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Medicinal Mushrooms: Their therapeutic properties and current medical usage with special emphasis on cancer treatments

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Biography

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PREAMBLE

Many clinically important drugs, such as aspirin, digitoxin, progesterone, cortison and morphine, have been derived directly or indirectly from higher plants. Less well-recognised but of great clinical importance are the widely used drugs from fungi such as the antibiotics, penicillin and griseofulvin, the ergot alkaloids and cyclosporin.

During the last two decades there has been an increasing recognition of the role of the human immune system for maintaining good health. Diseases now associated with immune dysfunction such as cancer, chronic fatigue syndrome, AIDS/HIV, hepatitis and autoimmune conditions are increasingly coming to the forefront and being given special attention from medical researchers and clinicians alike. Historically, the larger fungi, the mushrooms, have had a long and successful medicinal use especially in traditional Chinese clinical medicine for many forms of immune disorders. Chinese Pharmacopeias document the use of well over 100 species of mushroom by practitioners of traditional Chinese medicine, for a wide range of ailments. Many of these mushroom-derived medicinal products are now produced by major Japanese, Korean and Chinese pharmaceutical companies. Many of these products are being used worldwide by holistically oriented physicians, chiropractors, herbalists and naturopathic physicians in a clinical environment. To date, Western, medicine has made little use of these products in part due to their complex structure and lack of acceptable pharmaceutical purity.

Mushrooms are not a taxonomic group but do include well over 12,000 species which have macroscopic fruit-bodies, the mushrooms, which are large enough to be seen by the naked eye. Mushrooms are increasingly being evaluated in the West for their nutritional value and acceptability as well as their

pharmacological properties. Increasingly, many are being viewed nutritionally as functional foods as well as a source of physiologically beneficial and non-invasive medicines, while others are distinctly non-edible but considered purely as a source of medicinally beneficial compounds. Some of the most recently isolated and identified compounds originating from the medicinal mushrooms have shown promising immunomodulatory, antitumour, cardiovascular, antiviral, antibacterial, antiparasitic, hepatoprotective and antidiabetic properties. Modern scientific studies of the medicinal mushrooms have expanded exponentially during the last two decades primarily in Japan, Korea and China but also in the USA and scientific explanations of how these compounds function in the animal and human systems are increasingly appearing in peer-reviewed scientific and medical journals.

Mushroom-derived polysaccharides are now considered as compounds which are able to modulate animal and human immune responses and to inhibit certain tumour growths. While mushroom glucans are mostly non-cytotoxic, the same is not true of glucan-protein complexes. All of these compounds, however, have been shown to potentiate the host's innate (non-specific) and acquired (specific) immune responses and activate many kinds of immune cells that are important for the maintenance of homeostasis, *e.g*. host cells (such as cytotoxic macrophages, monocytes, neutrophils, natural killer cells, dendritic cells) and chemical messengers (cytokines such as interleukins, interferons, colony stimulating factors) that trigger complement and acute phase responses. Also, they can be considered as multi-cytokine inducers able to induce gene expression of various immunomodulatory cytokines and cytokine receptors. Lymphocytes governing antibody production (β-cells) and cell-mediated cytotoxicity (T-cells) are also

stimulated. However, for most of the mushroom-derived anti-cancer compounds, a detailed understanding of their exact mode of action has not yet been elucidated.

While many mushroom-polysaccharides have been shown to have considerable antitumour activity in several xenographs only a limited number have undergone clinical trials. At present the main products submitted for clinical testing include Lentinan from *Lentinus edodes* fruit-bodies, Schizophyllan from *Schizophyllum commune* mycelial broth, PSK and PSP, from mycelial cultures of *Trametes versicolor* and Grifron-D from fruit-bodies of *Grifola frondosa*. All have been through Phase I, II and III clinical trials mainly in Japan and China but now in US. However, in many cases the standards of these trials may not meet current Western regulatory requirements. In many cases there have been significant improvements in quality of life and survival. Increasingly, several of these compounds are now used extensively in Japan, Korea and China, as adjuncts to standard radio- and chemotherapy. While most of these clinical studies have used extracts from individual medicinal mushrooms, some recent studies from Japan have shown that mixtures of extracts from several known medicinal mushrooms, when taken as a supplement, have shown beneficial effects on the quality of life for some advanced cancer patients.

Perhaps the most encouraging observations from most of these studies is the ability of the mushroom-derived polysaccharides when taken prior to and during radiotherapy and/or chemotherapy to significantly reduce the side-effects of these treatments.

The safety criteria for the mushroom polysaccharides have been exhaustively studied with little evidence of any toxicity. In Phase I clinical trials, these compounds demonstrate remarkably few adverse reactions. Several purified mushroom

polysaccharides have been in clinical use in Japan, Korea, China and more recently in the USA for several years with no reports of any short-term or long-term toxicity.

Clinical efficacy of the mushroom polysaccharides will depend on understanding their precise scope of activity verifiable through *in vitro* and *in vivo* animal and tissue culture tests and human clinical trials, dose range, extraction methods, source and purity of the raw fungal material, duration and frequency of administration, and accuracy in matching the extracts to each particular patient based on traditional and modern diagnostic methods.

This Report, originally commissioned by the Cancer Research Campaign, aims to give a detailed and comprehensive appreciation of this complex area, derived from Oriental and Western literature together with the practical experience of the authors. It is to be hoped that Western oncologists will now have the opportunity to assess this area of cancer treatment and to judge whether it will have a realistic role in Western cancer research programmes.

Finally, from a holistic consideration, the consumption of whole edible medicinal mushrooms or extracts or concentrates (dietary supplements) may well offer novel, highly palatable, nutritious and health benefiting ingredients to the Western diet as functional foods.

EXECUTIVE SUMMARY

1. This literature study commissioned by the Cancer Research Campaign in November 2000, entailed searching computerized databases of published literature (e.g. Medline, BIDS/WOS, Embase, Science Citation Index, British Library Net) and searching of relevant specialised journals (as outlined in the proposal), many of which are not included in the computerized databases. Many original and peer-reviewed papers were obtained from the Document Supply Centre of British Library Net and scanning reference lists of appropriate review articles, books and other relevant publications (including symposia and conference proceedings). Consultations were achieved with key informants in the field, nationally and internationally. In addition to writing to many internationally-leading scientists in this field, Prof Smith spoke to a number of these scientists at an international conference held recently in Kiev where he presented an invited paper on this particular topic. However, while most of the aforementioned strategies proved successful, we were disappointed at the lack of response from some key scientific and medical centres in China and Japan who have specialised knowledge in this particular field.

We believe that this seminal literature study does contain the best up to date information on the therapeutic properties and current medical usage of medicinal mushrooms with special emphasis given on cancer treatment. It is proposed that the critical information in this report will be used to write reviews for appropriate journals. As a closing qualifying remark, while every effort was made to ensure that the best-published-data was gathered on the aforementioned, it must be appreciated that this particular field is enormous and a limited number of interesting papers may have been missed.

2. Scientific evidence supports the view that diet controls and modulates many functions of the human body and, accordingly, participates in the maintenance of the state of good health or homeostasis.

3. Arising from this awareness of the relationship between diet and disease has evolved the concept of functional foods and the development of functional food science. Foods as medicine underpins the paradigm of functional foods. The primary objectives of functional food science are to maintain good health, improve homeostasis and to create the conditions for disease reduction. It is seen to be quite distinct from the medical and pharmaceutical sciences where the objectives are mainly to cure or control diseases.

4. Mushrooms have long been valued as highly flavoursome and nutritional foods by many societies. In the Orient, there has long been the recognition that certain edible and non-edible mushrooms can have profound health benefits. When used as tonics the medicinal mushrooms are consumed whole or preferably as concentrated extracts and act as dietary supplements. A limited number of highly purified compounds derived from certain medicinal mushrooms are now being used in the Orient and the US as pharmaceutical-grade products in medicine – especially, but not exclusively, for cancer treatment.

5. Mycology is concerned with the study of the fungi, the term being derived from the Greek word *mykes*. They are heterotrophic, requiring organic carbon compounds of varying degrees of complexity for growth and reproduction. Most fungi exist as microscopic filaments or *hyphae* which form a complex *mycelium* or network. In some cases the mycelia form large complicated structures as exemplified in the mushrooms. This report deals

exclusively with large fleshy mushrooms, especially the medicinal mushrooms.

6. The use of psychotropic mushrooms by man dates far back into antiquity with the earliest records dating back to Palaeolithic times. There is an extensive literature implicating certain mushrooms in ancient religious beliefs and practices.

7. Consistent production of successful mushroom crops is built upon scientific knowledge and practical experience. To date about 35 mushroom species have been cultivated commercially with about 20 cultivated on an industrial scale. Most of these species are both edible and possess medicinal properties.

8. Mushroom cultivation involves several different operations each of which must be performed accurately if the enterprise is to be successful, *viz*. strain selection and maintenance, spawn production, mushroom production (log culture and enriched sawdust culture), and crop management for production. Mycelium production by liquid tank fermentation is now increasingly being used for the production of more uniform medicinal products. The ability to use pure substrates and controlled growth environments will aid in the final purity of the products.

9. The practice of using fungi, especially mushrooms, in Chinese Traditional Medicine (TCM), dates back into antiquity and has been recorded in ancient Chinese manuscripts. Increased scientific and medical research in recent decades, especially in Japan, Korea and China and more recently US, is confirming efficacy and identifying the bioactive molecules.

10. The main medicinal mushrooms both edible and non-edible are briefly depicted to identify their historical usage and their current commercial and

medical acceptance, *viz*. *Ganoderma lucidum* (Reishi or Ling Zhi), *Lentinus* (*Lentinula*) *edodes* (Shiitake), *Phellinus linteus, Porio cocos, Auricularia auricula, Hericium erinaceus, Grifola frondosa* (Maitake), *Flammulina velutipes, Pleurotus ostreatus* (Oyster mushroom), *Trametes (Coriolus) versicolor*, *Tremella fuciformis, Schizophyllum commune* and the non-mushroom *Cordyceps sinensis* (the caterpillar fungus).

11. Recent improvements in chemical technology have allowed the isolation and purification of the relevant compounds (especially the polysaccharides) which contain demonstrable anti-cancer activities. Most appear to act as immune system enhancers though some can have direct cytotoxic effects on cancer cells. Only a small number have progressed successfully to objective clinical assessment in trials.

12. The anti-tumour polysaccharides isolated from mushrooms (fruit-body, submerged, cultured mycelial biomass or liquid culture broth) are either water-soluble β-D-glucans, β-D-glucans with heterosaccharide chains of xylose, mannose, galactose or uronic acid or β-D-glucan-protein complexes - proteoglycans. Some are orally bioavailable.

13. Methods of extraction and purification of the various polysaccharides are now well worked out. The main medically important polysaccharide compounds that have undergone clinical trials include Lentinan (*Lentinus edodes*), Schizophyllan (*Schizophyllum commune*), PSK and PSP (*Trametes versicolor*) and Grifron-D (*Grifola frondosa*). Compounds from other medicinal mushrooms with proven anti-cancer properties have been studied in pre-clinical models and will increasingly be submitted for clinical trials.

14. Mushroom-derived glucan and polysaccharo-peptides can act as immunomodulators. The ability of these compounds to enhance or suppress

immune responses can depend on a number of factors including dosage, route of administration, timing and frequency of administration, mechanism of action or the site of activity. Several mushroom compounds have been shown to potentiate the host's innate (non-specific) and acquired (specific) immune responses and activate many kinds of immune cells that are important for the maintenance of homeostasis, e.g. host cells (such as cytotoxic macrophages, monocytes, neutrophils, natural killer cells, dendritic cells) and chemical messengers (cytokines such as interleukins, interferon, colony stimulating factors) that trigger complement and acute phase responses. They can also be considered as multi-cytokine inducers able to induce gene expression of various immunomodulatory cytokines and cytokine receptors. Lymphocytes governing antibody production (β-cells) and cell-mediated cytotoxicity (T-cells) are also stimulated.

15. Lentinan and Schizophyllan are T-cell oriented immunopotentiators and require a functional T-cell component for biological activity by way of increasing helper T-cell production, increased macrophage production leading to a stimulation of acute phase proteins and colony stimulating factors which in turn affect proliferation of macrophages, neutrophils and lymphocytes, and activation of the complement system.

16. PSK and PSP are potent immunostimulators with specific activity for T-cells and for antigen-presenting cells such as monocytes and macrophages. Their biological activity is characterised by their ability to increase white blood cell counts, interferon-y and interleukin-2 production and delayed type hypersensitivity reactions.

17. There have been extensive *in vivo* studies demonstrating the anti-cancer activity of the glucan polysaccharides and polysaccharide-peptides in animal

models. These studies strongly suggest an immunomodulating mode of action. However, in *in vitro* studies on various cancer cell lines, there is strong evidence for direct cytotoxic effects on the cancer cells for some, but not all, of the polysaccharides.

18. While all of the proprietary mushroom polysaccharides successfully used in animal and human cancer treatments are effective by i.v.route, several can also be effective orally.

19. Many of the mushroom polysaccharides have proceeded through Phase I, II and III clinical trials mainly in Japan and China but now in US. Lentinan (*L. edodes*) has demonstrated strong anti-tumour activity in a wide range of xenographty and with human clinical trials where it has proved successful in prolonging the survival especially those patients with gastric and colorectal cancer. Lentinan has been approved as a drug in Japan and is considered an important adjuvant treatment for several cancers. Schizophyllan (*S. commune*) has proved useful for recurrent and inoperable gastric cancer, as well as increasing survival times of patients with head and neck cancers. Neither of these compounds show any significant side-effects.

20. There are several on-going clinical trials with Grifron-D, GD (*G*. *frondosa*) on breast, prostate, lung, liver and gastric cancers underway in Japan and US. Results to date are promising. In *in vitro* studies GD appears to inactivate glyoxalase I, an enzyme believed to metabolise chemotherapeutic compounds used against cancer cells thus potentially enhancing their bioavailability.

21. Two compounds, PSK and PSP (derived from mycelial cultures of *T. versicolor*) have shown worthwhile anti-cancer properties when given with traditional chemotherapeutic agents with no increases in side-effects. PSK

has successfully been used in Phase I, II and III clinical trials with cancers of the stomach, oesophagus, nasopharynx, colon, rectum and lung, and with subsets of breast cancer. PSK gave protection against the immunosuppression that normally is associated with surgery and long-term chemotherapy. PSK continues to be used extensively in Japan as an adjunct to standard radio- and chemotherapy. PSP has been extensively studied by Chinese scientists and oncologists, with little evidence of side-effects. Clinical trials have shown efficacy in gastric, oesophageal and non-small cell (NSCLC) lung cancers, and PSP has been recognised as a drug by the Chinese Ministry of Public Health.

22. A significant observation from these studies is the apparent ability of all of the above mushroom-derived polysaccharides when administerded with radiotherapy and/or chemotherapy to significantly reduce the side-effects so often encountered by patients.

23. While the role of medicinal mushrooms in immunomodulation and anti-cancer activities represents the central theme of this Report it is pertinent to observe that many of the medicinal mushrooms have been highly valued for other medicinal properties including hypercholesterolemia, high blood pressure, diabetes, anti-viral, anti-bacteria, and antioxidant and free radical scavenging; each of these features is briefly discussed.

24. The safety criteria for mushroom-derived β-glucans have been exhaustively carried out in pre-clinical experiments. Acute, subacute and chronic toxicity tests have been carried out together with administration during pregnancy and lactation with no adverse effects. There were no anaphylactic reactions and no effects in mutagenicity and haemolysis tests, blood coagulation and a wide range of other regulatory tests. There was no evidence of genotoxicity.

Similar results have been obtained with other β-glucans. When applied to humans in Phase 1 clinical tests, the β-glucans demonstrate remarkably few adverse clinical reactions.

25. Current laws on dietary supplements in Europe, Japan and US are discussed with reference to herbal and mushroom products.

26. The safety of all medicinal mushrooms or their extracts cannot be guaranteed simply because they have been used for many centuries with apparent safety. Recent proposals have carefully examined historical usage and have set out reasons for adopting a more cautionary approach but at the same time indicating the way forward to ensure adequate safety and efficacy for an expanding use of mushroom dietary supplements and pharmaceutical products.

27. The main advantage of using mushroom products with respect to safety (when compared to herbal preparations) are:

- The overwhelming majority of medicinal mushrooms are cultivated commercially (not gathered from the wild). This guarantees proper identification and relatively pure, unadulterated products.
- Mushrooms are easily propagated vegetatively and, thus, kept to one clone. The mycelium can be stored for a long time and the genetic and biochemical consistency may be checked over time.
- The ability to grow most medicinal mushrooms as mycelium in fermenters under controlled conditions with consequent improved product purity. This may well be an important future trend in medicinal mushroom product formation.

28. Several purified mushroom polysaccharides have been in clinical use in Japan, China and the US for several years with no reports of any significant short-term or long-term adverse effects.

29. In view of the great interest in medicinal mushrooms and the absence of a specialised journal in this field, a special journal dedicated to medicinal mushrooms – "The International Journal of Medicinal Mushrooms (IJMM) was established in 1999 by Begell House (USA) (www.begellhouse.com). The IJMM highlights new perspectives in the field of mycology and medicine. JES is a Senior Editor. In September 12-14, 2001, an International Conference "Perspectives of Medicinal Mushrooms in Health Care and Nutrition in the 21st Century" was held in Kiev, Ukraine. Three hundred and forty eight scientists from 38 countries presented their results of this fascinating and growing science.

CHAPTER 1 INTRODUCTION

Synopsis

This chapter briefly examines the relationship of diet to health and defines the concept of functional foods. A dietary supplement is considered as an addition to the diet to enhance health. Foods as medicine underpins the paradigm of functional foods. The recognition of medicinal mushrooms as functional foods or as dietary supplements is fully discussed especially in the concept of Chinese holistic medicine and modern immunology.

In the developed nations of this world many causes of death or disability such as coronary heart disease, strokes, diabetes, atherosclerosis, obesity and certain forms of cancer can, in considerable part, be attributed to diet (Barasi, 1997). Poor food selection and restricted dietary intake can affect the nutritional status of an individual at any stage of life and can lead to impairment of long term health. Increasingly, scientific evidence is supporting the view that diet controls and modulates many functions of the human body and accordingly participates in the maintenance of the state of good health or homeostasis necessary to reduce the risk of many chronic diseases (Carter, 1993). Over the last few decades the science of nutrition has progressed from being largely epidemiologically based to the greater understanding of the physiological and genetic mechanisms by which diet and individual food components influence health and disease. It is indeed a paradox that nutrition is essential to support life but can also be considered as a causation of many chronic diseases.

Arising from the awareness of the relationship between diet and disease, has evolved the concept of “functional foods” and the development of a new scientific discipline “functional food science” (Sadler and Saltmarsh, 1998). A food may be considered to be functional if it contains a food component (whether a nutrient or not)

which affects one or more identified functions in the body in a positive manner. Correspondingly, it can also include foods in which potentially harmful components have been removed by technological means. The US Academy of Science has defined functional foods as those that “encompass potentially healthful products” including “any modified food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains” (Thomas and Earl, 1994).

Functional foods come in a plethora of name forms, e.g. dietary supplements, nutra- or nutri-ceuticals, medical foods, vita foods, pharmafoods, phytochemicals, mycochemicals, biochemopreventatives, designer foods and foods for specific health uses (Hasler, 1996; Head *et al*., 1996). Such complex designations could well be an impediment to their rightful maturation and consumer acceptance (Zeisel, 1999). There continues to be much confusion over these names especially in the commercial world. However, the term dietary supplement (DS) is now being more widely accepted and recognised. The term DS was formally defined by the US administration in 1994 as a product intended to supplement the diet to enhance health. A DS includes *one or more* of the following dietary ingredients: a mineral, amino acid, vitamin, herb or other botanical; or it is a dietary substance used to supplement the diet by increasing the total dietary intake and is intended for ingestion in the form of a capsule, powder, softgel or gel cap and not represented as a conventional food or as a sole item of a meal or the diet (Dietary Supplement Health and Education Act, Public Law 103-417, 1994).

However, foods as medicine underpins the paradigm of functional foods. Functional foods cannot claim to cure diseases but, increasingly, evidence is being produced that supports the role of some functional foods in disease prevention (Steinmetz and Potter, 1991). The concept of foods as medicine does not fit easily

within the current expertise of either pharmaceutical or food companies and the full creative development of functional foods may well require new alliances between these companies with respect to regulatory issues.

Functional food science is now considered as a part of nutritional science in which the primary objectives are to maintain good health, improve homeostasis and to create the conditions for disease risk reduction. In this way it should be seen to be quite distinct from the medical and pharmaceutical sciences where the objectives are mainly to cure or control diseases (Saris *et al*. 1998; Diplock *et al*. 1998). In many ways conventional medicine seeks to eliminate disease rather than to fortify the patient. In essence, functional food science aims: 1) to identify beneficial interactions between the presence or absence of a food component (macronutrient, micronutrient or so-called non-nutrient) and a specific function or functions in the body; 2) to understand their mechanisms so as to support hypotheses to be treated in protocols relevant for human studies. This will require multidisciplinary research programs containing the expertise of scientific partners including biochemists, nutritionists, the medical profession and process technologists.

Functional foods are set to play an increasingly important role in national efforts in developed nations to curtail medical expenditure and also to improve the dietary habits of the populace. Consumers are becoming increasingly more health conscious and discerning in the types of foodstuffs that are purchased. It is now not possible to overlook the critical role that diet, including functional foods, can play in general health and well-being. Many types of cancer can now be linked to inappropriate diets. In contrast, regular consumption of fruits and vegetables (now viewed as classical examples of functional foods) are now considered as essential ingredients in cancer prevention programmes (Steinmetz and Potter, 1991).

Medicinal mushrooms

Mushrooms have long been valued as highly tasty and nutritional foods by many societies throughout the world (Chang and Miles, 1989). Early civilisations, by trial and error built up a practical knowledge of those suitable to eat and those to be avoided, e.g. poisonous or even psychotropic. In many parts of the world, especially Europe, wild mushrooms are regularly collected and used directly as a main source of food or added to soups, stews and teas. Mushrooms are considered to be a good source of digestible proteins with protein content above most vegetables and somewhat less than most meats and milk. Protein content can vary from 10-40% on a dry weight basis. Mushrooms contain all the essential amino acids, but can be limiting in the sulphur-containing amino acids, cystine and methionine (Chang, 1991; Breene, 1990). Fresh mushrooms contain 3-21% carbohydrates and 3-35% fibre on a dry weight basis. Thus, a considerable proportion of the carbohydrate of mushrooms consists of dietary fibre which cannot easily be digested by humans and whichl function essentially as dietary fibre; in this way the calorific value of most mushrooms is low. Mushrooms probably contain every mineral present in their growth substrate including substantial quantities of phosphorous and potassium, lesser amounts of calcium and iron. Mushrooms appear to be an excellent source of vitamins especially thiamine (B_1), riboflavin (B_2), niacin, biotin and ascorbic acid (VitC). Vitamins A and D are relatively uncommon although several species contain detectable amounts of β-carotine and ergosterol which converts to active vitamin D when exposed to ultraviolet irradiation. While crude fat in mushrooms contains all the main classes of lipid compounds including free fatty acids, mono-, di- and tri-glycerides, sterols, sterol esters and phospholipids, levels are generally low, around 2-8% of dry weight (Breene, 1990). Without doubt, edible mushrooms in fresh,

cooked or processed forms are a nutritionally sound, tasteful food source for most people and can be a significant dietary component for vegetarians (Breene, 1990). In China, the term Yakuzen is generally used for medicinal food dishes of mushrooms.

However, in the Orient several thousand years ago, there was the recognition that many edible and certain non-edible mushrooms could have valuable health benefits (Bensky and Gamble, 1993; Hobbs, 1995). The edible mushrooms which demonstrate medicinal or functional properties include species of *Lentinus (*Lentinula*), Auricularia, Hericium, Grifola, Flammulina, Pleurotus* and *Tremella* while others are known only for their medicinal properties, e.g. *Ganoderma* and *Trametes (Coriolus)* – these are definitely non-edible due to their coarse and hard texture or bitter taste. The historical evolution of usage of these essentially scarce, forest-obtained medicinal mushrooms would most certainly not have been as whole mushrooms but as hot water extracts, concentrates, liquors or powders and used in health tonics, tinctures, teas, soups and herbal formulae. Nowadays, almost all of the important medicinal mushrooms have been subjected to large-scale artificial cultivation, thus removing the historical scarcity factor. This also ensures accuracy of identification and increased reliability and consistency of medicinal products. Also many of the edible species of medicinal mushrooms are gaining worldwide popularity because of their unique flavours, textures and amenability to culinary inclusion. Indeed, most people in the West who enjoy the unique organoleptic features of the Shiitake mushroom (*Lentinus edodes*) are singularly unaware of its possible health benefits. Regular consumption of whole medicinal, edible mushrooms could introduce a functional or medicinal contribution within the individual's diet. The extent of the health beneficial effect will be dependent on the level and regularity of

consumption and the relevance of whole fresh medicinal mushrooms and concentrates will be discussed in later chapters.

When used for a therapeutic intention the medicinal mushrooms are normally consumed as powdered concentrates or extracts in hot water, and the extract concentrated and used as a drink or freeze-dried or spray-dried to form granular powders which allow easier handling, transportation and consumption (Mizuno *et al*. 1995). As such, these liquid concentrates or dried, powdered mushrooms contained in capsules can be considered as *dietary supplements* or *mushroom nutriceuticals* with potential health benefits (Chang and Buswell, 1996). Mushroom nutriceuticals are usually crude mixtures and should not be confused with pharmaceuticals which are almost invariably a defined chemical preparation, the specifications for which are listed in pharmacopoeia. Regular intake of these concentrates is believed to enhance the immune responses of the human body, thereby increasing resistance to disease and in some cases causing regression of the disease state (Jong *et al*. 1991).

These mushroom dietary supplements are used extensively in traditional Chinese medicine in various combinations, often with other herbal products, to treat many medical conditions. A limited number of highly purified polysaccharide compounds derived from certain medicinal mushrooms are now being used, particularly in Japan, as pharmaceutical grade products and are discussed in later chapters.

Immune system modulation has long been a feature of Chinese holistic medicine and is referred to as Fu Zheng therapy. Fu Zheng can be considered as the Oriental equivalent of Western immunotherapy. Compounds derived from certain medicinal mushrooms are used extensively in the Orient to increase disease

resistance and to normalise body functions. Such extracts are used to treat deficient principles or qi or ch'i, the 'vital' or life energy, blood and yin (fluid) and yang functionality (especially the kidney).

Cancer and its treatment by conventional therapies as chemotherapy and radiotherapy are known to have adverse effects on the human immune system. Cancer immunology has become a rapidly growing field in basic cancer research. Ways are now being sought to promote host antitumour immune cell activity and to overcome the ability of the cancer cell to evade immune surveillance (Curt, 1998; Cassileth, 2000). Immunostimulating agents would possibly be useful adjuncts to conventional treatments of cancer if they do not interfere with the ability of the conventional treatment to kill tumour cells. These approaches, like chemo- and radiotherapy, are designed to cause the destruction of tumour cells but to be much more tumour-specific than present treatments and, consequently, less harmful to normal cells.

As will be shown in later Chapters, one of the most noticeable features of extracts derived from many medicinal mushrooms is their ability to function as immunomodulators. As such, the physiological constitution of host defence mechanisms are improved by the intake of these mushroom compounds which restore homeostasis and enhance resistance to disease. A central premise in Oriental medicine is to regulate homeostasis of the whole body and to return the diseased individual to the normal state.

It is interesting to note that several of the medicinal mushrooms and their concentrates are becoming particularly popular in the US – the movement began with a drive towards "healthy food" in the 60s-70s; now it is towards "healthy medicine". People are interested in the medicinal mushrooms because they appear

to have been used with considerable effect for hundreds of years in the Orient while many modern widely used pharmaceuticals while offering undoubted health benefits can also in some cases have serious side-effects. Furthermore, it is now increasingly being recognised that diet is intimately associated with optimal health and the tenet of Hippocrates *c* 400 B.C. "*Let food be your medicine and medicine be your food"* is fast becoming a truism for many people.

Over the last 2-3 decades scientific and medical studies have been carried out in Japan, China, Korea and more recently US which have increasingly demonstrated the potent and unique health enhancing properties of compounds extracted from a range of medicinal mushrooms. Explanations of how such compounds function in animal and human systems are now regularly appearing in peer-reviewed scientific and medical journals.

This review will aim to give a detailed analysis of the history and present state of knowledge of these organisms, methods of cultivation, range of organisms, methods of extraction, and detailed examination of medical implications with special emphasis on immuno-stimulation and cancer treatment. The review will also examine international regulations related to the use of such compounds (dietary supplements) with particular emphasis on safety. While the review will concentrate largely on their applications in the treatment of cancer, a brief overview of the wide range of other medical uses will also be included.

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CHAPTER 2 THE NATURE OF FUNGI WITH SPECIAL EMPHASIS ON MUSHROOMS

Synopsis

This chapter aims to give a basic understanding of fungi, their structure and mode of growth with specific emphasis in the mushroom fungi. The role of mushrooms in nature is outlined with reference to the main forms of nutrition. The historical uses of psychotropic mushrooms in early forms of religion are outlined together with the use of other mushrooms as items of food and medicine.

Introduction

Mycology is concerned with the study of the Fungi, the term being derived from the Greek word *mykes*, meaning a fungus. The Fungi were, until comparatively recent times, regarded as members of the Plant Kingdom but are now recognized as a very distinct and separate group of organisms. They are eukaryotes having well-defined membrane-bond nuclei with a number of definite chromosomes and, as such, clearly distinguishable from bacteria. They are heterotrophic, requiring organic carbon compounds of varying degrees of complexity which distinguishes them from plants which manufacture their own organic food by photosynthesis. All but a few fungi have well-defined cell walls through which all their nutrients must pass in a soluble form and, in this respect, they differ from animal cells which lack defined cell walls.

The number of species of fungi is a matter of speculation but recent estimates have strongly suggested that their numbers could be well in excess of 1.5 million. The fungi show immense differences in size, structure and metabolic activities. The smallest, such as the yeasts, grow as loose aggregates of single detached microscopic cells while most fungi exist as microscopic filaments or hyphae which extend at the tip, branching and fusing (anastomosing) to form a complex mycelium or network. As such mycelial networks increase in size they become visually

apparent and, indeed, in some cases the mycelia form large complicated structures exemplified by the large fruit bodies known colloquially as mushrooms. While many fungal species do grow and function in aqueous environments, the vast majority, by the nature of their apical growth patterns, are best adapted to growth over and through solid substrates, especially in terrestrial environments. Fungi function extensively in the soil environment breaking down dead organic matter but can also extensively grow in plants, animals and man causing decay and disease. Many fungi negatively attack manufactured products of all kinds including foodstuffs, fabrics, leather, timber, cosmetics, pharmaceuticals, aviation fuel etc., while, on the other hand, they make a huge contribution in biotechnology producing wines, beers, spirits, fermented food products such as cheeses, antibiotics, industrially-important organic acids and now many other important medicinal compounds. In themselves many edible mushrooms form the basis of huge commercial processes.

In mycology, as in other sciences, increased knowledge has resulted in complexity and, eventually, the division of the science into a number of branches with the resultant increase in specialization. What is termed pure mycology concerns the detailed structure, cytology and modes of development of fungi while taxonomic studies examine structure with a view to classifying fungi so as to show relationships and facilitate identification of the myriad of types. Medical mycology deals with the fungi which cause diseases in man and as well as the toxic effects of mycotoxins, fungal metabolites formed by filamentous fungi growing mainly in cereal grains and oilseeds. Several of these mycotoxins are now recognized as powerful carcinogens of man. *Such cancer-forming mycotoxins present in the human diet deserve greater awareness in the medical profession*. Field mycologists are particularly concerned with fungi found in fields and woods, particularly the larger forms, the mushrooms,

the mycelium of which colonises the field and forest litter. Some attack and colonise standing trees, producing large outgrowths or bracket fungi.

In the context of this report, attention will be drawn mainly to the larger fleshy fungi or mushrooms since almost all of the important edible and/or medicinal fungi are to be found within these species. In mycological terminology such mushrooms belong almost exclusively to the Basidiomycete and Ascomycete subdivisions. In both the Basidiomycetes and Ascomycetes there is a process of sexual reproduction occurring originally in the underground mycelial stage and finally manifesting in the large above-ground macroscopic fleshy fruit-bodies – the mushrooms (Fig. 1).

Such mushroom structures in their multifarious forms and colours are there primarily to disseminate the spores or units of propagation. The main difference between the two types is in the final mechanism of sexual spore release. In the Ascomycetes, often termed ‘sac fungi’ as within the mushroom mass they produce sac-shaped capsules (the asci) that actively release the spores to the atmosphere to be dispersed by the wind. In contrast the Basidiomycetes or ‘club-fungi’ produce spores attached to club-shaped structures, the basidia (Latin name for club). Some of the mushrooms are prized by the epicure, others are shunned as amongst the deadliest of poisons but, most important of all, there is the increasing recognition that many contain a Pandora’s box of intriguing medicinally important compounds. Some of the important British species of edible, poisonous, hallucinogenic mushrooms are listed in Tables 1-3, and medicinal mushrooms, mostly Oriental, are listed in Table 4 (Philips, 1994; Miles and Chang, 1997).

