IMMUNOMODULATION BY DIETARY MUSHROOM COMPOUNDS

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SUMMARY

Mushrooms are long used food supplements that display profound immunomodulatory activities, due to the presence of both polysaccharides (β-glucans) and proteins (FIP). These compounds work either alone or in concert and both target different aspects of the immune system. While β-glucans are considered to bind to innate receptors and activate innate cells (dendritic cells and monocytes/macrophages), immunomodulatory proteins will be taken up, processed and presented on antigen-presenting cells to CD4+ Th cells in the context of MHC class II molecules. The outcome is different and results in the activation of both innate and antigen-specific adaptive immune reactivity. The combined presence in a whole extracts is therefore expected to be of more use than either active compound alone. Here we will describe some of the immunomodulatory activities present in such extracts from edible mushrooms.

INTRODUCTION

Immunomodulation

Immunomodulation is the manipulation of the immune system by augmenting or decreasing the magnitude of the immune responsiveness. The augmentation of the immune response is known as immunostimulation or immunopotentiation, while suppression of immune responsiveness is called immunosuppression. The necessity of suppression of the function of the immune system is well recognized in the areas of transplantation and immunopathological disorders like autoimmunity. Conversely, augmentation of the immune response has been a target for increasing the host’s resistance to infections and diseases. Specific immunomodulation is limited to a single antigen such as a vaccine and thus immunopotentiation is used for the development of resistance against particular diseases. Non-specific immunomodulation implies for a more generalized change in the immune responsiveness leading to altered host reactivity to many different antigens. Prevention and treatment of diseases are generally achieved by a wide range of antibacterial, antiviral, antiparasitic and antifungal agents and vaccines. The impact of chemotherapy and vaccination on many complex diseases, however, has reached a plateau and if further progress is to be made, different strategies have to be developed. Immunomodulation is one of the most important alternatives in order to control...
diseases associated with the environment with additional advantages of amplifying specific responses to vaccines still needed to control severe, life-threatening diseases. The immunomodulation offers the additional benefit of reversing immunosuppression caused by various cancers, stress, infection, food-related problems, reproductive problems, and chronic inflammatory conditions.

Diet

A well-balanced diet is beneficial for a good immune function. The minerals zinc, copper, iron and selenium and the vitamins A, C and E have been established to be essential for a normal immune response (Cunningham-Rundles et al., 2005). Recently, food ingredients, such as fish oil, are being studied for potential immunomodulatory properties, and functional foods that influence the immune function are being developed, including pre- and probiotics (Field and Schley, 2004; Salminen et al., 1998).

It may be less well known that mushrooms have immunomodulating properties as well (Wasser, 2002). Some 140,000 species exist on earth of which some 14,000 species are described. Generally, mushrooms contain by weight approximately 90% water, 10-40% protein, 3-28% carbohydrate, 2-8% fat, 3-32% fibre and 8-10% ash (Breene, 1990). For millennia, they have been valued as tasteful foods and as medicinal substances. The knowledge and practice of the medicinal use of mushrooms originates from traditional eastern medicine and in Japan, China, Korea, and other Asian countries modern clinical practice still utilises mushroom-derived preparations. Over the past three decades scientific research has been performed on the medicinal properties of a growing number of mushroom species. Mushrooms are claimed to exhibit antiviral, antibacterial, cholesterol-lowering, blood pressure-lowering and hypoglycaemic effects. Mushroom products are commercially available to contribute to the treatment of infectious diseases, cardiovascular disorders, diabetics, and to prevent various diseases (Wasser and Weis, 1999; Zaidman et al., 2005).

Several major substances with immunomodulatory and/or anti-tumour activity have been isolated from mushrooms. These include mainly polysaccharides (in particular β-D-glucans), 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 haematopoietic 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.

The most extensively studied application of medicinal mushrooms is their anti-tumour activity. Polysaccharides, mostly β-glucans, have been established to be one of the most potent anti-tumour-active compounds (Borchers et al., 1999, 2004). While most regular chemotherapeutic agents are based on direct cytotoxicity to cells, β-glucans mediate their anti-tumour activity by stimulating the immune system (Brown and Gordon, 2003). Unfortunately, regular cancer therapy often has many serious side effects and can be unsuccessful. In addition, infectious diseases
become increasingly difficult to treat, as pathogens are becoming resistant to many antibiotics (Sheldon, 2005). β-glucans may contribute to the solution of these problems by stimulating the immune system to enhance anti-tumour and anti-infective responses.

**IMMUNOREGULATION AND CHRONIC INFLAMMATORY IMMUNE-MEDIATED DISEASES**

Properties of many diseases, particularly systemic auto-immune diseases characterised by persistent inflammation, strongly support the involvement of helper T-lymphocytes (Abbas and Lichtman, 2005). For example, pathogenic auto-antibody responses generally are of high affinity IgG class, after having undergone affinity maturation, which requires helper T cells. The protein antigens to which many auto-antibodies are directed generally require T cell help. Many of the successful therapies, e.g. cyclosporin A, act primary on T cells. Besides roles as helpers, T cells may directly provoke cellular injury during inflammatory phases of the disease process. T cells, and in particular CD4+ helper T cells produces effector molecules, called cytokines, upon activation. A multiplicity of cytokine abnormalities has been associated with various auto-immune and immune-mediated diseases. It is thus becoming common practice to analyse the role of helper T cells and the cytokines they produce in studying the immunopathological basis of particular diseases, to aid in the unambiguous diagnosis of the disease, to rationally design T cell and/or cytokine based immunotherapy protocols, and to provide parameters to monitor the efficacy of treatment.

