fr.) Sing. – Bioscience Biotechnology and Biochemistry **57**: 901-906.

Zhuang, C., Mizuno, T., Ito, H., Shimura, K., Sumiya, T. & Kawade, M. 1994a. Antitumor activity and immunological property of polysaccharides from the mycelium of liquid-cultured *Grifola frondosa.* – Nippon Shokuhin Kogyo Gakkaishi **41**: 724-732.

Zhuang, C., Mizuno, T., Ito, H., Shimura, K., Sumiya, T. & Kawade, M. 1994b. Chemical modification and antitumor activity of polysaccharides from the mycelium of liquid-cultured *Grifola frondosa.* – Nippon Shokuhin Kogyo Gakkaishi **41**: 733-740.