Fig. 1 The Basidiomycete mushroom life cycle (Stamets, 2000)

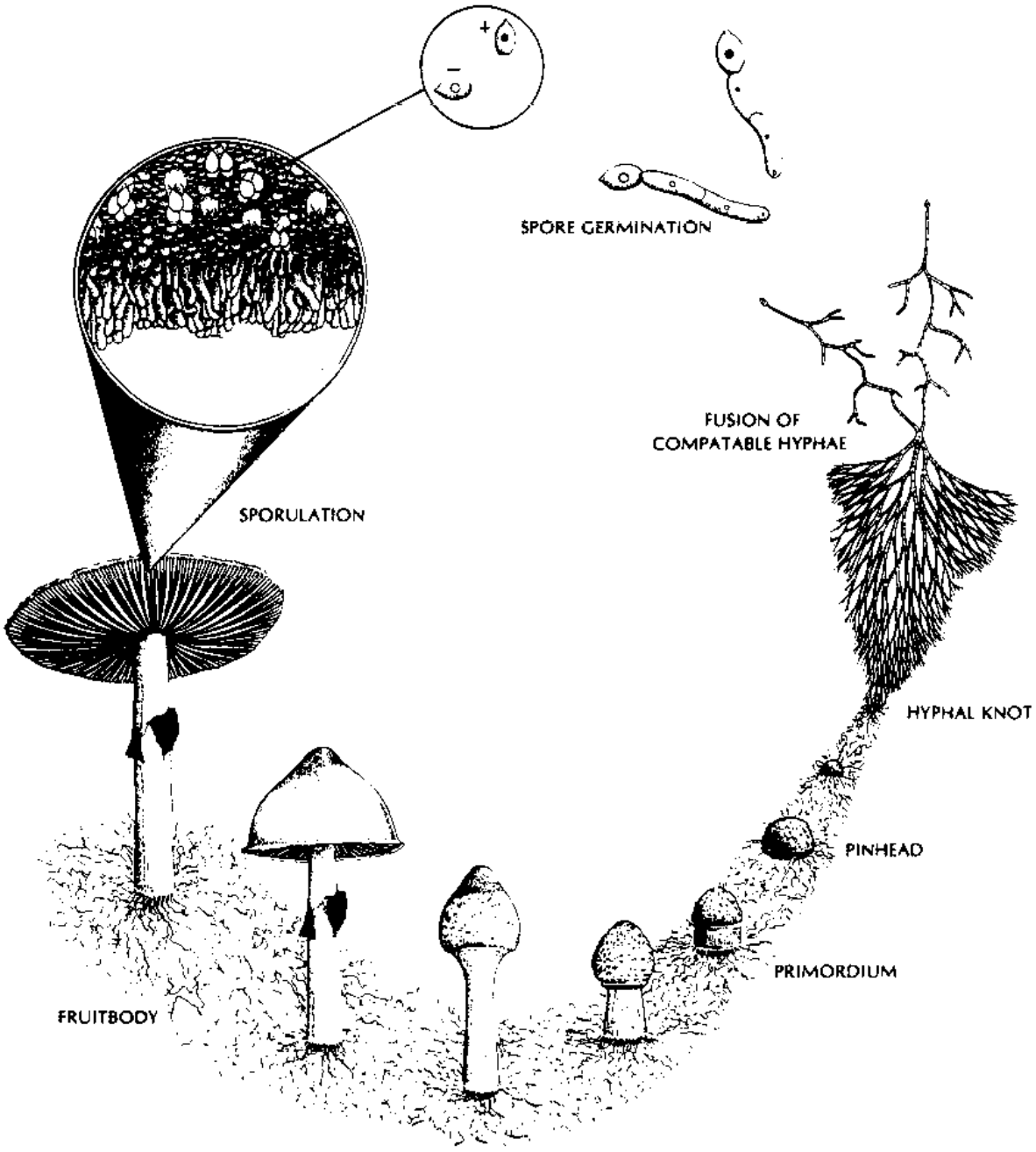

Table 1 Edible mushrooms in Britain

Agaricus compestris	The field mushroom
Boletus edulis	Cep
Cantharellus cibarius	Chanterelle
Coprinus comatus	Shaggy inkcap
Cratarellus cornuopiodes	Horn of Plenty
Hydnum respondum	Hedgehog fungus
Laetiporus sulphureus	Chicken of the Woods
Lepiota procera	Parasol mushroom
Lepiota saeva	Field blewit
Marasimus oreandes	Fairy Ring Champignon
Morchella esculenta	Morel
Sparassis crispa	Cauliflower fungus
Tuber aestivum	Truffle

Table 2 Some poisonous mushroom species in Britain

Amanita phalloides
Amanita virosa
Amanita pantherina
Inocybe patouillardii
Cortinarius speciosissimus
Cortinarius orellanus
Gyromitra esculenta

Table 3 Some of the main psychoactive mushrooms

Lycoperdon spp.
Psilocybe spp.
Paneolus spp.
Gymnopilus
Conocybe spp.
Amanita muscaria
Amanita pantherina

Table 4 Important medicinal mushrooms

Auricularia auricula
Trametes (Coriolus) versicolor
Flammulina velutipes
Ganoderma lucidum
Grifola frondosa
Hericium erinaceous
Lentinus edodes
Schizophyllum commune
Tremella fuciformis
Poria cocos

How mushrooms grow in nature

From an ecological and also an eventual cultivable perspective, mushrooms can be considered in three distinctive modes of growth, viz. as saprophytes, parasites or in a mycorrhizal association (Stamets, 1993).

The saprophytic mushrooms belong to the group of primary recyclers in nature, enzymatically breaking down the complex organic matter of dead organisms, especially those with a woody structure, and setting in motion the ultimate return of the organic building blocks to the ecosystem for reuse. Within this group there are three separate but overlapping groups characterized by their enzymatic capabilities. In nature, the *primary decomposers* are largely decomposers of woody structures and are characterized by possessing enzymes able to degrade the complex macromolecules such as lignin and cellulose. Decaying logs and tree stumps are most often colonized by colourful mushroom types. Many of the Oriental medicinal mushrooms, such as *Lentinus edodes* have their historical origin from such locations. Once such mushroom mycelium has been established and with the initial breakdown of the raw material, other microorganisms, including bacteria and other mushrooms, can further exploit the partially broken down nutrient environment. These are the *secondary decomposers* and are exemplified in the mushrooms by *Agaricus campestris* – the button mushroom. Finally, as the organic material is extensively broken down or decomposed, the *tertiary decomposers* are now able to grow. In nature, the three groups are not clearly separated but rather occur in the same location or habitat. Thus, in any one particular site, there can be a succession of mushroom types depending on the state of decomposition. The recognition of the growth needs of a mushroom in nature can hasten an eventual large-scale commercial cultivation procedure.

True *parasitic mushrooms* can attack living trees causing immense ecological damage and huge financial losses in forestry. While in most cases the mycelial growth within the body of the tree will be limited, in some cases it can lead to death of the trees. However, most parasitic mushrooms can also exist on dead tree material as *facultative parasites*. The medicinal mushroom *Pleurotus ostreatus* is a good example. The spread of such mushrooms from tree to tree and through the soil can be quite extensive. It is interesting to note that American scientists have calculated, using DNA measuring technology, that a single pure colony of the parasitic mushroom *Armillaria bulbosa* covered 37 acres, weighed at 222,000 lbs and had an estimated age of 1500 years – and is still growing. With the exception of certain Sequoia forest trees this is the largest living organism on the planet!

Undoubtedly, one of the largest groups of mushroom species are the *mycorrhizal mushrooms* (myco = mushroom, rhizzal = roots) which contain many of the important gourmet mushrooms, viz. the Truffles, Matsutake, Ceps and Chantarelles (Table 1). In these cases the underground mycelium of the mushroom grows extensively around the root tips of specific trees forming a protective sheath with some mycelium penetrating into the root tissue. The mycelium grows also in the soil mass and, eventually, appears at the surface as typical mushroom fruit-bodies or underground as solid fungal masses, i.e. the truffles. The fungal sheath greatly increases the absorption of essential nutrients, especially minerals, into the tree improving its vigour and disease resistance. In return, the mycorrhizal mushroom receives organic material of unknown composition. Many mycorrhizal mushroom species have been cultured on selected media as saprophytes but others such as the Truffles and the Chantarelles have, so far, defied true axenic, artificial cultivation. A huge commercial market awaits their inevitable cultivation. In many parts of the

world the collection, distribution and sale of such gourmet mushrooms from natural habitats is a multi-million pound industry.

The health of most forests is directly related to the presence, abundance and variety of mycorrhizal associations. Most mycorrhizal medicinal mushrooms still need to be gathered from the wild. As will be discussed later, fermenter cultivation of mycorrhizal mycelium may help to overcome the difficulty of successful total cultivation.

Mushrooms and historical uses

Many mushrooms have long been valued as tasty, nutritious food by different societies worldwide. To the ancient Romans they were “the foods of the Gods” resulting from bolts of lightening thrown to the earth by Jupiter during thunder storms; the Egyptians considered them as “a gift from the God Osiris”; while the Chinese viewed them as “the elixir of life”.

Throughout history many cultures have built-up a practical knowledge of which mushrooms were suitable to eat and those that were poisonous. Many cultures, especially in the Orient, identified that certain mushrooms could have profound health-promoting benefits (Hobbs, 1995).

However, there does exist an insidious fear of mushroom poisoning in many cultures which can even approach phobic levels. Such profound mycophobic reactions are evident in the UK, Ireland and much of North America while, in sharp contrast, mycophilic or fungus-loving societies can be witnessed throughout Asia, much of Europe, Poland and Russia where wild mushrooms are extensively collected or purchased for food to be incorporated into soups, stews and teas. Catholic countries, in general, are more mycophilic and it has been suggested that this may have arisen because they are not allowed to eat meat on Fridays and mushrooms

could be a good alternative (Taylor-Hawksworth, 2001). Notwithstanding, in some societies where gourmet mushrooms were regularly consumed at feasts and banquets, it was relatively easy to add in a few deadly poisonous mushrooms e.g. *Amanita phalloides* (Table 2) with dire consequences ensuing. Claudius II and Pope Clement VII are strongly believed to have died in this way. Symptoms and death normally came many hours later which allowed the perpetrator to be many "leagues" away before the onset of the symptoms. Some legends even suggest that the Buddha died in this way.

Perhaps the most fascinating aspect of ancient mushroom usage is related to the psycho-active, hallucinogenic properties of some mushrooms (Table 3). There is an extensive literature implicating certain mushrooms in ancient religious beliefs and practices (Arora, 1985). Investigations have demonstrated extensive past use of the tiny psycho-active hallucinogenic mushrooms *Psilocybe* spp. and *Panaeolus* spp. in Meso America and *Amanita muscaria* in Northern Europe/Siberia and in the Sahara region dating back to Paleolithic times. The accumulated, detailed data gathered to date strongly implicates the use of powerful hallucinogenic mushrooms in primitive forms of religion. There are numerous Meso American mushroom stones which date back to 3000 BC (Fig. 2). These stones were found at Mayan excavation sites in Guatemala and it is known that these "mushroom cults" were strongly persecuted by the Spanish when they arrived in the New World. These may represent the sacramental mushroom called Teonanc-tl (meaning flesh or food of the Gods) by the ancient Aztecs (Wasson, 1978).

Fig. 2 Meso American mushroom stones circa 3000 years BC from the Pacific Slopes of Guatemala

The oldest archaeological evidence of apparent mushroom use in the African continent can be seen in the Tassili images discovered in rock cave paintings in Algeria, dated at least 5000 B.C. (Fig. 3). The dancer is about 80 cm in height with the mask and stance typical of that historical period of rock paintings. The dancer is probably a shaman and the repetitive mushroom symbols hallucinogenic mushrooms (Samorini, 2001). In Europe, an effigy of a mushroom, not unlike *Amanita*, inserted in a scene with shamanistic connotations, has been found in a rock engraving of Mount Bego, France, dating back to 1800 B.C. Further important archaeo-ethnomycological documentation is to be found in ancient Greek culture (Samorini, 2001).

Fig. 3 Tasseli cave art from Northern Algeria, circa 5000 years BC.

Aristotle, Plato and Sophocles are believed to have participated in religious ceremonies which involved, in part, the consumption of mushroom decoctions. Furthermore, it has been strongly postulated that the mysterious SOMA in the Vedic

literature, a red fruit causing spontaneous enlightenment for those who ingest it could only be the red Fly Agaric, *Amanita muscaria* (Wasson, 1976) (Fig. 4). It is surprising that there has been such limited modern scientific study of the psychoactive compounds produced by such mushrooms. The extensive representation of mushrooms in a religious context would strongly imply their hallucinogenic potential and ingestion could induce mental experiences generally interpreted in terms of enlightenment and mystic-religious visions (Samarini, 2001).

Fig. 4 *Amanita muscaria* – the Fly Agaric

The recent discovery of the “Iceman” in the Italian Alps, who is believed to have died 5300 years ago, brought further intriguing evidence of ancient mushroom

use. Among his accessories was a string of Birch Polypore Mushrooms (*Piptoporus betulinis*). Such mushrooms have long been used as tinder for starting fires, as medicine for treating wounds, and for producing an invigorating and immune stimulating tea (Hobbs, 1995, Stamets, 2000).

Why, might we ask, do so many British people have this very deep-felt and tenaciously-held fear of mushrooms? Robert Graves in "Food for Centaurs" (1956) has constructively discussed the use of hallucinogenic mushrooms in ancient religious ceremonies. It has been seriously considered that the British phobic attitude may be a deeply ingrained tradition from Druid times that mushrooms contain magical properties and may only be eaten under the control of the Druids themselves. Yet all round us nations relish eating mushrooms of diverse kinds!

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CHAPTER 3 MEDICINALLY IMPORTANT MUSHROOMS

Synopsis

Many edible and non-edible mushrooms have long been used worldwide, especially in the Orient, for medicinal purposes. This Chapter gives a brief summary of the most important and widely used species. In each case their historical and current traditional use is considered together, where appropriate, with their commercial and modern medical applications. Important pharmaceutical products with proven medical applications have been derived from *Ganoderma* spp., *Lentinus edodes, Schizophyllum commune, Tremella fusiformis, Trametes versicolor*, and *Grifola frondosa,* and more recently *Phellinus* and *Hericium erinaceus*.

In addition to their nutritional value, many edible large mushrooms have long been used in the Orient for medicinal purposes. Many non-edible species have also gained important medicinal usage. An old Chinese proverb states that “medicine and food have a common origin”. At present there are at least 270 species of mushroom that are known to have various therapeutic properties (Ying *et al*., 1987). The practice of using fungi, especially mushrooms, in Chinese herbal medicines has been recorded in early records of the “Materia Medica”. The earliest book on medicinal materials in China, the “Shen Noug’s Herbel” (Shen Noug Pen Ts’ao Jing) (100-200AD), recorded the medicinal effects of several mushrooms including *Ganoderma lucidum, Poria cocos, Tremella fuciformis* and others. The most outstanding work on traditional Chinese medicines “Pen Ts’ao Kang Mu” (Compendium of Materia Medica) compiled by Li Shi-Zhen of the Ming Dynasty and published in 1575 documented more than 20 mushroom species, together with a non-mushroom insect-infesting fungus *Cordyceps senensis* which continues to be a major Chinese medicinal fungus (Bensky and Gamble, 1993).

Medicinal mushrooms have become even more widely used as traditional medicinal ingredients for the treatment of various diseases and related health problems largely due to the increased ability to produce the mushrooms by artificial methods. As a result of large numbers of scientific studies on medicinal mushrooms especially in Japan, China and Korea, over the past three decades, many of the traditional uses have been confirmed and new applications developed (Table 1, Wasser and Weis, 1999a). While much attention has been drawn to various immunological and anti-cancer properties of these mushrooms they also offer other potentially important therapeutic properties including antioxidants, anti-hypertensive, cholesterol-lowering, liver protection, anti-fibrotic, anti-inflammatory, anti-diabetic, anti-viral and anti-microbial. These properties will be examined in a later chapter. Clearly, many pharmaceutical companies in the Far East are viewing the medicinal mushrooms as a rich source of innovative biomedical molecules. Many polysaccharide-bound proteins produced by Basidiomycete fungi have been classified as anti-tumour chemicals by the US National Cancer Institute (Jong and Donovick, 1989). Some of the more important and leading medicinal fungi used in the Far East will be briefly summarised. For fuller details of each medicinal mushroom reference should be made to Hobbs (1995), Stamets (1993, 2001) and Mizuno (1995). A recent general paper by Wasser and Weis (1999b) gives detailed general mycological information on several of the most important medicinally valuable Basidiomycetes mushrooms, including biological and ethnomycological properties, taxonomy, morphology, anatomy, description, cultural characteristics, and distributions.

TABLE 1 Cross index of medically active higher Basidiomycetes mushrooms and their medicinal properties (Wasser and Weis, 1999a)

	Antifungal	Antiinflammatory	Antitumour	Antiviral (e.g. anti-HIV)	Antibacterial & Antiparasitic	Blood pressure regulation	Cardiovascular disorders	Hypercholesterolemia, hyperlipidemia	Antidiabetic	Immunomodulating	Kidney tonic	Hepatoprotective	Nerve tonic	Sexual potentiator	Chronic bronchitis
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Auriculariales			+			+	X	X							X
Auricularia auricula-judas (Bull.) Wettst.															
Tremellales		+	+					+	+	+		+			X
Tremella fuciformis Berk.						+									+
Tremella mesenterica Rits.:Fr.															
Polyporales															
Schizophyllum commune Fr.:Fr.		X	X		X					X	X	X			
Dendropolyporus umbellatus (Pers.:Fr.)			X							X		X			X
Jül.	+		X	X	X	X			X	X		+			+
Grifola frondosa (Dicks.:Fr.) S.F. Gray															
Fomes formentarius (L.:Fr.) Fr.			+		+										
Fomitopsis pimicola (Schw.:Fr.) P. Karst.		+	+		+							+			
Trametes versicolor (L.:Fr.) Lloyd			X	X	X						X	X			
Piptoporus betulinus (bull.:Fr.) P. Karst.	+		+		+										
Hericium erinaceus (bull.:Fr.) Pers.			+							X			X		X
Inonotus obliquus (Pers.:Fr.) Bond.et Sing.		X	X							X		X			
Lenzites betulina (L.:Fr.) Fr.			+				+								
Laetiporus sulphurous (Bull.:Fr.) Murr.	+		+												
Ganodermatales															
Ganoderma lucidum (Curt.:Fr.) P.Karst		X	X	X	X	X	X			X	X	X	X	X	X
Ganoderma applanatum (Pers.) Pat.			+	+	+					+					
Agaricomycetideae															
Agaricales s.l.															
Pleurotaceae															
Lentinus edodes (Berk.) Sing.		X	X	X	X	X		X	X	X	X	X		X	
Pleurotus ostreatus (Jacq.:Fr.) Kumm.			+	+	+			+					+		
Pleurotus pulmonarius (Fr.:Fr.) Quél	+		+					+							
Tricholomataceae															
Flammulina velutipes (Curt.:Fr.) P.Karst.	+	X	X	+						X					
Oudemansiella mucida (Schrad.:Fr.) v. Höhn.	X														
Armillariella mellea (Vahl.:Fr.) P.Karst.	+					X	X						X		
Hypsizygus marmoreus (Peck) Bigel.			X												
Marasmius androsaceus (L.:Fr.) Fr.		X											X		
Agaricaceae															
Agaricus bla\ei Murr.			X												
Agaricus bisporus (J.Lge) Imbach			+							X	X				
Pluteaceae															
Volvariella volvacea (Bull.:Fr.) Sing.			+	+	+			+							
Bolbitiaceae															
Agrocybe aegerita (Brit.) Sing.	+		+					+					+		

X = commercially developed mushroom product (drug or dietary supplement)

\+ = non commercially developed mushroom product.

Ganoderma lucidum and Ganoderma tsugae:

G. lucidum and related species have the longest historical usage for medicinal purposes, dating back at least four millennia (Zhao and Zeuny, 1994). In Japan it is called *Reishi* or *Mannetake* (10,000 year mushroom) and in China and Korea it is variously called *Ling Chu, Ling Chih* and *Ling Zhi* (Mushroom of Immortality). It is the mushroom most depicted in ancient Japanese, Korean and Chinese Art and has been extensively depicted in Chinese royal tapestries. Reishi is also widely used in the Orient as a talisman to protect a person or home against evil. The fungus grows in many parts of the world and in Japan is to be found mainly on old plum trees. Originally, rare and expensive it can now be artificially cultivated, which makes it more accessible and affordable.

The mushroom and mycelium contain steroids, lactones, alkaloids, polyssacharides and triterpenes. Pharmacologically, a number of the water-soluble polysaccharides have demonstrated antitumour and immunostimulating activities. At least 100 different alcohol-soluble triterpenes have been identified including highly oxidised lanostane-type triterpenoids such as ganoderic, ganoderenic, lucidenic, and ganolucidic acids. These triterpenoids have been shown to possess adaptogenic and antihypertensive as well as anti-allergic properties.

Fig. 1a *Ganoderma lucidum* growing naturally on tree stump

Fig. 1b Reishi motif on pavilion door in the Forbidden City, Beijing (Willard 1990**)**

Fig. 1c Contemporary Chinese painting depicting the Phoenix bird holding a Reishi mushroom: both Ancient Chinese symbols of longevity (Willard, 1990)

Fig 1d *G. tsugae*, antler form growing on sterilised sawdust media (Willard, 1990**)**

Fig. 1e *G. lucidum* growing on sterilised sawdust media (Willard, 1990)

This mushroom possesses many different medicinal properties dependent on the stage and environment of its growth (Jong and Birmingham, 1992, Liu, 1999). Traditionally, it has been widely used in the treatment of hepatopathy, chronic hepatitis, nephritis, hypertension, arthritis, neurastheine, insomnia, bronchitis, asthma and gastric ulcers. Scientific studies have confirmed that substances extracted from the mushroom can reduce blood pressure, blood cholesterol and blood sugar levels as well as inhibit platelet aggregations (Table 2). Reishi extracts have been highly effective in alleviating altitude sickness and also in treating myotonia dystrophica. Several major biochemicals such as polysaccharides, proteins and triterpenoids with potent immuno-modulating action have been isolated from *Ganoderma* spp. The major immuno-modulating effects of these active substances include mitogenicity and activation of immune effector cells such as T cells, macrophages and natural killer cells resulting in the production of cytokines, including interleukins, tumour necrosis factor-α and interferons. The therapeutic action of *G. lucidum* as an anti-cancer and anti-inflammatory agent has been associated with its immuno-modulating properties (Wang *et al*., 1977). While the extensive range of traditional medical treatments with this mushroom have not yet been fully substantiated by modern scientific standards they are being extensively scrutinised in the Far East and the USA (Chang, 1995, 1999, Chen and Miles, 1996). In view of its bitter taste and indigestible structure (often similar to varnished wood in appearance) this is not an edible mushroom but, in hot water extracted form, it is available worldwide in tablet and liquid products (Stamets, 1999).

Table 2 Pharmacological effects of whole Reishi extracts *in vivo* and *in vitro* (for references see Hobbs, 1995)

- Analgesic
- Anti-allergic activity
- Bronchitis-preventative effect, inducing regeneration of bronchial epithelium
- Anti-inflammatory
- Antibacterial, against *Staphylococci, Streptococci,* and *Bacillus pneumoniae* (perhaps due to increased immune system activity)
- Antioxidant, by eliminating hydroxyl free radicals
- Antitumor activity
- Antiviral effect, by inducing interferon production
- Lowers blood pressure
- Enhances bone marrow nucleated cell proliferation
- Cardiotonic action, lowering serum cholesterol levels with no effect on triglycerides, enhancing myocardial metabolism of hypoxic animals, and improving coronary artery hemodynamics
- Central depressant and peripheral anticholinergic actions on the autonomic nervous sytem reduce the effects of caffeine and relax muscles
- Enhanced natural killer cell (NK) activity *in vitro* in mice
- Expectorant and antitussive properties demonstrated in mice studies
- General immunopotentiation
- Anti-HIV activity *in vitro* and *in vivo*
- Improved adrenocortical function
- Increased production of Interleukin-1 by murine peritoneal macrophages *in vitro*
- Increased production of Interleukin-2 by murine splenocytes *in vitro*

Key active constituents:
Beta and hetero-Beta-glucans (antitumour, immunostimulating)
Ling Zhi-8 protein (anti-allergenic, immuno-modulating)
Ganodermic acids – triterpenes (anti-allergenic agents, cholesterol and blood pressure reducing)

Estimates place the annual value of *G. lucidum* products worldwide at more than US $ 1.6 billion (Chang and Buswell, 1999).

Lentinus edodes

This fungus is indigenous to Japan, China and other Asian countries with temperate climates. It is to be found in the wild on fallen deciduous trees especially

Fig. 2a *Lentinus edodes* growing naturally on fallen timber

Fig. 2b *L. edodes* fruiting on an oak log (Stametes 1993)

Key active constituents:

Acidic polysaccharides (glucuronoxylomannan) (antitumour, immunostimulatory, antidiabetic, skin enhancing)

Cordyceps sinensis and C. sobolifera

The fungi grow as *parasites* in larvae of Lepidoptera, gradually taking over the entire larval body. The diseased larvae bury themselves in the soil and die. Later the fungal mass or *stroma* grows out of the pupa and can be identified and collected.

The caterpillar fungus or *Tochukaso* has been highly regarded in Chinese medicine for many centuries. It is not a mushroom type fungus and the fruiting structure cannot be cultivated or cultured. The complete structure can be used in many forms, whole, powdered or extracted and has many applications in Chinese medicine (Hobbs, 1995; Halpern, 1999). Anti-cancerr polysaccharides have been isolated from several species of C*ordyceps* and some have been shown to have hypoglycaemic activity as well (Jones, 1997; Itami and Yahagi, 1990; Kun 1998). A major concern with herbal medicine using *Cordyceps* collected from nature is quality and safety.

However, the pure mycelium of these parasitic fungi can now be easily cultivated in fermentors and is attracting considerable interest as an agent to treat fatigue and improve motor function (Mizuno, 1999). The major chemical, pharmacological and toxicological studies on *Cordyceps sinensis* have been reviewed for English and Chinese literature by Zhu *et al*. (1988a,b). These studies show that the main activities of the fungus are in oxygen-free-radical scavenging.

Fig. 12 *Cordyceps* spp. stroma growing out of colonised insects

With this particular fungus it is clear that there will be increased usage of fermenter-produced mycelium. Such methods use selected media under aseptic conditions, providing better quality and homogeneity through process control.

Key active constituents:

Galactomannans (antitumour, immunostimulating)
Cordycepin
Sterols

Schizophyllum commune

This is a small, whitish fungus with no stalk which grows on dead trees throughout the year. It is a very common fungus and has worldwide distribution (Hobbs, 1995). Pharmacologically it is extremely important because it produces the polysaccharide Schizophyllan which shows considerable anti-cancer activity in xenograph and clinical practice. There have been numerous clinical trials with Schizophyllan which will be discussed later (Ooi and Liu, 2000).

Key active constituents:

Beta-glucans (antitumour and immunomodulation).

Fig. 13a *S. commune* growing naturally on dead deciduous tree

Fig. 13b *S. commune* view of underside of fruitbody

Agaricus blazei

This mushroom was first discovered in the USA in the 1940s but its main commercial cultivation now occurs in Japan and Brazil. In Japan it is called *Himematsutake* and is one of the most expensive medicinal mushrooms. A novel polysaccharide-protein complex has been shown to be highly active against a variety of xenographs (Ito *et al*., 1997).

Key active constituents:

Beta (1,3)-D-glucan, Beta (1-4)-D-glucan, Beta (1-6)-D-glucan (antitumour and immune enhancing)
Proteoglucans (antitumour).

Fig. 14 *Agaricus blazei*, Himematsutake or the Almond Portobella, grown in cased leachate cow manure (Stamets, 2000)

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Recent studies have shown that polysaccharides and polysaccharide-protein complexes from this mushroom have significant anti-cancer activity (Hishida *et al*., 1988, Kurashige *et al*., 1997). A limited number of clinical studies in Japan and the USA have shown that a purified fraction of polysaccharide is highly effective against cancers of the breast, lung, liver, prostate and brain. Details of clinical trials will be discussed later. Other fractions from *G. frondosa* exhibit immunological enhancement together with properties of anti-HIV, antihypertension, antidiabetic, and antiobesity (Zhuang and Mizuno, 1999). It is interesting to note that the β-glucan fractions from this mushroom are now being used by over 3,000 health professionals in the US for the prevention and treatment of :

Flu and common infection (bacteria and viruses)

AIDS (HIV)

Diabetes mellitus

Hypertension

Hypercholesterolemia

Urinary tract infections (particularly for women) (Professor Konno – personal communication).

Capsules with dried Maitake form widely accepted dietary supplements and apart from the Far East are now being extensively marketed in US and in Europe. Other examples are Maitake tea, whole Maitake powder and a Maitake drink.

Key active constituents:

1,3 and 1,6 Beta glucans (antitumour and immunomodulating)
Commercial product “Grifolan”

Flammulina velutipes

This is one of the most popular edible mushrooms in China and Japan where it is known as *Enokitake*. In nature it grows on stumps or decayed wood of hardwood trees as a typical mushroom. It is now mostly produced by artificial cutivation from jars of sawdust mix. After growth through the sawdust medium and as the primordia form on the surface, a plastic collar is placed around the neck of the jar and with special environmental conditions, results in the formation of elongated stipes and tiny mushroom heads. While they may be cooked in various ways they can also be used directly in salads. This is a major edible mushroom. It can be slightly salty and bitter in taste and is used in traditional Chinese medicine to treat liver diseases and gastric ulcers. Polysaccharides from this mushroom have been shown to inhibit the growth of cancers in a number of xenograph models. Flammulin, a basic simple protein from *F. velutipes* is able to markedly inhibit tumour cells (Komatsu *et al*., 1963). Flammulin has been purified to a crystalline state and clinical trials are now in progress (Zhang *et al*., 1999). The first scientific paper stating that edible mushrooms were effective against a solid tumour was with *Flammulina*.

A new antitumour glycoprotein has been isolated from cultured mycelium of this fungus - Proflamin. It is useful in combination therapy with other chemotherapy agents (Ikekawa, 1995).

Furthermore, an epidemiological study in Nagano Prefecture, Japan showed that the cancer death rate among farmers producing *F. velutipes* was remarkably lower than that of other people in the Prefecture and in Japan overall (Ikekawa, 2001).

Key active ingredients:

Beta-glucan-protein (antitumour and immunomodulating)
Beta-glycoprotein-Proflamin (antitumour)

Fig. 8a *F. velutipes* growing naturally on tree stump

Fig. 8b *F. velutipes* growing artificially on sawdust mix

Pleurotus ostreatus

The fruit-body of this mushroom is oyster-shaped and hence the common name Oyster Mushroom. It grows in layered clusters on deciduous trees in many parts of the world. It is one of the easiest to grow, most often on straw or sawdust

Fig. 9a *P. ostreatus* growing on decayed timber

logs, and has become one of the most popular edible mushrooms with a pleasant odour and taste. In the Sung dynasty (A.D. 420-479) it was referred to as “the mushroom of flower heaven” (Stamets, 1993, Hobbs, 1995).

Fig. 9b *P. ostreatus* growing on sawdust mixture

The medicinally beneficial effects of *Pleurotus* spp. were discovered independently on different continents. The awareness of their medicinal properties comes not only from Asia but from the folklore of central Europe, South America and African (Gunde-Cimerman, 1999). While first artificially cultivated in USA, production is now worldwide. There have been a number of studies suggesting a

role in numerous diseases with its anti-cancer activity, immunomodulating effects, and antiviral, antibiotic and anti-inflammatory activities. The major cause of death in the Western hemisphere is coronary artery disease with hypercholesterolemia as a primary risk factor. Drug therapy for lowering cholesterol has made considerable use of the pharmacologic agent lovastatin (mevinolin) and its analogues. Species of the genus *Pleurotus* are excellent producers of lovastatin and as such, *Pleurotus* could be considered as a functional food with natural cholesterol-lowering ability (Gunde-Cimerman, 1999). However, large scale production of lovastatin from fruit-bodies is not deemed commercially viable because of variability in fruit-body composition. Lovastatin is normally found only in the lamella and basidiospores and not in the stipe and cap. Mycelial cultivation could be the way ahead.

Key active constituents:

Beta-glucans (antitumour, immunomodulation)
Lovastatin (cholesterol-lowering)

Trametes (Coriolus) versicolor

This is a fungus with many synonyms but *Trametes* is now the accepted genus name. The multicoloured cap resembles a 'turkey tail' and occurs as overlapping clusters on dead logs in most parts of the world. This is not an edible fungus but hot water extracts have been used in traditional Chinese medicine from historical times for a wide range of ailments (Ying *et al*., 1987). Modern studies have produced two extremely important compounds, PSK or "Krestin", a water-soluble protein-bound polysaccharide and PSP, a polysaccharide-peptide both derived from mycelial cultures of the fungus. PSK has been shown to act directly on tumour cells (cytostatic and cytotoxic) as well as indirectly in the host to boost cellular

Fig. 10 *Trametes versicolor* growing naturally on fallen timber

immunity (Tsukagoshi, 1984). PSK also shows antiviral activity through stimulation of interferon production. PSP is a powerful immunostimulant and anti-cancer agent (Yang, 1993, Ng,1998). There have been a wide range of clinical trials for a range of human cancers. In most cases when taken with traditional chemotherapy or radiotherapy there have been significant increases in patient longevity. In 1987 “Krestin” accounted for 25% of the total expenditure of anti-cancer agents in Japan (Fukushima, 1989). A polysaccharopeptide isolated from this mushroom has been shown to inhibit the HIV-1 (Collins and Ng, 1997) while a polysaccharide showed chemopreventitive activity in an *in vitro* model (Kun *et al*., 1999). PSP and PSK are

just beginning to be available in the US and Europe. These compounds will be extensively discussed in later Chapters.

Key active constituents:

Beta-glucan-proteins (antitumour, antiviral, immunomodulating)
Ergopsterol (provitamin D2)

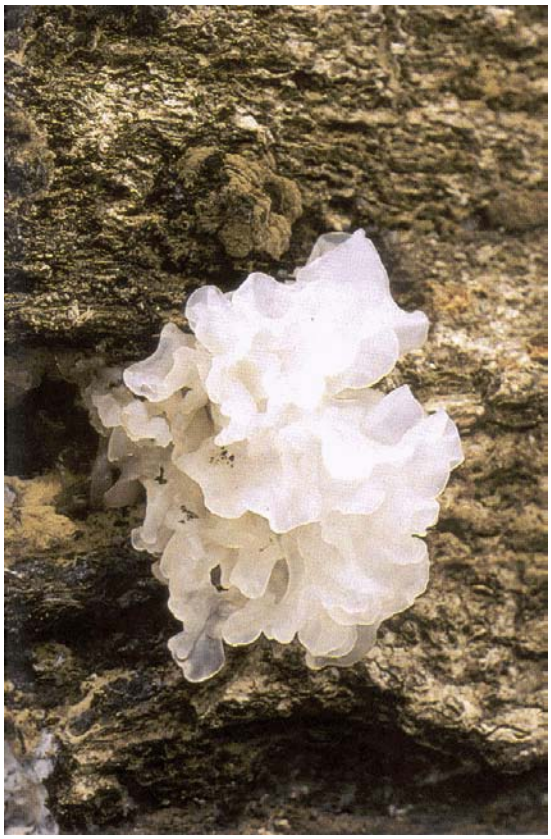
Tremella mesenterica and T. fuciformis

This fungus is commonly known as the “white auricularia” or “white jelly fungus”, and in Japan, *Shirokikurage*, with a jelly-like, translucent fruiting-body which usually grows on deciduous trees in warm climates worldwide. It can now be grown artificially and is being increasingly consumed in Asia.