Naive CD4+ helper T cells (Th) develop into functionally mature effector cells upon stimulation with relevant antigenic peptides presented by major histocompatibility complex (MHC) class II molecules on antigen presenting cells (APC). Based on the characteristic set of cytokines produced, Th cells are commonly segregated into at least two different subpopulations: Th1 cells producing exclusively interleukin-2 (IL-2), interferon-gamma (IFN-γ) and lymphotoxin (O’Garra and Arai, 2000). Th2 cells on the other hand, produce IL-4, IL-5, IL-6, IL-10 and IL-13. These Th1 and Th2 subsets appear to be extremes in cytokine production profiles and within these polarised subsets, individual Th cells exhibit differential rather than co-ordinated cytokine gene expression. The Th-1 and Th-2 subsets appear to cross-regulate each other’s cytokine production profiles, mainly through IFN-γ and IL-10. From this concept it was rationalised that disturbances in the balance of these two subsets may result in different clinical manifestations. IL-12 is a dominant factor promoting Th1 differentiation, and is produced by dendritic cells and macrophages. Moreover, IL-12 induces IFN-γ production by T cells and NK cells. It was reported that IL-18 acts synergistically with IL-12 to induce Th1 development while polarisation of Th2 cells is critically dependent on the presence of IL-4 produced by Th cells, basophiles and mast cells. APC-derived IL-6 has also been shown to induce small amounts of IL-4 in developing Th cells. IL-10 and APC-derived prostaglandin E2 (PGE2) inhibit IL-12 production and Th1 priming (see Figure 1).
Self-tolerance is induced in the T cell recognition repertoire by clonal deletion, anergy or silencing in the thymus. These processes are, however, not complete and thus potentially autoreactive T cell can escape into the periphery where such T cells are continuously suppressed by cells that act functionally as suppressor cells. These cells, able to suppress other cells by cell surface mediated activity and/or production of suppressive cytokines, like IL-10 and (transforming growth factor) TGF-β are now called regulatory cells. Regulatory T cells mediate active suppression of various immune responses. These T cells comprise classical Th2 cells, capable of inhibiting Th1 responses, but also alternative T cell populations. Two main populations are distinguished based on molecular and cellular differences: naturally occurring CD4⁺CD25⁺ naturally occurring regulatory T cells (Treg) that activate the Foxp3 transcription factor and that primarily detectable in the periphery. Alternatively, antigen-induced regulatory T cell populations (Tr, Th3) were identified based on their high secretion of IL-10 and TGF-β and their relation with tolerance induction on mucosal surfaces in the gastro-intestinal and the respiratory system. The mechanism of peripheral tolerance has been focused mainly on the suppression of classical cell-mediated (IFN-γ producing) Th1 responses and in animal (models of) diseases based on excessive activity of Th1 cells. It is now clear that such tolerance induction is also active in humoral type 2 responses. One of the primary mechanisms of tolerance induction is via secretion of immunosuppressive cytokines, like IL-10, IL-4 and TGF-β. As mentioned before, regulatory T cells have been isolated from in vitro cultures, which appeared to produce low levels of IL-2, no IL-4, but high levels of IL-10 and TGF-β. This demonstrates the importance of cytokines in regulating and dampening
the immune response. It will thus be of crucial importance to determine whether the immunomodulating capacity of herbal and fungal polysaccharides in many diseases act via induction of these regulatory T cell subsets.

Recent progress has provoked a breakthrough in our understanding of basic mechanisms underlying the development of chronic inflammatory immune-mediated diseases by showing that T cells are important to sustain T-cell tolerance. This is achieved via the production of cytokines, like IL-10. T cells are involved in the suppression of both Th1 (auto-immune) and Th2 (allergic) diseases. The role of T cells is currently a focus in allergic disease model systems, as in mice. This research will tell much on the immunological mechanisms that are at the basis of the development of allergies, and provide opportunities for effective immune therapy based on induction of T cells (Boonstra et al., 2000; Van der Velden et al., 2001).

DEVELOPMENT OF ORAL TOLERANCE

Induction and/or maintenance of oral tolerance to orally ingested antigens also require microbial colonization of the alimentary tract in early life. As the intestine is the first line of defense from the environment, and must integrate complex interactions between diet, external pathogens, and local immunological and non-immunological processes, it is critical that protective immune responses are made to potential pathogens yet it is equally important that hypersensitivity reactions to dietary antigens are minimised. Thus, the gut immune system must distinguish not only between self and non-self, but also between potentially dangerous foreign antigens and common harmless foodstuffs to which it is constantly exposed. Such suppressive mechanisms to avoid local and peripheral overreaction (hypersensitivity) against innocuous substances bombarding the mucosal surfaces are referred to as ‘oral tolerance’ when induced via the gut against dietary antigens (Brandzaeg, 2006). Similar down-regulatory mechanisms apparently operate against antigens from the commensal micro flora.

In physiological circumstances tolerance towards the indigenous intestinal microbiota is established and maintained. Different doses of orally administered antigens may induce energy in antigen-specific T cells or may stimulate the production of cytokines, like TGF-β, that inhibit lymphocyte proliferation, resulting in suppression of the immune response. Moreover, since TGF-β also induces isotype switching to IgA antibody production, the mucosal immune system is further protected (Abbas and Lichtman, 2005). It is unclear why soluble proteins in large doses induce systemic T cell tolerance, whereas oral immunisation with attenuated poliovirus vaccines induces protective T-cell dependent antibody responses and long-lived memory.

Permeability of the intestinal barrier is greatly enhanced during foetal life, but gut closure starts before birth and is considered more or less complete by 33 weeks of gestation. Paracellular permeability can thereafter be increased, resulting in sensitisation to dietary antigens, large enough to be presented by DC to T-cells and resulting in a pro-inflammatory response. Pro-inflammatory cytokines, like IFN-γ and TNF-α, induce paracellular leakiness, while IL-10 and TGF-β promote tight junc-
tion formation and particularly these cytokines play a central role in oral tolerance (Adams et al., 1993; Planchnon et al., 1994).

Besides, there is evidence indicating that IL-10, a tolerogenic cytokine, is produced in response to microbial stimuli: Mice with defective IL-10 production infected with Helicobacter hepaticus developed Th1-type intestinal inflammation, whereas normal mice produced IL-10 and remained healthy. Interestingly, IL-10 deficient mice have decreased levels of resident lactobacilli in the neonatal period, and normalising the amount of these bacteria in the colon prevented the development of intestinal inflammation. In humans, carriers of H. pylori infected individuals posses Treg that can in vitro suppress responses of antigen-specifically stimulated T cells. In vivo it was suggested that the induction of H. pylori specific Treg cells suppress protective antigen-specific responses and contribute to the persistence of the infection (Penner et al., 2005).

IMMUNOMODULATION BY MUSHROOM-DERIVED β-GLUCANS

Structure of β-glucans

The interest in β-glucans as anti-infective and anti-tumour agents originates from the early 1900s, when an insoluble yeast (Saccharomyces cerevisiae) cell wall particle, named zymosan, was developed. Experiments showed that intravenous injection of zymosan could stimulate the immune system. Later β-glucan was identified as the biologically active constituent. β-glucans are a heterogeneous group of glucose polymers, mostly consisting of a backbone of β(1→3)-linked β-D-glucopyranosyl units with β(1→6)-linked side chains of varying distribution and length (see Figure 2).

