Mediators of Inflammation • 2005:2 (2005) 63–80 • PII: S0962935105412223 • DOI: 10.1155/MI.2005.63

INVITED REVIEW

Antiinflammatory and Immunomodulating Properties of Fungal Metabolites

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Received 22 December 2004; accepted 25 January 2005

We discuss current information on the ability of extracts and isolated metabolites from mushrooms to modulate immune responses. This can result in a more enhanced innate and acquired disease resistance. The major immunomodulating effects of these active substances derived from mushrooms include mitogenicity and activation of immune effector cells, such as lymphocytes, macrophages, and natural killer cells, resulting in the production of cytokines, including interleukins (ILs), tumor necrosis factor alpha (TNF)-α, and interferon gamma (INF)-γ. In particular, the ability of selective mushroom extracts to modulate the differentiation capacity of $CD4^+$ T cells to mature into T_H1 and/or T_H2 subsets will be discussed. As a consequence these extracts will have profound effects in particular diseases, like chronic autoimmune T_H1-mediated or allergic T_H2-mediated diseases. Immunosuppressive effects by mushroom components have also been observed. The therapeutic effects of mushrooms, such as anticancer activity, suppression of autoimmune diseases, and allergy have been associated with their immunomodulating effects. However, further studies are needed to determine the molecular mechanisms of the immunomodulating effects of mushrooms metabolites both individually and in complex mixtures, for example, extracts.

INTRODUCTION

The number of different mushroom species on earth is estimated at 140 000, of which may be only 10% are known. 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 [1, 2, 3, 4]. Mushrooms have long been attracting a great deal of interest in many areas of foods and biopharmaceuticals. They are well known for their nutritional and medicinal values [1, 4, 5, 6, 7, 8, 9]. In accordance to Breene [10] the gross composition of mushrooms is water (90%), and from the dry matter: protein (10%–40%), fat (2%–8%), carbohydrates (3%–28%), fiber (3%–32%), and ash (8%–10%) (the ash percentage is the fraction of dry matter that remains after incineration of the organic material in a sample, and is mainly composed of salts, metals, and so forth). Many species of mushrooms are cultivated worldwide. Global production increased to about 6.2 million tons in 1997, with a more than 12% increase annually from 1981 to 1997 [11]. Mushroom extracts have been increasingly sold as dietary supplements. The market value of mushroom dietary supplement products worldwide is about US$5–6 billion per year [12].

Medicinal mushrooms have an established history of use in traditional oriental therapies. Historically, hot-water-soluble fractions (decoctions and essences) from medicinal mushrooms were used as medicine in the Far East, where knowledge and practice of mushroom use primarily originated [4, 13, 14]. Mushrooms such as *Ganoderma lucidum* (Reishi), *Lentinus edodes* (Shiitake), *Inonotus obliquus* (Chaga), and many others have been collected and used for hundreds of years in Korea, China, Japan, and eastern Russia [4].

Mushroom metabolites are increasingly being utilized to treat a wide variety of diseases, particularly as they can be added to the diet and used orally, without the need to go through phase-I/II/III trials as an ordinary medicine, and they are considered as a safe and useful approach for disease treatment. A lot of scientific

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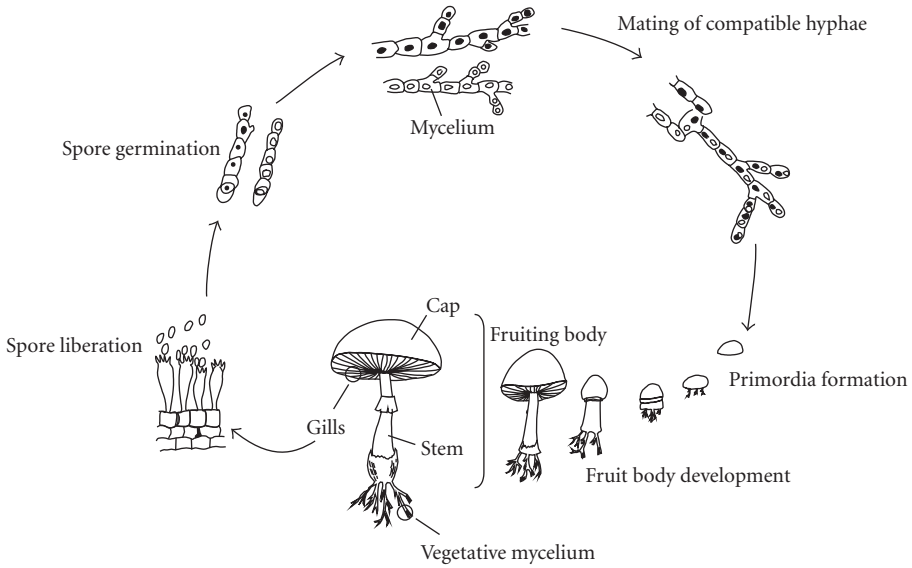


Figure 1. Diagrammatic representation of mushroom life cycle.

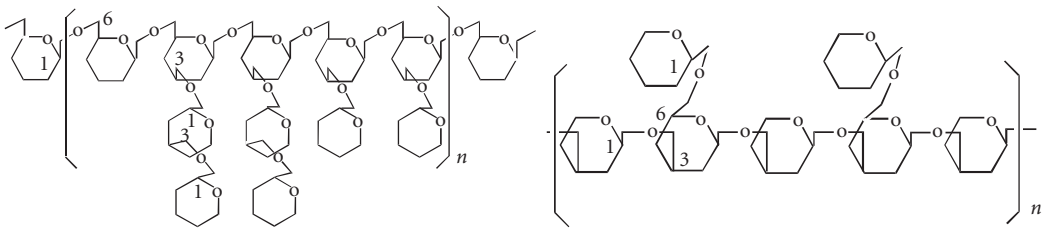
investigations have been performed to discover possible functional properties, which could be efficient in possible treatments of diseases like allergic asthma [15, 16, 17], food allergy [18, 19], atopic dermatitis [20], inflammation [21, 22], autoimmune joint inflammation such as rheumatoid arthritis [23], atherosclerosis [24, 25], hyperglycemia [26], thrombosis [27], human immunodeficiency virus (HIV) infection [28, 29], listeriosis [30], tuberculosis [31], septic shock [32], and cancer [33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55].

In the last years many researchers have studied the possibility that extracts and isolated metabolites from mushrooms stimulate or suppress specific components of the immune system. Immunomodulators can be effective agents for treating and preventing diseases and illnesses that stem from certain immunodeficiencies and other depressed states of immunity [56]. Synonymous terms for immunomodulators include biological response modifiers, immunoaugmentors, or immunorestoratives [57]. Those metabolites which appear to stimulate the human immune response are being sought for the treatment of cancer, immunodeficiency diseases, or for generalized immunosuppression following drug treatment, for combination therapy with antibiotics, and as adjuvants for vaccines [58]. Those metabolites that suppress immune reactions are potentially useful to mitigate autoimmune or certain gastrointestinal tract diseases (eg, Crohn's) [59].