It has a long historical use in traditional Chinese medicine as an immune tonic and for treating debility and exhaustion together with many other ailments including skin-care. It contains acidic polysaccharides especially glucuronoxylomannan, readily extracted with hot water giving a smooth and stable solution used in Oriental cuisine. The polysaccharides of this fungus show anti-cancer activity and can enhance immune functions (Hobbs, 1995). Clinical trials have shown it to be effective in treating radio- and chemo-therapy-induced leukopenia, boosting immunological functions and stimulating leukocyte activity (Hu and But, 1987). Med Myco Ltd. (Israel) have developed a submerged fermentation method to produce Tremellastin from *T. mesenterica* mycelium which contains 50% glucuronoxylomannan, together with proteins rich in amino acids, dietary fibre and vitamins of the B group. Dietary supplements from *Tremella* are only now beginning to expand into the Asian market, and they will certainly be of special significance in the cosmetic industry.

Fig. 11a *T. mesenterica* growing naturally on deciduous tree

Fig. 11b *T. fuciformis* growing naturally on deciduous tree



CHAPTER 4: TECHNOLOGY OF MUSHROOM CULTIVATION

Synopsis

This Chapter deals with the principles and practices of gourmet and medicinal mushroom cultivations. It stresses that successful cultivation involves the interaction of scientific knowledge and practical experience. Annual world production of gourmet and medicinal mushrooms is now estimated in excess of 14 billion US dollars. The operations essential to successful cultivation involve: selection of mushroom spores or strains, maintenance of mycelial cultures, development of spawn or inoculum, preparation of growing medium, inoculation and colonization, and crop management for optimum production. However, for greater pharmaceutical acceptance it is increasingly being recognized that product formation by way of fermenter-grown mycelial biomass will be the preferred option in many cases.

Introduction

Mushroom science is the discipline that is concerned with the principles and practices of mushroom cultivation. As is true in any branch of science, it is essential to establish the facts upon which principles can be derived for future developments of the discipline. Consistent production of successful mushroom crops will be built upon scientific knowledge and practical experience (Chang and Miles, 1989).

Rhere are at least 12,000 species of fungi that can be considered as mushrooms with at least 2,000 species showing various degrees of edibility (Chang, 1999a). Furthermore, over 200 species of mushroom have been collected from the wild and utilized for various traditional medical purposes mostly in the Far East. To date, about 35 mushroom species have been cultivated commercially and of these, about 20 are cultivated on an industrial scale (Table 1). The majority of these cultivate species are both edible and possess medicinal properties. However, two of the major medicinal mushrooms, viz. *Ganoderma lucidum* and *Trametes (Coriolus)*

spp. are distinctly inedible. Overall, the world production of cultivated edible and/or medicinal mushrooms was recorded as 4,909.3 x 10^3 tons in 1994 increasing to 6,158.4 x 10^3 in 1997 with an estimated value in excess of 14 billion US dollars (Chang, 1999b).

Mushroom cultivation is a worldwide practice (Table 2). In percentage terms, output yield of the leading 10 species cultivated made up *c* 92% of total world production of these six species, viz: *Agaricus bisporus* (31.8%); *Lentinus edodes* (25.4%), *Pleurotus* spp. (14.2%), *Auricularia auricula* (7.9%), *Flammulina velutipes* (4.6%), and *Volvariella volvaceae* (7.9%), made up 87% of the total production. It can be further observed that by late 1994, of these six species only *Agaricus* and *Pleurotus* were cultivated worldwide to be joined in 1997 by *Lentinus*. The other three of the major six species are grown almost exclusively in Asia (Chang, 1999b).

World production of mushrooms over the last two decades has shown a phenomenal pattern of growth (Table 1), with a 5 times increase in tonnage. While the white button mushroom (*Agaricus bisporus*) still retains the highest overall world production, its relative contribution is decreasing due to the dramatic increase in the other species, viz: *Lentinus* and *Pleurotus* in particular. In 1981, *Agaricus* production represented 72% of world production but by 1997 this had dropped to 32%. Overall, world production of mushrooms is increasingly being dominated by species that are both edible and have medicinal properties.

Table 1 World Production of Cultivated Edible and Medicinal Mushrooms in Different Years (Chang, 1999b)

	1981		1986		1990		1994		1997	
	Fresh Wt X 10^3		Fresh Wt X 10^3		Fresh Wt X 10^3		Fresh Wt X 10^3		Fresh Wt X 10^3	
Species	**Metric tons**	**%**	**Metric tons**	**%**	**Metric tons**	**%**	**Metric tons**	**%**	**Metric tons**	**%**
Agaricus bisporus/ bitorquis	900.0	71.6	1,227.0	56.2	1,420.0	37.8	1,846.0	37.6	1,955.9	31.8
Lentinus edodes	180.0	14.3	314.0	14.4	393.0	10.4	826.2	16.8	1,564.4	25.4
Pleurotus spp.	35.0	2.8	169.0	7.7	900.0	23.9	797.4	16.3	875.6	14.2
Auricularia spp.	10.0	0.8	119.0	5.5	400.0	10.6	420.1	8.5	485.3	7.9
Volvariella volvacea	54.0	4.3	178.0	8.2	207.0	5.5	298.8	6.1	180.8	3.0
Flammulina velutipes	60.0	4.8	100.0	4.6	143.0	3.8	229.8	4.7	284.7	4.6
Tremella spp.	-	-	40.0	1.8	105.0	2.8	156.2	3.2	130.5	2.1
Hypisizygus spp.	-	-	-	-	22.6	0.6	54.8	1.1	74.2	1.2
Pholiota spp.	17.0	1.3	25.0	1.1	22.0	0.6	27.0	0.6	55.5	0.9
Grifola frondosa	-	-	-	-	7.0	0.2	14.2	0.3	33.1	0.5
Others	1.2	0.1	10.0	0.5	139.4	3.7	238.8	4.8	518.4	8.4
Total	1,357.2	100.0	2,182.0	100.0	3,763.0	100.0	4,909.3	100.0	6,158.4	100.0
Increasing %			73.6		72.5		30.5		25.4	

It is pertinent to note that world production of mushrooms is now dominated by China with over 64% of total production. China has become a major producer and consumer of both edible and medicinal mushrooms. Furthermore, China is also the major producer of the non-edible medicinal mushrooms, e.g. *Wolfiporia (Poria) cocos* (10,000 tons) and *Ganoderma lucidum* (4,000 tons) (see Chapter 3). At least 10 new species of edible or medicinal mushrooms have been brought into cultivation in China in recent years and although as yet on a small scale, the potential, especially for mushrooms of medicinal value, is quite significant. Because of their historical background in the use of wild mushrooms, both as food and in Chinese traditional medicines, it is to be expected that China will continue to develop methods for cultivation of an increasing number of, as yet, uncultivatable mushrooms for medicinal exploitation. The traditional acceptance of mushrooms in herbal medicine and in expanding pharmaceutical industries, will ensure that China will continue to be a major exploiter of medicinal mushroom technology (Yamanake, 1997). China is also investing heavily in fermenter technology for growing mushroom mycelium of medicinal species.

Historically, mushrooms were gathered from the wild for consumption and for medicinal use. China has been the source of many early cultivations of mushrooms, e.g. *Auricularia auricula* (600 AD), *Flammulina velutipes* (800 AD), *Lentinus edodes* (1000AD) and *Tremella fuciformis* (1800). *Agaricus bisporus* was first cultivated in France in *c* 1600 while *Pleurotus ostreatus* was first grown in US in 1900. While mushroom cultivation now spans many centuries, it is only over the last 2-3 decades that there have been major expansions in basic research and practical knowledge leading to the creation of a major worldwide industry (Chang and Miles, 1989).

Table 2 World population of cultivated edible and medicinal mushrooms in 1997 (Metric tons) (Chang, 1999b)

	China	Japan	Rest of Asia	North America	Latin Amreica	EU	Rest of Europe	Africa	China	Total	%
Agaricus bisporus	330000	-	68400	425300	51600	875000	115200	36000	54400	1955000	31.8
Lentinus edodes	1397000	115300	47400	3600	300	500	300	-	-	1564400	25.4
Pleurotus spp.	760000	13300	88400	1500	200	6200	5800	200	-	875600	14.2
Auricularia spp.	48000	-	5300	-	-	-	-	-	-	485300	7.9
Volvariella volvacea	12000	-	60800	-	-	-	-	-	-	180800	3.0
Flammulina spp.	15000	10900	25700	-	-	-	-	-	-	284700	4.6
Tremella spp.	13000	-	500	-	-	-	-	-	-	130500	2.1
Hypsizygus marmoreus	2100	7200	100	-	-	-	-	-	-	74200	1.2
Pholioto nameko	3100	24500	-	-	-	-	-	-	-	55500	0.9
Grifola frondosa	2000	3100	-	-	-	-	-	-	-	33100	0.5
Hericium erinaceus	800	-	-	-	-	-	-	-	-		
Coprinus comatus	500	-	-	-	-	-	-	-	-	520800	8.4
Others	514900[a]	2900	800	400	-	200	100	-	200		
Total	3918300	368000	297400	430800	52100	881900	121400	36200	54600		
%	63.6	6.0	4.8	7.0	0.8	14.3	2.0	0.6	0.9	6160800	100

The cultivation of *Agaricus bisporus* is an outstanding example of a biotechnological enterprise that challenges the combined skills of industrial and biological technologies. *A. bisporus* cultivation in Western countries has achieved its current pre-eminence in the mushroom industries because of a solid foundation in basic scientific research in all aspects of *Agaricus* biology (genetics, physiology, biochemistry), bioprocess technology and, above all, the use of modern management principles (Chang *et al*., 1996). This foundation made possible a highly technical approach involving the creation and utilization of specialized equipment and advanced engineering technology. While *Agaricus bisporus* is a highly tasty and nutritious mushroom, it does not appear to have been used for any specific medical conditions. However, much fundamental knowledge has been acquired in recent years which will be of considerable value for other cultivations.

Mushroom cultivation technology

Mushrooms can be cultivated through a variety of methods. Some methods are extremely simple and demand little or no technical expertise. On the other hand, cultivations which require aspects of sterile handling technology are much more technically demanding (Chang and Miles, 1989). In the context of the present report, the simple and advanced methods for the cultivation of the Shiitake mushroom (*Lentinus edodes*) will be highlighted (Stamets, 1993, 2000; Stamets and Chilton (1983). This is a leading medicinal mushroom and, furthermore, many of the other important medicinal mushrooms are wood utilisers and have been easily grown by modifications of these methods. Detailed descriptions of the many growing techniques can be found in the Royce *et al*. (1985), Przbylowicz and Donoghue (1989) and Kozak and Krowczy (1999).

Mushroom cultivation involves several different operations each of which must be performed properly if the enterprise is to be successful. Failure of any phase will result in a decreased harvest or total loss (Table 3).

Table 3 Operations involved in mushroom cultivation

Selection of mushroom spores or strains
Maintenance of mycelial cultures
Development of spawn/inoculum
Preparation of growing medium
Spawn inoculation and colonisation of substrate
Crop management for mushroom production

Strain selection and maintenance:

The first stage in any mushroom cultivation process is to obtain a pure mycelial culture of the specific mushroom strain. Such cultures are now readily purchased from mushroom specialists, mushroom enterprises or mushroom institutes (Stamets, 1993). Such cultures have originally been derived from single or multi-spore cultures or by tissue culture from a mushroom of a high yielding and vigorous strain. Many strains have been developed by considerable genetic breeding programmes. Each type of mushroom culture generally requires unique substrate formulation for propagation and maintenance of purity. This information is normally freely available in the literature. Most growers will obtain spawn cultures from reputable production centres – ensuring purity, vigour and supply when required.

Spawn production:

For large scale production of mushrooms, large quantities of the specific inoculum are required (often 1-5% of the final mushroom production medium). In mushroom growing technology the inoculum is known as the “spawn”. Spawn is a medium that is impregnated with mycelium made from a pure culture of the chosen mushroom strain. Spawn production is a fermentation process in which the mushroom mycelium will be increased by growing through a solid organic matrix under controlled environmental conditions. In almost all cases the organic matrix will be sterilised grain, e.g. millet, rye or wheat. The purpose of the grain spawn is to boost the mycelium to a state of vigour such that it will rapidly colonise the selected bulk growing substrate. The grain is an important nutrient support as well as a vehicle for the eventual even distribution into the growing medium of the mushroom inoculant. Each individual grain becomes coated with the mycelium and in fact becomes a mycelial capsule (Fig. 1). All operations from pure culture isolation through spawn preparation must be conducted under sterile techniques and performed as rapidly as possible to lessen the possibility for contamination to occur.

An extensive technology has been developed throughout the world to ensure the production of high quality mushroom spawn. Many companies now specialize only in the production of mushroom spawn and can ship active spawn to growers when required. Most spawn is now prepared and shipped in autoclavable polypropylene bags with breathing patches. For natural log production of mushrooms the inoculum or spawn can also be in the form of wood chips coated with the specific mushroom strain.

Fig. 1 Colonisation of grain at 3 and 8 days after inoculation (Stamets and Chilton, 1983)

Mushroom production – Log Culture:

Most medicinally important mushroom species can grow as saprophytes on dead wood – primary decomposers. For this reason, *Lentinus edodes* will be used as the model system. Log culture for *Lentinus* or Shiitake was developed in Japan and China over 1000 years ago and is still widely used by small growers in Asia for sale in local markets. The advantage of log culture is that it is a simple and natural method but with the disadvantages that the process is labour-intensive and slow in comparison to growing mushrooms in sterilised sawdust mixtures. Log cultivation is not technically demanding and is relatively easy to carry out – but is seasonal and cannot meet demands for high productivity.

The logs (c 3' long x 6-8" diameter) are cut in winter or early spring from fast growing deciduous species, e.g. alder, poplar, oak, cottonwood which have thick outer bark. Logs can be inoculated with spores or with mycelial plugs inserted into drilled holes and then stacked in piles with mild to heavy soaking. Mycelial growth

through the log will occur over several months and the logs are then placed in an upright position partly embedded in the soil. Mushroom production will then occur primarily in the cooler spring and autumn months. Since this is an open, non-sterile procedure contamination with other wood rotting mushroom species can also occur (Fig. 2). Increased environmental control can be achieved by having overhead protection or even growth in greenhouses.

This is still a traditional method especially in China employing thousands of people but has only been used to a limited extent in USA and Europe where it is not commercially worthwhile. However, it generally produces high quality mushrooms and can be an interesting amateur method for producing gourmet and medicinal mushrooms. While the original recognition of the medicinal properties of these mushrooms came from natural growing systems in the forests most production for edible and medicinal purposes is now derived from the artificial log method.

Mushroom production – enriched sawdust culture:

While many wood utilising gourmet and medicinal mushrooms have traditionally been cultivated on hardwood logs outdoors in the natural environment an alternative, more intensive and regulated cultivation technique has been developed in several Asian laboratories over the last 2-3 decades. The success of this new approach largely reflects the major increase in world production of wood utilising mushrooms (Table 1).

In this innovative approach various hardwood sawdust or wood chips supplemented with nitrogen-rich additives such as rice bran (though other cereal brans work adequately) are mixed together and then compacted into special autoclavable polypropylene bags of various dimensions (Stamets, 1993, 2000; Yamanake, 1997). The bags are then autoclaved to ensure complete internal

Fig. 2 *Lentinus edodes* fruiting on oak logs (Stamets and Chilton, 1983)

sterility, allowed to cool to c. 20^{o}C and then aseptically inoculated with the desired amount of spawn. This stage demands complete sterile handling techniques and any relaxation of standards allows microbial contamination with concomitant financial losses. The inoculated bags – sometimes known as space bags or artificial logs, can then be moved to growing rooms with computer controlled environments giving accurate humidity and temperature conditions.

While formulations of the sawdust/supplement media are easily obtained from the literature, successful producers retain a strong element of secrecy with the exact composition of the supplements. Following inoculation the bags are stacked on trays or suspended from wires for several weeks during which time the mycelium grows

through the sawdust mix secreting enzymes which degrade the complex macromolecules of the substrate – lignin, cellulose, hemicellulose – the breakdown products being absorbed by the advancing mycelium. When the mycelium has reached maturity the log is given a cold temperature shock for 12-24h, restacked and the bag opened and within a few days the mushrooms develop (Fig. 3).

Overal, this new method greatly shortens the production time and gives much higher yields. Using natural log cultivation the time from spawning to harvesting of mushrooms can be between 8 months to one year with complete exhaustion of the log up to 3 years. 100 kg natural log can produce c. 10-15 kg fresh mushrooms over this period. In contrast, with the synthetic log, mushrooms can be harvested about 80 days after spawning and completion of economic production from then takes less than 6-8 weeks. 100 kg sawdust plus supplement can produce c. 80 kg fresh weight of mushrooms, with even higher yields regularly possible. This represents at least an 8-10 fold greater production than the natural log method.

Fig. 3 Production of *Lentinus edodes* from artificial logs (Stamets, 2000)

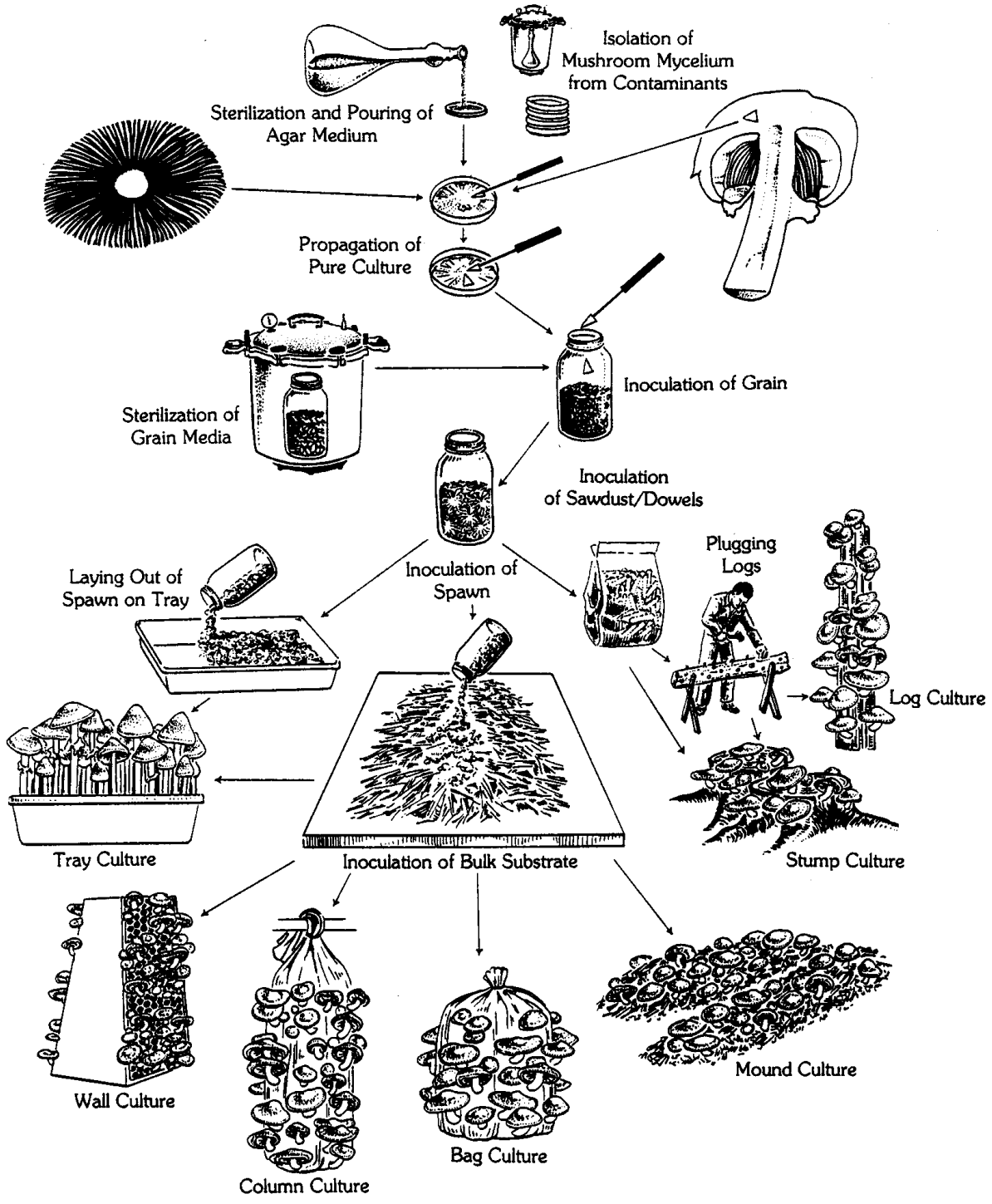
Crop management for production:

The whole process of mushroom production requires continued and careful attendance. Correct control of ambient temperature and humidity are highly critical together with a full understanding of potential microbiological contamination especially from other fungi. In many parts of the world pesticides will be employed – especially fungicides. However, the high premium paid for organic mushrooms necessitates that good management practices must be in operation to avoid the need for pesticides. This is especially important when concentrated powdered mushrooms are consumed as nutriceuticals.

At present most wood utilising gourmet and medicinal mushrooms are imported from Europe and the Far East especially China. In many cases, quality standards are dubious. However, a new production facility has now been established in Kent, which is now producing high quality fresh *Lentinus edodes* to the market and a wide range of other gourmet and medicinal mushrooms are planned.

At present the medicinal mushrooms are available worldwide as fresh mushrooms produced by either of the two main growing methods. Such mushrooms can be dried or extracted in various ways to obtain concentrated extracts of the potent and unique health enhancing medicinal products. Further purification has produced several pharmaceutical grade products now used for cancer therapy procedures in Japanese hospitals. An overview of the various techniques for growing mushrooms is shown in Fig. 4.

Fig. 4 Diagram illustrating overview of general techniques for the cultivation of mushrooms (Stamets and Chilton, 1983).



Mycelial production – liquid tank fermentation

In this approach the need for the mushroom fruitbody is bypassed with the mycelium of the medicinal mushroom being cultivated in deep-tank liquid

fermentation culture. This is a relatively new approach and if the important medicinal compounds can be produced in this way it will lead to major innovations and product diversity. Furthermore, the ability to use pure substrates and controlled growth environments will aid in the final purity of the products. How essential the formation of the complex fruitbody is in the final determination of product type and variety has still to be shown. However, it has already been shown that the medicinally important polysaccharides can be produced in this way as seen with PSK and PSP from *Trametes versicolor*. The growth of filamentous fungal mycelium in fermenters is well understood especially in the antibiotic industry. However, Basidiomycetes do have slower growth rate and lower yields when compared with, for example, *Penicillium* and *Streptomyces*.

A further advantage of this approach would be the mycelial cultivation of medicinal mushroom species that so far have defied axenic culture, e.g. many mycorrhizal species. It is now clear that liquid fermentation methods are becoming an important means of producing more uniform mycelial biomass from several types of medicinal mushrooms for product extraction and purification. This will generate nutraceutical and pharmaceutical products from medicinal mushrooms that can achieve higher quality standards and safety (see Chapter 9 for full details).

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CHAPTER 5: EXTRACTION, DEVELOPMENT AND CHEMISTRY OF ANTI-CANCER COMPOUNDS FROM MEDICINAL MUSHROOMS

Synopsis

The main antitumour compounds presently isolated from mushroom fruit-bodies, submerged cultural mycelial biomass or liquid culture broth have been identified as either water soluble β-D-glucans with heterosaccharide chains of xylose, mannose, galactose or uronic acid or β-D-glucan-protein complexes – proteoglycans. Methods of extraction and purification are outlined. Levels of anti-cancer activity are related to molecular weight, degree of branching and solubility in water of the respective molecules. The main medically important polysaccharide compounds to have achieved clinical relevance, viz. Lentinan, Schizophyllan, PSK and PSP, and Grifron-D are discussed.

Hot water extracts of many mushrooms used in traditional Chinese medicine and other folk medicines have long been said to be efficacious in the treatment of various diseases including many forms of cancer. The use of medicinal mushroom extracts in the fight against cancer is well known and documented in China, Japan, Korea, Russia and now increasingly in the USA (Mizuno *et al*., 1995). However, it is only within the last three decades that chemical technology has been able to isolate the relevant compounds and use them in controlled experiments. They have been extensively screened for medical properties especially for anticancer application (Mizuno, 1999). Many species of mushrooms have been found to be highly potent immune system enhancers, potentiating animal and human immunity against cancer (Wasser and Weis, 1999a, Borchers *et al*., 1999, Kidd, 2000; Ikekawa, 2000; Feng *et al*., 2001). While at least 30 mushroom species have yielded compounds with pronounced anticancer actions in xenographs only a small number have taken the next step, viz. objective clinical assessment for anticancer potential in humans.

Polysaccharides

Polysaccharides are a structurally diverse group of biological macromolecules of widespread occurrence in nature. They are composed of repetitive structural features that are polymers of monosaccharide residues joined to each other by glycosidic linkages. In this way they differ structurally from proteins and nucleic acids. Polysaccharides present the highest capacity for carrying biological information since they have the greatest potential for structural variability. The amino acids in proteins and the nucleotides in nucleic acids can interconnect in only one way while the monosaccharide units in oligosaccharides and polysaccharides can interconnect at several points to form a wide variety of branched or linear structures (Sharon and Lis, 1993). As a consequence, this enormous potential variability in polysaccharide structure allows for the flexibility necessary for the precise regulatory mechanisms of various cell-cell interaction in higher organisms such as man.

Many, if not all, Basidiomycete mushrooms have been shown to contain biologically active antitumour and immunostimulative polysaccharides. In a recent review Reshetnikov *et al*. (2001) have listed 650 species and 7 intraspecific taxa from 182 genera of higher Hetero- and Homo-basidiomycetes that contain pharmacologicaly active polysaccharides that can be derived from fruit-bodies, culture mycelium and culture broths. In general, there is normally a higher level and number of different polysaccharides extracted from fruit-bodies than from the other cultural sources. As discussed in Chapter 9 an important direction for future studies on mushroom polysaccharides will be by submerged fermenter culture to produce reliable, consistent and safe products.

The first definitive studies on these anticancer substances came in the late 1960s with the reports by Ikekawa *et al.* (1968, 1969) and Chihara *et al*. (1969, 1970). They demonstrated that extracts of several different mushroom species exhibited remarkable host-mediating antitumour activities against xenographs, e.g. Sarcoma 180. These observations brought immediate public attention. In both studies the compounds were easily extracted with hot water, and shown to be various types of polysaccharides. The polysaccharides are non-toxic and appear to affect tumours indirectly following administration, suggesting that the anticancer action is mainly host-mediated. The species xenograph, suitable dosage and schedule, are essential to achieve the anti-tumour effects (Jong and Donovick, 1989, Jong *et al*., 1991).

Antitumour polysaccharides isolated from mushrooms (fruit-body, submerged cultured mycelial biomass or liquid culture broth) are either water-soluble β-D-glucans, β-D glucans with heterosaccharide chains of xylose, mannose, galactose, and uronic acid or β-D-glucan-protein complexes – proteoglycans (Table 1). As a general rule the protein-linked glucans have a greater immunopotential activity than the corresponding glucans. Polysaccharide antitumour agents that have been developed commercial in Japan are shown in Table 2 and Fig. 1 (Mizuno, 1999).

Table 1 Antitumour active polysaccharides isolated from medicinal higher Basidiomycete mushrooms (from Wasser and Weis, 1999b).

Taxa	Fruiting body	Submerged cultured mycelial biomass	Liquid cultured broth
1	2	3	4
Phragmobasidiomycetes			
Auriculariales			
Auriculariacea			
Auricularia auricula-judae (Bull.) Wettst.	(1-3)- β-glucan	-	-
Tremellales			
Tremellaceae			
Tremella fuciformis Berk.	Glucuronoxylomannan, T-7, T-19 (exopolysaccharides), mannose, xylose, glucuronic acid	Glucuronoxylomannan	Xylose, glucuronic acid, mannose
T. mesenterica Ritz.:Fr.	β-D-glucuronosyl (epitope)	-	
Homobasidiomycetes			
Aphyllophoromycetideae			
Ganodermatales			
Ganodermataceae			
Ganoderma lucidum (Curt.:Fr.) P. Karst.	FI-1a (β-glucan), FIII-2b (hetero-β-glucan), acidic heteroglucan, chitin xyloglucan	-	β-glucan
G. applanatum (Pers.) Pat.	FI-1-B-1 (β-glucan)	F-1a-1-b (β-glucan), heteroglucans, peptidoglucans	-
G. tsugae Murr.	Heteroglucan, heterogalactan, β-glucan, glucan	Heteroglucan, α-glucan	-

Taxa	Fruiting body	Submerged cultured mycelial biomass	Liquid cultured broth
1	2	3	4
Polyporales			
Schizophyllaceae			
Schizophyllum commune Fr.:Fr	-	-	Sonifilan, SPG or Schizophyllan (β-glucan)
Polyporaceae			
Dendropolyporus umbelliatus (Pers.:Fr.) Jül.	GU-2, GU-3, GU-4, AP (β-glucan)	-	β-glucan
Grifola frondosa (Dick.:Fr.) S.F. Gray	Grifolan (β-glucan), Fa-1a-β (acidic β-glucan), FIII-2c (hetero-β-glucan), xyloglucan, mannoglucan, fucomannoglucan	Heteroglucan protein, manogalactofucan, heteroxylan, fucoxylan, galactomannoglucan	-
Fommes fomentarius (L.:Fr.) Fr.	β-glucan	β-glucan	-
Fomitopsis pinicola (Schw.:Fr.) P.Karst	F-1a-2-β (β-glucan) α-(1-6)-linked	α- and β-glucan	-
Albatrellus confluens (Alb. et Schw.:Fr.) Kotl. et Pouz.	(1-3)- β-D-glucan	(1-3)- β-D-glucan	-
Trametes versicolor (L.:Fr.) Lloyd	β-glucan	Coriolan, PSK, Krestin (β-glucan -protein)	-
Lenzites betulinus (L.:Fr.) Fr.	β-glucan	-	-
Wolfiporia cocos (Schw.) Ryv. et Gilbn.	Pachymaran (β-glucan)	-	-
Hericium erinaceus (Bull.:Fr.) Pers.	β-glucoxylan, glucoxylan protein, galactoxyloglucan protein	-	-
Ionotus obliquus (Pers.:Fr.) Bound.et Sing.	Polysaccharide fraction in the Allium-test	-	-

Taxa	Fruiting body	Submerged cultured mycelial biomass	Liquid cultured broth
1	2	3	4
Gasteromycetideae, Phallaceae			
Dictyophora indusiata Fisch.	T-2 HN (O-acetylated-(1-3)- β-D-mannan), T-3-M[1] (α-(1-3) linked D-mannan) , T3-G, T-4-N, T-5-N (three kinds of β-D-glucans), T-3 Ad (Neutral heterogalactan)	-	-
Phallus impudicus L.:Pers.	Pl-2 (glucomannan)	PI-2 (glucomannan)	-
Lentinus edodes (Berk.) Sing.	Lentinan (β-D-glucans)	KS-2-a-mannan-peptide, LEM, LAP (heteroglucan-protein), EP3	LEM, LAP (heteroglucan-protein), EP3
Pleurotus ostreatus (Jacq.:Fr.) Kumm.	Acidic polysaccharide fraction, HA (β-glucan)	-	β-glucan, heteroglucan
P. chitrinopileatus Sing.	Heteroglucan, β-glucan-protein, glycoprotein (FI, FII, FIII)	-	-
P. pulmonarius (Fr.:Fr.) Quél. (=P.sajor-caju Fr.:Fr.) Tricholomataceae	Xyloglucan, xylanprotein	-	-
Panellus serotimus (Pers.:Fr.) Kühn.	Heteroglucan, (1-6)- β-d-glucosyl-branched (1(2-3)- β-D-glucans	-	-
Omphalina epichysium (Pers.:Fr.) Quél	EL-2 (β-glucan)	-	-
Flammulina velutipes (Curt.:Fr.) P.Karst.	EA_6, EA_6-PII (β-glucan-protein)	Proflamin (glycoprotein)	-

Taxa	Fruiting body	Submerged cultured mycelial biomass	Liquid cultured broth
1	2	3	4
Leucopaxillus giganteus (Fr.)Sing.	Mannoxyloglucan, heteroglucan, glucan, xyloglucan, xylogalacetoglucan, galactoxyloglucan	-	-
Hypsizygus marmoreus (Peck) Bigel.	β-(1-3)-D-glucan	-	-
Agaricaceae			
Agaricus blazei Murr.	FI_1-a-β (β-glucan), FIII2-β (β-glucan-protein), FA-1a-β (hetero-β-glucan), FA-2b-β (RNA), FV-1 (insoluble β-glucan)	ATOM (glucomannan-protein)	AB-FP (mannan-protein)
A. bisporus (J.Lge) Imbach	β-glucan	-	-
Pluteaceae			
Volvariella volvacea (bull.:Fr.) Sing.	VVG (β-1-3)-D-glucans, α-manno-β-glucan	-	-
Strophariaceae			
Pholiota nameko (T.Ito) S.Ito et Imai	Galacto-β-glucan	-	-
Crepidotaceae			
Crepidotus mollis (Schaeff.:Fr.) Kumm.	CPS (β-glucan)	-	-
Bolbitiaceae			
Agrocybe aegerita (Brit.) Sing.	α-(1-3)- β-glucans	-	-

Table 2 Polysaccharide antitumor agents developed in Japan (immunotherapeutical drugs as biological response modifiers, BRM) (Mizuno, 1999)

Name of drug	Krestin	Lentinan	Sonifilan
Abbreviation	PSK	-	SPG
Common Name	Krestin	Lentinan	Schizophyllan
Company	Sankyo, Kureha	Ajinomoto, Yamanouchi, Morishita	Taito, Kaken
Marketed date	May 1977	December 1985	April 1986
Fungus (origin)	*Trametes versicolor* (mycelium)	*Lentinus edodes* (fruit body)	*Schizopyllum commune* (medium product)
Polysaccharide Structure	β-glucan-protein -1,6- branching -1,3: 1,4-main chain	β-glucan -1,6-branching -1,3-main chain	β-glucan β-1,6-branching β-1,3-main chain
MW	100,000	500,000	450,000
Specific rotation	-	+ 14-22^{o} (NaOH)	+ 18-24^{o} (water)_
Pharmaceutical	1-g sack	1-mg vial	20-mg ampoule (2 ml)
Price	¥ 1,000	¥ 9,500	¥ 9,500
Dose route	p.o.	i.p., i.v.	i.p., i.v.
Cancer treated	Cancer of digestive organ, lung and breast	Cancer of stomach	Cervical cancer

Exopolysaccharides in culture media can be extracted by simply adding 96% ethanol (volume ratio 1:1), the precipitate collected by centrifugation, dissolved in distilled water and dialysed against distilled water for 2 days. The homogeneity of the exopolysaccharides can then be analysed by gel filtration through Sephadex G-200 (Babitskaya *et al*., 2000).