These polysaccharides are major cell wall structural components in fungi and are found in plants and some bacteria as well. As they are not present in animals, they are recognized by the innate immune system and are considered to be classic pathogen-associated molecular patterns (PAMP). β-glucans mostly show a triple-strand right winding helix structure. Various β-glucans have been isolated from diverse mushroom species showing different immunological activity, which could be correlated with their solubility in water, molecular weight, conformation (tertiary structure) and degree of branching. Lentinan (Chihara et al., 1970; Yap et al., 2001) is isolated from the fruiting body of Lentinus edodes (shiiitake mushroom) and consists of five β(1→3)-β-D-glucopyranosyl units in a linear linkage and two β(1→6)-linked side chains (degree of branching: 0.4). The molecular weight is about 4-8 x 10^5 g/mol. Grifolan has a degree of branching of 0.33 and a molecular weight of approximately 5 x 10^6 g/mol. The compound is isolated from the liquid-cultured mycelium of Grifola frondosa (maitake mushroom). Another β-glucan has been extracted from this mushroom: Maitake D-fraction (Kodama et al., 2003). This is a high molecular weight polysaccharide. In contrast with other β-glucans, which have a β(1→3) main chain, D-fraction consists of a β(1→6) main chain with β(1→3) branches. Schizophyllan is obtained from a culture medium of Schizophyllum commune (split gill fungus) (Hashimoto et al., 1991). Its branching rate is 0.33 and the molecular weight is approximately 4.5 x 10^5 g/mol. SSG is a compound isolated from the culture filtrate of Sclerotinia
sclerotiorum (white mould) and has a branching rate of 0.5 and a molecular weight of approximately $2 \times 10^6$ g/mol. As this field of research is growing new mushroom species are being investigated for their immunostimulating glucans, including Agaricus blazei, Sparassis crispa, Ganoderma lucidum, Pleurotus ostreatus, and Sclerotium glucanicum (Brown and Gordon, 2003; De Ruiter et al., 1992; Karacsonyi and Kuniak, 1994; Kulicke and Lettau, 1997; Dong et al., 2002). Several animal studies were performed on the use of $\beta$-glucans in foods to modulate natural disease resistance (Charampopoulos et al., 2002; Chesterman et al., 1981; Gibson and Roberfroid, 1995) and to study the influence of $\beta$-glucans to reproductive and pregnancy-related problems (Cozens et al., 1981a; 1981b; 1981c).

**Effects of $\beta$-glucans on the immune system**

Particular mushroom $\beta$-glucans have an immunomodulatory and anti-tumour effects (Bohn and BeMiller, 1995). These substances are regarded as biological response modifiers. This basically means that they cause no harm and place no additional stress on the body; they help the body to adapt to various environmental and biological stresses; and they exert a non-specific action on the body, supporting some or all of the major systems, including nervous, hormonal, and immune systems, as well as regulatory functions.

Three $\beta$-glucans are used as biological response modifiers: Lentinan from Lentinus edodes, D-fraction from Grifola frondosa, and schizophyllan from Schizophyllum commune. Lentinan and schizophyllan are approved in Japan for clinical use to improve the immunity of cancer patients. $\beta$-glucans are used as a mild and non-invasive form of treatment of cancer and other diseases, and in the prevention of metastasis spread of tumours. In cancer treatment they can be used as a co-treatment in conjunction with other forms of therapy, such as chemotherapy and surgery.

The anti-tumour action of $\beta$-glucans is largely mediated via activation of the immune response; the polysaccharides do not attack cancer cells directly (Arihara et al., 1992a; 1992b; Baba et al., 1986; Chihara et al., 1970; Maeda et al., 1988; Minato et al., 1999; Ng et al., 2002; Oka et al., 1992, 1996; Ooi et al., 2000; Sudate, 1996; Taguchi et al., 1980, 1987; Usui et al., 1983; Wada et al., 1987; Wasser, 2002; Zaidman et al., 2005; Zhang et al., 2002, 2005). Experiments showed that the anti-tu-
mour effect was lost in neonatal thymectomised mice or after administration of anti-lymphocyte serum. These results suggest that the anti-tumour action is T-lymphocyte dependent. In addition, macrophages play an important role, because the anti-tumour activity can be inhibited by pre-treatment with anti-macrophage agents. The production of various cytokines is induced by β-glucans, which results in the proliferation, maturation, and differentiation of immune cells, such as NK cells and T-lymphocytes.

Besides anti-tumour activity, many β-glucans are thought to possess anti-infective activity against various bacterial, viral, and parasitic infections (Irinoda et al., 1992). For example, schizophyllan demonstrated protective effects against *Staphylococcus aureus*, and *Escherichia coli* infections in mice. SSG reduced the number of live intracellular *Mycobacterium tuberculosis* in macrophage cultures when it was incubated together with the bacteria (Markova et al., 2003; Hetland et al., 2002).

Little is known about the biological effects of β-glucans after oral administration (Rice et al., 2005). Oral administration of glucan phosphate led to a significant, but modest, increase in the serum level of the pro-inflammatory cytokine IL-12. No changes were observed in serum levels of other tested cytokines. Oral administration of glucan phosphate to mice one day before challenge with *Staphylococcus aureus* or *Candida albicans* led to increased long-term survival.

The precise mechanism which mediates the effects of β-glucans on the immune system is not totally clarified yet. However, research is starting to shed some light on the cellular receptors and molecular mechanisms involved (Brown et al., 2003; Falch et al., 2000; Hamano et al., 1999; Hamuro et al., 1974; Kataoka et al., 2002; Kerekgyarto et al., 1996; Kodama et al., 2003; Liu et al., 1999; Masihi et al., 1997; Murata et al., 2002; Oka et al., 1992, 1996; Ooi and Liu, 2000). The immune response triggered by β-glucans was primarily designed for the control of fungal pathogens. The immune response to pathogens relies upon the cooperation between the innate and the adaptive immune system. Innate immunity is the first line of defence against infections. Cells of the innate immune system recognise structures that are characteristic for microbes, such as lipopolysaccharides in Gram-negative bacteria and β-glucans in fungi. The receptors for these structures, called pattern recognition receptors (PRR), include scavenger, lipopolysaccharide, mannose, β-glucan, and Toll-like receptors (TLR). Innate immunity to fungi is mainly mediated by neutrophils and macrophages. They liberate fungicidal substances, like reactive oxygen intermediates and lysosomal enzymes, and phagocytose fungi for intracellular killing. Helper T cells and cytotoxic T cells cooperate in the adaptive immune response against fungal infections.

Role of β-glucan receptors

To date, four different β-glucan receptors have been identified: Complement receptor type 3 (CR3), lactosylceramide, scavenger receptors and Dec tin-1. They have been reported on monocytes, macrophages, neutrophils, eosinophils, natural killer (NK) cells, certain lymphocytes, as well as on non-immune cells including endothelial cells, alveolar epithelial cells, and fibroblasts.

CR3 is a heterodimeric integrin receptor, consisting of CD11b and CD18 chains and is expressed on monocytes, neutrophils, NK cells, and selected lymphocytes. It functions as a phagocytic receptor for iC3b-opsonized par-
articles (iC3b is an inactive complement protein), including opsonised particulate glucans, and it possesses a lectin domain, which recognises a variety of β-glucans directly. As leukocytes lacking CR3 can still bind and respond normally to β-glucans, the receptor does not seem to be indispensable. On the other hand, CR3-dependent cytotoxicity to tumour cells is stimulated by β-glucan priming: Experiments have shown that incubation of NK cells or neutrophils with small soluble β-glucans primed the CR3-receptor to enhance the cytotoxicity against iC3b-opsonised target cells that were otherwise resistant to CR3-mediated cytotoxicity. In addition, it plays a role in the recruitment of leucocytes to sites of inflammation by binding to adhesion molecules on endothelial cells.