At least 651 species and 7 infraspecific taxa representing 182 genera of hetero- and homobasidiomycetes mushrooms contain antitumor or immunostimulating metabolites [4]. Bioactive metabolites can be isolated from fruiting bodies (Figure 1), pure culture mycelia, and culture filtrate (culture broth). Nowadays many attempts are being made to obtain bioactive metabolites from mycelia through submerged fermentation culture. The cultivation of mushrooms to produce fruiting bodies is a long-term process requiring from one to several months for the first fruiting bodies to appear. The growth of mushroom cell cultures in submerged conditions in a liquid culture medium accelerates the process, resulting in biomass yield within a few days and allows to obtain standardized nutriceutical substances.

Several major substances with immunomodulatory and/or antitumor activity have been isolated from mushrooms. These include mainly polysaccharides (in particular β-D-glucans (Figure 2)), polysaccharopeptides (PSP), polysaccharide proteins, and proteins. Furthermore, other bioactive substances, including triterpenes, lipids, and phenols, have been identified and characterized in mushrooms with proven medicinal properties. The major immunomodulating effects of these active substances derived from mushrooms include mitogenicity and activation of immune cells, such as hematopoietic stem cells, lymphocytes, macrophages, dendritic cells (DCs) and natural killer (NK) cells, resulting in the production of cytokines. The therapeutic effects of mushrooms, such as anticancer activity, suppression of autoimmune diseases, and allergy have been associated in many cases with their immunomodulating effects.

Whilst it is known that mushroom extracts have immunomodulatory and/or antitumor activity, the standard approach has been to isolate, characterize, and administer the pure active constituents. However, different components in a mushroom extract may have synergistic activities [49, 60]. There are several reports of mushrooms containing more than one polysaccharide with antitumor activity. The responses to different polysaccharides are likely to be mediated by different cell surface receptors, which may be present only on specific subsets of cells and may



(a) (b)

Figure 2. Repeating unit of immunomodulatory β-glucans (a) from *Grifola frondosa* (D-fraction, MW: 1000 kD) and (b) from *L edodes* (lentinan, MW: 500 kD).

Table 1. Immunomodulatory activities of mushroom compounds on hematopoietic stem cells.

Species	Compound	Immune effects	Reference
Grifola frondosa	MD-fraction	↑ BMCs growth and differentiation into CFU-GM ↑ recovery of CFU-GM response after DOX induced hematopoietic suppression	[61]
Lentinus lepideus	PG101	↑ CFU-GM, BFU-E, IL-1β, IL-6, GM-CSF ↓ TNF-α in irradiated mice	[63]
Sparassis crispa	SCG	↑ granulocytes, monocytes, $\gamma\delta$T cells and NK1.1 cells in the peripheral cells in CY-induced leukopenia	[64]

trigger distinct downstream responses. A combination of such responses involving different cell subsets could conceivably provide greater tumor inhibition than could be induced by a single polysaccharide [49].

EFFECTS OF MUSHROOM METABOLITES ON HEMATOPOIETIC STEM CELLS

Various metabolites, especially carbohydrates isolated from mushrooms, were reported to affect bone marrow cells (BMCs), and to induce hematopoiesis (Table 1). Recently, Lin et al [61] reported that Maitake MD-fraction (obtained by further purification of D-fraction), an extract isolated from the fruit body of *Grifola frondosa* whose active component is an isolated β-glucan, a protein-bound polysaccharide compound, caused direct enhancement of the colony-forming units-granulocytes/macrophages (CFU-GM) response of BMCs progenitors and enhanced recovery of the CFU-GM response after doxorubicin (DOX) induced hematopoietic suppression. These studies suggest that MD-fraction has the potential to reduce hematopoietic suppression induced by chemotherapy.

PG101, a water-soluble extract that consists of protein-bound polysaccharides, isolated from cultured mycelia of *Lentinus lepideus* [62], is a potent immune modulator that recovers the radiation-damaged bone marrow system very efficiently. In PG101-treated mice, the number of CFU-GM and erythroid burst-forming units (BFU-E) were increased to almost the levels seen in nonirradiated control as early as 8 days after irradiation. Radiation is known to result in serious dysregulation of cytokine expression. PG101 increased the levels of IL-1β, IL-6, and granulocyte macrophage-colony-stimulating factor (GM-CSF) over the 24-day period. PG101 significantly reduced the level of TNF-α. TNF-α, which is increased as a consequence of tissue injury and anemia due to radiation, is thought to be a key mediator for the pathogenesis of radiation damage. Thus, PG101 showed great potential as a supplement or a major therapeutics in immunocompromised or immunosuppressed individuals whose bone marrow system is damaged [63].

SCG, a β-(1→3)-D-glucan with β-(1→6) branches isolated from fruit bodies of *Sparassis crispa*, enhanced the hematopoietic response in cyclophosphamide- (CY-) induced leukopenic mice by intraperitoneal routes over a wide range of concentrations. Monocytes and granulocytes in the peritoneal cavity, liver, spleen, and bone marrow recovered faster than in the control group. The ratio of NK cells and $\gamma\delta$T cells in the liver, spleen, and peritoneal cavity was also increased. These results suggest the usefulness of *S crispa* in cancer immunotherapy [64].

EFFECTS OF MUSHROOM METABOLITES ON THE INNATE IMMUNE SYSTEM

Macrophages

The recognition of microbes by macrophages and neurophilic granulocytes leads to phagocytosis of the microbes and activation of the phagocytes to kill the ingested microbes. Recognition is mediated by toll-like receptors (TLR) that are specific for different components of microbes. TLR-2 binds lipogycans, TLR-4 binds bacterial

lipopolysaccharide (LPS), TLR-5 binds flagellin, and TLR-9 binds unmethylated CpG nucleotides present in bacteria. As a consequence of recognition and phagocytosis several enzymes are activated, including oxidases and inducible nitric oxide synthase (iNOS), resulting in the production of bacteriocidal reactive oxygen intermediates (ROI) and nitric oxide (NO).

The effects of mushroom extracts and metabolites on macrophages have been extensively studied in vitro and in vivo. Some mushroom metabolites activate macrophages to produce various mediators, even in normal mice. Activities are summarized in Table 2.

Water extracts of the mycelial culture and fruiting bodies of *Agaricus blazei* Murill induced TNF-α secretion by macrophages derived from rat bone marrow. Fractions B-4 and B-5 obtained from ethanol precipitation of fruiting bodies markedly induced TNF-α secretion. Similar effects were observed in IL-8 secretion by macrophages. Regarding NO, fraction B-5 induced a significant increase in NO secretion and fractions B-4 and B-6 slightly induced NO secretion. Northern blot analysis showed that the increases in cytokine and NO secretion were due to an increase in cytokine mRNAs or NO synthase mRNA [65]. Thus *A blazei* Murill contains certain components which activate macrophages contributing to the immune response in vitro.