Fig. 1 Three mushrooms from which the antitumour polysaccharide agents have been developed in Japan and China. A: Krestin (PSK) from *Trametes versicolor* (mycelium); B: Lentinan from *Lentinus edodes* (fruit body); and C. Schizophyllan from *Schizophyllum commune* (medium product) (Mizuno, 1999).

Extraction, fractionation, purification and chemical modification

There is a broad similarity in the various methods that have been developed to extract the anti-cancer polysaccharides from mushroom fruit-bodies, mycelium and liquid media (Mizuno, 1999).

In the initial step dried mushroom powder or mycelium is repeatedly heated in 80% ethanol to extract and eliminate low molecular weight substances. Crude fractions 1, 11 and 111 are obtained from the remaining ethanol extract residue by extraction with water (100^{o}C, 3h), 1% ammonium oxalate (100^{o}C, 6h) and 5% sodium hydroxide (80^{o}C, 6h) in that order (Fig. 2). Further purification of the polysaccharides are achieved by a combination of techniques including ethanol concentration, fractional precipitation, acidic precipitation with acetic acid, ion-exchange chromatography, gel filtration and affinity chromatography (Fig. 3).

There is a growing interest in increasing the activity of medicinal mushroom polysaccharides by various chemical modifications and perhaps creating a range of semi-synthetic compounds not unlike the penicillin story. Chemical modification can be achieved by oxido-reductohydrolysis (Smith degradation) and also by formolysis. Some positive improvements in activity have been recorded but it is still at a very early stage (Mizuno, 1999).

A recent study by Yap and Ng (2001) has established a more efficient procedure for the extraction of β-D-glucans from *Lentinus edodes* (Fig. 4). The β-D-glucan was isolated through ethanol precipitation and freeze-drying in liquid nitrogen. Purity testing, using a carbohydrate analysis column, gave 87.5% purity. From a commercial aspect this method is less time-consuming, more efficient and of relatively low cost when compared to the original Chihara *et al*. (1970) and Mizuno (1999) methods (Table 3).

Fig. 2 Fractional preparation of polysaccharides from mushrooms (Mizuno, 1999).

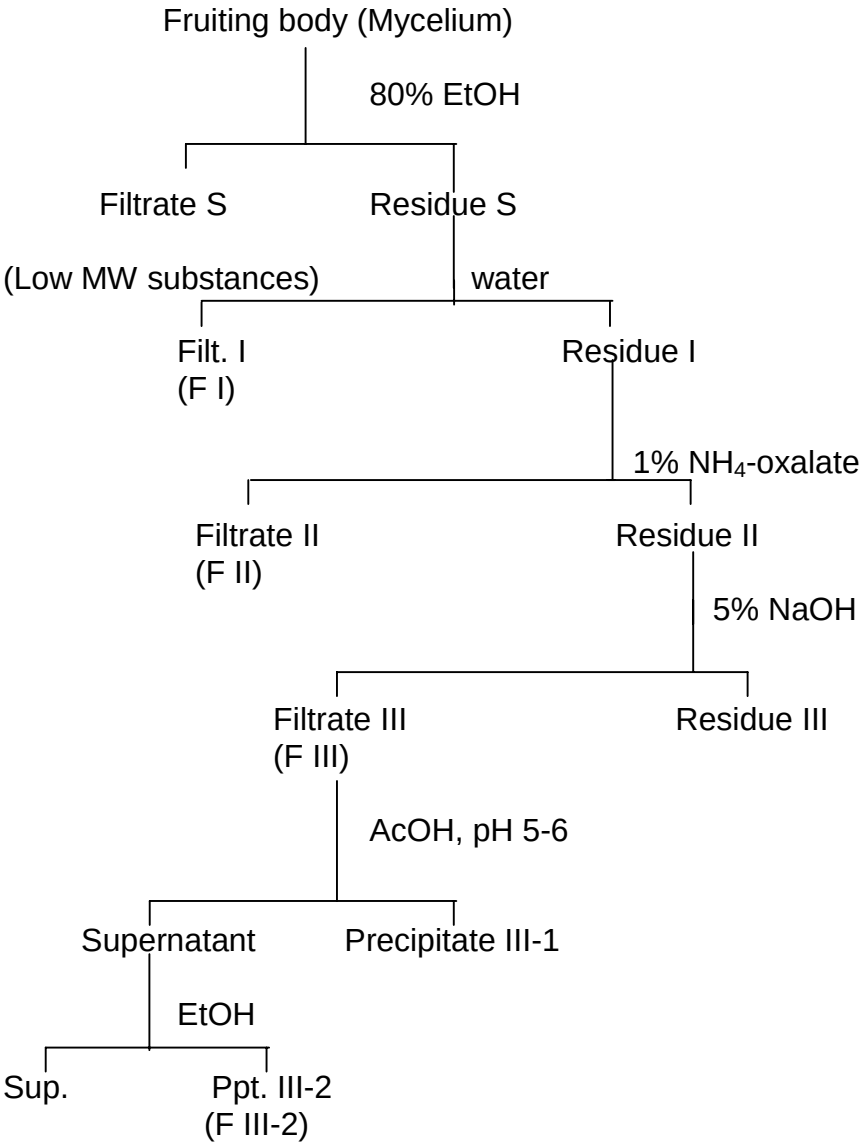


Fig. 3. Fraction purification of polysaccharides by chromatography (Mizuno 1999).

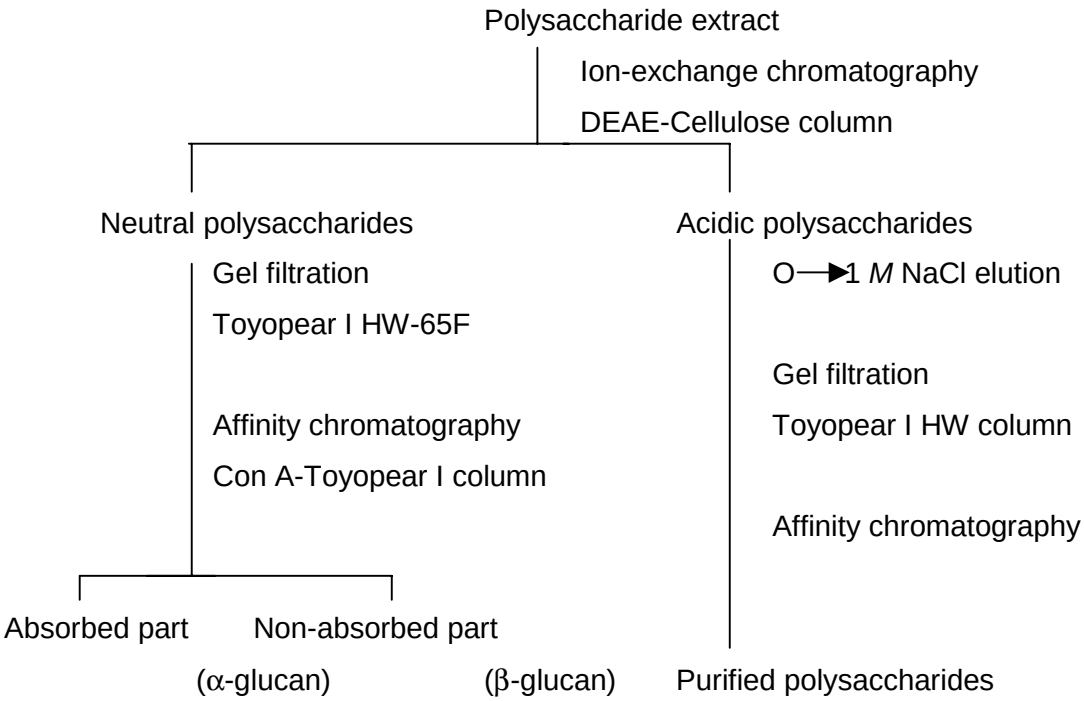


Fig. 4 New method for extracting lentinan from *Lentinus edodes* (Yap and Ng, 2001).

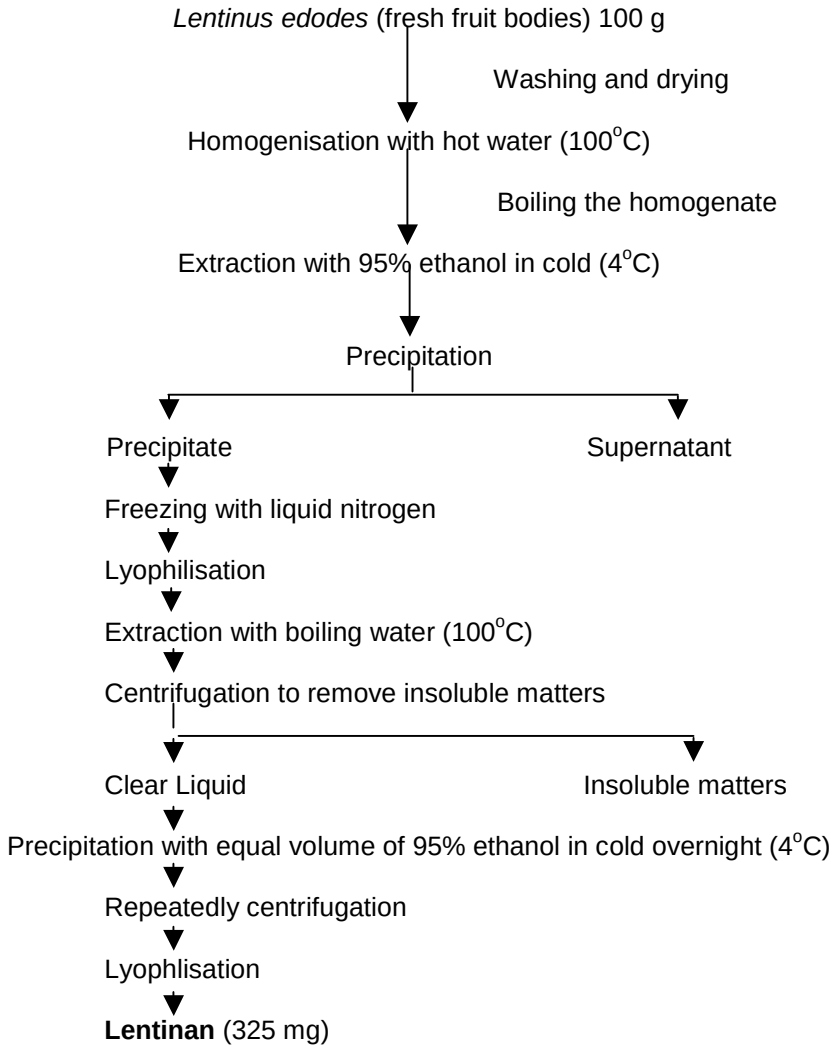


Table 3 Comparison of two methods of preparation of β-D glucan from *Lentinus edodes* (adapted from Yap and Ng, 2001)

Characteristics of methods	Method of extracting lentinan	
	Chihara's method	**New biochemical method**
Number of days taken to prepare extract	14	5
Requirement of sophisticated equipment or rarely used chemicals	Many	None except liquid nitrogen
Cost of preparation	High	Low
Total yields from 100g of fresh mushrooms	4 mg	325 mg
Percentage concentration of lentinan in extract produced (%)	96.03	87.50
Purity obtained	99.23	87.65

β-D-glucans

The basic β-D-glucan is a repeating structure with the D-glucose units joined together in linear chains by beta-bonds (β). These can extend from carbon 1 of one saccharide ring to carbon 3 of the next (β1-3), from carbon 1 to carbon 4 (β1-4) or from carbon 1 to carbon 6 (β1-6). Mostly there is a main chain which is either β1-3, β1-4 or mixed β1-3, β1-4 with β1-6 side chains. The basic repeating structure of a β1-3 glucan with β1-6 side chains is shown in Figs, 5 and 6. Levels of anticancer activity are related to their molecular weight, branching and solubility in water. The study of their steric structures by NMR analyses and X-ray diffractions clarified that active β-D-glucan shows a triple-stranded right-winding helix structure (Bluhm and Sarco, 1977). Not all β-D-glucans contained in fungi exhibit antitumour activity. The extent of occurrence of this activity seems to be influenced by solubility in water, size of molecules, and the β-(1-6)-bonding system in the β-(1-3) major chain. Some of the water insoluble β-glucans are soluble in dilute alkali and then can show marked antitumour activity (Bohn and BeMillar, 1995).

Lentinan from *L. edodes* and Schizophyllan from *S. commune* are the two best studied and commercially available β-D-glucans and have been shown to have strong immunomodulating and anticancer properties (see Chapters 6 and 7). They consist of a main chain of (1->3)-linked β-D-glucopyranosyl units with β-D-glucopyranosyl branch units linked 1->6 at, on average, an interval of three main chain units, degree of branching (DB 0.33), and have average molecular weights of 500,000 and 450,000 respectively (Sasaki and Takasuka, 1976). Within each batch of these β-D-glucans there can be considerable variation in molecular size. It has been suggested that immune response to β-D-glucans could be in part non-specific and determined by size rather than by chemical structure (Bohn and BeMillar, 1995).

Individual species-derived β-D-glucans have unique molecular structures (Ohno *et al*., 1988) and it has been surmised that the higher ordered structures (triple helices) of high molecular weight β-D-glucans could be responsible for the considerable immunomodulatory activity (Maeda *et al*., 1988). Only higher molecular weight molecules apparently form triple helical structures which are stabilised by the β-D-glucopyranosyl branch units (Saito *et al*., 1991). There is good evidence to propose that both Lentinan and Schizophyllam are active only when they exist in a single helical structure (Saits *et al*., 1991).

Clinical use of Lentinan and Schizophyllan as immunotherapeutic agents for cancer treatment will be discussed in Chapter 7. From a structure-activity concept it has been suggested that the antitumour activity of (1->3)- β-glucans resides in the helical conformation of the glucan backbone, possibly triple-stranded, but perhaps, even more important, is the presence of hydrophyllic groups located on the outside surface of the helix. Furthermore, increased water solubility favours enhanced

antitumour activity while the location of substituent groups would also be important (Bohn and BeMillar, 1995).

Recent studies have demonstrated that the concentration of polysaccharides in certain medicinal mushroom species can be related to the stage of development of the mushroom fruitbody and also to the time after harvest and subsequent storage conditions (Minato *et al*., 1999, 2001). Immunomodulating activities of extracts from *L. edodes* decreased rapidly when the mushrooms had been stored at 20^{o}C for 7 days while no decrease occurred at low temperature storage (1^{o} and 5^{o}C). The decrease in activity was related to the decrease in concentration of Lentinan which was degraded by internal β-glucanase activity (Minato *et al*., 1999). A similar series of experiments on the immunomodulating activity of extracts from *L. edodes* and *G. frondosa* showed, in each case, an increase in activity during growth and development of the fruitbody followed by a decrease at the final stages of maturation. These activities were paralleled by similar concentration changes in Lentinan and Grifron, the respective β-glucans (Minato *et al*., 2001).

These observations are highly significant both from a pharmaceutical and functional food point of view. It becomes imperative that medicinal mushrooms should be harvested at the optimum β-glucan concentration in the fruitbody and also that the harvested fruitbodies should be stored at correct temperature conditions before processing or consumption. Such results must surely compromise the use of medicinal mushrooms derived for distant parts which must involve inadequate environmental conditions and subsequent loss of β-glucans. As a result of these studies it is obvious that the pattern of polysaccharide formation in other medicinal mushrooms should be examined. Where polysaccharides are produced by

fermentation processes it is much easier to then harvest at optimum production points as is already practised in other fermentations such as with antibiotics.

Heteropolysaccharides and Glycoproteins

While water-soluble β-D-glucans are widely distributed in mushroom species, many species also contain β-D-glucans with heterosaccharide chains of xylose, mannose, galactose and uronic acid which can be extracted by salt and alkali treatments. Other species can contain polysaccharide-peptides or glycoproteins which are polypeptide chains or small proteins to which polysaccharide β-D-glucan chains are stably attached (Boldizsar *et al*., 1998) (Fig. 7).

Hot water extracts from *Grifola frondosa*, the Maitake mushroom, contain the D-Fraction which appears to be a highly active anticancer agent for both animals and humans (Jones, 1998; Maitake Products Inc., 1998). The D-Fraction is obtained from the hot water crude extract by deproteination. Maitake D-Fraction contains mainly β-D-glucan with 1-6 main chains and 1-4 branchings together with the more common 1-3 main chains and 1-6 branching.

Ganoderma lucidum, the Reishi mushroom, contains β-D-glucan in hot water extracts together with glucuronoglucan, xyloglucan, unannoglucan, xylomannoglucan and other active heteroglucans and protein complexes. Purifications involve using salts, alkali and DMSO (Mizuno *et al*., 1984).

Fig. 5 Primary molecular diagram of mushroom beta-D-glucan (Kidd, 2000)

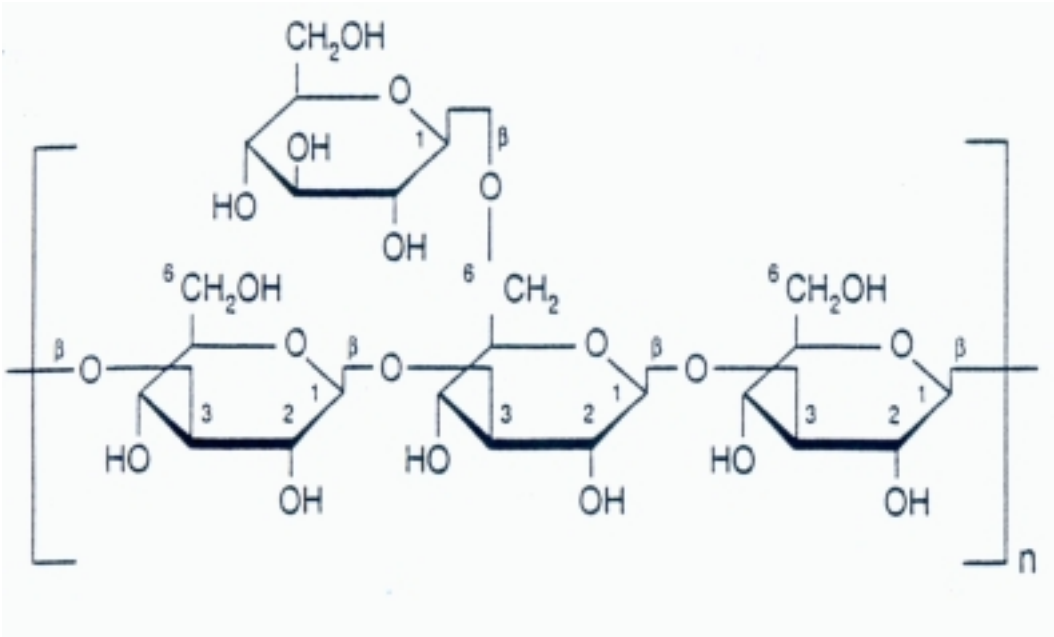


Fig. 6 Molecular model of the right-handed triple spiral helix of antitumour-active-beta-D-glucan (Schizophyllan) (Mizuno, 1999).

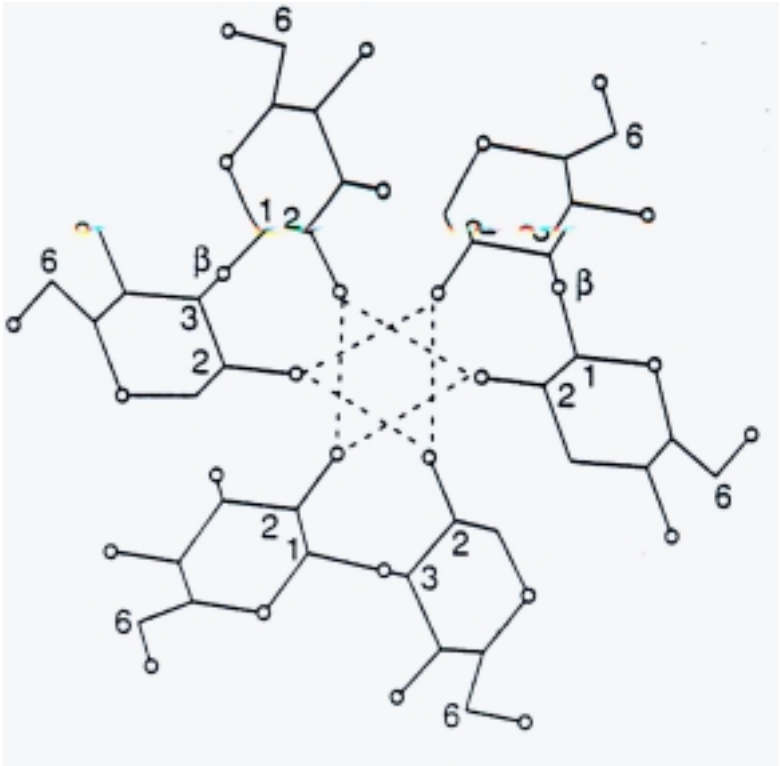
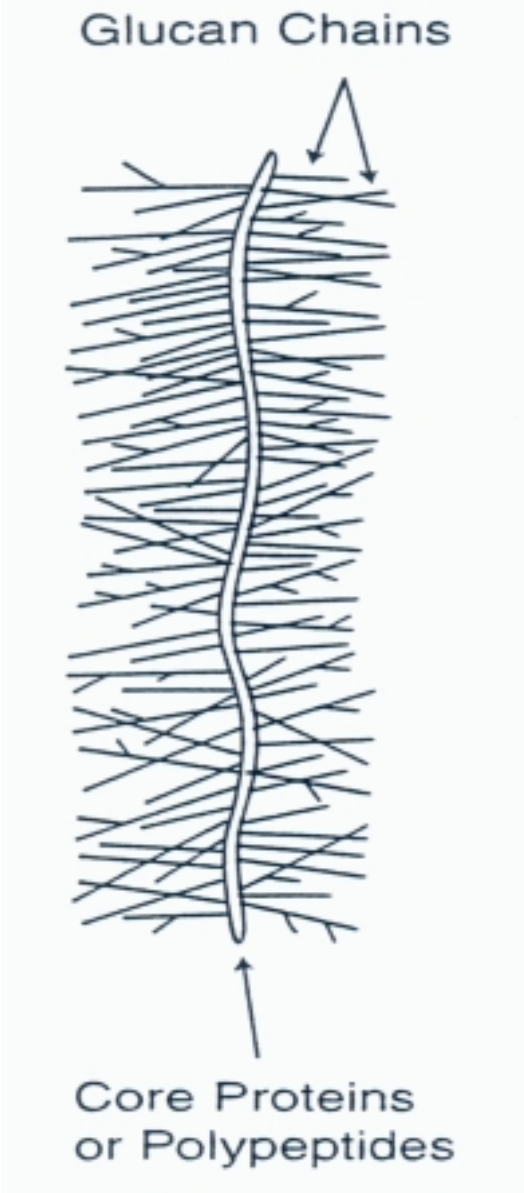


Fig. 7 The molecular plan of a mushroom proteoglycan



Hot water extracts from cultured mycelium of *Lentinus edodes* contain polysaccharide KS-2, an α-mannan peptide containing the amino acids serine, threonine, alanine and proline.

LEM and LAP extracts are derived from *L. edodes* mushroom mycelium and culture media respectively and are glycoproteins containing glucose, galactose, xylose, arabinose, mannose and fructose. LEM also contains nucleic acid derivatives, vitamin B compounds and ergosterol. LEM and LAP both demonstrate

strong antitumour activity by i.p., and p.o. in animals and humans. LEM is prepared from a hot water extract of powdered mycelia, incubated for 50-60 h at 40-50^{o}C and partially hydrolysed by endogenous enzymes. The residue was extracted with water, 60^{o}C, and the filtrate freeze dried. The final light brown powder was LEM. The yield of LEM is about 6-7 g/kg medium. LAP is obtained as the filtrate of a water solution of LEM by adding 4 volumes of ethanol. The yield of LAP is approximately 0.3 g/g LEM. An immunoactive substance EP3 has been obtained by further fractionation of LEM. The active substance is considered to be a water soluble lignin containing numerous carboxyl groups (Susuki *et al*., 1990). LEM and LAP are, therefore, complex mixtures of compounds which are now being further purified (Hobbs, 2000).

An antitumour active β-glucan-protein (EA_6) has been isolated from the fruit-body of *Flammulina velutipes* while a new antitumour glycoprotein has been isolated from cultured mycelium. This glycoprotein, “Proflamin” (mw = 16,000) is water soluble and contains 90% protein and 10% saccharide and has activity against allogeneic and syngeneic tumours (Zhang *et al*., 1999).

PSK (polysaccharide-K) and PSP (polysaccharide-peptide) have been derived from *Trametes* (*Coriolus*) *versicolor*. PSK is extracted from a mycelial strain CM-101 and is approximately 62% polysaccharide and 38% protein. The glucan portion of PSK consists of a β1-4 main chain and β1-3 side chain, with β1-6 side chains that bond to a polypeptide moiety through O-N-glycosidic bonds. The polypeptide portion is rich in aspartic, glutamic and other amino acids and has a molecular weight ranging from 94,000-100,000 daltons and is orally bioavailable (Sakagami and Aoki, 1991). This compound has been systematically tested against a wide range of human cancers with some considerable success (Ikuzawa *et al*., 1988, Kidd 2000).

PSP was first isolated from cultured deep-layer mycelium of the COU-1 strain of *Trametes versicolor* in 1983 (Yang, 1999). PSP may contain at least four discrete molecules, all of which are true proteoglycans. PSP differs from PSK in its saccharide makeup, lacking fucose and containing arabinose and rhamnose. The polysaccharide chains are true β-glucans; mainly 1-4, 1-2 and 1-3 glucose linkages together with small amounts of 1-3, 1-4 and 1-6 galactose, 1-3 and 1-6 mannose, and 1-3 and 1-4 arabinose linkages. The molecular weight of PSP is approximately 100,000 daltons and can be easily delivered by oral route. PSP is rapidly gaining recognition with many successful human cancer trials (Jong and Yang, 1999) (Chapter 7). Although the molecular weights of PSK and PSP are approximately 100,000 daltons, PSP does not contain fucose and PSK lacks arabinose and rhamnose (Yang and Ying, 1993). Saphadex gel chromatography, DEAE-cellulose column chromatography and HPLC reveal that the polysaccharides and peptides of PSP are clearly bound and not separated. Where there is polysaccharide there is polypeptide. PSP polysaccharide is connected with a small molecular weight protein. Up to now at least 10 kinds of 'protein bound' polysaccharides have been isolated, e.g. coriolan I and II - most are covered by US and Japanese patents. However, only PSK and PSP have been used in clinical trials. It should be noted that Japanese and Chinese scientists still prefer to use the *Coriolus* generic name instead of *Trametes*.

Active Hexose Correlated Compounds (AHCC)

This is a proprietary extract prepared from the co-cultivation of several Basidiomycete mushrooms including *Lentinus edodes, Trametes versicolor* and *Schizophyllum commune* grown on rice (Ghoneum *et al*., 1995). However, there is no data available on the exact species complement or on methods of preparation. It

is apparently a hot water extract following enzyme treatment, and the extract contains polysaccharides, amino acids and minerals and is orally bioavailable. The glucans present are stated to have low molecular weight, c. 5,000 daltons and are α-1-3 type. These details are surprising since typically low molecular weight material is normally inactive and α-glucans have minimal immuno-potentiating activity. However, there have been limited studies and reports suggesting an interesting level of efficacy against hepatocellular carcinoma (Kamiyama, 1999). Ghoneum (1998) found that a derivative, arabinoxylane, derived from this fermentation increased human NK activity by a factor of 5 over two months.

Dietary Fibre

High molecular weight compounds excreted without digestion and absorption by humans are called dietary fibres. Mushrooms contain dietary fibres belonging to β-glucans, chitin and heteropolysaccharides (pectinous substances, hemicellulose, polyuronides etc), making up as much as 10-50% in the dry matter. Much of the active polysaccharides, water soluble or insoluble, isolated from mushrooms, can be classified as dietary fibres (i.e. β-glucan, xyloglucan, heteroglucan, chitinous substance) and their protein complexes. Many of these compounds have carcinostatic activity and by physicochemical interactions they will absorb possible carcinogenic substances and hasten their excretion from the intestine. Thus, mushrooms in general may have an important preventative action for colorectal carcinoma (Mizuno, 1996).

In summary - while a variety of polysaccharides from various sources have been shown to enhance the immune system the most active appear to be branched (1-3)-β-D-glucans. All have a common structure, a main chain consisting of (1-3)-

linked β-D-glucopyranosyl units along which are randomly dispersed single β-D-glucanopyranosyl units attached by 1-6 linkages giving a comb-like structure with various conformations. The (1-3)- β-D-glucan backbone is essential and the most active immune stimulating polymers have degrees of branching between 0.20 and 0.33. Information has been accumulating both that triple helical structures formed from high molecular weight polymers are possibly important for immunopotentiating activity and that activity is independent of any specific ordered structure. Immunopotentiating activity depends mainly on a helical conformation and on the presence of hydrophilic groups located on the outside surface of the helix. Most of the active (1-3)- β-D-glucans have been isolated from Basidiomycetes (Bohn and BeMiller, 1995).

While most attention has been given to studies demonstrating the medicinal effects of the polysaccharides from single mushroom species, several studies are suggesting that the human and murine immune systems can be given greater stimulation by using mixtures of polysaccharides from several proven medicinal mushrooms (Ghoneum *et al*., 1995; Wedam and Haynes, 1997; Sawai *et al*., 2002). A complementary effect of each mushroom component on enhancing immunological function can be expected from mixed medicinal mushroom extracts (see also Chapters 6 and 7).

Terpenoids

Certain terpenoids and their derivatives have been isolated from mushroom species from the Polyporales and Ganodermatales and have been shown to be cytotoxic. At least 100 different triterpenoids have been identified from fruiting bodies and mycelium of *Ganoderma lucidum* and *G. applanatum* and include

ganoderic, ganoderenic, lucidenic acids- and several ganoderals (for references see Wasser and Weis, 1999b). A cytotoxic tricyclic sesquiterpene, illudin, isolated from *Omphalotus olearius* and *Lampterimyces japanicus* shows interesting anticancer properties. Furthermore, the semisynthetic illudin analog, 6-hydroxy-methylcylfulvene (HMAF) has inensity profiles of a tumour growth inhibitor. HMAF is undergoing phase I human clinical trials and could well be a promising new anticancer drug (Weis, 1996).

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CHAPTER 6 IMMUNOMODULATORY ACTIVITIES OF MUSHROOM GLUCANS AND POLYSACCHARIDE-PROTEIN COMPLEXES IN ANIMALS AND HUMANS

Synopsis:

This chapter focuses on the ability of extracts from medicinal mushrooms to stimulate or modulate the host immune responses. Numerous bioactive polysaccharides or polysaccharide-protein complexes from medicinal mushrooms are described that appear to enhance innate and cell-mediated immune responses, and exhibit antitumour activities in animals and humans. Stimulation of the host immune defence systems by bioactive polymers from medicinal mushrooms has significant effects on the maturation, differentiation and proliferation of many kinds of immune cells in the host. Many of these mushroom polymers were reported previously to have immunotherapeutic properties by facilitating growth inhibition and destruction of tumour cells. Recent research has also shown that some of these mushroom-derived polymers may possess direct cytotoxic effects on cancer cells. Whilst the mechanism of their antitumour actions is still not completely understood, stimulation and modulation of key host immune responses by these mushroom polymers appears central. Although somewhat controversial, recent evidence suggests that mushroom polymers (β-glucans) may trigger the stimulation of many kinds of immune cells in animals and humans by binding to a specific cellular receptor known as complement receptor type 3 or CR3.

Overview of the human immune system

A general overview of the immune system is provided in Appendix I. This has been provided so that an appreciation can be gained of how biological molecules from certain medicinal mushrooms may modulate the immune responses. Readers who are not familiar with the workings and complexities of innate (non-specific) and acquired (specific) immunity are advised to consult Appendix I before reading this chapter.

Medicinal mushrooms and the human immune response

While the historical and traditional usage of the medicinal mushrooms, especially in the Far East, is almost limitless (Hobbs, 1995), this chapter will largely focus on presenting well-investigated findings from the last three decades. Indeed, the oldest written record of mushrooms as medicinals is in an Indian medical treatise from 3000 BC (Kaul, 1997). Of significant relevance and importance is the ability of particular mushroom-derived compounds to modulate the human immune response and to inhibit certain tumour growths (Wasser and Weis, 1999a,1999b).

Medicinal mushroom research has focused on discovering compounds that can modulate positively or negatively the biologic response of immune cells. Those compounds which appear to stimulate the human immune response are being sought for the treatment of cancer, immunodeficiency diseases, or for generalised immunosuppression following drug treatment; for combinational therapy with antibiotics; and as adjuncts for vaccines (Jong *et al*., 1991). Those compounds that suppress immune reactions are potentially useful in the remedy of autoimmune (an abnormal immune response against self-antigens) or certain gastro-intestinal tract diseases (e.g. Crohns) (Badger, 1983). Several classes of compounds, such as proteins, peptides, lipopolysaccharides, glycoproteins, and lipid derivatives, have all been classified as molecules that have potent effects on the immune system (Tzianabos, 2000). Whilst polysaccharides are generally considered to be classic T-lymphocyte-dependent antigens that do not elicit cell-mediated immune responses (host defences that are mediated by antigen-specific T lymphocyte cells and various non-specific cells of the immune system), certain polymers have recently been shown to act as potent immunomodulating agents (Tzianabos, 2000). Compounds that are capable of interacting with the immune system to upregulate or downregulate specific aspects of the host response, can be classified as immunomodulators. Whether immunomodulators enhance or suppress immune

responses can depend on a number of factors such as dosage, route of administration, and timing and frequency of administration (Tzianabos, 2000). The type of activity these compounds exhibit can also depend upon their mechanism of action or the site of activity.