Lactosylceramide is a glycosphingolipid present in the plasma membranes of many cells. It has been suggested that the interaction of β-glucans with this receptor can induce macrophage inflammatory protein-2 and the activation of the nuclear transcription factor NF-κB and can enhance the neutrophil oxidative burst and antimicrobial functions. The mechanisms are still unknown. There are indications that macrophage scavenger receptors can recognise β-glucans as well.

Dectin-1, a receptor first discovered in mice, seems to have an important role in mediating the biological response to β-glucans (Gantner et al., 2003; Herre et al., 2004). It is a transmembrane receptor with an immunoreceptor tyrosine-based activation (ITAM) motif in its cytoplasmic tail. Upon β-glucan binding to the extracellular side of Dectin-1 at the cell surface, the ITAM motif becomes phosphorylated, generating a signal which induces phagocytosis and the respiratory burst (the production of reactive oxygen intermediates). It is present on monocytes, macrophages, and neutrophils, and at lower levels on dendritic cells and a subpopulation of splenic T lymphocytes. Human Dectin-1 differs from its murine counterpart in that it is alternatively spliced, in a cell-specific manner, giving rise to several isoforms of which only two are functional. They are sometimes referred to as the β-glucan receptor and are similar in structure and function to murine Dectin-1. Dectin-1 also recognises an endogenous ligand on activated T cells in a β-glucan independent manner and may act as a T cell co-stimulatory molecule. Zymosan, a particle from yeast cell wall which consists of a variety of compounds, including β-glucans, mannans, mannoproteins and chitin, was found to interact with Toll-like receptors (TLR). Both TLR2 and TLR6 are required for activation of NF-κB in macrophages and dendritic cells, leading to the production of the pro-inflammatory cytokines TNF-α and IL-12 in response to zymosan. The adaptor protein MyD88 mediates the intracellular signalling to NF-κB. The binding of β-glucans to Dectin-1 alone does not stimulate the production of TNF-α and IL-12. However, Dectin-1 enhances the production of these pro-inflammatory cytokines when TLR2 is stimulated. TLR2 was found not to recognise β-glucans, but some other component of zymosan. This suggests that the strong pro-inflammatory activities reported for β-glucans in many studies, might be partly caused by unidentified TLR2 triggering contaminants in impure extracts. This hypothesis is supported by the results of a study performed by Kataoka et al. (2002) who found that branched (1→3)β-glucans, like lentinan and schizophyllan, could not stimulate NF-κB activity in macrophages. Taken together, for the activation of NFκB and the induction of a pro-inflammatory...
response Dectin-1 needs to cooperate with TLR-2. The activation of Dectin-1 alone by β-glucans is sufficient to stimulate the respiratory burst and phagocytosis (see Figure 2). It seems that TLR2 does not recognise β-glucans but other compounds present in zymosan.

Recently, new insights in the role of Dectin-1 in the response to β-glucans were provided by Rogers et al. (2005) and by Palma et al. (2006). Mouse B cell hybridoma cells (which normally cannot bind zymosan) were transduced with Dectin-1. These Dectin-1 transduced cells were now able to bind β-glucans of zymosan and produced IL-2 and IL-10. This shows that cytokine production is also occurring independent of TLR signalling. The same research group clarified part of the signalling pathway of Dectin-1. They discovered that a kinase called Syk was recruited upon β-glucan binding to Dectin-1. They also found that single phosphorylation of the membrane-proximal tyrosine (position 15) in the ITAM-motif is sufficient to recruit Syk and to couple to downstream IL-2 and IL-10 responses. Experiments were performed with Syk-deficient dendritic cells from chimaeric mice and it was found that these cells were completely unable to produce IL-2 or IL-10 in response to zymosan but could still bind zymosan and produce normal levels of IL-12 and IL-6. It can be concluded that Syk is required for zymosan-induced IL-2 and IL-10 production, but not for IL-12 synthesis, which could be signalled via the TLR2 pathway. IL-10 induction can occur independently of TLR signalling; IL-2 production is enhanced by TLR signalling (see Figure 3).

The importance of the Dectin-1/Syk pathway lies in the biological function of IL-2 and IL-10 during an infection. IL-2 and IL-10 stimulate the development of regulatory T cells thereby limiting immunopathology to a local infection, allowing persistence of immunity and resistance to re-infection, and maintaining immunological memory. In addition, IL-10 acts on activated macrophages to terminate their inflammatory responses and returning the immune system to its resting state. The Dectin-1/Syk pathway could therefore be exploited therapeutically in allergy, autoimmunity, and graft rejection. Normally, resistance to yeast infections is characterised by a strong T helper1-type response, in which IL-12 and TNF-α play an important role. In this way the infection can be cleared effectively.

In summary, it seems that β-glucans induce the production of IL-2 and IL-10 and the development of regulatory T cells via the Dectin-1/Syk pathway, while other compounds present in zymosan (and probably other fungal compounds) stimulate a pro-inflammatory T helper1-type response with production of IL-12 and TNF-α. Dectin-1 is responsible for phagocytosis and induction of the respiratory burst as well. Furthermore, CR3, lactosylceramide, and scavenger receptors mediate the stimulatory effects of β-glucans on the immune system. It should be noted that the amount or influence of the cytokines produced via the Dectin-1/Syk pathway might be small, because in animal and in vitro experiments a pro-inflammatory cytokine pattern is usually found, in which IL-12 and TNF-α predominate, at least for lentivirus. This may be explained by the influence of other β-glucan receptors or by contaminants binding to Toll-like receptors.

Oral delivery and gastro-intestinal absorption

To understand if and how oral delivery of β-glucans can have a biological
Figure 3: Zymosan can interact with Dectin-1 leading to production of IL-2 and IL-10. Triggering of TLR2 induces IL-12 and TNF- production and cooperative signalling of Dectin-1 with TLR2 augments this (From Rogers et al., 2005).