Wang et al [66] reported that after treatment of macrophage cultures with a polysaccharide from fresh fruiting bodies of *G lucidum*, the levels of IL-1β, TNF-α, and IL-6 were 5.1-, 9.8-, and 29-fold higher than in cultures of untreated cells. In addition, the release of INF-γ from T lymphocytes was also greatly enhanced in the presence of this polysaccharide. This proinflammatory cytokine response is suggested to facilitate the antitumor activity of this extract.

Grifolan (GRN), an antitumor β-glucan isolated from *G frondosa* induced the release of IL-1, IL-6 and TNF-α from macrophages [67, 68]. Ishibashi et al [69] reported that an insoluble as well as a high-molecular-mass soluble form of GRN are required for TNF-α production by macrophages.

The effect of Maitake D-fraction was studied by Sanzen et al [70] on the iNOS-mediated NO production in RAW264.7 macrophages with special reference to antitumor activity of MD-fraction against human hepatoma-derived huH-1 cells and the data suggested that MD-fraction is a novel inducer for iNOS which contributes at least in part to antitumor activity of MD-fraction.

Kodama et al [30] examined the effects of Maitake D-fraction on the treatment of *Listeria*-infected mice in combination with vancomycine (VCM). In mice administered with both D-fraction and VCM, macrophages produced 2.7 times as much IL-1β as that of nontreated control mice. The bactericidal activity of splenic T cells was also enhanced by 2.6 times of that of nontreated control mice. These results suggest a clinical benefit of D-fraction in the case of antibacterial treatment for patients with high risks.

Monocytes/macrophages seem to be the major target cell type responsive to PG101. Jin et al [62] proposed that PG101 interacts with macrophages or related cells and results in the activation of the transcription factor nuclear factor kappa B (NF-κB), which sets off a series of reactions producing a variety of proinflammatory and antiinflammatory cytokines (TNF-α, IL-1β, IL-10, IL-12, GM-CSF, IL-18) in a sequential manner. Inflammatory-cytokine-induced phosphorylation of a degradative motif in IκB triggers IκB proteolysis, liberating NF-κB from the inactive heterodimer and NF-κB transcription which in turn prevents cytokine-induced death of inflammatory cells. Despite its significant biological effect on various cytokines, PG101 remained nontoxic in both rats and human peripheral blood mononuclear cells (hPBMCs) even at a biological concentration approximately 20 times greater. PG101 demonstrates great potential as a therapeutic immune modulator.

A galactomannan isolated from a polar extract of *Morchella esculenta* carpophores enhanced macrophage activation. At 3.0 μg/mL the galactomannan polysaccharide (about 2.4% protein) increased NF-κB-directed luciferase expression in THP-1 human monocytic cells to levels of 50% of those achieved by maximal activating concentration (10.0 μg/mL) of LPS [71].

By administering PL, an acidic polysaccharide isolated from *Phellinus linteus*, the production of NO and tumoricidal activity were increased in murine peritoneal macrophages in vivo and in vitro. PL has been claimed to cause the inhibition of tumor growth and metastasis of murine B16F10 melanoma cells [72]. Such properties of PL may be related to its ability to induce the production of the tumoricidal effector molecule NO through protein tyrosine kinase (PTK) and protein kinase C (PKC) [73]. Considering the main role that proinflammatory cytokine production plays in the pathogenesis of septic shock, Kim et al [32] examined how the in vivo administration of PL can modulate circulating cytokine responses in LPS-treated mice. Administration of PL in vivo decreased IL-2, IFN-γ, and TNF-α production in splenocytes and enhanced spontaneous cell apoptosis in macrophages and lymphocytes stimulated with LPS in vitro. Thus, part of the antiinflammatory effects of PL treatment in vivo may result from the enhanced apoptosis of a portion of the activated macrophages and lymphocytes. The ability of PL to significantly reduce TNF-α production indicates the potential of the polysaccharides in possible therapeutic strategies that are based on down regulation of TNF-α [32].

The methanol extract of fruit bodies of *Cordyceps pruinosa* inhibited IL-1β, TNF-α, NO, and prostaglandin E_2 (PGE_2) in vitro and in vivo. The extract inhibited these inflammatory mediators in LPS-stimulated murine macrophage cell line RAW264.7 and primary macrophages, by suppressing gene expression of IL-1β, TNF-α, iNOS, and cyclooxygenase-2 (COX-2) through the inhibition of NF-κB activation. Administration of the extract significantly decreased the plasma level of these

Table 2. Immunomodulatory activities of mushroom products on macrophages.

Species	Product	Immune effects	Reference
G frondosa	D-fraction	↑ IL-1β	[30]
L lepideus	PG101	↑ TNF-α , IL-1β, IL-10, IL-12, GM-CSF, IL-18	[62]
A blazei	Water extracts mycelia and fruit bodies	↑ TNF-α	[65]
	Fractions B-4 and B-5	↑ TNF-α , IL-8, NO	
G lucidum	Polysaccharide	↑ IL-1β , TNF-α, IL-6	[66]
G frondosa	GRN	↑ IL-1, IL-6, TNF-α	[67, 68, 69]
G frondosa	MD-fraction	↑ iNOS	[70]
M esculenta	Galactomannan	↑ macrophage activity	[71]
P linteus	PL	↑ NO ↓ IL-2, IFN-γ, and TNF-α production in splenocytes ↓ apoptosis of a portion of the activated macrophages and lymphocytes in LPS-treated mice	[72, 73]
C pruinosa	Methanol extract	Inhibit IL-1β, TNF-α , NO, PGE_2	[74]
S aspratus	Fucogalactan	↑ TNF-α, NO	[75]
A cylindracea	Ubiquitin-like peptide	↑ NO	[77]
T mongolicum	Lectins (TML-1, TML-2)	↑ TNF-α, Nitrite ions	[78]

inflammatory mediators in LPS-injected mice. These results suggest that the *C pruinosa* methanol extract suppresses inflammation through suppression of NF-κB-dependent inflammatory gene expression, suggesting that the *C pruinosa* extract may be beneficial for treatment of endotoxin shock or sepsis [74]. Also, the methanol extract of fruit bodies of *Pleurotus florida* showed anti-inflammatory and antiplatelet-aggregating activities but the exact mechanism for these activities is unknown [21].

A fucogalactan, isolated from *Sarcodon aspratus*, elicited the release of TNF-α and NO in macrophages of mice in vitro. TNF-α production induced with 50 μg/mL of fucogalactan was significantly higher than that induced by lentinan (500 μg/mL) by approximately 4.3-fold. Mizuno et al [75] suggested that the immunomodulating activity of this fucogalactan on TNF-α and NO productions might contribute to antituor activity in tumor-bearing hosts as well as various immunomodulating effects.

In mice treated with an immunosuppressive carcinogen, administration of a mushroom-enriched diet containing *L edodes*, *G frondosa*, and *Pleurotus ostreatus* restored the normal level of the chemotactic activity of macrophages and the capability of lymphocytes to proliferate in response to mitogen [76].