Immunomodulating polysaccharides derived from a variety of diverse microbial genera include *Streptococcus* spp. (hyaluronic acid), *Bacteriodes fragilis* (Polysaccharide A), *Candida albicans* (Mannan) and *Saccharomyces cerivisiae*, have shown significant promise in the treatment of infectious diseases (Garner *et al*., 1990; Shapiro *et al*., 1986; Muller *et al*., 1997; Schrager *et al*., 1998). Antitumour effects were another promising biopharmacological activity of polysaccharides from these sources. The earliest bacterial-derived polysaccharide reported to have antitumour activity was attributed to *Serratia marcescens* and became known as Shear's polysaccharide (cited in Ooi and Liu, 2000). This polysaccharide could cause extensive cytotoxic damage to Sarcoma 37 tumours, but as it had serious side-effects, clinical trials have not been performed. Although many other polysaccharides from bacteria such as *Escherichia coli*, *Streptococcus pyogenes* (OK-432), *Proteus vulgaris*, *Acetobacter xylinum* and *Salmonella typhimurium* have also been reported to exhibit cytotoxicity against solid tumours (Whistler *et al.,* 1976); however most of these bacterial polysaccharides belong to endotoxic lipopolysaccharides.

One of the most significant factors of many of the derived bioactive polymers from medicinal mushrooms is their role as immunomodulators. Whilst information will be presented in this report that demonstrates the ability of certain medicinal mushroom (MM) extracts to modulate key components of the immune system, it is appropriate at this point to mention a number of important immune responses that are stimulated by some of these bioactive polymers. As described in Appendix I, the

immune system plays an important role in the body's defence against infections and tumour formation. Moreover, the body's defence against viral attack and against spontaneously arising malignant tumour cells comprises a dynamic orchestrated interplay of innate and acquired immune responses. Innate immunity (having macrophages, neutrophils, NK and dendritic cells as gatekeepers), is regulated by chemical-messengers or cytokines and by activation of inflammatory and acute phase responses (Chihara, 1992). The mononuclear phagocyte system (e.g., macrophages and monocytes), dendritic cells and certain lymphocytes (e.g., natural killer cells) serve a number of important roles including the recognition and destruction of abnormal cells.

Stimulated macrophages and Natural Killer (NK) cells produce cytokines such as interferons, interleukins and others that are targeted towards destroying cancer cells. These are regarded as the first line in the host defence system, and may themselves successfully eliminate infected or transformed cells prior to the establishment of fully-fledged humoral and CMI responses (Borchers *et al*., 1999). As described in Appendix 1, specific immunity to abnormal cells or tissues includes humoral (e.g., generates antibodies) and cell-mediated immunity (also promotes inflammatory responses and ultimately kills infected or abnormal cells). As a fully functional immune response is critical to the recognition and elimination of tumour cells, the identification of mushroom derived compound(s) that are capable of stimulating components of innate or acquired immunities may be of potential benefit for cancer treatment.

Thus these immunological activities play a governing role in host recognition, targeting and destroying unwanted tumour-potentiating viruses and abnormal or cancerous cells. Induction and expression of cellular immunity in host resistance to cancer and persistant microbial infections is contingent upon a myriad of complex

interactions between antigen, macrophages, and lymphocytes (Borchers *et al*., 1999). It is through the orchestrated interplay of many of these innate and specific immune responses that abnormal cells are targeted and destroyed. For example, the key immune mechanisms that are involved in Lentinan (a polysaccharide from *L. edodes*) mediated destruction of cancer cells are illustrated in Figure 1 (Chihara, 1992). Tumours may develop when transformed cells escape immunological host defence mechanisms (Ooi and Liu. 1999, 2000). Indeed, the increased incidence of spontaneous tumours in immunosuppressed individuals (as well as those congenital or acquired immunodeficiencies), indicates that the immune system can provide a significant mechanism for host resistance against cancer and infectious diseases (Jong *et al*. 1991).

The ability of bioactive polysaccharides and polysaccharide-bound proteins to modulate so many important immune cells may due to the structural diversity and variability of these macromolecules. Unlike proteins and nucleic acids, polysaccharides contain repetitive structural features which are polymers of monosaccharide residues joined to each other by glycosidic linkages (Ooi and Liu, 2000). Among these macromolecules, polysaccharides offer the highest capacity for carrying biological information because they have the greatest potential for structural variability. For example, the number of possible permutations for four different sugar monomers can be up to 35,560 unique tetrasaccharides, whereas four amino acids can form only 24 different permutations (cited in Ooi and Liu, 2000). Therefore, this enormous potential variability in polysaccharide structure gives the necessary flexibility for the precise regulatory mechanisms of various cell-cell interactions in Higher organisms.

Immuno-modulating effects of *Lentinus edodes* mycelium (LEM) extract and Lentinan

Of all the mushroom immune modulators investigated, bioactive polymers from *Lentinus edodes* has been studied extensively for interesting biological effects. Moreover, *L. edodes* is the source of two preparations with well-studied pharmacological effects – *Lentinus edodes* mycelium (LEM) extract and lentinan (Hobbs, 2000). Lentinan (a cell wall constituent extracted from fruiting bodies or mycelium) is a highly purified, high molecular weight polysaccharide in a triple helix structure containing only glucose molecules with mostly β-(1→3)-Glc linkages in the regularly branched backbone, and β-(1→6)-Glc side chains (Aoki, 1984, Hobbs, 2000). The configuration of the glucose molecules in a helix structure is thought to be important for the biological activity (Hamuro et al., 1971). Lentinan is protein-free as it is completely devoid of any nitrogen, phosphorous, sulphur or any other atoms of carbon, oxygen, and hydrogen (Hobbs, 2000). Lentinan is water-soluble, heat stable, acid stable and alkali labile (Hobbs, 2000). LEM is a preparation of the water-soluble material from powdered mycelia extract of *L. edodes* harvested before the mushroom fruiting bodies develop. The major active constituent of LEM is reported to be a heteroglycan protein conjugate, that is, a protein-bound polysaccharide. It contains about 24.6% protein and 44% sugars, in addition to nucleic acid derivatives and vitamins (Breene, 1990, Iizuka, 1997). Other active polysaccharides and protein-polysaccharide complexes and water-soluble lignins were isolated from LEM (Tabata et al., 1992).

Lentinan does not attack cancer cells directly, but produces its antitumour effect by activating different immune responses in the host. Recent research has shown that LEM and Lentinan are true immuno-potentiators, as administration of these bioactive polymers had a clearly augmenting effect on the proliferation of

peripheral mononuclear cells (PMNCs) from healthy donors, (Aoki, 1984, Hobbs, 2000). Indeed, Lentinan and LEM appear to act as a host defense potentiator that is able to restore or augment the responsiveness of host cells to lymphocytokines, hormones, and other biologically active substances. Evidence suggests that this immune-potentiation occurs by stimulating the maturation, differentiation or proliferation of cells involved in host defense mechanisms. Thus, Lentinan has been shown to increase host resistance against various kinds of cancer and has the potential to restore the immune function of affected individuals (Chihara, *et al*., 1989, 1992). Many of these immune pathways stimulated by Lentinan are illustrated in Figure 1.

Lentinan has displayed various kinds of immune activities in both animals and in humans (Table 1). Until recently, the interactions of Lentinan with many kinds of immune cells were not known. An insight into receptor-binding in immune cells by β-glucans from fungi was provided by Ross et al. (1999). These authors showed that β-glucans from yeast bind to iC3b-receptors (CR3, CD11b/CD18) of phagocytic cells and natural killer (NK) cells, stimulating phagocytosis and/or cytotoxic degranulation. Further information of receptor-base binding and affinity for many kinds of immune cells is provided later in this chapter. Thus, research has shown previously that Lentinan stimulates various kinds of immune cells including macrophages, NK-cells and lymphocytes (T and B cells).

The anti-tumour activity has been shown to be abolished in neonatally thymectomised mice and was decreased by the administration of antilymphocyte serum. Both practices reduce or eliminate T lymphocyte production that is central to cell-mediated immunity. This supports the concept that Lentinan requires

Table *1* ***Immune effects of Lentinan in vitro and in vivo in animals and humans*** *(Hobbs, 2000)*

Activity	Experimental Animal System		Human System	
	in vitro	**in vivo**	**in vitro**	**in vivo**
Humoral factors				
Inhibition of immunosuppressive factors	–	++	–	++
Immunopotentiative factors, increased production	–	++	–	–
C3-splitting activity	–	+	–	–
Antibody production	–	+	–	+
Opsonin production	–	–	–	+
Colony-stimulating factor production	+	–	–	–
Production of lymphocyte-activating factor (interleukin-1)	+	+	+	+
Inhibition of prostaglandin release	–	+	–	–
Interferon production	–(?)	+	–±	–
Tumor necrosis factor production increased	–	+	–	–
Complement C3 production	–	–	–	+
Cellular factors				
Polymorphonuclear leukocyte activation	–	+	+	+
Peritoneal macrophage activation	–	+	–	–
Natural killer cell activation	+	+	±~+	++
Activation of helper T cells	–	+	+	++
Activation of killer T cells	+	+	+	–
Inhibit suppressor T-cell activity	–	+	–	–
Activation of cytotoxic macrophages	–	+	–	+
Delayed-type hypersensitivity reaction	+	+~++	–	–
Mitogenicity	–	–	±~+	++

immunocompetent T-cell compartments (Maeda *et al*., 1971; Maeda and Chihara, 1973). The effect of Lentinan was also inhibited by anti-macrophage agents such as carrageenan. Unlike other well-known immuno-stimulants, Lentinan is in a unique class of DT-cell-oriented assistants, in which macrophages play some part. For example, Lentinan can activate NK-cells *in vitro* in the same concentrations that are achieved in the blood plasma of patients treated clinically with Lentinan. NK cell activity is involved in tumour suppression and while these cells do not stimulate certain T-killer cell activity, or do so only under certain conditions, they are strong T-helper cell stimulants both *in vitro* and *in vivo*. Lentinan can inhibit prostaglandin synthesis, which can slow T-cell differentiation in animals and humans, as well as inhibiting suppressor T-cell activity *in vivo* (Aoki, 1984), and in addition, increase the

ratio of activated T cells and cytotoxic T cells in the spleen when administered to gastric cancer patients with chemotherapy (Hobbs, 2000).

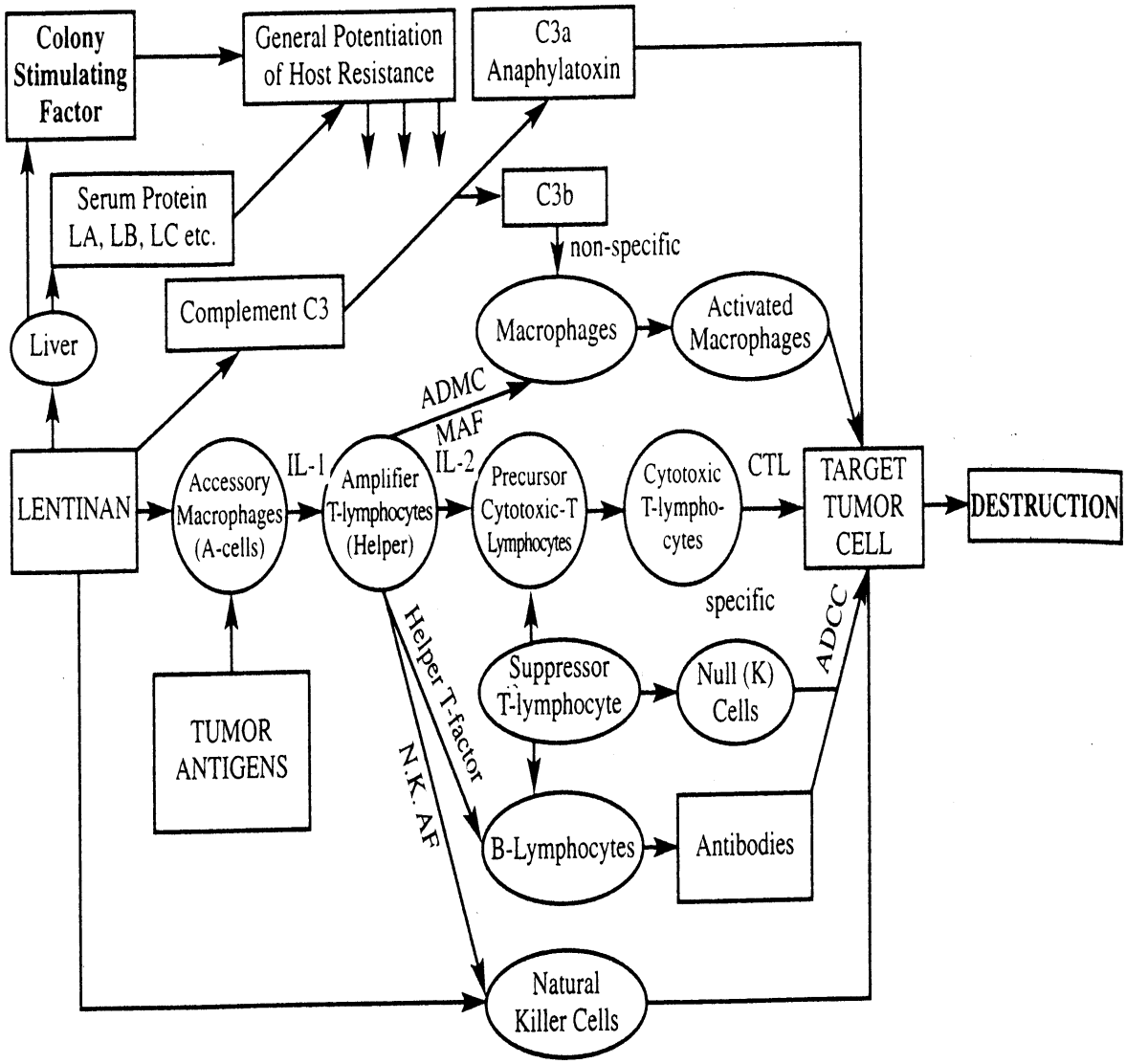
Using the blood of healthy donors and cancer patients, Lentinan has been shown to stimulate peripheral blood lymphocytes *in vitro* to increase interleukin-2-mediated LAK-cell (lymphokine-activated killer cell) and NK cell activity at levels achievable *in vivo* by administration of clinical doses of Lentinan. Lentinan has also been shown to inhibit suppressor T cells activity *in vivo* and to increase the ratio of activated T cells and cytotoxic T cells in the spleen when administered to gastric cancer patients undergoing chemotherapy.

Many interesting biological activities of Lentinan have been reported (Figure 2), including:

- An increase in the activation of non-specific inflammatory response such as acute phase protein production (Suga *et al*, 1986)
- Vascular dilation and haemorrhage-inducing factor *in vivo* (Maeda *et al*, 1991)
- Activation and generation of helper and cytotoxic T cells (Chihara *et al* 1992)

Augmentation of immune mediators like interleukin-1 (IL-1) (Fruehauf *et al*., 1982), IL-3 (Izawa *et al*., 1984), IL-6 (Maeda *et al*., 1992), colony stimulating factor(s) (Izawa *et al*., 1984), and others. These serum factors are mainly produced by macrophages or T-lymphocytes and act on lymphocytes, hepatocytes, vacular endothelial cells, and other cells. Lentinan has been shown previously to increase the capacity of peripheral blood mononuclear cells of patients with gastric cancer,

Figure 1. ***Host immune responses involved in Lentinan-mediated destruction of cancer cells*** **(**Chihara *et al*., 1992)



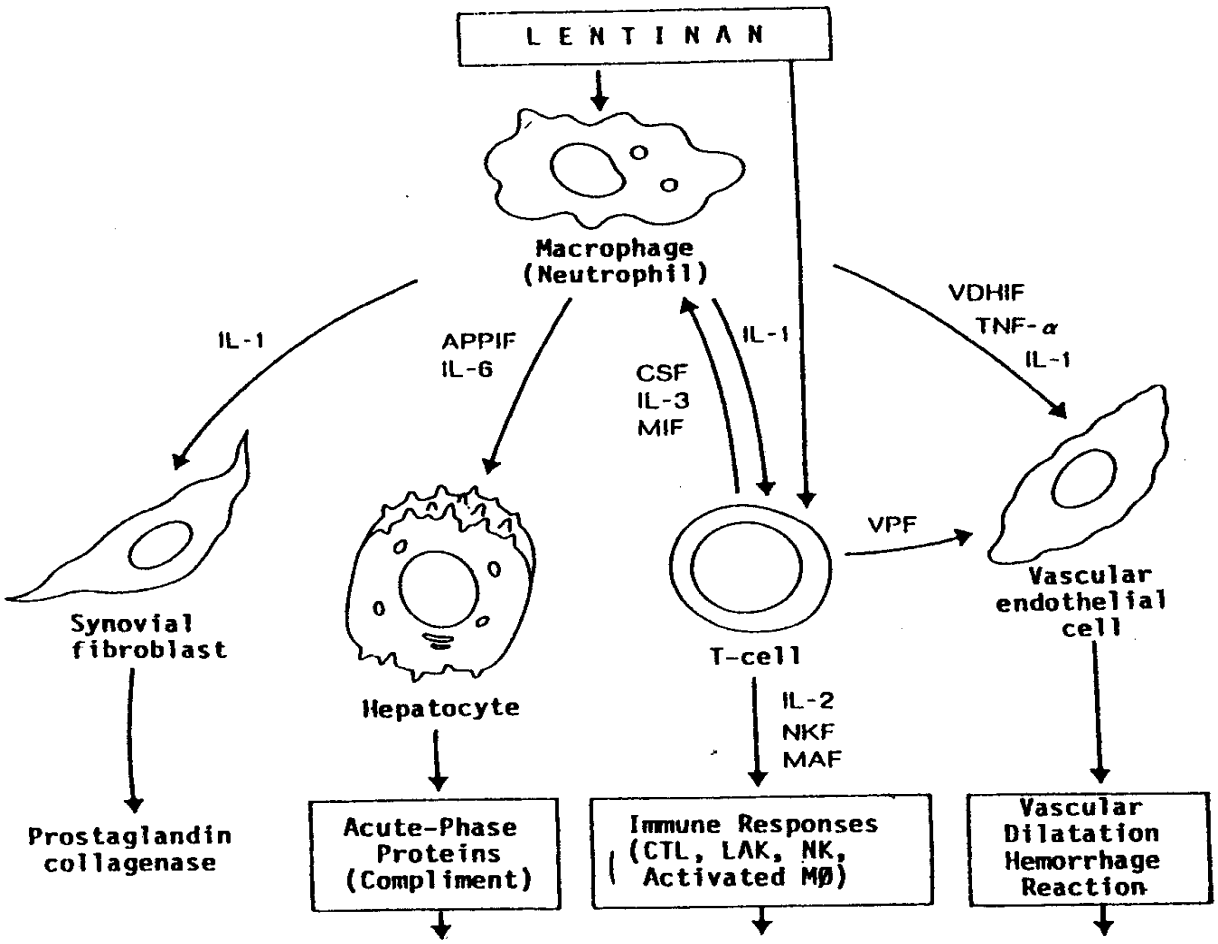
resulting in the production of IL-1α, IL-1β and TNF-a (Chihara *et al*., 1992). In its role as a host defence potentiator, Lentinan triggers the increased production of colony stimulating factors (CSFs) and IL-3, which correlates with the IL-1-producing activities of macrophages (Izawa *et al*., 1984; Chihara *et al*., 1989). Increased production of IL-1 results in augmented maturation capable of inducing IL-2, natural killer activating factor, and macrophage-activating factor (MAF). IL-1 also amplifies

maturation of immature effector cells to mature cells and augments responsiveness to lymphcytokines such as IL-2, MAF and others.

Lentinan's immune-activating ability may be linked with its modulation of hormonal factors, which are known to play a role in tumour growth. The anti-tumour activity of Lentinan is strongly reduced by administration of thyroxine or hydrocortisone. Lentinan can also restore a tumour-specific antigen-directed delayed-type hypersensitivity (DTH) response. Indeed, Lentinan is not formally included among the non-specific immunostimulants (RES stimulants), but it augments the induction of antigen-specific cytotoxic T-lymphocytes, macrophages, and other non-specific immune responses. An overview of the host immune responses involved in Lentinan-mediated recognition and destruction of cancer cells is presented in Figure 1. Interestingly, accumulating evidence suggests that Lentinan-stimulation of dendritic cells (antigen-presenting cells that are found in lymph nodes, spleen and thymus; follicular and interdigitating dendritic cells, skin: Langerhans cells, and other tissues; interstitial dendritic cells) has an important impact on immunomodulation and anti-tumour activity. Moreover, dendritic cell tumour-infiltration in association with killer cytotoxic T cell stimulation and activation have been shown to have a governing role in tumour attack and elimination (Chihara, 1997).

Bohn and BeMiller (1995) reported that the likely mode of immunopotentiation by this (1-3)-β-D-glucan involves activation of cytotoxic macrophages, helper T cells and NK cells, and the promotion of T cell differentiation. Macrophages are one of the many critical components in the immune system, co-operation between which is necessary for tumour rejection. Bohn and BeMiller (1995) also reported that macrophages have a highly selective cytotoxicity towards cancer cells *in vitro*; and

Figure 2. Early phase of the mechanism of action of Lentinan and possible pathway for inflammatory and immune reactions. IL-1, IL-2, IL-3, and IL-6: interleukin-1, -2, -3, and –6. APPIF: acute phase protein-inducing factor VDHIF: vascular dilation and hemorrhage-inducing factor; CSF: colony-stimulating factor; MIF: migration inhibition factor;' TNF: tumor necrosis factor; VPF: vascular permeability factor; NKF: natural killer cell-activating factor; MAF: macrophage-activating factor; CTL: cytotoxic T lymphocyte; LAK: lymphokine-activated killer cell (Maeda *et al*. 94)



there is evidence that they may also destroy malignant cells *in vivo*. T cell competence appears necessary for selection of macrophage resistance, which suggests that these two cell types interact in the intact host in response to a tumour challenge.

Thus, Lentinan has been shown to restore or augment the ability of host cells to respond to lymphocytokines or other intrinsic bioactive factors and protect patients

from infectious disease or cancer metastases (Chihara, 1971). Lentinan can also improve the physiological constitution of host defence mechanisms by restoring homeostasis and enhancing intrinsic resistance to disease. Homeostasis is a term given to cellular processes, by which both negative and positive control are exerted over the values of a variable or set of variables, and without which control the system would fail to function. To summarise, Lentinan may restore and augment immunological responsiveness of host cells, but it has no direct cytotoxicity against tumours.

Immunomodulating effects of Ganoderma lucidum

Ganoderma lucidum has been used extensively as "mushrooms of immortality" in China and other Asian countries for 2000 years (Shiao *et al*, 1994). Several major substances with potent immuno-modulating action have been isolated from this mushroom, including polysaccharides (in particular β-D-glucan), proteins (e.g., Ling Zhi-8) and triterperoids (Jong and Birmingham, 1992; Gao and Zhou, 2001). Other components such as steroids and organic gemanium also play an important role in the immuno-modulating activity of *G. lucidum*. The major immuno-modulating effects of these active substances derived from *G. lucidum* include mitogenicity and activation of immune effector cells such as macrophages, NK and T cells (Gao and Zhou, 2001). Stimulation of these immune effector cells results in the production of cytokines such as interferon (INF), interleukins (IL) and tumour necrosis factor (TNF)-α.

More than 100 types of polysaccharides have been isolated from *G. lucidum*. β-D-glucans (i.e., polysaccharides producing D-glucose by acid hydrolysis) have been shown to be biologically active (Mizuno *et al*., 81; Mizuno *et al*., 1982; Gao, 2000). Modification of D-glucosyl groups of side chains of β-D-glucans enhanced

anti-tumour activity. There is evidence that the β-D-glucans induce biological response by binding to membrane complement receptor type three (CR3, $\alpha_M\beta 2$ integrin, or CD11b/CD18) on immune effector cells such as macrophages (Battle *et al*., 1998; Mueller *et al*., 2000). The β-glucan binding site of CR3 has been mapped to a region of CD11b located at the C-terminus of the I-domain. The ligand-receptor complex can be internalised, and the intercellular events that occur after glucan-receptor binding have been determined (Muller *et al*., 1996). Preliminary evidence shows that the NF-κB is activated (Battle *et al*., 1998). β-D-glucan can also help override the normal resistance of iC3b-opsonized tumour cells to the cytotoxic activation of phagocyte and NK cell CR3, allowing this important effector mechanism of the complement system to function against tumour cells (Ross *et al*, 1999; Xia *et al*., 1999).

More than 100 triterpenoids have been isolated from the fruiting body and mycelia of *G. lucidum*, which include highly oxidised lanostane-type triterpenoids (e.g., ganoderic acid, lucidenic acid, ganodermic acids, ganoderenic acids, lucidone, ganoderal, and ganoderols) (Kim and Kim, 1999; Wasser and Weis, 1999a). Some of these triterpenoids have shown immuno-modulating activity (Kim and Kim, 1999). Immunomodulating proteins, such as Ling Zhi (LZ)-8, has been isolated from *G. lucidum*. (Tanaka *et al*., 1989). The major biological activities of LZ-8 resemble lectins, with mitogenic activity towards mouse spleen cells and human peripheral lymphocytes and agglutination of sheep red blood cells *in vitro*. Recently, immuno-modulatory protein (Fip-gts) has been purified from *G. tsugae* (Lin *et al*., 1997).

Extracts from *G. lucidum* containing poylsaccharides and LZ-8 have shown mitogenic effects on human peripheral blood mononuclear cells (PBMC) (King *et al*., 1989). Both *in vitro* and *in vivo* studies in mice have shown that water-soluble

extracts from *G. lucidum* can stimulate the production of interleukin (IL)-2 by splenocytes in the presence of hydrocortisone (Zhang *et al*., 1993).

Crude, water-soluble extracts from *G. lucidum* have been previously shown to be potent activators of human T lymphocytes, where they induce the production of cytokines such as IL-1β, INF-γ, TNF-α, IL-2, IL-6 and IL-10 (Wang *et al*., 1997; Mao *et al*., 1999). A polysaccharide fraction from *G. lucidum* (GLB) was shown to promote the production of IL-2 in a dose-dependent manner. GLB augmented the toxicity of cytotoxic T lymphocytes by as much as 100% when administered at a concentration of 200 μg/ml (Lei and Lin, 1992). LZ-8 also mediates T cell activation via cytokine regulation. Haak-Frendscho *et al*. (1993) showed that stimulation of PBMC by LZ-8 results in the production of IL-2 and a commensurate up-regulation of IL-2 receptor expression. LZ-8 also induces aggregate formation in PBMC, which is correlated to a marked rise in ICAM (intercellular adhesion molecule)-1 expression and to an increased production of INF-γ, TNF-α and IL-1β. The addition of neutralising monoclonal antibodies to IL-2 receptor and TNF-α blocks cellular aggregate formation and proliferation, and ICAM-1 expression.

A water-extracted polysaccharide fraction from *G. lucidum* enhanced the cytotoxicity of splenic NK cells in tumour-bearing mice (Won *et al*., 1989, Lee *et al*., 1995b). Murine and human macrophages are also activated by polysaccharides from *G. lucidum* (Lee *et al*., 1995b). The macrophage responses (such as the release of cytokines, nitric oxide and other mediators) are associated with anti-tumour and anti-inflammatory effects. CR3 receptors on human macrophages bind β-D-glucans and become internalised. This initiates a cascade of events including the production of IL-1β, IL-6, INF-γ and TNF-α which cause anti-proliferation and the induction of apoptosis in HL-60 and U937 leukemic cells (Lee *et al*., 1995b; Wang *et al*., 1997). Antibody neutralisation studies have shown that INF-γ and TNF-α

released from macrophages act synergistically to inhibit the growth of leukemic cells (Li *et al*., 2000).

A β-D-glucan (Ganoderan) and a protein-polysaccharide fraction (GLB) from *G. lucidum* are potent stimulators of mice and chicken macrophages. Ganoderan and GLB have been shown to increase the expression of MHC class II molecules on these antigen-presenting macrophages (Oh *et al*, 1998). There is also evidence to suggest that extracts from *G. lucidum* can influence humoral or B cell immunity. *In vivo* studies have shown that repeat administration of LZ-8 at 8-12 mg/kg reduces antibody production in mice (Kino *et al*, 1991). Lee *et al*. (1990) showed that an alkali extract from *G. lucidum* activated both the classical and alternative pathways of the complement system. This extract also activated the reticuloendothelial system and increased haemolytic plaque forming cells in the spleen of mice. A clinical study in aged patients with insomnia and palpitations recently showed that the consumption of *G. lucidum* extract for up to 6 weeks increased their serum C3 levels (Yang and Pai, 2000).

Various substances from *G. lucidum* (e.g., polysaccharides, triterpenoids, and proteins) have been shown to have marked immuno-modulating effects such as augmenting the activity of effector T cells, NK cells and macrophages. To-date, no pure β-glucan product derived from this particular mushroom has been commercially available. However, β-glucan appears to be one of the first biological response modifiers for which the cellular mechanism of action has been initially defined at the specific receptor level. As the cytotoxic host defence function of β-glucan is specific for target cells bearing iC3b, its' action in promoting host defense relies on the specificity of the antibody.

Immunomodulating effects of polysaccharides PSP and PSK from **Trametes versicolor**

Protein bound polysaccharides PSK (Krestin) and PSP have been isolated from the mushroom *Trametes versicolor*. These compounds are chemically relatively similar and have a molecular mass of about 100 kDa. The polysaccharide component is made up of monosaccharides with α-(1-4) and β-(1-3) glucosidic linkages. PSK and PSP differ mainly in the presence of fucose in PSK and rhamnose and arabinose in PSP. Both PSK and PSP are potent immunostimulators with specific activity for T-cells and for antigen-presenting cells such as monocytes and macrophages. The biologic activity is characterized by their ability to increase white blood cell counts, IFN-γ and IL-2 production and delayed type hypersensitivity reactions (Tzianabos, 2000). Numerous reports have documented the ability of PSK and PSP to activate cellular and humoral components of the host immune system. In addition, these polysaccharides have been shown to inhibit the growth of tumour cell lines and to have in vivo anti-tumour activity (Tzianabos, 2000). As there is considerable information on the immunomodulating activities of both PSP and PSK, they will be discussed separately.

The effect of PSP on the phagocytic functions has been tested in normal ICR mice. It was determined that the carbon clearance rate of the groups given oral (p.o.) doses of 0.5-1.5 g PSP/kg or intraperitoneal (i.p.) injections of 100, 200, 400 mg/Kg was similar to that of groups treated with acanthopanax (300 mg/kg). Regardless of the route of administration PSP increased the carbon clearance rate in mice, suggesting that PSP can appreciably increase the phagocytic function of normal animals (Jong and Yang, 1999; Yang *et al*., 1993). *In vitro* experiments with spleen T-lymphocytes cultured in solutions containing various concentrations of PSP showed that, when compared to the physiological saline control group,

concentrations in excess of 100 μg/ml produced an increase by a factor of 1.5 to 4 times in T-lymphocytes. It was also determined that PSP can appreciably increase the secretion of IL-2 in mice (Yang *et al*., 1993). Human white blood cells (WBCs) were cultivated in solutions containing different concentrations of PSP. Using vesicular stomatis virus as the challenge virus, the PSP induced interferon in human WBCs. γ-interferon levels in the PSP group were twice those of the control group, while α-interferon levels were two to four times higher. PSP can promote the expression of the IL-6 gene of peripheral blood lymphocytes (PBL) in humans and hence induce the production of interleukin 6 (IL-6) (Yang *et al*., 1993; Yu *et al*., 1996).

It has been recently reported that PSP in different concentrations promoted the proliferation of T-lymphocytes both in human peripheral blood and mouse splenocytes (Li *et al*., 1999). It appeared that human T-cells were more sensitive to PSP than that of mouse lymphocytes, a finding that was corroborated by Ling and Wang (1996). PSP augmented T-helper cell (CD_4^+) activation, and also increased the ratio of CD4+/T suppressor (CD8+) production. Li (1999) also showed that while the chemotherapeutic drug cyclophosphamide (CPA) inhibited the delayed type hypersensitivity (DTH) response in mice, administration of PSP and CPA together restored the DTH response in mice to normal levels, suggesting that PSP negates that inhibitory action of CPA in treated mice. CPA was first reported in 1958 and has become the leading drug of the alkylating class in the clinical treatment of cancer particularly for lymphomas, leukemias and a variety of solid tumours (Struck *et al*., 1995; Yule *et al*., 1996). Alkylating agents including CPA are cytotoxic, rapidly kill dividing neoplastic and normal cells and have drastic effects on cells on the immune system. Both humoral and cellular immune function may be inhibited to a varying degree by CPA (Awward *et al*., 1995). Indeed, it has been documented that CPA

reduces the ability of B lymphocytes to mount an appropriate antibody response *in vivo* and *in vitro*. CPA also reduces circulating levels of leukocytes and induces immunological function disturbances such as impaired phagocytic capability, release of lytic enzymes and inhibition of NK cell activity (Turk and Poulter, 1972; Dumont, 1974; Eremin; 1992, Qian *et al*., 1999).

PSP was shown to enhance B cell function (humoral immunity) in normal and tumour bearing mice that had been challenged with foreign antigen (i.e., sheep red blood cells). PSP increased the levels of haemolysin (antibody) in treated mice, this response was particularly pronounced in tumour bearing–mice (Zhou *et al*., 1988). PSP influenced macrophage response as it significantly increased both the clearance index and phagocytic index of mice that were intravenously challenged with charcoal particles. Enhanced production of IL-1, IL-6 and INF was also observed in activated macrophages (such as) from charcoal-challenged mice. While the use of PSP had no significant effect on NK activity in radio-labelled K562 tumour cells, it did increase NK activity in tumour cells that had been treated with cyclophosphamide. Treatment of K562 tumour cells with cyclophosphamide alone was shown to inhibit NK activity. This may explain in part, why PSP has been shown to improve the immune condition of patients receiving chemotherapy. Indeed, clinical studies showed that PSP treatment increased the activity of NK cells by an average of 64.5% in 138 cancer patients (Yang and Li, 1993).