cal effect it is important to know if they are absorbed in the gastrointestinal tract, as humans cannot digest them. Rice et al. (2004, 2005) examined the absorption, pharmacokinetics, and biological effects of the three watersoluble β-glucans glucan phosphate, laminarin and scleroglucan that were administered by oral gavages to rodents. Laminarin (molecular weight: 7.7 x10³; degree of branching (DB): 1/10; single helix) and scleroglucan (MW: 1.02 x10⁶; DB: 1/3; triple helix) both have a backbone of (1→3)-β-D-glucopyranosyl units and β(1→6)-linked side chains and mainly scleroglucan is comparable to the mushroom β-glucans described above. Oral administration produced measurable plasma levels of the three β-glucans. Laminarin showed two peak plasma levels: one at 3 hours and one at 12 hours. Scleroglucan plasma levels peaked twice as well: at 15 minutes and at 3 hours. It is interesting that scleroglucan, the largest β-glucan, was absorbed most rapidly. The maximum plasma level was 115 ng/ml for laminarin and 355 ng/ml for scleroglucan when rats were given an oral dose of 1 mg/kg. It is noteworthy that most in vitro studies are performed with higher concentrations of β-glucans (approximately 5-500 µg/ml), so it is questionable if the low plasma levels after oral administration are sufficient for immunostimulation. In contrast, Tani et al. (1992) performed an in vitro study with macrophages and NK cells and stated that 25 to 100 ng/ml was the optimal concentration of lentinan to improve cytotoxicity. The bioavailability of laminarin and scleroglucan was 4.9 and 4.0%, respectively. A water-insoluble, particulate glucan preparation was not detected in plasma. A possible explanation is that particles are phagocylosed and transported by macrophages. Fluorescently labelled β-glucans were used to determine which cells in the gastrointestinal tract could bind them. It was demonstrated that gut-
sociated lymphoid tissue (GALT) cells, isolated from Peyer’s patches, can bind β-glucans. Macrophages showed an increase in Dectin-1 expression and dendritic cells increased their TLR2 expression. In an experiment with intestinal epithelial cells it appeared that only a subpopulation (10%) of these cells incorporated β-glucan. These cells were found not to express Dectin-1 receptor, so uptake of β-glucans by this cell type could not have been mediated by this receptor in contrast to GALT cells. Hashimoto et al. (1991) suggested that high molecular weight β-glucans may be taken up by microfold cells in the intestine, where they interact with the GALT. It is possible that the subpopulation of epithelial cells in Rice’s experiment consists of microfold cells. The data support an active uptake mechanism for β-glucans.

A different explanation might be a prebiotic effect of β-glucans on the gut flora. A prebiotic is a food ingredient that is not hydrolysed by the human digestive enzymes in the upper gastrointestinal tract and beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve host health. Oat β-glucan has been reported to selectively support the growth of lactobacilli and bifidobacteria in rat experiments and in in vitro studies. To date, it is not known if mushroom β-glucans could have a similar effect. In addition, there could be an effect of contaminating substances like proteins or fats in impure lentinan extracts.

It should be noted here, that the choice to use laminarin and glucan phosphate for these experiment by Rice et al. (2004,2005) is unusual, because laminarin is generally considered inactive and the biological effects of glucan phosphate are not clear. Taken together, these experiments show that orally administered β-glucans interact with a variety of gastrointestinal cells, enter the systemic circulation, and persist in the plasma up to 24 hours.

**Distribution, metabolism, and excretion**

Suda et al. (1996) studied the distribution and metabolism of i.p. administered, labelled SSG (MW: 2x10⁶) in mice. Following administration, the concentration of SSG was first high in peritoneal exudate and blood, but these concentrations lowered sharply in 48 hours. In contrast, concentrations rose mainly in liver and spleen, and a slight increase was found in the kidney, intestine and faeces. After one month about 30% of administered SSG was present in the liver and about 10% in the spleen.

Because of the absence of (1→3)-β-D-glucanase in mammals, these glucans are thought to be metabolised by oxidative degradation by macrophages (Miura et al., 1995, 1996). However, the majority of SSG in liver and spleen was recovered from the non-cellular fraction and not from macrophages. These results suggest that β-glucans are not easily taken up by macrophages to degrade and exclude them from the body. Even five weeks after administration, the metabolites of SSG extracted from the liver of the mice retained significant anti-tumour effects. Nevertheless, the molecular weight of SSG in the liver was lowered with time, and it became about 1x10⁵ g/mol. It was suggested that the degree of branching decreased as well. In contrast, no significant change was observed in the molecular weight and degree of branching of SSG in the spleen.

The elimination of β-glucans from the blood is quite slow (Sortwell et al., 1981). After a single dose (intravenous: 1 mg/kg) administered to rats, the elimination half-life was 2.6 hours for
laminarin and 3.1 hours for scleroglu-
can. The administration (i.p.) of multi-
ple doses of β-glucans (0.25 mg/week) to
mice led to saturation of the liver
and spleen and circulation of glucan in
the blood, as blood glucan concen-
trations were high all the time.

Not much is known about the clear-
ance of β-glucans from the whole
body. Lower molecular weight β-glucans (such as glucan phosphate, MW: 1.3 x 10^5) are possibly cleared by
glomerular filtration in the kidney. High molecular weight glucans (such as lentinan) are retained mainly in the
liver and degraded by liver macro-
phages, the Kupffer cells, which may
take several weeks.

### EFFECTS OF LENTINAN ON THE IMMUNE SYSTEM

The immunomodulatory effects and
the applications of lentinan are various. Lentinan is well known for its anti-tu-
mour effect, and recently the anti-in-
fective properties are being appreciated
as well. Lentinan influences many
components of the immune system:
Several cells as well as molecules. Chihara et al., (1969) reported the anti-
tumour activity of lentinan after con-
ducting an experiment with Sarcoma
180 transplanted in CD-1/ICD mice.
These results were confirmed by other
research groups, like Baba et al (1986),
who found a rapid decrease in the
number of tumour cells from sarcoma
180 tumours in ICR mice and an ac-
cumulation of polymorphonuclear leu-
kocytes (now called neutrophils) in the
tumour after i.p. lentinan injection.
Later, lentinan also showed anti-tu-
mour activity to syngeneic and au-
tochthonous tumours. Tumour regres-
sions of up to 100% were reported in
experimental animals in different
studies. Clinical studies with lentinan
in cancer therapy followed, although
very few are placebo-controlled and
double-blind, and showed that lentinan
was particularly effective in patients
with gastric and colorectal cancer. A
follow-up, randomised controlled study
in patients with advanced and recurrent
stomach cancers showed a prolonga-
tion of life. The results of clinical
studies also gave indications that lenti-
nan treatment increased NK and LAK
cell activity and induced IL-1 and
TNF-α production by human mono-
cytes/macrophages. LAK cells (lymp-
phokine activated killer cells) are IL-2
activated NK cells. There are also indi-
cations that lentinan could prevent
chemical and viral oncogenesis. Fur-
thermore, i.p. injection of lentinan in
mice induced vascular dilatation and
haemorrhage dose-dependently. This
response is probably mediated by T
cells and macrophages and correlates
well with anti-tumour activity. Re-
cently, many other possible applica-
tions for lentinan besides tumour sup-
pression have been investigated. Lenti-
nan may be useful in boosting the im-
mune response against various infec-
tions. Lentinan has shown antiviral
(e.g. human immunodeficiency virus,
*Herpes simplex*), antibacterial (e.g.
*Mycobacterium tuberculosis*, *Listeria
monocytogenes*), antiparasitic (*Schisto-
soma* spp.), and antifungal effects
(*Candida albicans*). Irinoda et al.
(1992) treated mice with lentinan
(i.ntranasal or intravenous) before
giving them an aerosol of virulent
influenza virus. Significant protection
was achieved by administrating 200 µg
of lentinan intranasally.