Proteins and peptides from mushrooms are also known to activate macrophages. A ubiquitin-like peptide isolated from fruiting bodies of the mushroom *Agrocybe cylindracea* enhanced NO production in murine peritoneal macrophages with a potency comparable to that of LPS [77]. Two lectins isolated from the mushroom *Tricholoma mongolicum* (TML-1 and TML-2) stimulated the production of nitrite ions and TNF-α by macrophages in normal and tumor-bearing mice [78].

Natural killer cells

Natural killer cells are a class of lymphocytes that rapidly respond to intracellular infections with viruses or bacteria, by killling the infected cells and by producing the macrophage-activating cytokine, IFN-γ.

Some mushroom metabolites exhibit stimulating effects on NK cells (Table 3). Innate immunity is in the critical arms of immune surveillance against tumor development. Moreover, in the innate immune system, NK cells, which do not express T-cell receptors that recognize specific peptides presented on the major histocompatibility complex (MHC), rather than T cells, seem well suited for this role. NK cells can recognize the surface changes that occur on a variety of tumor cells and virally infected cells [79]. NK cells have two relevant functions, related to the natural immune response against pathogens [80]. One is cytotoxicity, mediated by the recognition and lysis of target cells such as virus- and bacteria-infected cells. The second NK cells function is to produce cytokines such as IFN-γ, TNF-α, and GM-CSF, that can modulate natural and specific immune responses. Additionally, infected or activated DCs and macrophages produce cytokines and chemokines such as IFN-α/β, IL-12, IL-15, and IL-18 that stimulate NK cells to rapidly produce other cytokines (including IFN-γ, TNF-α, and GM-CSF) and chemokines (such as ATAC/lymphotactin, mig, and MIP-1α) [81].

Kodama et al [41, 82] monitored levels of NK cell cytotoxic activity in cancer patients receiving D-fraction. Elevated levels of cytotoxic activity were maintained for one year. To elucidate the mechanisms underlying long-term activation of NK cells during treatment with D-fraction, the authors examined tumor volume and levels of IFN-γ and TNF-α in MM46-bearing C3H/HeN mice to which D-fraction was administered for 19 days. D-fraction

Table 3. Immunomodulatory activities of mushroom products on NK cells.

Species	Compound	Immune effects	Reference
G frondosa	D-fraction	↑ TNF-α, IFN-γ released from spleen cells; TNF-α expressed in NK cells in tumor-bearing mice ↑ NK cells activity (INF-γ) indirectly through IL-12 produced by macrophages and DCs in normal mice	[41, 82, 83, 84]
A blazei	Hot-water extract	↑ NK activity of spleen cells in naïve BALB/c mice	[85]
A blazei	*n*-hexane Dichloromethane Methanol	Maintain NK activity of spleen cells in tumor-bearing mice	[87]
A blazei	ABMK	↑ NK activity on cancer patients	[88]

markedly suppressed tumor growth, corresponding with increases in TNF-α and IFN-γ released from spleen cells and a significant increase in TNF-α expressed in NK cells. Furthermore, D-fraction increased macrophage-derived IL-12, which serves to activate NK cells. Thus, NK cells are not only responsible for the early effects of D-fraction on tumor growth, but also for the long-term tumor-suppressive effects of D-fraction through increased IL-12 released from macrophages. D-fraction was capable of enhancing and maintaining peripheral blood NK cell activity in patients with lung and breast cancer [41]. In addition, Maitake D-fraction, stimulated the natural immunity related to the activation of NK cells indirectly through IL-12 produced by macrophages and DCs in normal mice [83]. IFN-γ production by splenic NK cells increased significantly 3 days after D-fraction administration. In a recent study, Kodama et al [84] reported the activation of macrophages and DCs in normal mice as well. Therefore, administration of D-fraction to healthy individuals may serve to prevent infection by microorganisms.

Treatment with hot-water extracts of *A blazei* fruiting bodies increased NK activity of spleen cells in naïve BALB/c mice [85]. In meth A-bearing BALB/c mice, the same extracts enhanced the induction of antigen-specific cytotoxic T lymphocytes (T_C) and IFN-γ production. Up regulation of NK and T_C activity is triggered by IL-12-dependent activation [86]. It is not yet clear whether oral administration of *Agaricus* extracts enhances IL-12 production in vivo [85].

Ehrlich-carcinoma-bearing mice treated with the *n*-hexane, dichloromethane, or methanol extracts from *A blazei* fruiting bodies were able to maintain the NK activity of spleen cells during the first 10 days after tumor implantation. The NK activity of these groups was similar to that of normal controls and higher than that of tumor-bearing mice treated with water. The results of NK activity on the 30th day after the injection of tumor cells suggest that none of the three extracts was able to maintain the lytic activity against Yac-1 target cells. It is possible that after 30 days the production of soluble factors like prostaglandins, TGF-β, or IL-10 by Ehrlich carcinoma cells was enough to prevent the increase of NK activity by the *n*-hexane extract [87].

Ahn et al [88] investigated the beneficial effects of the consumption of an extract of *A blazei* Murill Kyowa (ABMK) on immunological status and qualities of life in cancer patients undergoing chemotherapy. They observed that NK cell activity was significantly higher in the ABMK-treated group and suggested that ABMK treatment might be beneficial for gyneacological cancer patients undergoing chemotherapy.

The medicinal fungus water extract (FWE) consists of equal amounts of *Coriolus versicolor*, *Cordyceps sinensis*, *L edodes*, *A blazei*, and *G lucidum*. Zhang et al [89] reported that FWE enhanced the phagocytosis of peritoneal macrophages, promoted NK activity in mice, and suppressed the growth of B-16 melanoma. FWE had significantly promoted mouse NK activity at the dose of 400 mg/kg, which suggests that FWE may possess the ability to activate NK to directly kill tumor cells, induce NK to secrete cytotoxic agents to elicit the apoptosis of tumor cells, or remove tumor cells by other pathways.

Dendritic cells

DCs are antigen-presenting cells (APC) with a unique ability to induce primary immune response of both helper (T_H) and T_C [90]. Beside activating naive T cells, DCs can directly activate naive and memory B cells. DCs at different stages of differentiation can regulate effectors of innate immunity such as NK cells and NK T cells. The induction of tumor immunity can be initiated by the effectors of innate immunity and further developed by cells of adaptive immunity, with DCs playing a central regulatory role.

Cao and Lin [91] studied the regulatory effects of Gl-PS, *G lucidum* polysaccharides (GLPS), on maturation and function of cultured murine bone-marrow-derived DCs in vitro. Gl-PS could promote not only the maturation of cultured murine bone-marrow-derived DCs, but also the immune response initiation induced by DCs.