Recent studies showed that PSP stimulates lymphokine-activated-killer (LAK) cell proliferation, and reduces the concentration of IL-2 needed to produce a cytotoxic response (Jian *et al*, 1999). Qian *et al*. (1999) also showed that PSP (2g/kg/day) possessed immunopotentiating activities, being effective in restoring cyclophosphamide (CPA) induced immunosuppression such as depressed lymphocyte proliferation, NK cell function, production of white blood cells and the

growth of spleen and thymus in rats. In addition, PSP increased both IgG and IL-2 production where CPA had significant inhibitory effects. PSP effectively stimulated the generation of INF-α reaching levels of 800 – 1000 IU/ml when the concentration of PSP was 100 μg/ml, and improved yields of INF-γ were reported (Yang *et al*., 1999). PSP in concentrations of 50-100 μg/ml promoted the proliferation of phytohaemagglutinin (PHA) - activated human peripheral blood lymphocytes (Liang *et al*., 1999). These researchers observed a greater increase in the CD_4^+ cell group levels compared with CD_8^+ cells, thereby raising CD_4^+/CD_8^+ ratio.

Macrophages play a pivotal role in non-specific immunity and can be activated by invading microorganisms, lymphokines, endotoxin and various cell mediators and regulators. An increase in the production of reactive nitrogen intermediates, reactive oxygen intermediates (superoxide anions) and TNF was reported by Liu *et al*. (1999) in peritoneal macrophages collected from C57 mice that had received PSP in drinking water for 2 weeks. Northern blot analysis of DNA also demonstrated that PSP activated the transcription of the tumour necrosis factor gene in these cells, indicating the PSP immunomodulatory effect on defensive cells. Liang *et al*. (1999) recently addressed the question as to whether polysaccharide-bound proteins (e.g., PSP) act by exerting cytotoxicity on tumour cells or by regulating the immune responses of effector cells such as macrophages. This showed that PSP at concentrations of 2.5-10 μg/ml did not exert any cytotoxicity on cultured mouse peritoneal macrophages nor on five tumour cell lines consisting of two macrophage-like cells (PU5 and P338D1), a human choriocarcinoma cell line (JAR), a mouse melanoma (B16) and sacroma (S180) cell lines. The molecular basis for the tumouricidal activity of activated macrophages is not clearly known, but their secretary products such as TNF and reactive nitrogen and oxygen intermediates may play an important role in this process (Nathan and Hibbs, 1991). Reactive

nitrogen intermediates suppress mitochondrial respiration of tumour cells, hence exhibiting their cytotoxicity against target cells (Takema *et al*., 1991). However, it is not known whether PSP directly modulates cytokine action or participates in signal transduction within macrophages.

PSK has been shown to have no substantial effect on immune responses of the host under normal conditions (Ehrke *et al*., 1983; Tsukagoshi *et al*., 1984). It can restore the immune potential to the normal level after the host was depressed by tumour burden or anticancer chemotherapeutic agents (Tsukagoshi *et al*., 1984; Dong *et al*., 1996, 1997). In ICR mice, antibody production against trinitrophenyl that had depressed the immunity in Sacroma 180-bearing mice can be restored by PSK administration. Oral administration of PSK can improve the impaired antitumour CD4+ T-cell response in gut-associated lymphoid tissue of specific-pathogen free mice (Harada *et al*., 1997). PSK enhances the cytotoxic activity of peripheral blood lymphocytes (PBLs) *in vivo* and *in vitro*. On a related issue, it may accelerate the interaction of PBL with tumour cells such as T24 human urinary bladder tumours when effector cells and target cells are exposed to PSK simultaneously.

After intra-tumoural adminstration, PSK may come into close contact with tumour cells, whereupon local inflammatory responses occur and result in the non-specific killing of these abnormal cells. Consequently, local administration of PSK is more efficient than systemic use (Mizutani and Yoshida, 1991). It has been reported that PSK induces gene expression of some cytokines such as TNF-α, IL-1, IL-8, and IL-6, *in vivo* and *in vitro* (Kato *et al*., 1995; Liu *et al*., 1996). These cytokines, produced by monocytes, macrophages, and various other cell types, mediate multiple biological effects by direct stimulation of cytotoxic T cells against tumours, enhancement of antibody production by B lymphocytes and induction of IL-2 receptor expression on T lymphocytes. The induction of TNF-α by PSK would

contribute, in part, to potent tumouricidal effects of this agent, as the administration of neutralizing antibody against TNF-α significantly attenuates the anti-tumour activity of PSK in the murine model (Kato *et al*., 1995). Interestingly, recent studies indicate that PSK exerts tumourcidal activity by inducing T cells that recognise PSK as an antigen and kill tumour cells in an antigen-specific manner (Okazaki *et al*., 1995).

The anti-tumour activity of medicinal mushrooms has been evaluated in Japan for prevention of esophageal, gastric, and lung cancers with promising results (Ng, 1998). In phase II and phase III trials in China, PSP significantly enhanced immune status in 70 to 97% of patients with cancer of the stomach, esophagus, lung ovary and cervix. In these studies, PSK and PSP increased the number of immune cells and facilitated dendritic and cytotoxic T-cell infiltration of tumours. The polysaccharides were well-tolerated and compatible with chemotherapy and radiation treatment.

Immunomodulatory activities of compounds from other medical mushrooms

Schizophyllan from *Schizophyllum commune*

In addition to the intensively researched mushrooms described previously, glucans and polysaccharide-bound protein complexes from many other medicinal mushrooms have been shown to exert immunomodulating activities *in vivo* and *in vitro*. Schizophyllan, from *Schizophyllum commune*, is relatively similar to Lentinan in composition and biological activity, and its mechanism of immunomoduation and anti-tumour action appears to be quite similar (Jong *et al*., 1991). Recently, the induction of gene expression of cytokines by schizophyllan has been studied *in vitro*

and *in vivo* (Nemoto *et al*., 1993; Okazaki *et al*., 1995). After schizophyllan is administered intraperitoneally to ICR mice, the kinetics of gene expression of cytokines is different in peritoneal exudate cells, splenocytes, and hepatocytes (Ooi and Liu, 1999). It is generally accepted that protein synthesis and gene expression of cytokines are regulated separately. Therefore, the antitumour activity of Schizophyllan is due mainly to host-mediated immune responses (Nemoto *et al*., 1993; Okazaki *et al*., 1995).

Neither Schizophyllan nor Lentinan demonstrated any anti-tumour activity against Sarcoma 180 in an *in vivo* experiment with cyclosporin A as a T cell suppressor, which suggests that an immunocompetent T cell component is necessary for developing anti-tumour activity (Kraus and Franz, 1991 and 1992). These results indicate that Schizophyllan and Lentinan are T-cell oriented immunopotentiators and, therefore, require a functional T cell component for its biological activity and that the action of (1-3)-β-D-glucans on the host's immune system might: (1) increase helper T cell production, (2) increase macrophage production, (3) bring about a non-immunological increase of the host defence mechanisms through stimulation of acute phase proteins and colony stimulating factors, which in turn effects proliferation of macrophages, PMNC, and lymphocytes and activation of the complement system (Bohn and BeMiller, 1995).

Grifolan from *Grifola frondosa*

Another (1-3)-β-glucan, Grifolan, from *Grifola frondosa* is similar to schizophyllan in primary structure (Adachi *et al*., 1990). Enhancement of mRNA levels of IL-6, IL-1 and TNF-α of macrophages by Grifolan treatment is detected *in vitro* by reverse transcription-polymerase chain reaction (RT-PCR), showing that grifolan is a novel macrophage activator that increases cytokine production (Adachi

et al., 1994; Ooi and Liu, 1999). A novel polysaccharide-bound protein (PSPC) (Mol. Wt. 15.5 KDa) has been isolated from cultured mycelia of *Tricholoma mongolicum* Imai (Wang *et al*., 1996). PSPC activated both lymphocytes and macrophages from BALB/c mice and showed no direct cytotoxic activity against fibroblasts, hepatoma cells, and choriocarcinoma cells. Similarly, an immunomodulatory and anti-tumour PSPC with a molecular weight of about 154 x 10^3 has been purified and characterised from the culture filtrates of *Tricholoma lobayense* Heim (Liu *et al*., 1995, 1996). It inhibited the growth of Sacroma 180 implanted in mice intra-peritoneally or subcutaneously, with no sign of toxicity *in vivo* (Liu *et al*., 1995). PSPC has been able to restore the phagocytic function of peritoneal exudate cells and the mitogenic activity of T cells of tumour-bearing mice. Moreover, the induction of gene expression of nine out of seventeen cytokines and five out of six cytokine receptors in peritoneal exudate cells and splenocytes by administration of PSPC prepared from *Tricholoma lobayense* has been confirmed by RT-PCR and *in situ*-hybridization (Liu *et al*., 1996a). This suggests that the immune cells are responding to PSPC through gene expression and the production of immunomodulatory cytokines that might mediate immunopotentiation of this agent *in vivo* (Liu *et al*., 1999).

Immunopotentiation effected by binding of mushroom β-glucans or polysaccharide-protein complexes includes activation of innate defences (such as cytotoxic macrophages, neutrophils and NK cells) and stimulation and proliferation of humoral (B cells) and cell-mediated immune systems (such as helper T cells, promotion of T cell differentiation), and activation of alternative complement pathway. Pharmacologically, these mushroom compounds are classified as biological response modifiers and have antitumour activity, a result of activation or augmentation of the host's immune system or immunocompetency rather than direct

cytotoxicity. However, recent evidence suggests that some mushroom polysaccharides may also possess cytotoxic properties. In search for a more effective treatment for hormone-refractory prostate cancer, the potential antitumour effect of Grifron-D (a unique β-glucan from the Maitake mushroom *Grifola frondosa*) on androgen-independent prostatic cancer PC-3 cells was investigated (Fullerton *et al*., 2000). A dose-response study showed that almost complete (>95%) cell death was attained in 24 h with ≥ 480 μg/ml Grifron-D. Combinations of Grifron-D in a concentration as low as 30 to 60 μg/ml with 200 μM vitamin C were as effective as GD alone at 480 μg/ml, suggesting that vitamin C acts synergistically to potentiate Grifron-D activity. Significantly elevated lipid peroxidation levels and positive *in situ* hybridization staining of Grifron-D treated cells indicated oxidative membrane damage resulting in apoptotic cell death. These seminal findings have shown that this bioactive β-glucan from the Maitake mushroom has a cytotoxic effect, presumably through oxidative stress, on prostatic cancer cells *in vitro*, leading to apoptosis.

The information presented here illustrates the distinct biologic properties associated with mushroom polysaccharides and polysaccharide-bound protein complex immunomodulators. While the activity of some of these polymers has been well-documented, the lack of defined structural and mechanistic information for promising compounds from other mushrooms is limiting efforts to study their potential for clinical use. Recent investigations have led to a more detailed understanding of structural aspects of polysaccharides that influence biologic function and host immune responses (Bohn and BeMiller, 1995). Continued advancements in our understanding of particular structure-function relationships and polysaccharide-specific receptors should provide a foundation for the further development of these compounds that have novel immunomodulatory activities.

In conclusion, a fundamental principal in Oriental medicine is to regulate homeostasis of the whole body and to bring the diseased person to his or her normal state (Chihara *et al*., 1992). Potentiating the physiological constitution in favour of host defence results in the activation of many vitally important cells for the maintenance of homeostasis. We here report that a wide variety of medicinal mushrooms fit the criteria of host defence potentiators where many were shown previously to possess novel characteristics associated with the immune and other systems (such as nervous and endocrine). A variety of polysaccharides from a variety mushrooms have the ability to enhance the immune system, i.e., behave as immunomodulators. All have shown significant anti-tumour activity, a result of activation of the host's immune system, rather that direct cytotoxicity. The most active immunomodulators come from mycelia, fruiting bodies and from culture fluids of fungi and warrant further investigation. The mushroom polysaccharides appear to be well-tolerated and compatible with chemotherapy and radiation therapy. However, studies that identify the molecular mechanisms that occur in specific immuno-modulation by MMs, such as receptors and what downstream events are triggered by the binding of these polymers to their target cells, are urgently needed.

Evidence for β-glucan receptor binding of immune cells

Ross and co-workers (1999) showed recently that β-glucans from fungi bind to specific iC3b-receptors (CR3, CD11b/CD18) of phagocytic cells and NK cells, stimulating phagocytosis and/or cytotoxic degranulation. The iC3b-receptor, CR3, known also as Mac-1 or $\alpha_M\beta_2$-integran, has two major functions. As Mac-1 adhesion molecule, it mediates the diapedesis of leukocytes through the endothelium and it

stimulates phagocytosis and degranulation in response to microorganisms or immune complexes opsonised (i.e., coated with) iC3b (Ross et al., 1999).

Most β-glucan that have immuno-modulatory properties are derived from yeast and fungi (mushrooms) and have a backbone structure of linear β-1, 3-linked D-glucose molecules with β-1, 6-linked sied chains (Bohn and BeMiller, 1995). Although somewhat controversial (Czop and Kay, 1991; Zimmerman et al, 1998), recent data suggest that CR3 serves as the major, if not only receptor for β-glucans with human (Thornton et al, 1996) or mouse (Xia et al., 1999) leukocytes, and therefore, may be responsible for all reported functions of β-glucans *in vitro* and *in vivo*. These β-glucans polymers specifically target macrophages, neutrophils, and NK cells to tumours that are opsonised with antibody and C3 (complement), and therefore, β-glucan appears to have the same specificity as opsonising antibody (Ross, 1999).

As stated by Hobbs (2000) “This research has particularly shown the therapeutic value in mice of small soluble β-glucans (5 – 20 Kda) that bind to CR3 with high affinity and prime, the receptor for subsequent cytotoxic activation if, and only if, CR3 subsequently comes in contact with an iCR3-opsonised target immune cell”. Furthermore, particulate β-glucan and high molecular weight, soluble β-glucans (such as Lentinan and Schizophyllan) that have been used for patient therapy in Japan have been shown to be large enough to cross-link membrane CR3 of neutrophils and monocytes, triggering respiratory bursts, degranulation, and cytokine release (Ross, 2000).

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CHAPTER 7 THE ROLE OF POLYSACCHARIDES DERIVED FROM MEDICINAL MUSHROOMS IN CANCER

Synopsis

This Chapter sets out the current information on the use of various mushroom polysaccharides in cancer treatment. Many human cancer cell-lines have been studied and in some cases direct cytotoxic effects have been demonstrated. Many of the mushroom polysaccharide compounds have proceeded through to Phases I, II and III clinical trials and several are used extensively in Asia to treat various cancer. It is anticipated that such proprietary mushroom compounds will mainly be used as complementary or adjunctive therapies to be used in adition to mainstream care.

INTRODUCTION

It has been generally recognised that in the treatment of cancer surgery with or without radiotherapy remains the modus operandi for most cancer cures. Radiotherapy is used quite successfully for many forms of cancer while chemotherapy has become an integral part of a multi-disciplinary treatment of cancers and has served also as a palliative measure in cases of advanced cancer.

However, in almost all cases, a major cause of treatment failure has been the development of distant metastases. While surgery and radiotherapy are all means of eradicating loco-regional disease they are of little value with distant metastases. For such distant metastases chemotherapy is the recommended approach but effectiveness is limited by toxic side-effects at high doses. Furthermore, within the holistic approach of clinical cancer therapy there is now increasing emphasis being given to patient quality of life (QOL) following these above classical treatments. Survival should not be the sole criterion for assessing the treatment results. Thus, it has increasingly become an accepted practice that the oncologist should combine all

available disciplines that could contribute to patient welfare after the main treatment(s) has attempted to destroy the primary cancer site.

It is also well-recognised that both radiotherapy and chemotherapy invariably damage or weaken the patient's immunological defenses which may also have been damaged by the cancer itself. From these observations there has now developed a new awareness in cancer therapy, viz. is it possible to modify the host biological response to malignant invasion? As discussed in the previous chapter, Biological Response Modifiers have now evolved as the fourth method of cancer treatment in addition to surgery, radiotherapy and chemotherapy. Such treatments with BRMs are considered more biological than directly cytotoxic.

This chapter will set out the current information available on the use of various mushroom polysaccharides in cancer treatment procedures. In all cases these compounds have demonstrated pre-clinical efficacy, including direct cytotoxicity. However, many drugs can be effective in the laboratory but fail in clinical practice due either to inherent toxicity when used at effective dose rates or lack of efficacy.

While the vast majority of the published studies on the use of medicinal mushroom polysaccharides in oncology have appeared in Oriental Journals, there has been a major increase in publications in peer-reviewed Western Journals by Asian scientists and a perceptible change in the attitude of Western medical doctors and scientists towards the pharmaceutical developments derived from traditional Chinese medicines (Kidd, 2000).

While all of the mushroom polysaccharides successfully used in animal and human cancer treatments have been administered intravenously, several can also be effective by oral (p.o.) administration. Delivering anticancer agents by oral methods is becoming increasingly important in cost reduction of the regime for a

disease that requires protracted treatment and for the patient's increasing preference and improved quality of life. Orally formulated chemotherapy is increasing in contemporary oncology practice driven not only by a preference for outpatient treatment but also by the potential for improved quality of life. Since cytostatic therapy often requires protracted drug administration, the use of a self-administered oral formulation is to be preferred (Demario and Rateim, 1998; Sulkes *et al*. 1998).

As discussed later in this section, two mushroom polysaccharides (Lentinan and Schizophyllan), both large molecules, are only effective by i.v. or i.p. administration. Furthermore, in this context, it is pertinent to note that a recent study with the antitumour β-1,6 glucan from *Agaricus blazei* with mice showed that i.v. administration gave highly satisfactory results while no effect was seen with oral administration. However, a simple acid treatment of the whole β-1,6 glucan produced molecular masses of *c* 10k Da which when administered orally to mice demonstrated activity (Fujimiya *et al*. 2000). This study could well have significant application with the other large β-glucans and so improving their oral bioavailability and increased use as immunonutriceuticals.

In a recent survey of the clinical testing of new oncology drugs, it was pertinent to note the large number of immunological research programmes as well as a number of studies examining drugs that stimulate apoptosis (aimed at inducing programmed cell death in cancer cells)(Pigache, 2001). In almost all the examples that will be discussed in this chapter the polysaccharides act mainly as immune-stimulants *with little or no adverse drug reactions*. Furthermore, several of these extracts have been shown to stimulate apoptosis in cancer cells (e.g. Fullerton *et al*., 2000).

Many of the mushroom polysaccharides have proceeded through Phase I, II and III clinical trials. With the exception of Lentinan (*L. edodes*), PSK and PSP (*T. versicolor*) where many hundreds of cancer patients have been subjected to clinical trials the other compounds have only been assessed in small numbers of patients. In Japan and China, Phase I clinical trials have little significance since no maximum tolerated dose was reached. Recently, the FDA in US has exempted Maitake-polysaccharides from Phase I study because of limited side-effects. While there are examples where the mushroom polysaccharides have shown efficacy against specific types of cancer as monotherapy the overwhelming successes have been demonstrated when they function together with proven and accepted chemotherapeutic agents. The degree to which medicinal mushrooms have been tested for *in vitro* and *in vivo* activity varies. In some cases, such as with Polysaccharopeptide (PSP) extensive *in vitro* activity has been demonstrated against a variety of cell lines (human leukemia cell line, S180/H238 sarcoma, P388 leukemia, etc.) and a number of xenografts (nasopharyngeal carcinoma, Lewis lung, etc.) (extensively reviewed in Xu, 1999). However, there still remains the need to carry out more systematic studies to complement the promising clinical data.

Lentinus edodes

There is an immense literature related to the anticancer effects of Lentinan on animals and humans and only the more relevant and recent medical studies will be presented here. Lentinan was first isolated and studied by Chihara *et al*. (1970) who demonstrated that its anti-tumour effects were greater than other mushroom polysaccharides and was active for some, but not all, types of tumours (Maeda *et al*., 1974). The purified polysaccharide has been shown in numerous xenographs to cause tumour regression and in some cases even a complete response (for

extensive review of animal studies, see Hobbs, 1995, Wasser and Weis, 1999). The cytostatic effect of Lentinan is due to the activation of the host's immune system. Also, pre-clinical and clinical toxicity with Lentinan is rarely noted. Accumulated information on anti-tumour activity, prevention of metastasis, and suppression of chemical and viral oncogenesis in animal models by Lentinan are summarised in Table 1 (Wasser and Weis, 1999).

While Lentinan is a pure polysaccharide composed only of atoms of carbon, oxygen and hydrogen, LEM and LAP, also present in mycelial extracts of *L. edodes*, are glycoproteins, and have demonstrated antitumour activity in xenograft models and clinical trials. Again, both LEM and LAP activate the host immune system (Mizuno, 1995). In Japan Lentinan is presently classified as a medicine whereas LEM and LAP are considered as food supplements (nutriceuticals).

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

Lentinan has proved successful in prolonging the overall survival of cancer patients, especially those with gastric and colorectal carcinoma (Furue *et al*., 1981, Taguchi *et al*., 1985a,b). In patients with inoperable or recurrent gastric cancer, tumour responses and prolonged median survival were also noted. In a randomised controlled study of patients treated with tegafur or a combination of Lentinan and tegafur overall survival was significantly prolonged in the Lentinan plus tegafur group. Of 145 patients, 68 received tegafur alone, and 77 received Lentinan plus tegafur. The respective 50% survival times for the two groups were 92 days

Table 1 Lentinan – pre-clinical animal models (Wasser and Weis, 1999)

Model	Model	Dose of Lentinan (mg/kgxdays)	Tumour inhibition ratio (%)	Complete regression of tumour	Decreased tumour occurrence
1	2	3	4	5	6
Allogeneic					
Sarcoma 180	CD-1/ICR	0.2 x 10	78.1	6/10	
		1 x 10	100.0	10/10	
		25 x 10	88.2	0/8	
		80 x 5	-8.5	0/8	
	SWM/Ms	1 x 10	100.0	10/10	
	A/J	4 x 5	96.5	9/10	
	C3H/He	4 x 5	36.2	0/6	
	C57/BI/6	4 x 5	51.8	0/6	
Syngeneic					
A/Ph.MC.S1	A/Ph(A/J)	1 x 10	100.0	18/18	
DBA/2.MC.CS1	DBA/2	1 x 10	76.5	2/7	
P-815	DBA/2	5 x 4	89.0	2/8	
L-5178Y	DB/2	10 x 3	84.0	3/9	
MM-46	C3H/He	5 x 2	100.0	9/9	
Autochthonous					
MC-induced primary	DBA/2	1 x 10	80.5	2/5	
Inhibition of metastasis					
DBA/2.MC.CS-T	DBA.2	1 x 10	94.2		
MH-134	C3H/He	1 x 14	100.0		
Madison-109	BALB/c	25 x 2			
Prevention of oncogenesis					
MC-induced	SWM/Ms	1 x 10			83→31%
MC-induced	DBA/2	1 x 10			78→37%
Adenovirus	C3H/He	10 x 3			79→40%

Note: all tumours were solid, transplanted s.c. Route of Lentinan injection was i.p., except i.v. for P-815, L-5178Y, and MM-46. Tumor inhibition ratio = (C-T)C x 100, where C = average tumour weight of control mice and T = that of Lentinan-treated mice.

(tegafur alone) and 173 days (Lentinan plus tegafur). Sub-group analysis was also carried out by: (1) tumour extension, (2) histology; and (3) Borrman classification. With each prognostic factor the addition of Lentinan significantly prolonged 50% survival (Table 2).

Overall more patients with the combined therapy appeared to survive longer: 19.5% survived more than one year, 10.4% more than two years and 6.5% more than three years. Using the criteria of the Japan Society for Cancer Therapy for Evaluation of Clinical Effects of Cancer Chemotherapy on Solid Tumors patients treated with

Lentinan had a significantly higher response rate (14.9%) than patients in the control arm (2.0%).

Table 2 Prolongation of life by various prognosis factors (Ajinomoto Co. 1984)

Background		Treatment	No. of cases	50% survival (day) 100 150 200 250 300 350 400
Extension of tumour	Abdominal localisation	Tegafur group	13	166 days
		LENTINAN _ tegafur group	19	237 days
	Hepatic or peritoneal metastasis	Tegafur group	47	68 days
		LENTINAN + tegafur group	48	169 days — P < 0.01
	Distant metastasis	Tegafur group	8	170 days
		LENTINAN + tegafur group	9	133 days
Histolgy	Well-differentiated adenocarcinoma	Tegafur group	31	105 days
		LENTINAN + tegafur group	34	223 days — P < 0.01
	Poorly-differentiated adenocarcinoma	Tegafur group	34	91 days
		LENTINAN + tegafur group	40	169 days — P < 0.01
Borrmann classification	Borrmann types 1 and 2	Tegafur group	7	119 days
		LENTINAN + tegafur group	7	391 days
	Borrmann types 3 and 4	Tegafur group	32	100 days
		LENTINAN + tegafur group	41	163 days — P < 0.01

Lentinan combined with other chemotherapeutic agents appears to have efficacy in a variety of settings (Matsuoka *et al*., 1995). Furthermore when patients responded well to Lentinan treatment there was a significantly larger response (2.5 x) in their killer T cell/suppressor T cell ratio ($CD11^{-}$ $CD8^{+}$/$CD11^{+}$ $CD8^{+}$) in peripheral blood. The ratio of NK cells with higher activity to NK with moderate activity ($CD57^{-}$

$CD16^+/CD57^+$ $CD16^+$) was higher in the responders than in the non-responders and correlated well with survival times. However, these results remain controversial as a later study suggested that lymphocyte subset changes in peripheral blood did not necessarily correlate with the lymphocyte subset changes that were taking place in the tumour (Matsuoka *et al*., 1997).

Few adverse reactions to Lentinan have been noted. In a detailed study of 469 patients, 32 (6.8%) experienced an adverse reaction – none serious; the total number of episodes was 46 (9.8%) (Table 3). Only 2 patients required discontinuation of treatment due to unacceptable tolerance. Perhaps the most intriguing aspect of Lentinan use in conjunction with chemotherapy is its apparent ability to greatly reduce the debilitating effects of the chemotherapy, *e.g*. nausea, pain, hair loss and lowered immune status. Although there have been few formal quality of life studies this anecdotal evidence has been noted as a feature of many of the mushroom polysaccharides.

Table 3 Adverse reactions attributable to Lentinan (Ajinomoto Co., 1984)

Number of patients evaluated for adverse reactions		469
Number of patients with adverse reactions (%)		32 (6.8)
Number of episodes of adverse reactions (%)		46 (9.8)
		Incidence (%)
Type of adverse reaction (with an incidence greater than 0.5%)	Rash/redness	1.9
	Chest pressure sensation of oppression	1.7
	Nausea/vomiting	1.7
	Headache/headache dull	0.6
	Feeling of warmth	0.6
	Diaphoresis	0.6

Other adverse reactions:

Fever, transient hot flushes of face, anorexia, leukopenia, 2 episodes each (0.5%); dizziness, decreased PBC, decreased haemoglobin, pharynx strangled sensation of pharyngitis, 1 episode each (0.2%) (from Ajinomoto Technical Document).

Schizophyllum commune

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

Schizophyllan has been shown to be cytostatic in Sarcoma 180 tumours xenographs. The survival of Sarcoma 180 xenographs was not affected by pre-treatment with Schizophyllan, while combined pre- and post-treatment and post-treatment alone resulted in increased survival. Schizophyllan had no effect on the survival of Sarcoma 37, Ehrlich carcinoma – or Yoshida sarcoma ascites tumours (Wasser and Weis, 1999).

Various clinical trials have been carried out in Japan, although many are not blinded. Despite this Schizophyllan has been approved for clinical use in Japan. Early clinical studies with Schizophyllan in combination with conventional chemotherapy (tegafur or mitomycin C and 5-fluorouracil) in a randomised controlled study of 367 patients with recurrent and inoperable gastric cancer resulted found a significant increase in median survival (Furne, 1985). However, a similar study was unable to confirm this apparent success with Schizophyllan (Fugimoto *et al*., 1984). Recently Schizophyllan has also been shown to increase overall survival of patients with head and neck cancers (Kimura *et al*., 1994).

In a randomised controlled study of Schizophyllan in combination with radiotherapy, Schizophyllan significantly prolonged the overall survival of Stage II cervical cancer patients but not Stage III (Okamura *et al*., 1986, 1989). In a

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

Grifola frondosa extracts

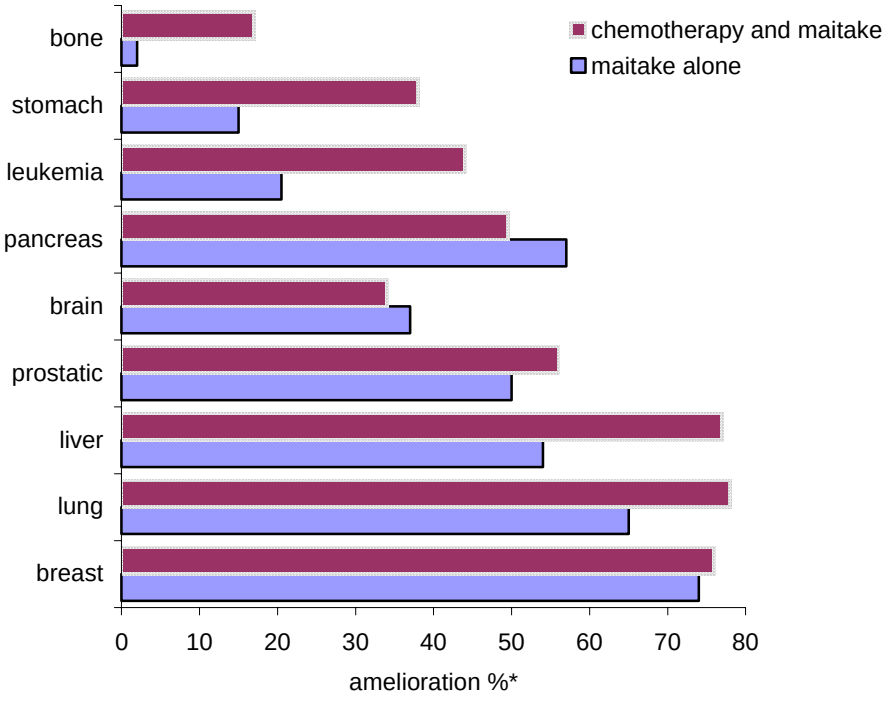
Several studies have shown that β-D-glucan and glycoprotein complexes derived from this mushroom (also known as Maitake) have strong antitumour activity in xenographs (Kurashiga *et al*., 1997) and there have also been limited number of clinical trials. More recently, a highly purified extract, β-glucan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

prostate cancer cells (PC9) *in vitro*, possibly acting through oxidative stress, and causing 95% cell death by apoptosis (Fullerton *et al*., 2000). Vitamin C addition reduced the effective level of GD required. Simultaneous use with various anticancer drugs showed little potentiation of their efficacy except for the carmustine/GD combination (90% reduction in cell viability). This potentiation of GD action by vitamin C and the chemosensitising effect of GD on carmustine may well have significant clinical implications. Unpublished studies by the same authors (manuscript in preparation) again using prostate cancer cells *in vitro* have shown that the cytotoxic effects of the anticancer drug was significantly potentiated or enhanced with GD, possibly mediated through the inactivation of glyoxalase I, a vital detoxifying enzyme responsible for detoxification of cytotoxic metabolites / substances. This study suggests that GD may be useful with some anticancer drugs to improve the efficacy of ongoing clinical chemotherapy. The Maitake D-fraction is a relatively new compound and there are a number of clinical trials in breast, prostate, lung, liver, and gastric cancers underway in the US and Japan. Most of these are at an early clinical stage (phase I / II).

Early pilot studies from China published in abstract form involving 63 cancer patients reported a response rate (partial and complete) against solid tumours at 95% and for leukaemia (type not specified) 90% (Jones, 1998). A recent Japanese non-randomised clinical study using the D-fraction has been carried out in a variety of advanced cancer patients (n=165). Patients took either oral D-fraction plus crude Maitake powdered tablets, or D-fraction plus placebo tablets in addition to chemotherapy (Nanba 1997a). Tumour regression or significant symptomatic improvement were observed in 11 out of 15 advanced hepatocellular carcinomas with D-fraction plus Maitake. When D-fraction plus Maitake was combined with

chemotherapy, the overall response rates were increased by 12-28% when results from all cancer types were combined.

Fig. 1 Effects of Maitake D-Fraction on Cancer Patients
(reproduced from Nanba 1997)



*Definite tumour regression and/or significant symptomatic improvement

As the authors of this study observed chemotherapy itself could also significantly lower the immune system of patients. They reported that many of the patients recovered from the severe side-effects caused by chemotherapy when D-fraction was given, although this conclusion appears to be an anecdotal observation. In a similar manner to Lentinan, there are now increasing examples of synergism between Maitake D-fraction and crude Maitake powder and conventional chemotherapy.

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

Phellinus linteus

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

become available in Japan for sale as a functional food (an immunity activation substance).

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

Active Hexose Correlated Compound (AHCC)

The components of this proprietary extract have been considered elsewhere in this Report, and the full details of preparation and content are not available. In contrast to the other anticancer glucans, the glucans of AHCC are low molecular weight, alpha-1,3 structures. As such, they should have low-immunopotentiating activity but still retain their tumouro-static activity.

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

The AHCC Research Association was formed in 1996 to advance the awareness of AHCC as an anticancer therapy. They state that of 300 cancer patients administered AHCC, 58 patients experienced same effect, 46 showing complete or partial responses. The participants in these studies had cancers of the

lung, breast, stomach, oesophagus, colon, liver etc. To date, the published evidence of the efficacy of this complex preparation must be treated with some scepticism until more detailed controlled studies are forthcoming.

Ganoderma lucidum

Over the last ten years there have been numerous reports of pre-clinical anti-tumour activity of *G. lucidum* extracts in a variety of tumours (Lee *et al*., 1995; Wang *et al*., 1997). Such extracts effectively inhibited metastasis in animal (mouse) models and increase survival when administered as monotherapy or in combination with conventional chemotherapy (Hwang *et al*., 1989; Furusawa *et al*., 1992; Lee *et al*., 1995). Some preclinical studies have suggested that the anti-tumour action of *G. lucidum* polysaccharides could be a result of its biological response modifying effects (Chang, 1996). Ganopoly (an aqueous extract of *G. lucidum*) has been shown in *in vitro* systems and in xenographs to have immunomodulating effects, through the activation of macrophages, T-lymphocytes, and natural killer cells (Gao, 2000).