The protective effects of lentinan
against *Mycobacterium tuberculosis*
infection were studied by Markova et
al. (2003) in *in vitro* and *in vivo* mouse
models. The administration of lentinan before infection at a dose of 1 mg/kg three times at 2-day intervals could reduce mycobacterial infection. Peritoneal macrophages from animals treated with lentinan were greatly stimulated. Wierzbicki et al. (2002) studied the effect of the addition of lentinan to an orally administered vaccine against human immunodeficiency virus (HIV) envelope glycoprotein. Lentinan was found to increase envelope glycoprotein-specific T-helper 1 type cytokine production (IL-2 and IFN-γ) and cytotoxic T-lymphocyte activities but had no effect on humoral responses.

**Influence at the cellular level**

Lentinan exerts its influence on different cells of the immune system. An *in vitro* assay showed that phagocytosis by mouse macrophages was enhanced. In addition, *in vivo* lentinan administration to mice led to a higher number of macrophages, a higher percentage of activated macrophages, and enhanced antibody-dependent macrophage-mediated cytotoxicity compared with controls. In tumour-bearing mice the cells responsible for the anti-tumour activity of lentinan were studied. T cells played a role in the specific cytotoxicity and NK cells contributed to the a-specific cytotoxicity to tumours (Borchers et al., 1999).

In an *in vitro* experiment human peripheral blood mononuclear cells were cultured with lentinan (25 to 1000 ng/ml). Cytotoxicity of macrophages and NK cells was increased. The optimal concentration of lentinan was from 25 to 100 ng/ml, which is equivalent to the plasma concentration obtained after clinical doses of this agent (Tani et al., 1992).

In 15 patients with gastric carcinoma, peripheral blood mononuclear cells were obtained before and 3, 5, and 7 days after lentinan administration (2 mg i.v.). The ability to generate lymphokine activated killer (LAK) cell activity, tested by *in vitro* activation of blood cells with IL-2 was significantly augmented 5 days after lentinan injection, when compared with before administration. LAK cells are IL-2 activated NK cells. NK cell activity was significantly enhanced after seven days. The conclusions of this study can be questioned, because it was performed without control patients receiving a placebo, and the data of only 9 of 15 patients are presented without an explanation for the missing data (Arinaga et al., 1992).

In an *in vivo* study tumour-bearing mice received chemotherapy with or without lentinan (0.1 mg/day i.p.). Additional lentinan treatment induced increased intra tumour CD86+ dendritic cell (DC) infiltration and splenic DCs were more potent stimulators of T cell proliferation. In addition, the activity of splenic cytotoxic T cells was increased. Furthermore, the survival period of the mice treated with lentinan was significantly longer than that of mice treated with chemotherapy alone. It should be noted here, that the immunohistochemical staining for CD86 is not very specific for DCs, as other cells also possess CD86 (Musiake et al., 2005).

The effect of lentinan on B cells, neutrophils, eosinophils, basophils and mast cells is not clarified yet. However, it is known that neutrophils can bind lentinan. Monocytes bind lentinan stronger, but lymphocytes bind lentinan only minimally. This suggests that the effect of lentinan on lymphocytes is mediated by the stimuli of DCs and monocytes/macrophages, such as cytokine production. Lentinan is able to restore the suppressed activity of helper T-cells to their normal state in tumour-bearing hosts, leading to restoration of humoral immune responses. In addi-
tion, lentinan promotes a skewing of the Th1/Th2 balance towards Th1 (Oka et al., 1996).

**Influence on Th1/Th2 balance**

A very important feature of lentinan is that it affects cytokine production. Lentinan influences the cytokine production of different cells, including macrophages and T helper cells. Activated macrophages produce cytokines which have a role in inducing inflammatory reactions and in stimulating T cells. In this way T helper cells are stimulated to differentiate, and their cytokines activate macrophages and B cells.

CD4+ T cells can differentiate into different subsets, of which T helper 1 (Th1) and T helper 2 (Th2) cells are very important (see Figure 1). The two subsets are distinguished by the cytokines they produce: IFN-γ is the signature of Th1 cells; and IL-4, IL-5, IL-10, and IL-13 are produced by Th2 cells. These cytokines determine the effector functions and promote growth or differentiation of their own respective subset. The development of Th1 cells is induced by IL-12 produced by activated macrophages and dendritic cells and is antagonised by IL-4 and by IL-10. IL-4 favours induction of Th2 cells. Th1 cells are important for the intracellular destruction of phagocytosed pathogens, including bacteria, parasites, yeasts and viruses and the elimination of cancer cells. IFN-γ acts on macrophages to stimulate phagocytosis and killing of pathogens and on B-lymphocytes to produce opsonising antibodies. Th1 cells also produce TNF-α, which activates neutrophils and stimulates inflammation, and IL-2 which acts as an autocrine growth factor. The principal function of Th2 cells is in eradicating helminths and other extracellular parasites by activating mast cells and eosinophils, and stimulating IgE-production. If uncontrolled, Th1 cells can mediate immunopathology and autoimmune diseases. Over-activation of Th2 cells can lead to allergic manifestations.

Murata et al. (2002) found that lentinan administered i.p. to C57BL/6 and DBA/2 mice could skew the T-helper response toward Th1. Peritoneal macrophages isolated from lentinan-treated animals could produce more nitric oxide and IL-12 in response to stimulation, while the production of IL-6, IL-10, and Prostaglandin E2 (PGE2) was decreased. PGE2 is known to function as an endogenous immunosuppressive mediator. Nitric oxide is considered an effector molecule of cytotoxic macrophages against tumours. The lowered amount of IL-6 contributes to the inhibition of Th2 induction. IL-12 strongly stimulates Th1 development and the lowering of IL-10 neutralises the inhibition of activation of Th1 cells by IL-10. To study the effect on the Th cells, lentinan (5 mg/kg) or saline (0.5 ml) was injected i.p. on days 1, 3 and 5 and spleens were harvested the day after the final injection. The culture supernatants of purified splenic CD4+ T cells stimulated for 24 h with coated anti-CD3 antibody were analysed for IFN-γ and IL-4. IFN-γ was significantly increased, but IL-4 levels did not change significantly. This indicates a polarisation toward Th1, which can be related to the cytokine pattern induced in macrophages. Skewing of Th1/Th2 balance to Th1 favours cellular immune responses against tumours and intracellular pathogens.

Experiments performed with other cell types also give indications for a Th1 polarisation. Liu et al. (1999) administered lentinan i.p. to mice and the cytokine gene expression levels of IL-1α, IL-1β, TNF-α, IFN-γ, and monocyte colony stimulating factor (M-CSF) were analysed in peritoneal exudate
cells (PECs) and splenocytes. The biological effects of IL-1 are similar to those of TNF: they both induce inflammation. The expression of all five cytokines was up-regulated in PECs of treated mice; in splenocytes only IL-1α expression was not up-regulated. This cytokine pattern was also found in a study by Arinaga et al. (1992a,b), in which patients with gastric carcinoma received intravenous administration of lentinan. The ability of monocytes to produce IL-1α, IL-1β, and TNF-α in vitro in response to LPS stimulation was significantly augmented as compared with before treatment. It should be noted that this study was not placebo controlled.