PL induced maturation of bone-marrow-derived DCs and readies them for T-cell-mediated immune responses. PL significantly increased membrane molecules, including MHC class I, II, CD80, and CD86, and IL-12p70 in DCs. Also, PL markedly reduced the endocytic activity of DCs and augmented their capacity to promote the proliferation of naïve allogeneic T cells [92]. PL enhanced

the phenotypic and functional maturation of DCs *via* TLR-2- and/or TLR-4-mediated NF-κB, ERK, and p38 MAPK signal pathways. It is the first article reporting that a polysaccharide from mushrooms can activate a TLR signaling [93]. Kim et al [94] reported that the administration of PL induced antitumor and immunomodulating activities via maturation of $CD11c^+CD8^+$ DCs in tumor-bearing mice. The inhibitory effect of PL on the growth of MCA-102 tumor cells was associated with its immunoregulatory properties, including the induction of IL-12 and IFN-γ production leading to a T_H1 dominant state. Therefore, PL would be useful in preventing tumor growth, and it also has the advantage of having no side effects.

The existence of a strongly immunosuppressive state in cancer-bearing individuals inhibits DCs maturation. Kanazawa et al [95] reported that a protein-bound polysaccharide K (PSK) isolated from the cultured mycelium of *C versicolor* promoted both the phenotypic and functional maturation of DCs derived from human $CD14^+$ mononuclear cells. PSK has also been reported to resolve the immunosuppressive state of a cancer-bearing host and might be associated with DCs maturation directly [95]. Activities of mushroom metabolites on DCs are summarized in Table 4.

Complement

Activation of complement by either the classical or alternative pathway results in the generation of a wide spectrum of biological activities with the potential to modify immune responses [96, 97]. Particularly, the activation of complement via the alternative pathway is important in natural immunity to bacterial infections [98, 99].

Although there are a few reports concerning the relationship between complement-activating and tumor-regressing activity of glucan including lentinan, the positive correlation between the two activities was found by Okuda et al [100]. They observed a correlation between the ability to activate complement via the alternative pathway in vitro and inhibition of tumor growth in vivo. However, the opposite result, no correlation, was found by Hamuro et al [101]. Thus there is no consistent view on the correlation between the two antagonozing activities.

ABP-F and ABP-M, fine particles of *A blazei* Murill fruiting body and mycelium, respectively, prepared by mechanical disruption, activated the human complement system via the alternative pathway in human serum (Table 5). When particles from fruiting bodies of *A blazei* Murill (ABP-F) were 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. Further, 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 [102].

An alkali extract from cultured mycelium of *G lucidum* activated both classical and alternative pathways of complement [103]. Min et al [104] reported that triterpenoids such as ganoderiol F, ganodermanondiol, and ganodermanontriol from *G lucidum* had a potent anticomplement activity against the classical pathway with IC_{50} values of 4.8–4.17 μM. A clinical study in elderly patients with insomnia and palpitation has shown that taking *G lucidum* essence for 4–6 weeks increased their serum C3 levels [105].

Also, LELFD, a β-(1→3)-glucan, obtained from liquid-cultured mycelium of *G frondosa*, could activate the alternative complement pathway [106].

Anticomplementary activity of 61 strains of higher fungi from Korea was screened for immunostimulation [107]. Extracts from 11 of 61 strains, including 5 of *G lucidum*, 3 of *L edodes*, 2 of *Cordyceps sp*, and 1 of *Agaricus campestris*, showed higher anticomplementary activity than Krestin from *C versicolor*. The most potent anticomplementary activity was found with an extract from *L edodes* IY105, that reduced complement capacity by 31.7%.

EFFECTS OF MUSHROOM METABOLITES ON ADAPTIVE IMMUNE SYSTEM

T lymphocytes

T lymphocytes include T-helper (T_H) cells and cytotoxic T (T_C) cells. T_H cells interact with B cells and help them to divide, differentiate, and make antibody or interact with mononuclear phagocytes and help them destroy intracellular pathogens. T_H cells generate their effects by releasing soluble cytokines and/or by direct cell-cell interactions. The T_C cells destroy target host cells that have been infected by pathogens.

T_H cells

$CD4^+$ cells secrete a number of cytokines that are important in the activation of B and other T cells, as well as cells of the innate immune system. Based on the types of cytokines these $CD4^+$ cells produce, they are classified into a number of T_H types (0, 1, 2, or 3). T_H1 cells produce IL-2, IFN-γ, and TNF-β (LT), and introduce cellular immunity to mainly intracellular infections organisms. T_H2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13, and activate humoral immunity, mainly directed against extracellular infections. Precursor or T_H0 cells produce IL-4 and IFN-γ concomitantly. Less is known about the physiological role of T_H0 type cells. Thymus-derived regulatory T-cell populations, including naturally occurring $CD4^+CD25^+$ T cells and inducible IL-10 or TGF-β-producing T_R/T_H3 cells, develop in the periphery from T_H cells depending on the tolerance-inducing microenvironment in which these T cells reside. By blocking activation of other lymphocytes and APC either directly (by CTLA4-CD28 interaction) or indirectly (by cytokines like IL-10 and TGF-β), these cells ensure self-tolerance mechanisms. In diseased states, however, the presence and/or

Table 4. Immunomodulatory activities of mushroom compounds on DCs.

Species	Compound	Immune effects	Reference
G lucidum	Gl-PS	↑ proliferation of one-way MLC induced by DC ↑ phenotypic and functional maturation of DC ↑ membrane molecules, including MHC I, II, CD80, and CD86, and IL-12p70 in DC ↓ endocytic activity of DC ↑ capacity of DC to promote the proliferation of naïve allogenic T cells and readies them for T-cell-mediated immune responses	[91]
P linteus	PL		[92]
C versicolor	PSK	Promoted both the phenotypic and functional maturation of DC derived from human $CD14^+$ mononuclear cells	[95]

Table 5. Immunomodulatory activities of mushroom compounds on complement.

Species	Compound	Immune effects	Reference
A blazei	Fine particles of fruiting body: ABP-F, and mycelium: ABP-M	Activation of the human complement system via the alternative pathway in human serum	[102]
G lucidum	Alkali extract	Activation of both classical and alternative pathways of complement	[103]
G lucidum	Triterpenoids	Anticomplement activity	[104]
G frondosa	LELFD	Activation of the alternative complement pathway	[106]

activity of these cells is often reduced leading to enhanced immunopathology, characteristic of chronic inflammatory diseases, like auto-immune and allergic diseases.

The downstream immune response is chosen depending on which subtype of T cell is activated, which means that the proportion of the activated sub-types influences phylaxis immunity and antitumor immunity. This control system is also affected by the production of IL-1β, IL-12, and IL-18 by APC [108, 109]. The development of T_H1 or T_H2 types from naïve cells to effector cells is regulated by the presence of specific cytokines in the microenvironment at the time of T cell priming. For the T_H1 type, IL-12 is a necessary cytokine of differentiation [110], whereas for the T_H2 type, IL-4 and IL-10 are critical [111]. Recent study shows that many immune disorders are attributable to the collapse of the system controlling the proportion of T_H1 to T_H2 cells [112]. Many diseases such as leprosy, allergy, multiple sclerosis, and responses to immunotoxic agents have pathology associated with aberrant T_H1 and T_H2 polarization. T_H1 cells may cause immunopathology and organ-specific autoimmune disease if dysregulated [113, 114, 115, 116]. Because cytokines produced by T_H2 cells, such as IL-4 and IL-5, can activate mast cells and eosinophils and in addition can result in elevated levels of IgE, they have been strongly implicated in atopy and allergic inflammation [117]. Restoration of the proper balance between T_H1 and T_H2 cells is generally considered essential in the treatment of tumors, which are generated when cellular immunity is affected by immunosuppressing factors.