Within the realms of traditional herbal medicine in China and in several Asian countries many cancer patients use *G. lucidum* proprietary extracts as adjunct to conventional treatment or as the sole therapy. What then can be said of the effectiveness of such products on human cancers? Relatively few clinical studies have so far been published in Chinese while no clinical trials with *G. lucidum* extracts against various human cancers have been published in English peer-reviewed journals (Gao, 2000). However, an extensive open, non-randomised clinical trial has recently been carried out on of patients with advanced cancers using a proprietary aqueous extract of *G. lucidum* – Ganopoly (Zhou *et al*., 2001). This compound is marketed as an over-the-counter product in Hong Kong, New Zealand, and Australia.

The clinical trial was carried out to evaluate the efficacy and safety of Ganopoly in 143 patients with advanced cancers of the lung, breast, liver, colorectum, prostate, bladder, brain and non-Hodgkin's lymphoma that had already been treated with conventional chemotherapy. This trial explicity follows many of the rules and conventions that define Western oncology trials. Eligibility criteria included confirmation of diagnosis, objective measurable disease, Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, life expectancy of 12 weeks or greater, no recent or concomitant anti-cancer therapy, and informed consent. All patients underwent evaluation of the extent of the disease, quality of life, hematologic, biochemical and selected immune function studies at baseline and after 6 and 12 weeks of Ganopoly therapy. Standard criteria were used to evaluate adverse events and responses. The extracts were given orally at 1800 mg three times daily. Patients were entered into the study from January 1997 through September 1998 if they met certain eligibility criteria and did not meet any of the recognised exclusion criteria. Eligibility and exclusion criteria were according to internationally accepted rules for clinical trials. WHO (1979) criteria were used to evaluate efficacy and toxicities were graded according to the Common Toxicity Criteria (Green and Weiss, 1992). A complete response (CR) was defined as the complete disappearance of all tumour masses without the appearance of any new lesions and normalisation of all clinical and laboratory signs and symptoms of active disease. A partial response (PR) was defined as a 50% or greater reduction of the products of the longest perpendicular diameters of the measured sentinal lesions without demonstrable new lesions elsewhere. Stable disease (SD) occurred when no new lesions appeared and no measurable lesions increased more than 25% in a cross-directional area. Progressive disease (PD) was defined as the appearance of

new lesions and/or an increase in the cross-sectional area of any previously known lesions by greater than 25%. Quality of life was quantified with the previously validated Functional Assessment of Cancer Therapy-General (FACT-G) scale (Cella *et al*., 1993).

The trial was designed to enrol 15 assessable patients for each of the 8 tumour types with an initial entry of 120 patients. Further accrual would be halted if no CR or PR were observed in each specific tumour type and the therapy would then be judged to be inactive. Should one or more CRs or PRs occur among the 15 patients, another 25 patients would be brought in. Tumour types with four or more CRs or PRs were to be targeted for further study. The planned accrual was designed to provide a 90% likelihood of rejecting treatment with the true response rate of 5% or less and a 90% probability of accepting treatment with a true response rate of 20% or more. Data compiled at baseline, 6 weeks and 12 weeks were analysed by two-way analysis of variance by ranks. Median values of quantities, such as age and days since diagnosis, were compiled using Wilcoxon's rank-sum test, and categorical values (of quantities such as primary tumour site and off-study reason) were compared using chi-squared tests and for 2X2 table, Fisher's exact test.

A total of 83 men and 60 women were enrolled and the median age of all patients was 61 years. Ninety three percent of the patients had stage IV disease. Twenty seven patients were not assessable for response and toxicity because they were lost to follow-up or refused further therapy before 12 weeks of treatment.

Of the 100 fully assessable patients, 46 patients (32.2%) had progressive disease (PD) before or at the 6 week evaluation point (range, 5 days – 6 weeks). Sixteen patients (11.2%) developed PD between 6 and 12 weeks of therapy. No

objective (partial or complete) responses were observed, but 38 of 143 patients (26.6%) had stable disease (SD) for 12 weeks or more (range 12 – 50 weeks). There was no significant changes in the FACT-G scores in 85 assessable patients. However, palliative effects on cancer-related symptoms, such as sweating and insomnia were observed in many patients. In the group of patients with SD, FACT-G scores improved in 23 patients, unchanged in 5 patients and declined in 1 patient. Within this group, the median change for the baseline score to the 6- and 12-week score was +7.6 and +10.3 score, both statistically significant ($P < 0.05$). No significant change of the selected immune function parameters were observed in 75 assessable patients. However, in the group of 32 patients with SD for 12 weeks or more, Ganopoly significantly increased lymphocyte mitogenic reactivity to concanavalin A and phytohemagglutinin by 48-52% ($P < 0.05$) and significantly enhanced natural killer cell activity by 75% ($P < 0.05$). Five adverse events (grade I) were recorded, 3 of which were gastrointestinal (nausea 2; diarrhoea, 1).

While objective responses were not observed with this study the results do indicate that this *Ganoderma* extract, Ganopoly, could well have an adjuvant role in the treatment of patients with advanced cancer (Cassileth, 2000; Jacobson *et al*., 2000).

Recently there has been a Phase II clinical trial with a herbal supplement PC SPES which includes, with other components, extracts of *G. lucidum*, of patients suffering from prostate cancer (Small *et al*., 2000). The treatment significantly reduced the serum prostate-specific androgen (PSA) levels in all 33 androgen-dependent prostate cancer patients with a duration of > 57 weeks. Further details are not yet available.

Trametes versicolor

Trametes versicolor is not an edible mushroom but since ancient times extracts has been used in traditional Chinese medicine for therapeutic effects including the treatment of cancer. TCM used the extracts that were derived from whole fruit-bodies. Today two compounds, PSK (polysaccharide-K) and PSP (polysaccharide-peptide) are purified from this fungus by deep tank fermentation of the mycelium using a variety of strains. PSK (Krestin) was first isolated in Japan in the late 1960s while PSP was isolated about 1983 in China. Each compound has shown remarkable anticancer properties with few side-effects. Remarkably by 1987 PSK accounted for more than 25% of total national expenditure for anti-cancer agents in Japan. Numerous clinical trials have been carried out over the years and are briefly summarised below:

PSK:

There have been several decades of successful clinical trials using PSK to treat head and neck, upper GI, colo-rectal and lung cancers with some reported success in treating breast cancer as well. Clinical trials with PSK have recently been extensively reviewed by Kidd (2000) and will be briefly summarised here. Almost exclusively, clinical trials have been carried out in Japan.

PSK and gastric cancer:

PSK has been used as a form of immunotherapy for more gastric cancer patients than any other cancer type. In early 1970s Kaibara's group began trialing PSK with their existing chemotherapy regimens for stage IV disease (Kaibara *et al*., 1976). After surgical resection (partial or full gastrectomies), PSK at 3g per day was

added to a chemotherapy regimen of Mitomycin C and 5-fluorouracil (5-FU) (n=66). When compared with a historical control group, the 2 year survival rate was more than double, a finding that was later confirmed by Fujimoto *et al*. (1979) in a larger prospective study (*n*= 230). Further studies by Hattori *et al*. (1979) (n=110) and Kodama *et al*. (1982) (*n* =450) suggested that PSK gave some protection against the immunosuppression that normally is associated with surgery and long-term chemotherapy.

One of the few double-blind randomised controlled trials (*n*=144) examining the role of single agent PSK found a significant increase in disease-free and overall survival. PSK had significant effects on these patients immune systems as measured by increased delayed-type hypersensitivity on skin tests and enhanced chemotactic migration of neutrophils (Kondo and Torisu, 1985). All these studies suggest that individuals with very low immunity are less likely to benefit from PSK therapy than individuals with a reasonably competent immune system. Other non-randomised trials in Japan have supported these findings (Mitomi and Ogoshi, 1986; Niimoto *et al*., 1988; Maehara *et al*., 1990; Nakazato *et al*., 1994). Tsujitani *et al*. (1992) had previously observed that dendritic cells could infiltrate gastric cancers in some patients and biopsy examination correlated this dendritic infiltration of their tumours with an increase in disease-free and overall survival post-surgery. It was concluded that patients with gastric cancer with limited dendritic cell infiltration prior to surgery when given PSK immunotherapy were more likely to have significant response. The most recent phase III 2 arm trial of PSK in the treatment of gastric cancer carried out by the “Study Group of Immunochemotherapy with PSK for Gastric Cancer of Japan” showed that combining PSK with conventional

chemotherapy significantly improved disease-free and overall survival (Nakazato *et al*., 1994).

PSK and other cancers

In a non-controlled, retrospective analysis of combined radiation, chemotherapy and immunotherapy (using PSK or OK-32, another immuno-potentiator) with 133 patients with oesophageal cancer, there were improvements in one-year and two-year survival (Okudaira *et al*., 1982). In another more recent study PSK improved overall survival in oesophageal cancer in patients with levels of pre-operative high α1-anti-chymotrypsin or sialic acid (Ogoshi *et al.,* 1995). In a small scale trial in Taiwan for nasopharngeal carcinoma PSK adjunct therapy had a small but significant impact on five-year survival (Go and Chung, 1989).

In a study of 185 patients with epidermoid carcinoma, adenocarcinoma or large-cell carcinoma ($\leq$ IIIb) given PSK as an immune system potentiator following radiotherapy, almost four times more patients who were treated with PSK had significant improvements in disease-free survival than those not given PSK (Hayakawa *et al*., 1993). PSK was clinically significant with more advanced patients with Stage III disease than Stage I and II patients. PSK had greater activity for older patients (> 70 years) and patients with small primary tumours.

Early studies with breast cancer patients seemed to imply that long-term PSK immunotherapy in conjunction with chemotherapy could have beneficial results (Suginachi *et al*., 1984). In a later much larger trial (914 patients) in-depth analysis implied that PSK significantly extended survival in ER-negative, Stage IIA patients without lymph node involvement (Toi *et al*., 1992). However, in a further large trial, Morimoto *et al*. (1996) could find no statistical evidence of any benefit from PSK. These contradictory studies may have been clarified by Yokoe *et al*. (1997) who

compared HLA B40 antigen positive patients treated with PSK against B40 negatives. It was found that B40-positive patients treated with PSK (3g/daily, two month course each year) in addition to chemotherapy had an improved 10 year overall survival rate compared to B-40 negative patients. Thus, HLA B40 may be a predictive factor for PSK response.

The foregoing studies give strong indications of the potential benefits of incorporating PSK into some cancer treatments as an adjunct to radio- or chemotherapy. Furthermore, PSK can improve immune status secondary to the side effects associated with traditional therapies. As stated by Kidd (2000) "*after a quarter century of trials indicating PSK can improve cancer survival, the cumulative human findings amount to a recommendation for its inclusion in standard anticancer protocols. With its risk for adverse effects virtually nonexistent, PSK's contribution to the benefit-risk profiles of these protocols can only be positive"*.

PSP and clinical trials

While PSK has been almost exclusively developed and tested within Japan, PSP in contrast is a product of China and continues to be assessed for efficacy safety by their scientists and oncologists. There is a close similarity between PSK and PSP polypeptides although PSP lacks fucose and instead contains arabinose and rhamnose. Since the first development of PSP in 1983 there has been rapid progress through human clinical trials. Phase I clinical trials were carried out by Xu (1993) and it was shown that an oral dose of up to 6g/day was well talented and lacking in side-effects. Patients showed improvement in appetite and general condition, together with a stabilisation of haematopoietic parameters.

The Phase II study by the Shanghai PSP Research Group with 8 hospitals in Shanghai was carried out using patients with cancers of the stomach, lung and oesophagus. The dosage was 1g three times daily to a total of 190g. Results confirmed the role of PSP as a biological response modifer improving the immunological status of the patients after surgery, radiotherapy and/or chemotherapy (Liu and Zhou, 1993). Following the success of the Phase II clinical trials, a Phase III trial was conducted in a large cohort of patients (650) in Shanghai hospitals. 189 were randomised to taking PSP and placebo; 461 patients were unblinded to their therapy (Liu *et al*., 1999). These trials showed that PSP improved disease-free survival of gastric, oesophageal and non-small-cell lung cancers while again substantially reducing the normal unpleasant side-effects of conventional treatments (Sun and Zhu, 1999; Sun *et al*., 1999). PSP had a protective effect on the immunological functions of conventionally-treated patients, thus demonstrating that PSP can be classified as a clinical biological response modifier. Other BRMs such as LAK cells, IL-2, α y IFN or TNF are also being used in the treatment of advanced cancer cases (Liu, 1999). Yet, these drugs at effective doses, in many cases, produce quite severe side-effects such as fevers, chills, rashes, arthralgia, hypotension, oliguria, pulmonary oedema, congestive heart failure and CNS toxicities. Mao *et al*. (1998) have shown dramatic anti-tumour effects when PSP was combined with IL-2. As side-effects of IL-2 are dosage and schedule dependent, it is reasonable to expect that with PSP, a lower dose of IL-2 could be used clinically with subsequent decrease in the severity of the side-effects (McCune and Chang, 1993). A further observation noted that PSP in combination with radiotherapy induced a significant increase in the percentage of apoptotic cells at 24h, compared with radiation alone, and it has been surmised that the antitumour mechanism of PSP

action may also involve the induction of DNA damage by apoptosis in the target cancer cells (Stephens *et al*., 1991).

A common adverse reaction of radiotherapy and chemotherapy is haematopoietic toxicity. Several studies have shown a strong amelioration of these toxic effects by PSP (Shiu *et al*., 1992; Sun *et al*., 1999).

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

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

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

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

Safety data

Pre-clinical

Lentinan

Toxicity tests using Lentinan have been carried out in a variety of species with dosing ranges of 0.0001-30 mg/kg for 5-6 week by iv administration. Some swellings and proliferation of reticuloendothelial cells were at dosages >25mg/Kg. Some species in the ≥ 2 mg/kg groups also developed gastrointestinal or urinary bladder haemorraghes with dermatological changes. All lesions occurred in high dose groups and tended to regress after discontinuing Lentinan administration.

Fertility of males was not affected at 0.1-1.0 mg/kg. No abnormalities were detected with doses 5.0-10 mg/kg during fetal organogenesis in rats and no abnormality at maximum dose of 5.0 ug/kg during perinatal and lactation period. There was little or no penetration into the foetus and no excretion into maternal milk (Ajinomoto Technical Document , 1988).

In antigenicity studies there were no anaphylactic reactions and no effect on allergic reactions. Lentinan had no effect in a mutagenicity test, haemolysis test, blood coagulation, ability to induce arthritis and no effect on adjuvant-induced arthritis.

The manufacturers of the other β-glucan products now being used in clinical work have carried out tests comparable to those with Lentinan and have obtained similar results.

PSP

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

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

1. Mutagenicity tests to assess genotoxicity of PSP using a special strain of *Salmonella typhimurium* – no evidence of mutagenic activity.
2. Cytotoxicity tests of PSP with V_{79} Chinese hamster cells *in vitro* – no toxic effects against the V_{79} cell line.
3. *In vivo* micronucleus tests to assess the cytogeno-toxicity on mammalian somatic cells – PSP showed no evidence of mutagenic potential when administered in this *in vivo* test.

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

Subchronic toxicity tests have been performed with various concentrations of PSP on rats by p.o. administration. PSP was administered at dosage rates of 1.5, 3.0 and 6.0g/kg body weight every day for up to 62 days. At the time of the final administration of PSP and 2 weeks after the last administration, the general conditions, i.e. blood indexes, serum biochemistry indexes and patho-histology indexes of the PSP groups were compared to the control group and no obvious differences were observed (Jiang *et al*., 1999). A further study with mice demonstrated that acute, chronic, genetic, reproductive and two-generation teratogenic toxicity were very low at 50-100 times the oral clinical dose (Jin, 1999 – contains many relevant references on PSP safety tests).

Other Medicinal Mushrooms

Recent studies have shown that crude extracts of *Ganoderma lucidum* and *G. lipsiense* do not exhibit genotoxic properties (clastogenicity and/or aneugenicity), at any dose level tested. Using the Cytokinensis – Blocked Micronucleus Assay (CBMA) on cultured human lymphocytes there was no evidence that the extracts contained clastogens (the micronuclei containing one or more acentric chromosome fragments) and anangens (the micronuclei containing one or more whole chromosomes) (Steinmetz *et al*., 2001). Similar studies should be performed for the other important medicinal mushrooms. Hot aqueous extracts of wild *Ganoderma* fruit-bodies were assessed for cytotoxicity and *in vivo* genotoxicity by both acute and subchronic oral exposure of mice (dose equivalent of 220 g fresh *Ganoderma* fruit-

body/kg body weight). No evidence was found for genotoxic chromosomal breakage nor cytotoxic effects by the extracts (Chiu *et al*. 2000). Previous suggestions that *Ganoderma* extracts had anti-mutagenic properties were not substantiated using Comet Assays.

A recent study by Badalian *et al*. (2001) examined certain pharmacological activities of *Flammulina velutipes* (an edible medicinal mushroom) and *Paxillus involutus* (poisonous mushroom) and *Tricholoma tigrinum* on mice, using methanol-soluble and water-soluble residues separated from methanol extracts of fruit-bodies. The fungal extracts did not show any particular analgesic effects while algogen activity and significant spasmolytic papavertine-like activities were observed for *P. involutus*. Both *P. involutus* and *T. tigrinum* showed effects on the central nervous system with increased dynamic activity and curiosity of the mice. *F. velutipes* showed little evidence of any of these pharmaceutical disturbances.

Clinical

In the clinical setting tens of thousands of patients have been treated with PSP. Many patients have been successfully taking PSP for over 10 years with no serious adverse effects (Yang, 1999).

The highly purified β-glucan (Grifron-D®) from the Maitake mushroom *Grifola frondosa* has also been approved by the FDA for trial under an Investigational New Drug Application (IND) for patients with advanced cancer and some clinical trials are now underway. Due to the absence of adverse reactions in previous trials and with no significant pre-clinical toxicity the FDA has exempted this polysaccharide from a Phase I study (Fullerton *et al*., 2000). Furthermore, a number of large phase III

trials using Lentinan found no adverse reactions or evidence of drug-drug interactions (Taguchi, *et al* ., 1985 and Furure, *et al*., 1981) .

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CHAPTER 8 ADDITIONAL MEDICINAL PROPERTIES

Synopsis

Extracts from many medicinal mushrooms have long been used for a wide range of ailments in traditional Chinese medicine. Modern scientific and medical studies are increasingly supporting many of these health claims. The main areas of medical studies include blood pressure-lowering, cholesterol lowering, liver protective, antifibrotic, anti-inflammatory, anti-diabetic and anti-microbial activites.

While the role of medicinal mushrooms in immunomodular and anti-cancer activities represents the dominating theme of this report, it is important to recognise that many of these mushrooms also show other quite significant medical properties, such as blood pressure- lowering, cholesterol lowering, liver protective, antifibrotic, anti-inflammatory, anti-diabetic, anti-viral and other anti-microbial activities (Ooi and Liu, 1999; Ooi; 2000, Wasser and Weis, 1999a, b, Hobbs, 1995; Gunde-Cimerman, 1999). Only a brief resume will be given here of the extensive additional medical properties of certain medicinal mushrooms which have been supported by recent scientific and medical studies.

Cardiovascular and hypercholesterolemia effects

A highly significant cause of death in most developed countries is coronary artery disease. The main risk factors are hypercholesterolemia and dyslipoproteinemia, disturbance in blood platelet binding, high blood pressure and diabetes. Increased blood levels of total cholesterol, low density lipoprotein (LDO) and very low density lipoprotein (VLDL) cholesterol as well as lowered levels of high density lipoprotein (HDL) cholesterol have been identified as major risk factors in the development of coronary artery disease (CAD)(Alberts *et al*., 1989). As much as 2/3rd of total body cholesterol in most individuals is of endogenous origin. Clinical

intervention studies have clearly demonstrated the therapeutic importance of correcting hypercholesterolemia.

The initial steps in the prevention and treatment of CAD and hypercholesterolemia is the modification of the nutritional regime with a diet low in fats and saturated fatty acids and rich in crude fibres. Mushrooms in general, and *Pleurotus, Lentinus* and *Grifola* in particular, because of their high fibre content, sterols, proteins, microelements and a low calorific value, are *almost ideal for diets designed to prevent cardiovascular diseases* as first suggested by Traditional Chinese Medicine (Breene, 1990; Hobbs, 1995).

When diet control is not successful the next step is drug therapy. Early attempts to identify inhibitors of cholesterol synthesis resulted in the development of inhibitors that could affect stages in the biosynthetic pathway for cholesterol formation. A major rate-limiting step in the pathway is at the level of the microsomal enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase; mevalonate $NADP^+$ oxidoreductase [CoA acylating] EC 1.1.1.34). HMG-CoA reductase occurs early in the biosynthetic pathway and is among the first committed steps to cholesterol formation that catalyses the reductions of HMG-CoA into mevalonate (Rodwell *et al*., 1976).

Mevinolin (lovastatin) produced commercially from the filamentous fungus *Aspergillus terreus* was the first specific inhibitor of HMG-CoA reductase to receive approval for the treatment of hypocholesteremia (Alberts *et al*., 1980). The genus *Pleurotus* of the medicinal mushrooms has several species that produce mevinolin (Gunde-Cimerman and Cimerman, 1995). *P. ostreatus* has been shown to produce the highest amount of lovastatin in the fruit-body, especially in the lamellae or gills.

Mevinolin has been detected in submerged fermentation broth of *P. saca* and in the surface fermentation broth of *P. sapidus* (Gunde-Cimerman *et al*., 1993).

The addition of 4% dried *Pleurotus* to a high cholesterol diet effectively reduced cholesterol accumulation in the serum and liver of experimental rats redistributing cholesterol in favour of HLDL, reduced production of VLDL and LDL cholesterol, reduced cholesterol absorption and reduced HMG-CoA reductase activity in the liver (Bobek *et al*., 1991). Limited clinical trials with 15-20g dried *Pleurotus* supplement in the daily diet over a one-month period reduced hypercholesterolemia in many but not all patients (Bobek *et al*., 1998). It has been suggested that *Pleurotus* mushrooms could be recommended as a natural cholesterol lowering substance within the human diet (Gunde-Cimerman, 1999). Somewhat similar results have been achieved with *Grifola frondosa* and *Auricularia auricula* (Ryong and Tertov, 1989).

Antilipemic effects of polysaccharides from *Tremella fuciformis* and *T. aurantia* have been shown to lower plasma cholesterol levels (Sheng and Chen, 1989; Kiho *et al*., 2000), while an antihypercholesterolemic agent has been produced from fruit bodies and mycelium of *T. aurantai* (Koichi and Takahiro, 1999).

It has long been recognised that eritadenine, a compound extracted from *Lentinus edodes* is able to lower blood serum cholesterol (BSC). Eritadenine reduces BSC in mice not by inhibition of cholesterol biosynthesis but by the acceleration of the excretion of ingested cholesterol and its metabolic decomposition (Susuki and Oshima, 1974). Various studies have shown that *Lentinus* mushrooms can lower both blood pressure and free cholesterol in plasma, as well as accelerate accumulation of lipids in the liver, by removing them from circulation (Kabir and Kumura, 1989). It has been suggested that high dosages of eritadenime may impair

the secretion of very low-density lipoprotein cholesterol and in a similar manner to soybean protein, eritadenine lowers cholesterol by decreasing the ratio of phosphatidylcholine (PC) to phosphatidylethanolamine (PE) in liver microsomes (Sugiyama and Yamakawa, 1996). Several small studies with *Lentinus* extracts in Japan have shown positive decreases in serum cholesterol in young women and people older than 60 years of age (Hobbs, 1995).

Nucleic acids from *L. edodes* also have significant platelet agglutinating inhibitory effects (antithrombotic activity) (Hokama and Hokama, 1981). PSK also causes decreases in LDL cholesterol in hyperlipidemia patients (Tsukagoshi 1984).

A recent review of literature by Francia *et al*. (1999) has collated how different fungal activities can reduce the effects of risk factors for cardiovascular diseases in experimental animals. Of the 17 species of macrofungi examined, including some well recognised medicinal mushrooms, 16 showed at least one of the following activities, i.e. ability to reduce hypercholesterolemia or to treat dyslipoproteinemia; possibility to decrease arterial hypertension or hyperglycemia, and the ability to cure disturbances in platelet aggregation (Tables 1-4). However, water extracts of fruitbodies of *L. edodes* have been shown to lessen the effectiveness of blood platelets in the process of coagulation and consequently those who bleed easily and who take anticoagulants should exert caution when chronically consuming extracts of *L. edodes* in therapeutic amounts or water-soluble fractions such as LEM (Yang and Jong, 1989). Nevertheless, the exact mechanisms of action remains to be elucidated before considering an eventual human treatment application for prevention or cure of cardiovascular diseases. This review contains an extensive list of relevant references.

References for Tables 1-4 can be found in Francia *et al*. (1999). Many of these extracts have long been used in traditional Chinese medicine for treating various cardiovascular disorders (Hobbs, 1995; Willard, 1990).

Table 1 Effects of macrofungi on lipids and cholesterol

[Seven fungi had an effect on lipids in general and cholesterol in particular]

1. Six species reduced total cholesterol level
   Auricularia auricula – judae
   Cordyceps sinensis: the activity could be due to a polysaccharide, the CS-F30, composed of galactose, glucose and mannose.
   Ganoderma lucidum
   Grifola frondosa
   Pleurotus ostreatus
   Tremella fuciformis

2. Two species reduced the 'bad cholesterol' level
   Auricularia auricula – judae
   Tremella fuciformis

3. Three species reduced the triglyceride level
   Cordyceps sinensis
   Grifola frondosa
   Lentinus edodes

4. *Agaricus campestris*: demonstrated no hypocholesterolemic activity.

Table 2 Macrofungi reducing blood platelet binding

[Six species reduced platelet binding (*in vitro*]

Auricularia auricula-judae
Calyptella sp: the active compound is the 5-hydroxy-3-vinyl-2 (5H) – furanone.
Ganoderma lucidum: the binding activity is due to adenosine.
Kuehneromyces sp: the active compound is kuehneromycine B.
Neolentinus adhaereus: the active compound is 2-methoxy-5-methyl-1,4 benzoquinone.
Panus sp: the activity is due to two compounds, panudial and nematolon.

Table 3 Macrofungi with an arterial blood pressure lowering effect

[Three fungal species reduced the arterial pressure]

Ganoderma lucidum *Grifola frondosa* *Tricholoma mongolicum*: the decrease of arterial pressure attributable to a lectin.

Table 4 Macrofungi reducing glycemia

[Six species appeared to decrease glycemia]

1	Four species were active in insulin-dependent-diabetes. *Agaricus bisporus* *Agrocybe aegerita*: the glycemia lowering was due to two polysaccharides: AG-HN1, a polysaccharide of high molecular weight composed of glucose and AG-HN2, a polysaccharide of low molecular weight composed of fructose, galactose, glucose and mannose. *Cordyceps sinensis*: could be due to the CS-F30, a polysaccharide composed of galactose, glucose and mannose. *Tremella aurantia*: the active compound is the TAP (*Tremella* acidic Polysaccharide).
2.	One species was active in non-insulin-dependent-diabetes. *Grifola frondosa*: this mushroom is able to diminish glycemia but also insulemia and the blood level of triglycerides.
3.	One species showed an activity only in non-diabetic animals. *Coprinus comatus*.

Due to their high content of fibre and proteins and low fat content, extracts of edible mushrooms have been considered to be ideal foods for dietetic prevention of hyperglycemia (Gunde-Cimerman, 1999). Extracts of several medicinal mushrooms, including *Tremella aurantia, 'Cordyceps sinensis', Ganoderma lucidum* and *Auricularia auricula-judae* have been shown to lower blood glucose (Kiho *et al*., 1995; Yan *et al*., 1998; Hikimo *et al*., 1989). The blood glucose and triglyceride (TG) lowering effects of water soluble extracts from *Lentinus edodes, Pleurotus ostreatus* and *Phellinus linteus* in the streptozotocin-induced diabetic model have been clearly

demonstrated (Kim *et al*., 1997, Kim *et al*., 2001). Such results strongly suggest that these mushrooms have potential preventive and therapeutic action in diabetes mellitus (type I and II).

Antimicrobial effects

Antimicrobial drugs have long been used for prophylactic and therapeutic purposes. Unfortunately the recent increase in the occurrences of drug-resistant bacterial strains is creating serious treatment problems. Consequently, the antimicrobial activity of various antitumour polysaccharides from medicinal mushrooms are being re-evaluated in terms of their clinical efficacy. Such compounds would be expected to function by mobilising the body's humoral immunity to ward off viral, bacterial, fungal and protozoal infections resistant to current antibiotics.

Many cancer and AIDS patients die of opportunistic infections because of immunosuppression (Table 5). Several mushroom polysaccharides have shown antiviral activity against ectromelia virus and cytomegalovirus infections (Jong and Donovich, 1990). Lentinan from *L. edodes* when used in conjunction with azidothymidine (AZT) suppressed the surface expression of HIV on T-cells more than AZT did alone. Lentinan and sulphated lentinan exhibited a potent anti-HIV activity resulting in inhibition of viral replication and cell fusion.

Lentinan has also shown: (a) antiviral activity in mice against VSV (vesicular stomatis virus), encephalitis virus, Abelson virus, an adenovirus type 12; (b) stimulated non-specific resistance against respiratory viral infection in mice; (c) conferred complete protection against an LD75 challenge dose of virulent mouse influenza A/SW15; (d) increased resistance to the protozoal parasites *Schistosoma japanicum*, *Sch. mansoni;* (e) exhibited activity against *Mycobacterium tuberculosis*

bacilli resistant to antituberculosis drugs, *Bacillus subtilis*, *Staphylococcus aureus, Micrococcus lenteus, Candida albicans* and *Saccharomyces cerevisiae*; (f) increased host resistance to infections with potentially lethal *Listeria monocytogenes* (for original references see Wasser and Weis, 1999a).

LEM and a new lignan-rich compound JLS-18 derived from LEM block the release of infectious *Herpes simplex* virus in animals (Sarkar, 1993) and it has been suggested because of its high activity that JLS-18 could be of value in the treatment of hepatitis B and AIDS patients (Yamamoto, 1997).

Sulfated Schizophyllan polysaccharide displayed strong anti-HIV activity while the anti-tumour effect was reduced or lost (Ito and Sugawara, 1990). Schizophyllan has also been reported to enhance protection against *Staphylococcus* sp. infection (Matsuyama *et al*., 1992).

The Japanese National Institute of Health and the US National Cancer Institute have both stated that sulfated *Grifola frondosa* extract are able to prevent as much as 97% HIV infected T-helper lymphocytes from being destroyed *in vitro*. This is important because measuring the T-helper cell count makes it possible to trace the progress of HIV to full blown AIDS (Ishikawa, 1991; US National Cancer Institute, 1992). Interestingly, *G. frondosa*, D-fraction together with dimethyl sulfoxide (DMSO) has also shown success in treating AIDS associated Kaposi sarcoma (Zhuang and Mizuno, 1999).

PSK has been shown to induce potent antimicrobial activity against *Escherichia coli*, *Listeria monocytogenes* and *Candida* (Tsukagoshi, 1984; Sakagami and Takeda, 1993).

In recent years Basidiomycetes and other higher fungi including some recognised as medicinal mushrooms have been re-investigated as sources of novel

antibiotics – mainly as a result of the increasing difficulty and cost of isolating novel bioactive compounds from the Actinomycetales such as *Streptomyces*.

Difficulties such as slow growth rate in fermenters of Basidiomycetes and the low yield of products derived from them compared with the Actinomycetes are now far outweighed by the opportunity of finding new antibiotics with novel structures types as well as compounds with new modes of action (Brizuela and Garcia, 1998). The fact that the Basidiomycetes have been insufficiently investigated coupled with the broad range of structural types of antibiotics which are produced by these organisms, suggests that they may well be a source of new and useful bioactive compounds (Anke, 1989).

A recent extensive examination of over 200 species of Basidiomycetes in Spain demonstrated that almost 50% had significant direct antibiotic activity against a range of test organisms. It was interesting to note that the bracket polypore *Piptoporus betulinus* carried by the historic Iceman (Chapter 2) displayed a high broad spectrum antibiotic activity! (Suay and Arenal, 2000).

Researchers have shown that a water extract of *L. edodes* demonstrated growth-enhancing effects on colon-inhabitating beneficial lactic acid bacteria, *Lactobacillus brevis* and *Bifidobacteria breve*. The effective factor in the extract is considered to be the disaccharide sugar, trehalose. The authors suggest that the *L. edodes* extracts can improve the beneficial intestinal flora of the gut and reduce the harmful effects of certain bacterial enzymes such as β-glucosidase, β-glucuronidase and tryptophanase as well as reducing colon cancer formation (Bae, 1997).

Clearly, the antimicrobial potential of extracts of several types of medicinal mushrooms and indeed other Basidiomycetes not yet exploited must warrant further examination. The proven immuno-modulatory effects of many of these mushroom

species will be of significance especially when such infections occur in individuals where the immune system is not functioning well such as young children, the elderly and with patients enduring major anaesthetic and surgical procedures.

Table 5 Spectrum of mycoses and mycetes related to AIDS (Wasser *and Weis*, 1999a)

Mycoses	Causative organisms/saprophytes	Main target issues	Incidence %
Dermatophytoses	Anthropophillic dermatophytes: *Trichophyton rubum, Epidermophyton floccosum*, and others	Skin and appendages	80-90
Candidoses	*Candida albicans, C. tropicalis, C. parapsilosis, C. guiliermondii, C. krusci,* and other species	Oral cavity; skin; vagina; oesophagus	70-90 25-30 20-25
Torulopsidoses	*Torulopsis glabrata, T. candida*	Intestinal tract; Parasitic; Saprobic Systemic, mainly brain	 1-2 70-90 <1
Trichosporosis	*Trichosporon cutaneum*		
Cryptococcosis Histoplasmosis	*Cryptococcus neoformans*	Brain (lungs, skin)	5-7

A series of studies has recently been carried out with a PGG-glucan on patients undergoing high-risk major abdominal and thoracic surgery or high-risk gastrointestinal surgery. PGG-glucan is a highly purified proprietary β-(1-3)-glucan with β-1,6 branches (poly 1-6 glucotriosyl-β 1-3 glucopyranose glucan) (Onderdonk *et al*., 1992). Three separate multicentre (including Harvard Medicial School), randomised, placebo-controlled, double-blind clinical trials were carried out. In the initial study patients receiving high doses of β-glucan (2.0 mg/kg) exhibited significantly fewer postoperative infections complications when compared with placebo (Babineau *et al*., 1994a). In a second study, patients given β-glucan had 1.4 infections per patient vs. 3.4 infections in the placebo group (Babineau *et al*., 1994b). In a further study involving 1,249 patients the βb-glucan-treated patients showed a

statistically significant (39%) reduction in serious infections and death compared with placebo (Dellinger *et al*., 1999). However, this final study was terminated before anticipated completion because of an increased incidence of adverse effects in patients receiving PGG-glucan. Since β-(1-3) glucans exhibit considerable structural diversity such trials should be repeated with β-(1-3) glucans derived from the medicinal mushrooms which have demonstrated no adverse human side-effects. With the increasing concern of hospital-derived postoperative microbial infections together with antibiotic resistance, such studies must warrant serious consideration, and further expansion with mushroom-derived β-glucans must be considered because of their proven antimicrobial effects.