Although most studies show a similar cytokine profile, the production of these molecules seems to be dependent on the genotype of the host and the specific health/disease status. Kerekgyarto et al. (1996) found that the cytotoxic activity and TNF secretion of murine macrophages was elevated by lentinan when applied in vitro or in vivo. The effectiveness of lentinan to induce these responses was highly influenced by the genotype of the host. In the experiment by Irinoda with mice infected with influenza virus (described above), TNF production could not be detected. The amount of IL-6 produced differed between uninfected and infected mice: in uninfected mice, IL-6 was higher in lentinan treated mice than in untreated mice; in infected mice, lentinan treated mice first showed higher IL-6 levels, later much lower levels than untreated mice. IL-6 has diverse actions including the stimulation of the synthesis of acute-phase proteins by the liver and the proliferation of antibody-producing cells.

A remarkable cytokine pattern was observed by Masihi et al. (1997) in their experiment with bacillus Calmette-Guerin (BCG)-primed mice. BCG is widely used as a vaccine against tuberculosis. It has also been recognized as an immune modulator and it induces local inflammation. Mice were pre-treated with lentinan and LPS was used to stimulate cytokine production. Lentinan induced an inhibition of up to 82% of TNF, a moderate reduction of 25% of IL-1β, and no significant differences in IL-6 or IL-10 levels, and a marked depression of chemiluminescence (respiratory burst) activity.

IMMUNE ACTIVITIES OF FUNGAL POLYSACCHARIDES

Mushrooms are abundant sources of a wide range of useful natural products (Zaidman et al., 2005; Borchers et al., 2004; Wasser, 2002; Ooi and Lui, 2000). Medicinal properties have been attributed to mushrooms for thousands of years, particularly in traditional Chinese and Japanese medicine.

Mushrooms have recently attracted much attention on account of their in vivo and in vitro immunomodulatory activity (Shamtsyan et al., 2004), which has been demonstrated for many mushrooms, including extracts and isolated compounds from the fruiting body, spores, mycelia, and culture medium of various mushrooms (Kodama et al., 2003, 2005). The major immunomodulating effects of these active substances include mitogenicity, stimulation of haematopoietic stem cells and activation of immune effector cells, such as helper T cells, cytotoxic T cells, macrophages, dendritic cells, endothelial cells, neutrophils, monocytes, and NK cells (Lull Nogiera et al., 2005).

One of the approaches to evaluate potential immunomodulating activity is the assessment of the capacity of ex-
tracts or pure compounds to influence the production of cytokines by immune cells. Cytokines are soluble glycoproteins which are critically involved in the immune response. The functions of these proteins are diverse and include roles in normal humoral and T cell-mediated immune response. Various pathologic conditions are accompanied by changes in cytokine levels and by disturbances in the cytokine-mediated interplay between innate and acquired immune responses (Stanilova et al., 2005).

For the last years a few studies have reported the immunomodulating activity of mushrooms in a human peripheral blood mononuclear cells (PBMC) assay. PBMC represent a heterogeneous population of immune cells (B cells, T cells, and various granulocytes) that arise from pluripotent haematopoietic stem cells in the bone marrow. PBMC account for cellular and humoral immune responses; some PBMC (B and T cells) have the inherent ability to proliferate rapidly after antigenic and mitogenic stimulation (Zempleni and Mock, 2000).

The immune activities of mushroom-derived polysaccharides are well documented and are considered to have function of promoting activities of antigen non-specific immune NK cells that are able to rapidly and effectively kill cancerous cells; placing a premium on the production of interferons that effectively prevent virus reproduction; increasing the activities of complement C3 that enhance animals’ disease resistance; increasing the number and activities of the phagocytes (neutrophilic granulocytes) that release H2O2 dissolving cancerous cells; protecting the normal cells and preventing the reduction of leucocytes (Wargovich et al., 2001). Therefore, mushroom polysaccharides can play an important role in the health care of human. Berović et al. (2003) studied the effects of extra- and intra-cellular polysaccharides isolated from mycelia of Ganoderma lucidum on the induction of IFN-γ and TNF-α, synthesis in primary cultures of human peripheral blood mononuclear cells (PBMC) isolated from a buffycoat. They found that the TNF-α inducing activity was comparable with romurtide, which has been used as a supporting therapy in cancer patients treated with radiotherapy and/or chemotherapy. Jin et al. (2003) treated PBMC with PG101, a water-soluble extract from cultured mycelia of Lentinus lepideus. PG101 increased levels of TNF-α, IL-1β, IL-10, and IL-12 by 100- to 1000-fold, whereas GM-CSF and IL-18 were activated by an order of magnitude. On the contrary, IFN-γ and IL-4 were not affected. Considering the type of affected cytokines, it is possible that PG101 could be used to enhance the immune system in immunosuppressed or immunocompromised individuals or to control the haematopoiesis of specific cell types or lineages.

In brief, as immunodulators, polysaccharides affect the growth of immune organs (bursa, thymus, and spleen), activities of immune cells (granulocyte, monocyte and macrophage), functions of both cellular immunity and humoral immunity as well as cytokines and complement system (Yuan and Shi, 2000). The effects of herb polysaccharides on immune system are summarized below according to Xue and Meng (1996):

- Stimulating growth of immune organs: The organs of the immune system are concerned with the growth, development, and deployment of lymphocytes. Herb polysaccharides, e.g. Isatidis radix, Ligustri fructus, Polypori scierotium, Astragali membranaceae radix, Tremella fuciformis, Cistanchea
herba and Cordyceps polysaccharide increase the weights of immune organs.

- Promoting activities of immune cells: Herb polysaccharides, e.g. *Isatidis radix*, *Codongopsis radix*, *Tremella fuciformis*, *Bupleuri radix*, *Angelicae sinensis radix*, *Astragali membranacea radix* and *Polypori sclerotium* polysaccharide, increase the number and activities of many interdependent cell types such as T, B lymphocytes, macrophage, NK cells that collectively protect the body from bacterial, parasitic, fungal, viral infections and from the growth of tumour cells.

- Enhancing functions of cellular immunity: Herb polysaccharides, e.g. *Isatidis radix*, *Tremella fuciformis*, *Astragali membranacea radix*, *Lycii fructus* and *Angelicae sinensis radix* polysaccharide, increased spleen and thymus index, rate of T-lymphocyte transformation and proliferation as well as production of IL-2, while decreased prohibition effects of serum, macrophage and suppressor T cell populations on T-cells’ function and against deformation and necrosis of lymphocytes in spleen, thymus and lymph nodes.