Some mushroom polysaccharides might induce a type 1 immune response, whereas others favor a type 2 polarization [49, 118]. Borchers et al [49] reported that the limited data available to date do not allow one to determine whether mushroom polysaccharides do so independently of the animal strain or species and disease state investigated or whether the nature of their immunomodulatory effects depends on the model to a greater extent than has been appreciated to date.

Lentinan has been described as a T-cell-oriented adjuvant [119]. The skewing of T_H1/T_H1 balance to T_H1 by lentinan (Table 6) is directed through the distinctive production of IL-12 versus IL-6, IL-10, and PGE_2 by peritoneal macrophages, depending on intracellular glutathione redox status [120]. Based on the intracellular content of glutathione, two classes of macrophages have been proposed with diverse functional consequences: reductive macrophages with high, and oxidative macrophages with low glutathione levels.

Sclerotinia sclerotiorum glucan (SSG) from *Sclerotinia sclerotiorum* IFO 9395 induced the development of T_H1 cells via the IL-12 pathway [118].

Inoue et al [121] investigated the antitumor functions of D-fraction in relation to its control of the balance between T lymphocyte subsets T_H1 and T_H2. D-fraction decreased the activation of B cells and potentiated the activation of T_H cells, resulting in enhanced cellular immunity. It also induced the production of IFN-γ, IL-12p70, and IL-18 by whole spleen cells and lymph node cells, but suppressed that of IL-4. These results suggest that D-fraction establishes T_H1 dominance which induces cellular immunity in the population that was T_H2 dominated due to the presence of this particular carcinoma [121]. In a later study, Harada et al [122] reported that D-fraction induces the differentiation into T_H1 cells of $CD4^+$ T cells in tumor-bearing BALB/c mice

Table 6. Immunomodulatory activities of mushroom compounds on T cells.

Species	Compound	Immune effects	Reference
F velutipes	Fve	T_H1 response	[18]
S sclerotiorum IFO 9395	SSG	T_H1 response	[118]
L edodes	Lentinan	T_H1 response	[120]
G frondosa	D-fraction	Enhances T_H1 dominant response through enhancement of IL-12p70 and IFN-γ produced by activated DCs	[121, 122]
V volvacea	Vvo	↑ T_H1-specific cytokines (IL-2, IFN-γ , LT), T_H2-specific cytokine (IL-4), TNF-α, and IL-2R	[123, 124]

in which the T_H2 response was dominant through enhancement of IL-12p70 production by DCs, when the ratio of $CD8\alpha^+$ DCs to $CD8\alpha^-$ DCs increased. In addition, examination of the tumor rejection effect of D-fraction-stimulated DCs loaded with tumor antigen revealed that tumor growth is inhibited completely by activating $CD4^+$ T cells and $CD8^+$ T cells. Furthermore, the level of TNF-α, which is produced by activated macrophages and NK cells and is cytotoxic for tumor cells, increased by D-fraction-DCs injection, indicating that D-fraction enhanced the protective immunity by DCs loaded with tumor antigen through activating macrophages and NK cells. Although the action of D-fraction on DCs and its intracellular signal transduction pathway remain unclear, D-fraction may be a useful stimulator of DCs, which induce the differentiation of $CD4^+$ T cells to T_H1 cells [122].

Vvo, a fungal immunomodulatory protein (FIP) purified from the edible mushroom, *Volvariella volvacea*, induced most T_H1-specific cytokines (IL-2, IFN-γ, and LT) and one T_H2-specific cytokine (IL-4) within 4 hours in mouse spleen cells. This result indicates that Vvo principally acts on T_H1 cells and to a lesser extent on T_H2 cells in the early event of activation. It is known that IL-4 acts on B cells to induce activation and differentiation, leading in particular to the production of IgE. The lower effect of Vvo compared with other FIPs on the prevention of systemic anaphylaxis may be attributed to the elevated expression of IL-4 [123, 124].

Fve, a FIP isolated from the fruiting body of *Flammulina velutipes*, selectively stimulates a T_H1 response in hPBMCs [18]. Recently Hsieh et al [18] have characterized the immunomodulatory effects of Fve in more detail and investigated the prophylactic use of Fve via the oral route in a murine model of food allergy. They have demonstrated that oral administration of Fve during allergen sensitization could induce a T_H1-predominant allergen-specific immune response in mice and protect the mice from systemic anaphylaxis-like symptoms after subsequent oral challenge with the same allergen. It is worth noting that Fve could be administered orally and retain its activity, while most protein drugs cannot. This characteristic greatly promotes the potential of immunoprophylactic use of Fve [18]. Liu et al [16, 17] have demonstrated the efficacy of local nasal immunotherapy (LNIT) for group 2 allergen of house dust mite *Dermatophagoides-pteronyssinus-* (Dp2-) induced airway inflammation in mice, using Dp2 peptide and Fve or LZ-8, a FIP isolated from *G lucidum*.

B cells

Three polysaccharides isolated from *G lucidum*, two heteroglycans (PL-1 and PL-4) and one glucan (PL-3) enhanced the proliferation of T and B lymphocytes in vitro to varying contents and PL-1 exhibited an immune stimulating activity in mice [125].

PGL, a complex β-D-glucan, has a strong effect on suppressing the antibody production [126].

GLIS, a proteoglycan isolated from the fruiting body of *G lucidum*, is a B-cell stimulating factor. This compound stimulated B lymphocyte activation, proliferation, differentiation and production of immunoglobulins. The activation of B cells by GLIS may be associated with the expression of PKC α and PKC γ in B cells [127]. GLIS stimulated the proliferation of mouse spleen lymphocytes, resulting in a threefold to fourfold increase in the percentage of B cells. GLIS also activated mouse spleen lymphocytes, and most of the activated cells were B cells [127].

PL selectively activates murine B cells but not T cells [128]. Since PL cannot penetrate cells due to its large molecular mass (approximately 15 kD), this selectivity may be caused by the surface binding of this molecule to receptors specifically expressed on B-cells but not on T cells. The B-cell receptor, BCR, consists of surface immunoglobulin and CD79a-CD79b. Upon BCR ligation, the BCR-associated kinase Lyn phosphorylates CD79a-CD79b. In addition, coreceptors such as CD19 and CD38 positively regulate BCR signaling. Complement receptor CD11b-CR3, or Mac-1, is expressed on the surface of macrophages and NK cells and has been identified as the receptor of β-glucans [129]. Although PL and β-glucans show different specificities on B and T cells, they may use the same receptor on B cells. A further complete investigation of the membrane receptors of PL should shed light on its selectivity for B cells.