- Promoting humoral immunity: Herb polysaccharides, e.g. *Eucommiae cortex*, *Cordyceps*, *Codongopsis radix*, *Astragali membranacea radix*, *Tremella fuciformis* and *Atractylodis macrocephalae rhizoma* polysaccharide, enhanced humoral immune response by increasing spleen and serum antibody production, antibody titres and plague forming cells (PFC).

- Inducing cytokine production and complement: Cytokines are central molecules that control host immune response to infectious agents. Cytokines, which are produced and secreted by activated T-cells and NK cells activated by antigens, are responsible for clonal T-cell proliferation and antibody production of B-cells, proliferation and activity of macrophages, and NK cells. Herb polysaccharides (e.g. *Phytolaccae radix*, *Ginseng radix*, *Tremella fuciformis*, *Poriae albae sclerotium* and *Eleutherococci radix* polysaccharide) increased cytokine production such as interferons (IFN-α, IFN-β and IFN-γ) and interleukin (IL-2), TGF-β, and TNF-α.

Cell wall fragments of higher plants and yeasts, such as from the brewing industry, are known to have an impact on the immune system and to be able to stimulate innate immunity. Such preparations are already applied in the feed industry, for instance in shrimp and broiler feed (*Thanardkit et al.*, 2002; *Von Wettstein et al.*, 2000).

From preliminary immuno-assays, it has become clear that mushroom extracts show superior immune-modulating properties when compared to for instance β-glucans form beer yeast or from herbs. Striking differences between fungal and yeast cell walls may exist, as fungi are reputed for their high content in amino-glucans (*Hamuro et al.*, 1974; *de Ruiter et al.*, 1992; *Usui et al.*, 1983; *Karácsonyi* and *Kuniak*, 1994; *Dong et al.*, 2002; *Wasser*, 2002). This phenomenon is unexplained yet, i.e. it is not known what e.g. the differences in chemical structure between cell wall components from fungi resp. yeast are in relation to immune modulation, or whether fungi produce additional biologically active immune-modulating substances. Modification of polysaccharide fragments may increase their activity (*Zhan and Cheung*, 2002).
FUNGAL IMMUNOMODULATORY PEPTIDES

Proteins and peptides from mushrooms are also known to activate macrophages. An ubiquitin-like peptide isolated from fruiting bodies of the mushroom Agrocybe cylindracea enhanced NO production in murine peritoneal macrophages with potency comparable to that of LPS. 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 tumour-bearing mice.

Vvo, a fungal immunomodulatory protein (FIP) purified from the edible mushroom, Volvariella volvacea, induced most Th1-specific cytokines (IL-2, IFN-γ, and LT) and one TH2-specific cytokine (IL-4) within 4 hours in mouse spleen cells. This result indicates that Vvo principally acts on Th1 cells and to a lesser extent on Th2 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. Fve, a FIP isolated from the fruiting body of Flammulina velutipes, selectively stimulates a Th1 response in hPBMCs (Hsu et al., 1997, 2003). Recently Hsieh et al. (2003) 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 Th1-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 pointing out 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. Liu et al (1999) 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.

In contrast to the polysaccharide metabolites of edible mushrooms, only little is known about the effect of their proteins on the immune system. Hsieh et al. (2003) studied at the possibilities of therapy for food allergy using an edible-mushroom derived protein. They used a Fungal Immunomodulatory Protein (FIP) isolated from the commonly eaten mushroom Flammulina velutipes, called FIP-fve. It was demonstrated that oral administration of FIP-fve during allergen sensitization could induce a Th1-predominant allergen specific immune response in mice which protected the mice from systemic anaphylaxis-like symptoms after subsequent oral challenge with the same allergen. They concluded that the FIP-fve could activate T cells and selectively stimulate a Th1 response. In addition they demonstrated that the suppression of allergen-specific IgE response may play a crucial role in this protection. FIP-fve might either activate primed type-1 T cells or drive naive T cells to a type-1 phenotype. This strategy is convenient in practice and may have the potential to be used clinically in young children for the prevention of allergic diseases, but the optimal dosage, efficiency, and adverse effects in humans should be determined. It is well established that many
mushrooms-extracted compounds are commonly used as immunomodulators or as biological response modifiers. The basic strategy underlying immunomodulation is to identify aspects of the responses that can be enhanced or suppressed in such a way as to augment or complement a desired immune response (Wasser, 2002; Zaidman et al., 2005). Indications for the latter are for instance the identification of an immune-modulating protein (Fip-vvo) from Volvariella volvacea (Hsu et al., 1997).

Utilization of a T cell polarization pulse, followed by comparison of relative cytokine production by intracellular cytokine staining under polarized conditions allows ex vivo assessment of the T cell polarization state in vivo (Cameron et al., 2005). Since strong and clear T cell polarization typically takes place in chronic disease states or exposure to (potentially toxic) agents, this technique provides an assessment of the current direction of polarization the T cells are heading towards during acute responses, on a population basis via relative cytokine production, and on a cell-by-cell basis via intracellular cytokine staining (ICS). Most importantly, the comparison of cytokine production by enzyme-linked immunosorbent assay must be made on a relative scale, as outlined since the potency of the key Th1 and Th2 cytokines, IFN-γ and IL-4 respectively, may not necessarily be equal. Similar relative comparisons have been made during micro-array analysis of Th1 and Th2 gene transcripts. Additionally, all polarizations must be compared to the results obtained from CD4+ T cells isolated from healthy controls polarized under identical conditions. The rationale for this is apparent when looking at the data, because some individuals are naturally Th2 biased in their cytokine profile, while others are naturally Th1 biased. The addition of ICS to this method most importantly allows for further assessment of the T helper population in experimental groups, to determine if a mixed cytokine profile detected via ELISA and comparison of relative cytokine production truly represents an undifferentiated Th0 response of naive cells, or rather if it is indicative of a heterogeneous population of simultaneously differentiating Th1 and Th2 cells. Additionally, it can allow for further characterization of the cytokine-producing subsets.

**CONCLUSIONS**

A wide variety of immune interactions can be identified by which components present in mushroom extracts can exert their immunomodulatory activity. Some of these interactions are preferred by β-glucan, particularly those associated with TLR interactions on innate immune cells and resulting in the induction of pro-inflammatory cytokine production. Others, like FIP will be presented by APC to T-cells and (in)directly modulate Th cell differentiation. By integrating the various mechanisms exploited by these mushrooms predictions can be made for their immunomodulating activity in vivo. Figure 4 summarizes these known and suggested activities of the various mushrooms used for dietary immune intervention in humans. From these known interactions in vitro extrapolations to the potential use of such extracts for the treatment of human diseases can be inferred and increasing research is performed according to this paradigm.
Figure 4: Summary of published interactions of isolated components or extracts of various edible mushrooms on the induction of immune responses. From: Lull Nogiera et al., 2005.

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