Table 7. Immunomodulatory activities of mushroom compounds on B cells.

Species	Compound	Immune effects	Reference
G lucidum	PL-1, PL-3, PL-4	↑ T and B lymphocytes proliferation ↑ antibodies	[125]
G lucidum	PGL	↓ antibody production	[126]
G lucidum	GLIS proteoglycan	↑ proliferation of mouse spleen lymphocytes ↑ B lymphocyte activation, proliferation, and differentiation and production of immunoglobulins	[127]
P linteus	PL	↑ murine splenic lymphocytes and activation of B cells	[128]
G lucidum	LZ-8	↓ antibody production	[130]
F velutipes	Fve	↓ antibody production	[131]

Evidence that FIPs suppress antibody production came from the result that the proportion of Arthus reaction-positive mice was reduced to 40% by LZ-8 [130]. Fve also suppressed antibody production as demonstrated by its effect in the hind paw edema test but the inhibition was not complete [131]. Activities are summarized in Table 7.

Figure 3 summarizes the targets for interaction between mushroom ingredients and various components of the adaptive immune system.

RECOGNITION AND RECEPTORS

Evidence for β-glucan receptor binding of immune cells

The innate immune system is the first line of defense against microbial invasion, and must immediately recognize and counter infections while the slower, more specific, adaptive response is mounted. The innate cellular response is comprised principally of phagocytic cells and is dependent on germline encoded receptors which recognize conserved microbial structures. The innate immune system identifies infectious agents or compounds by means of pattern-recognition receptors (PRR). These receptors recognize pathogen-specific macromolecules called pathogen-associated molecular patterns (PAMP).

Polysacharides cannot penetrate cells due to their large molecular mass, so the first step in the modulation of cellular activity is binding to immune cell receptors. Among all the immunomodulatory metabolites isolated from mushrooms, glucans and in particular β-glucans have been studied profoundly to identify its target receptor in immune cells. It has been postulated that glucans are fungal pattern-recognition molecules for the innate immune system [132, 133]. The mechanisms by which the innate immune system recognizes and responds to fungal cell wall carbohydrate is a very complex and multifactorial process [134]. The various activities of β-glucans may reflect the presence of multiple cellular targets or receptors [135]. To date several β-glucan receptors have been identified as candidates mediating these activities [136], namely, complement receptor 3 (CR3, $\alpha_M\beta_2$ integrin, or CD11b/CD18) [137], lactosylceramide [138], scavengers receptors [139], dectin-1 [140], and toll-like receptors TLR-2 and TLR-4 [141].

Dectin-1 is broadly expressed, with highest surface expression on populations of myeloid cells (monocyte/macrophage and neutrophil lineages) in the blood, bone marrow and spleen. DCs, and a sub-population of T cells , also expressed dectin-1 but at lower levels [142]. It is plausible that the expression of dectin-1, as a T-cell binding receptor, on a subset of T-cells may be part of a novel mechanism for the regulation of the T cell response by specific subsets of T cells as well as by APC [143].

Recently, Kim et al [93] have shown that PL, proteoglycan isolated from *P linteus*, could induce the phenotypic and functional maturation of DCs via TLR-2 and/or TLR-4. Shao et al [141] suggested that TLR-4 is also involved in GLPS-mediated macrophage activation. Rat antimouse TLR-4 monoclonal antibody (AB) inhibited the proliferation of BALB/c mouse B cells under GLPS stimulation. Combination of Abs against mouse TLR-4 and immunoglobulin achieved almost complete inhibition of GLPS-induced B-cell proliferation, implying that both membrane Ig abd TLR-4 are required for GLPS-mediated B cell activation.

Lowe et al [134] reported that a β-D-(1→3)- linked glucan polymer composed of seven glucose subunits is the minimum binding ligand for glucan PRR on a human monocyte cell line and indicated that all available monocyte glucan receptors will recognize the basic β-D-(1→3)-glucan structure with approximately the same affinity. However, as the glucan polymer becomes more complex it appears to be preferentially recognized by one glucan receptor versus another.

Additional studies are required to determine which receptor(s) are essential to the expression of the various immunobiological effects ascribed to β-glucans. The intracellular events that occur after glucan-receptor binding have not been fully determined. As long as it remains unclear what receptors are involved in and what downstream events are triggered by the binding of these glucans to their target cells, it will be difficult to make further progress in understanding their biological activities.

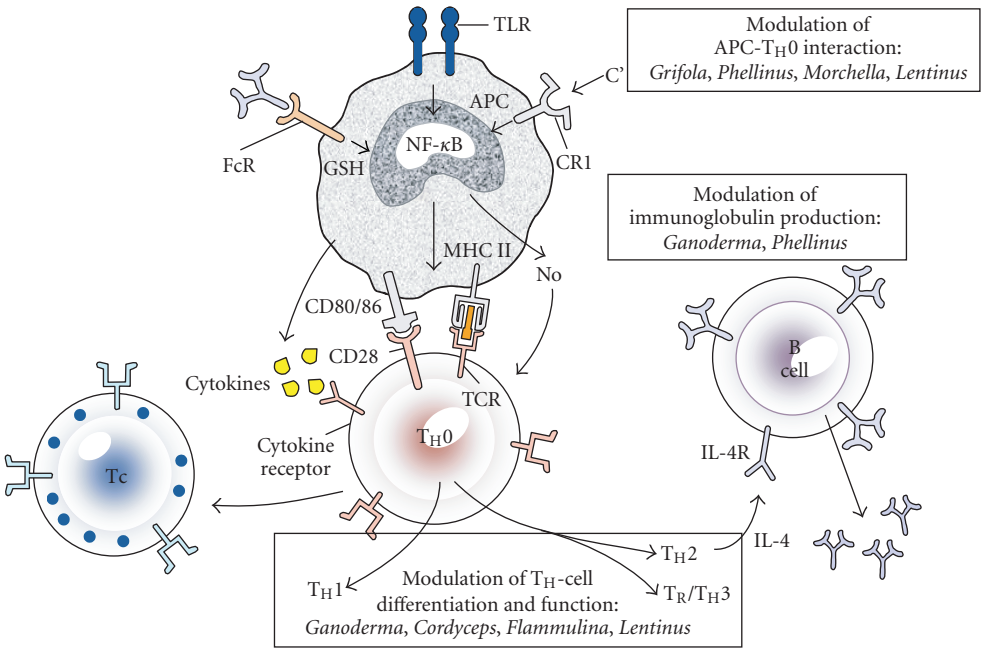


FIGURE 3. Schematic representation of the possible targets of the adaptive immune system for mushroom ingredients with immunomodulatory properties. APC: antigen-presenting cell; FcR: Fc receptor; TLR: Toll-like receptor; CR1: complement receptor type 1; C': activated complement; GSH: glutathione; MHC II: major histocompatibility complex class II; TCR: T-cell receptor; T_H: helper T cells; T_C: cytotoxic T lymphocytes; T_R: regulatory T cells; NO: nitric oxide; IL-4: interleukin-4; IL-4R: interleukin-4 receptor; CD: cluster designation.

CONCLUSIONS

The information presented here illustrates the distinct immunomodulatory properties associated with mushroom constituents. The discovery and identification of new safe drugs, without severe side effects, has become an important goal of research in the biomedical science. Medicinal effects have been demonstrated for many traditionally used mushrooms, with large differences in immunomodulatory properties. The species studied so far represent a vast source of immunomodulating and antitumor extracts and metabolites. Thus, the biochemical mechanisms that mediate the biological activity are still not clearly understood. Mushroom metabolites are known to stimulate different cells of the immune system. The major immunopotentiation effects of these active substances include mitogenicity, stimulation of hematopoietic stem cells, activation of alternative complement pathway, and activation of immune cells, such as T_H cells, Tc cells, B cells, macrophages, DCs, and NK cells.

Different profiles have been observed in relation to the activated immune cells, for example, GLPS activate mouse B cells and macrophages but not T cells [141], polysaccharides from *P linteus* can stimulate B cells, T cells, and macrophages [144], while lentinan is a stimulator of T cells and macrophages, but not B cells [145]. Some of them might promote a T_H1 response and others a T_H2 response [49]. In the particular case of glucans, despite the structural and functional similarities of some of them, they differ in their ability to elicit various cellular responses, particularly cytokine expression and production and in their effectiveness against specific tumors [5]. The relationship between polysaccharide origin, structure, and their immunomodulation activity remains to be further characterized [125, 146].

Mushroom products are obvious immunoenhancers that potentiate the immune system in multiple ways. Mushroom polysaccharides are among the emerging new agents that could directly support or enhance functional autologous hematopoietic stem cell recovery [61]. In preventive medicine, defense against invasion by foreign bodies is dependent on enhancing the natural immune system, including activation of macrophages and NK cells. Macrophages stimulated by mushroom products release several inflammatory cytokines, IL-1, IL-6, IL-8, TNF-α, and NO, all of which directly induce tumoricidal activity in macrophages. Macrophages produce also IL-1β, IL-10, IL-12, GM-CSF, and IL-18. In other cases mushroom extracts inhibit the production of NO, PGE_2, IL-1β, and TNF-α in LPS-stimulated macrophages and LPS-administer mice. This antiinflammatory effect occurs by down regulation of iNOS, COX-2, IL-1β, and TNF-α gene expression via the suppression of NF-κB activation. Thus, these mushroom extracts might be relevant for clinical use for inflammatory diseases, including endotoxemia or sepsis. Some mushroom metabolites like D-fraction

represent an important biological response modifier (BRM) due to the enhancement of NK cells activity in cancer patients. Mushroom polysaccharides induce regulatory effects on maturation and function of DCs and consequently enhance the capacity of DCs to promote the proliferation of naïve allogenic T cells and readies them for T-cell-mediated immune responses. Both classical and alternative pathways of complement have been activated by mushrooms and also anticomplementary activity has been detected in different mushrooms. T and B lymphocytes are also activated by mushrooms. Some mushroom polysaccharides stimulate the production of antibodies but others as PGL have a strong effect on suppressing the antibody production [126].

The immunomodulating action of mushroom metabolites is specially valuable as a means of prophylaxis, a mild and noninvasive form of treatment, prevention of metastatic tumors, and as a cotreatment with chemotherapy [4]. The enhancement or potentiation of host defense mechanisms has been recognized as a possible means of inhibiting tumor growth without harming the host, but other alternative mechanisms are possible, like targeting the *ras*-mediated signaling pathway [147]. Whether certain metabolites enhance or suppress immune responses can depend on a number of factors, including dose, route of administration, and timing of administrations of the compound in question. The type of activity these metabolites exhibit can also depend on their mechanism of action or the site of activity. Taken together, the present data suggest that mushroom extracts or metabolites should be selected and used properly for modulation of immune responses. Due to the differences in activities among various extracts and isolated metabolites, it is imperative to evaluate its biological properties before any suggestions for use of a particular product in clinical practice. For example, D-fraction enhanced rather than suppressed the development of collagen-induced arthritis (CIA) [148]. Administration of D-fraction stimulates immune function of normal and tumor-bearing mice [84]. GLIS from *G lucidum* has an effect on lymphocytes or purified B cells from tumor-bearing mice markedly stronger than on lymphocytes or purified B cells from normal mice [127]. It has also been reported that an extract from the deep layer of cultivated mycelia of the Cov-1 strain of *C versicolor* enhances the immune functions in old mice but not in young mice [149].

For some of the mushroom metabolites described, further research is needed to determine whether there are any in vivo benefits comparable to the in vitro effects reported. Although it is unlikely that high molecular weight polysaccharidse would be absorbed after oral administration, it is possible that it could exert a therapeutic effect by direct interaction with the mucosal immune system of the gastrointestinal tract. Thus, they could be developed as a preparation for use as a dietary supplement or pharmaceutical.

Some mushroom metabolites, such as the glucans lentinan and schizophyllan, or the polysaccharide-protein PSK, and the PSP, are used clinically for immune therapy [150, 151, 152, 153] and have been developed as pharmaceuticals in Japan and are now commercially available worldwide. PSK was commercialized by Kureha Chemicals, Japan. After extensive clinical trials, PSK was approved for use in Japan in 1977, and by 1985, it ranked 19th on the list of the world's commercially most successful drugs [154]. Annual Japanese sales of PSK in 1987 were worth US$357 million [154]. About 10 years after PSK, PSP appeared on the market. Both compounds have been isolated from *C versicolor*. In addition to clinically tested PSK and PSP, numerous other extract preparations of *C versicolor* are on the market as neutraceuticals and traditional medicines. Neutraceutical PSP preparations are sold worldwide in the form of capsules, ground biomass tablets, syrups, food additives, and teas [153].

Quality control of mushrooms poses significant challenges: small differences in genetics, soil, temperature, moisture, and time of harvesting can lead to significant differences in the concentration of important constituents. The cultivation of mushrooms to produce fruiting bodies is a long-term process requiring from one to several months for the first fruiting bodies to appear. Nowadays, more research is carried out in relation to submerged culture. Submerged culture has potential advantages for higher mycelial production in a compact space and for a shorter incubation time with a lesser chance of contamination. Further optimization of the culture medium composition and physicochemical conditions of growth allows regulation of fungal metabolism in order to obtain standardized nutriceutical substances in higher yield. Mycelia formed by growing pure cultures in submerged culture is the best technique for obtaining consistent and safe mushroom products [3, 12, 155]. Mushrooms are still far from being thoroughly studied.

ACKNOWLEDGMENT

The authors acknowledge the financial support of the Valencian authorities (Generalitat Valenciana; CTBPDC/2003/014) for Cristina Lull.

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