Antioxidant , anti-inflammatory, free radical scavenging activities and the ageing process

A wide variety of pathological damage, such as DNA, carcinogenesis and cellular degeneration, related to the ageing process and ageing itself can be caused by reactive oxygen species (ROS) produced by sunlight, ultraviolet and ionising radiation, chemical reactions and metabolic processes. Furthermore, there is a vast accumulation of studies that implicate oxygen derived free radicals such as superoxide, hydroxyl radicals and high energy oxidants such as peroxy nitrite as mediators of inflammation, shock and ischemia/reperfusion injury (Cuzzocrea *et al*., 2001). There is also growing evidence to show that production of ROS at the site of inflammation can contribute to tissue damage (Salvimini *et al*., 1996). Interventions against ROS could exert beneficial effects on inflammation and shock (Halliwell and Parihar, 1984). Several mushroom species have been studied for anti-inflammatory and antioxidant activities (Ukai *et al*., 1983) and patents have been established for these usages (Xiu, 1996).

Extracts of *G. lucidum* can apparently remove the hyperoxide radical believed to be a main factor in the human ageing process (Liu *et al*., 1997), and the ageing mouse model (Pan *et al*., 1999). In a clinical trial with 30 elderly people *Ganoderma lucidum* extract (GLE) was given oral 1.5 g 3 times daily for 30 days. Interleukin-2 and interferon (IFN) production by peripheral mononuclear cells (PBMC) and NK cell activity *in vitro* were respectively measured. Production of IL-2 and IFN were significantly increased after GLE treatment. Such results could suggest that GLE is a possible treatment to raise the cellular immunological activity in ageing people (Tao and Feng, 1991; Tao, 1993).

A *Ganoderma lucidum* polysaccharide GLB7 decreased the production of oxygen free radicals and antagonised the respiratory burst induced by PMA in murine peritoneal macrophages (Li and Lei, 2000). Such observations could imply that the polysaccharide-induced inhibition of oxygen free radicals in murine peritoneal macrophages play an important role in the anti-ageing effect of *Ganoderma* extracts.

PSK in a cell-free system consisting of hypoxanthine-xanthine oxidase rapidly quenched the superoxide radical, a property not shared by Schizophyllan (Sakagami and Aoko, 1991). PSK further repressed the mimetic activity of superoxide dismutase (SOD) and promoted oxidative stress relief for cancer-bearing hosts (Kobayashi and Kariya, 1994). PSK also gave protection to macrophages from lipoperoxide accumulation and foam cell formation created by oxidatively modified low-density lipoprotein (Yuan and Meiz, 1996). This protection is believed to be due to the induction of gene expression of antioxidative enzymes (Chen and Zhou,1997). PSP shows similar scavenging effects on superoxide and hydroxyl radicals (Hu and Chen, 1992). Significant superoxide and hydroxyl radical scavenging activities have

been demonstrated for several mushroom antitumour polysaccharides (Liu *et al*., 1997).

Hepatoprotective effects

Fruit-bodies of *Ganoderma lucidum* have long been a major factor in folk medicine for the treatment of chronic hepatitis (Willard, 1990). Ganoderic acids R and S were isolated from cultured mycelia and shown to have strong antihepatotoxic activity in galactosamine-induced cytotoxic tests with primary cultured rat hepatocytes (Hirotani and Ito, 1986). Another hepatoprotective compound, ganosporeric acid A, was isolated from the ether-soluble fraction of the spores of *G. lucidum* (Chen and Yu, 1991). The wide spectrum of medical efficacies of *Ganoderma lucidum*, including hepatoprotective activities, is shown in Table 6.

A polysaccharide fraction from *L. edodes* showed liver protective action in animals together with improved liver function and an enhance production of antibodies to hepatitis B (Mizuno, 1995). Lentinan and LEM have given favourable results in treating chronic persistent hepatitis and viral hepatitis B patients (Zhu, 1985; Amagase, 1987). Extracts of *G. lucidum* have shown good results in treating hepatitis, particularly in cases without severe liver impairment (Yan, 1987). A clinical study with lyophilised extract of *G. lucidum* showed highly beneficial results on quality of life with patients suffering from active hepatitis B (Soo, 1994).

There have been other interesting medical reports relating to marked improvement with patients suffering from cirrhosis of the liver and chronic hepatitis B with extracts from *Dendropolyprus umbellatus* (Bensky and Barolet, 1990), *Schizophyllan commune* (Kakuma, 1991), *Trametes versicolor* (Zhou, 1989), and *Poria cocos* (Guo, 1984). PSP may, thus, be useful in the wider context of the treatment of hepatitis (Yeung, 1995).

Table 6 Medical efficacies of *Ganoderma lucidum* (Kim and Kim, 1999)[see for relevant references].

Efficacy	Compound
Anti-HIV activity	Ganoderic acid α Ganoderic acid β Ganoderic acids B, C1, H Ganoderiols A, B, F Ganodermanondiol Ganodermanontriol Ganolucidic acid A Lucidumol B 3β, 5α-Dihydroxy-6β-methoxyergosta-7-diene
Antihypertension (ACE inhibitor)	Ganoderic acids B, D, F, H, K, S, Y Ganoderol B
Bitterness	Ganoderic acids A, C1, J Lucidenic acids A, D1 Lucidone A, C
Cytotoxicity	Ganonderic acids T, V, W, X, Y, Z 3β-Hydroxy-26-oxo-5α-lanosta-8,24-dien-11-one Ergosta-7,22=diene-3β, 3α,9α-triol
Enzyme inhibitor	
FPT inhibition[a]	Ganoderic acids A and C
PLA-2-inhibition[b]	Ganoderic acid T
DNA pol. β inhibition[c]	5,8-Epidoxy-5α,8α-ergosta-6, 22E=dien-3β-ol
Hepatoprotective	Ganoderic acids R, S Ganosporeric acid A
Histamine release inhibition	Cyclooctasulfur Ganoderic acids C and D
Hypocholesterolemic	Ganoderic acid Mf Ganodermic acid B Ganodermic acid T-O
Platelet aggregate inhibition	Ganodermic acid S

[a]FPT : farnesyl protein transferase
[b]PLA_2: phospholipase A_2
[c]DNA pol: DNA polymerase

This Chapter has only been a brief overview of the many other aspects of medical usage of the medicinal mushrooms which are being pursued worldwide. It, hopefully, shows the direction of medical research into these compounds and their undoubted value and significance in areas outwith cancer and immunotherapy.

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CHAPTER 9 REGULATORY AND SAFETY CRITERIA FOR FUNCTIONAL FOODS AND DIETARY SUPPLEMENTS AND PHARMACEUTICAL MEDICINES; THE ROLE FOR MEDICINAL MUSHROOMS

Synopsis

The regulatory and safety aspects of dietary supplements in general are reviewed with reference to European, USA and Japanese laws. Herbal extracts are given special consideration and consumer product information should give special mention of active ingredients, dosage, mode of administration etc.
Dietary supplements from medicinal mushrooms are analysed in detail and current approaches to safety are examined. A central feature of the purported medicinal or chemo-preventive role of mushroom extracts must be the undoubted synergistic interaction of the many constituents.

For time immemorial mankind has used traditional medicines for human healthcare with terrestrial plants occupying a significant therapeutic role (Pezzuto, 1997). Recently, the World Health Organisation has estimated that approximately 80% of the world's inhabitants still depend on traditional (mostly herbal but also including fungal) medicines for primary health purposes (Cragg and Newman, 2001), while plant-derived pharmaceutical products play an important role with the remaining 20% of the world's population in developed countries. At least 120 important drugs are obtained from plants (Farnsworth, 1988).

Many of the now clinically useful anticancer drugs are either natural plant products or derivatives of natural products, e.g. paclitaxel (Taxol®) from *Taxus brevifolia* L. and vincristine (Oncovin®) from *Cantharanthus roseus* G.Don. (Pezzuto, 1997). Plants continue to offer a wide range of compounds with diverse structures and activities which will continue to occupy an important role in modern cancer therapy, especially within the sphere of chemotherapy.

In a previous Chapter, the role of certain complex polysaccharides derived from various mushroom species has been examined with respect to their anticancer activities. Their mode of action is likely to be through stimulating the human immune system to attack the cancer cells, though there is some evidence that they can, in some cases, also act directly on the cancer cells. In contrast to the above-mentioned plant-derived anticancer compounds the mushroom compounds are extremely complex structurally and will seldom be completely chemically pure.

While the mushroom-derived polysaccharide anticancer compounds will be used at various levels of purity in clinical applications as adjuncts to existing chemotherapeutic compounds, yet another important role could be as functional foods either consumed whole, or as concentrated extracts, as dietary supplements. In this way they may have a role in disease prevention or cancer chemoprevention. As previously noted, certain Japanese growers of medicinal mushrooms who have been regular consumers of their produce, show a lower cancer incidence when compared with the national incidence of cancer. As stated by Pezzuto (1997) “In general terms, cancer chemoprevention may be considered as the prevention of cancer in human populations by ingestion of chemical agents that prevent carcinogenesis. It is important to differentiate the concept of cancer chemoprevention from primary cancer prevention, such as the cessation of cigarette smoking and cancer chemotherapy, the therapy used after the diagnosis of cancer”.

In most Western countries, cancer incidence increases gradually after the age of 30 and is greatest for the age group between 70 and 80. It is now well-recognised that the occurrence of cancer is strongly associated with ageing or the elongated lifespan of humans, and that a preneoplastic condition could often have already started in the cells many years earlier. Consequently, an important strategy for

preventing certain cancers could be to inhibit the development of the first clonal expansions and delay the clinical onset of tumour development (Muto *et al*., 1989).

Evidence is accumulating from human epidemiological studies and animal studies that dietary factors may reduce the incidence of some cancers, possibly by, as yet unknown, chemopreventive mechanisms (Hirayama, 1979; Boone *et al*., 1990; Havas *et al*., 1994; Zhang *et al*., 1994). There is an increasing number of compounds that may be viewed as chemopreventive and have been categorised as intentional food additives, non-nutrient food molecules, micronutrients, industrial reagents, hormones and antihormones, and pharmaceutical agents (Costa *et al*., 1990).

Before concentrating on the regulatory and safety aspects of putative chemopreventive mushroom products some important guidelines on herbal products and dietary supplements, in general, will be examined (Kingston, 2001, Wasser *et al*., 2000a,b).

Regulatory Environment for Dietary Supplements

While pharmaceutical products must have undergone clinical trials to demonstrate safety and efficacy prior to marketing, no such requirements are demanded of herbal products. This situation can be traced back to the 16th Century when Henry VIII passed laws controlling the possession of poisons but giving exemption to herbalists and their suppliers. Section 12 of the Medicines Act 1968 excludes herbal medicines from the normal regulatory process if not processed (other than crushing and/or drying), are not sold under a brand name, and no specific disease reduction or claims are made on their behalf. In this way these products do not require expensive clinical trials and since unprocessed herbal

products cannot be patented there can be little financial attraction for manufacturers to run their products through the medicine registration process.

WHO (1991) published a seminal report “Guidelines for the Assessment of Herbal Medicines” which set out “to define basic criteria for the evaluation of quality, safety and efficacy” of all herbal (including mushrooms) medicines. “As a general rule in this assessment, traditional experience means that long-term use as well as the medical, historical and ethnological background of those products shall be taken into account.” Depending on each country’s situation, “the definition of long-term use may vary, but would be at least several decades … Prolonged and apparently uneventful use of a substance usually offers testimony of its safety”. The Guidelines call for various assessments of quality, efficacy and the intended use, and reference should be made to pharmacopoeia monographs where they exist. If none exist, then the manufacturer should be required to produce a similar statement. Procedures should all correspond to Good Manufacturing Practices and include stability testing of the final product as packaged. With regard to safety “A guiding principle should be that if the product has been traditionally used without demonstrated harm, no specific restrictive regulatory action should be undertaken unless new evidence demands a revised risk-benefit assessment” (Alkerele, 1992). It is recommended that consumer product information should include a quantitative list of active ingredients, dosage, dosage form, indications, mode of administration, duration of use, any major adverse effects, contraindications, warnings, etc. (Wasser *et al*., 2000a).

The European Situation

On a European level EC Directive 65/65/EEC defines “any substance or combination of substances presented for treating or preventing disease … or which

may be administered with a view to making a medical diagnosis or to restoring, correcting or modifying physiological function" as a medical product requiring a license or marketing authorisation. Notwithstanding, in the case of herbal products, the distinction between medicine and non-medical products such as foods and cosmetics, is increasingly blurred with concomitant confusion across the EU.

Current EU regulations make few provisions for dietary supplements (DS), and are more concerned with the protection of consumers from unsafe products. DS that seek acceptance under current dietetic regulations must produce evidence to inspecting authorities that supports the statements claiming those special properties of the food that guarantee it to fulfil the purpose which, on the basis of the claim, the purchases will expect to fulfil. Medical claims, both explicit or implied, are subject to additional regulations and these products must hold a medical license (Ehrnreich, 2000).

The EU Novel Food Regulations will bring even more complexity as novel foods include food types or ingredients that have not been previously used for human consumption. Furthermore, for the near future, new functional food products must be submitted to each member state for consideration. However, it is anticipated that a new food authority will be set up with greater harmonisation potential, but for the present time, the overall thrust with novel foods is towards safety (Smith and Rowan, 2000).

The Working Party of the Pharmaceutical Committee of the European Commission has proposed a draft Directive on the regulation of herbal "medicines". These proposals would permit herbal medicines to be granted a license if the product, or a product with the same ingredient, dosage and oral route of administration, has been in "traditional use" over a period of 30 years for a particular

indication. Manufacturers would also be required to provide a bibliographic review of safety data and an expert report on those data. A significant aspect of this document would be to improve the current inadequate quality control in herbal products. Currently, herbal products are immensely variable in composition and the user has no reliable means of knowing exactly what they are consuming. Such products could well be contaminated with other organics (possibly toxic) as well as microbial presence and toxic metals (Kingston, 2001). Dosages are also problematic with identical remedies and may actually contain very different amounts of active ingredients. Furthermore, most often the amount of the active ingredient is not shown on the label. Indeed, it is often the case that the pharmacological active substance or substances have not been properly identified. The benefit from the herbal product may well rely on synergism between several ingredients.

As a consequence of the current lack of regulatory requirements reliable safety and efficacy data on herbal medicines is not easily available. Attempts at clinical trials for several plant-derived products have mostly been badly designed or the results poorly interpreted. Products were mainly not standardised, with different methods of preparation and administration which could lead to variation in the amounts of active principles reaching the patient (Kingston, 2001). Professor Ernst, the holder of the only Chair of Complementary Medicine in the UK at Exeter University, is planning to conduct objective evidence-based research to provide reliable data involving randomised controlled trials (RCTs). In these trials the herbal product is compared either with a placebo or an alternative drug on patients who do not know what treatment they are receiving. He has completed a number of these “meta-analyses” on several herbal remedies with some showing little or no efficacy but others showing some considerable promise (for references, see Kingston, 2001).

While there is some very supportive evidence of medical benefits with St John's wort preparations, patients currently taking other drugs should exert caution since complications can occur. This product has been shown to stimulate cytochrome P_{450} enzymes in the liver of some patients causing breakdown of other drugs.

The House of Lords Report on Complimentary and Alternative Medicine (2000) examined many aspects and issues including: evidence of efficacy; information available for both patients and doctors; training of CAM practitioners; regulations and risks; and possible provision of CAM on the National Health Service. With the aim of generating reliable data for regulation, it is planned "to attempt to build up an evidence base with the same rigor as is required of conventional medicine" by means of RCTs. Three central questions must be answered:

1. Does the treatment offer therapeutic benefits over placebos?
2. Is it safe?
3. How does it compare in terms of medical outcome and cost-effectiveness with other treatments.

Of the many areas of consultation by the Committee the views expressed by the group, Patient Concern, were thought-provoking, viz. that treatment with unproven therapies is not wrong if the patient is happy with the treatment, knows that it is unproven and has not been led to believe that it will definitely work! However, the report does stress that such products could be dangerous if they were relied upon to treat life-threatening diseases at the expense of other drugs.

Germany is by far the leading country in Europe for the consumption of herbal products. The German Commission E is an independent division of the German Federal Health Agency (Bundesgesund Leitsamt) which collects information on herbal medicines and evaluates them in relation to safety and efficacy.

Subsequently, they are published as brief monographs approving or disapproving the over-the-counter sale or use (Blumental, 1999). These monographs are believed to represent the most accurate information available worldwide on the safety and efficacy of herbs and phytomedicines (Tyler, 1998). These evaluations on efficacy are based on a doctrine of reasonable certainty which contrasts with the American FDA's insistence on a "doctrine of absolute proof". The FDA relies on information passively submitted to it from drug manufacturers!

The German Commission involves physicians, pharmacists, pharmacologists, toxicologists, representatives from the pharmaceutical industry and lawyers. Surely it is time for the UK government to emulate this creative initiative. It is planned that these monographs will all eventually be translated into English. It is anticipated that in the future mushroom-derived DSs will be subjected to this level of research on estimation of safety and efficacy (Wasser *et al*., 2000a,b).

The Japanese Situation

In Japan where consumers demand an increasing emphasis on safety and health, functional foods with proven clinical efficacy (now officially termed Foods for Specific Health Use (FOSHU)) are distinguished by their beneficial physiological effects. Such foods are designed to be consumed as a constituent part of a regular daily diet and to help promote and maintan health by regulating bodily functions and to protect against a range of conditions and diseases, including cancer, heart disease, diabetes, osteoporosis and hypertension. FOSHU status tends to carry claims that have the nuance of preserving or promoting health rather than to make specific claims.

In many ways the FOSHU regulatory system is possibly the most advanced in the world and is primarily designed to allow established and accepted ingredients to be used in food rather than to encourage development of new ingredients. The following requirements must be met to achieve a full FOSHU license: the product must contribute to the improvement of dietary habits and enhance health; the health benefits of the food or ingredient should have a clear medical basis; the food/ingredient must have definable levels of appropriate consumption based on medical knowledge; the food/ingredient should be safe as judged from experience; relevant information should be defined in terms of physiochemical properties; nutritional composition of the product should not differ greatly from that of ordinary foods; the product must be consumed regularly rather than occasionally; and the product must be in the form of ordinary food rather than pills or capsules. FOSHU approval differs from pharmaceutical approval in that FOSHU is for ordinary food with a "specific health benefit" that is already considered safe from prior experience (Ehrnreich, 2000).

The United States Situation

An important dilemma with respect to the regulation of functional foods is that they exist at the interface between foods and drugs (Kottke, 1998). In existing US food regulations there is no provision for foods consumed with the intention of preventing disease. The Federal Food Drug and Cosmetic Act (1938) considered such foods as drugs "articles intended for the diagnosis, cure, integration, treatment or prevention of disease" thus severely limiting labelling referring to disease-prevention or risk-reduction.

However, the Dietary Supplement Health and Education Act (DSHEA) (1994) allows many functional foods to be considered as dietary supplements which are

exempt from regulation as drugs. The Nutritional Labelling and Education Act (1990) permits health and disease prevention claims on a food label. A health claim is defined as “any substance that expressly or by implication characterises the relationships of any substance to a disease or health-related condition, e.g. fruits and vegetables and cancer prevention”.

With respect to DSHEA, Congress considered that there could well be a positive relationship between sound dietary practice and good health and that there could well be a connection between dietary supplement use, reduced healthcare expenses and disease prevention.

At present when DS manufacturers plan to market a new ingredient (ie. not marketed in US before 1974) information must be submitted 75 days in advance to the FDA that concludes that the new ingredient can be considered to be safe. Safe means that the new ingredient does not cause a significant or unreasonable risk of illness or injury under conditions of use recommended in the products labelling. This information will be in the public domain 90 days after receipt by the FDA (Wasser *et al*., 2000a). Having complied with DSHEA requirements, once a DS is marketed the FDA has the responsibility for showing that a DS is unsafe before it can take action to restrict the product’s use. The manufacturer is responsible for ensuring that the ingredient list is accurate and that the ingredients are safe. The content must match the amount declared on the label.

The DSHEA has created an Office of Dietary Supplements (ODS) at the National Institute of Health (NIH) to promote the scientific study of the benefits of DS for promoting health and preventing disease. The FDA has made major efforts to improve the labelling of DS products. All ingredients must be listed and for extracts

additional information may be provided including the solvent used and the concentration of the extract.

The FDA have recently issued the final regulations on structure/function (SF) claims for DSs under the DSHEA of 1994. There is a significant move in relation to SF claims for over-the-counter drugs, including DSs. In association with the American Herbal Products Association (AHPA) the FDA has expanded the range of SF claims by agreeing that some claims are not disease claims. Some are, instead, claims that deal with the structure or function of the body. In this way a DS can make claims as antacid, digestive aid, short-term laxative and many other uses previously not allowed for DSs.

Previously, the definition of disease was “any deviation from, impairment of, or interruption of the normal structure or function …”. Now the FDA will use the definition “damage to an organ, structure or system”. This change in definition will automatically reduce the range or number of health claims for DSs.

Dietary supplements from medicinal mushrooms

There are presently several types of DS derived from medicinal mushrooms being marketed (Wasser *et al*., 2000b).

1. Dried and pulverised naturally growing mushroom fruit-bodies in the form of capsules or tablets.
2. Artificially cultivated fruit-body powders, hot water or alcohol extracts from them, or the same extracts concentrated and their mixtures.
3. Dried and pulverised preparations of the combined substrate, mycelium and mushroom primordia following inoculation of edible semi-solid medium (usually grains).

4. Biomass or extracts of mycelium or the broth harvested from submerged liquid culture grown in bioreactors.

It has been estimated that worldwide sales of DSs from medicinal mushrooms is US$ 5-6 billion per year with the market value for Reishi DSs in 1995 estimated at US $ 1.628 billion (Chang and Buswell, 1999) (http://vm-cfson.fda.gw) . Shiitake products also have a very high profile. However, there is currently no standard protocols for guaranteeing medicinal mushroom DSs for product quality and efficacy.

Any compounds that will influence body functions such as blood pressure, immune response etc. are classified as pharmacological agents, and as such will invariably demonstrate toxicity at high dosage levels. Thus, a completely safe pharmacological agent would not have any biological activity (Huxtable, 1999).

In the case of the medicinal mushrooms they have been used for traditional medical purposes for long periods of time, in some cases for thousands of years. There are few documented examples of adverse effects to man and as such they can be considered as safe. However, from a pharmacological point of view, safety is a relative concept and it is clear that the safety of all mushroom-derived DSs cannot be guaranteed simply because they have mostly many centuries of usage.

Recently, Wasser *et al*. (2000a) have carefully examined this concept and have set out reasons for adopting a more cautionary approach but at the same time indicating the way forward to ensure adequate safety and efficacy for mushroom dietary supplements.

1. Considering the historic perspective "safety" in traditional terms is very different from that in modern times. Firstly, mortality patterns of developed societies today are very different from those of traditional ones … Secondly, traditional users rarely had the means to evaluate long-term or chronic toxicity

of agents; but we do have cautionary instances of plants and the mushroom *Paxillus involutus* that have been used medicinally for centuries and recently proved to carry delayed toxic effects (Huxtable, 1992; Schmidt *et al*., 1971).

2. Many supposedly traditional mushroom products are now marketed in ways markedly different from those in the past. Today larger amounts may typically be taken, the material is used more frequently, it is consumed in the form of enriched extracts and it may be taken simultaneously with synthetic drugs. The user of shiitake (*L.* edodes) in old China, for instance, could not ingest as much active polysaccharide (Lentinan) as a modern user taking it in pure form extracted from shiitake as a DS. Notably, 200 kg of fresh mushrooms are needed for extraction of 31 g of lentinan. This heightens the possibility of ill effects from traditionally “safe” mushrooms.
3. Also, many mushrooms or mushroom preparations traditionally taken as treatments for specific conditions are now often marketed for use as prophylactic agents. The idea of DSs themselves in many cases implies that they are taken in the absence of any indicated conditions to prevent disturbances of health.
4. Finally, reliance on traditional use as an indication of safety involves a danger, namely the poor information available to us from antiquity. Huxtable (1999) recently carried out an analysis of historical sources for different herbal medications and he clearly showed that the literary sources of such information are in many cases contradictory and vague.

Thus, it is clear that safety criteria for medicinal mushroom preparations should be based solely on modern scientific evidence and not to rely heavily on inadequate historical evidence. However, it is reassuring that when compared with

herbal preparations, mushroom preparations show little evidence of overt toxicity. The main advantage of using mushroom-based DSs with respect to safety (as opposed to herbal preparations) are the following as stated by Wasser *et al*. (2000a).

1. "The overwhelming majority of mushrooms used for production of DSs are cultivated commercially (and not gathered in the wild). This guarantees proper identification and pure and unadulterated products. In many cases it also means genetic uniformity. (However, it should be noted that the most highly prized and desired *Ganoderma lucidum* mushrooms are still collected from the wild).
2. Mushrooms are easily propagated vegetatively and thus keep to one clone. The mycelium can be stored for a long time and the genetic and biochemical consistency may be regularly checked.
3. Yet another important advantage … is the fact that many mushrooms are capable of growing in the form of mycelial biomass in submerged fermenter cultures."

The task of converting the very biodiverse raw materials of mushrooms into a consistent product will reflect industrial practices and standards and on methods of assessing efficacy. Mushrooms are complex structures both morphologically and physiologically with undoubted variations in chemical composition from batch to batch. The composition of a mushroom fruiting body will reflect substrate composition and ingredients which can vary considerably since the basic raw materials are normally of agricultural or forestry origin. Also the degree and uniformity of maturation can be critical, e.g. lovastatin levels in *Pleurotus* are highly dependent on the size and age of the fruiting body (Gunde-Cimerman, 1999). The cultivation for fruiting body production can be a long-term process taking from one to

several months for full production. Use of the complete mushroom fruiting body does imply that the standardisation of the DSs from medicinal mushrooms is in a rather poor state. Perhaps it is time that the mushroom DS manufacturing companies followed the recent example of the herbal industry who formed the Institute for Nutraceutical Advancement (INA) and within it the Methods Validation Program (MVP – the first organised effort to develop and validate botanics (www.nutraceuticalinstitute.com). The methods were also submitted to the US Pharmacopoeia (USP) and the American Organisation of Associated Chemists (AOAC) for possible publication (Lange, 1998).

The important way ahead for mushroom DSs and pharmaceuticals could be a greater use of pure culture mycelial cultivation in liquid or solid substrate fermenters. Such an approach offers several advantages:

1. Speed of growth with reduction in production time.
2. Optimisation of culture medium composition.
3. Optimisation of physico-chemical conditions to allow regulation of mushroom metabolism.
4. Improved yield of specific products.
5. Possible designed variation in product types.

Increasingly, industrial producers of DSs and pharmaceutical grade compounds such as Lentinan, LEM, Grifron, PSK, PSP and, more recently, glucuronoxylomannan from *Tremella* (Reshetnikov *et* al. 2001) are moving or have already moved over to fermenter produced products. Clearly, this may be the way ahead and it is only a matter of time before many of the main medical products from medicinal mushrooms are produced in this way.

The logical progression of medicinal mushrooms is the step across to pharmaceutical application as seen with the purified polysaccharides such as Lentinan, PSK etc. In the US, pharmaceutical companies are examining their ability to compete in the future FDA-approved herbal pharmaceutical market. Botanicals, which should include mushroom nutriceuticals, are complex mixtures that contain many chemical constituents and are marketed as dietary supplements with no regulatory control. When specific therapeutic claims are made then, as previously discussed, they become subject to standard national regulatory approval. The pharmaceutical development of such herbals or nutriceuticals creates major difficulties for the FDA which was originally set up to assess drugs that typically contain only one active ingredient (Glasser, 1999). The FDA has currently several investigational new drug (IND) applications for botanical products and may soon allow Phase II clinical trials. It is anticipated that there will be three levels of control: control of the raw materials (the plant or the mushroom); control of the manufacturing process which must be a validated and responsible process; and control of the final product which could include fingerprinting and biological assays to evaluate the active components and/or relevant chemical markers of clinical efficacy, when activities are not identified. Furthermore, there must be assurance of batch-to-batch consistency. It is believed that over 60% of the herbal products presently being marketed would fail any analytical tests for quality, identity of material or amount of active components when known!

Most of the mushroom DSs presently in the market place are highly diverse and there are currently few standard protocols to ensure product quality. There must be thorough analysis, improved quality and legal control which will, in turn, increase

and maintain consumer confidence and achieve the current and future standards set by national regulatory authorities.

A central feature of the purported medicinal or chemo-preventive role of crude herbal and mushroom extracts must be a synergistic interaction of the many constituents. In such synergistic systems it can be considered that activity results from the presence of multiple active principles which must be together to create the desirable response (Pezzuto, 1997). When such complex mixtures are submitted to fractionation the active ingredients are separated and consequently activity may be lost. The undoubted difficulties of studying this form of synergism suggests why this effect seen in herbal and related products remains a relatively unstudied aspect. Should modern medicine continue to be driven by the necessity to rely predominantly on pharmaceutically pure medicines?

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CHAPTER 10 CONCLUSIONS

The effect of purified polysaccharides on immunostimulation and cancer therapy

This Report has attempted to appraise, in particular but not exclusively, the therapeutic functions of mushroom polysaccharides and polysaccharide-protein complexes on animal and human systems. Amongst their many biopharmacological activities the most intriguing are those associated with immunomodulatory and anti-cancer effects. In mushrooms, they occur mostly as glucans with different types of glycosidic linkages such as (1-3), (1-6)-β-glucans and (1-3)-α-glucans, as true heteroglucans, while others bind to protein residues as polysaccharide-protein complexes.

Why then do these mushroom polysaccharides display such an array of biopharmacological activities? Polysaccharides, unlike proteins and nucleic acids, contain repetitive structural features that are polymers of monosaccharide residues joined to each other by glycosidic linkage. Consequently, these polysaccharides offer a high capacity for carrying biological information because of their increased potential for structural variability. The amino acids in proteins and the nucleotides in nucleic acids can only interconnect in one way while the monosaccharide units in the polysaccharides can interconnect at several points to create a wide array of linear and branched molecules. It has been calculated that the number of possible permutations from four different sugar monosaccharides could be up to 35, 560 unique tetrasaccharides, whereas four amino acids can form only 24 different permutations. This, then, creates a vast potential flexibility for the precise regulatory mechanisms of various cell-cell interactions in higher organisms. Is this then at least

a part explanation for the multivarious medical claims that have been made for the extracts from medicinal mushrooms used in TCM?

A fundamental principle in Oriental medicine is to regulate homeostasis of the whole body and to bring the diseased person to the normal state. Potentiating the physiological constitution in favour of host defence results in the activation of many vitally important T-cells for the maintenance of homeostasis. Of significant relevance and importance is the reported ability of particular mushroom-derived compounds to modulate human immune responses and to inhibit certain tumour growths. Currently, and in marked contrast, medicinal mushrooms are not yet employed in Western medicine practices despite the ongoing intensive research for new or complementary therapeutic solutions such as for the treatments of cancer, immmunodeficiency diseases, or for generalised immunosuppression following conventional treatment. A significant hurdle to their non-introduction has been, until recently, the lack of supporting scientific and medical data in peer-reviewed Western journals.

Medicinal mushroom research has focused on discovering compounds that can modulate positively or negatively the biological response of immune cells. Certain mushroom derived-glucans and polysaccharide-bound proteins have been shown to act as immunomodulators or biological response modifiers (BRMs), where these polymers interact with the immune system to upregulate or downregulate specific aspects of the responses of the host and this may result in various therapeutic effects. Whether certain compounds enhance or suppress immune responses can depend on a number of factors including dosage, route of administration, timing and frequency of administration, mechanism of action or the site of activity. Many mushroom-derived polysaccharides appear to fit the accepted criteria for BRM compounds. They cause no harm and place no additional stress on

the body, they assist the body to adapt to the various environmental and psychological stresses, and they have a non-specific action on the body, supporting all the major systems, including nervous, hormonal, and immune systems, as well as regulatory functions.

In this report, a wide variety of mushroom polysaccharides, including Lentinan (from *L. edodes*), Schizophyllan (from *S. commune*), PSK and PSP (from *Trametes versicolor*), and Grifron-D (from the Maitake mushroom *G. frondosa*) and others are described, and their properties are shown to satisfy the criteria for BRMs. Many of these mushroom-derived polymers potentiate the host's innate (non-specific) and acquired (specific) immune responses in a similar manner, where they activate many kinds of immune cells that are vitally important for the maintenance of homeostasis. Key innate responses that are stimulated by these mushroom derived-β-glucans or polysaccharide-protein complexes include host T-cells (such as cytotoxic macrophages, monocytes, neutrophils, natural killer cells, and dendritic cells) and chemical messengers (cytokines such as interleukins, interferon and colony stimulating factors) that trigger complement and acute phase responses. Moreover, mushroom polysaccharides or polysaccharide-protein complexes are considered as multi-cytokine inducers that are able to induce gene expression of various immunomodulatory cytokines and cytokine receptors. In addition, acquired responses are also enlisted, where lymphocytes that govern antibody production (B-cells) and cell-mediated cytotoxicity (T-cells) are stimulated. While the immune system is shrouded in tremendous complexity, our current understanding shows that it is regulated in an orchestrated dynamic manner.

Mushroom-derived polysaccharides have shown anti-tumour activities in both pre-clinical models and in clinical trials. Although the mechanism of their anti-tumour

action is still not completely clear, Lentinan, Schizophyllan, PSP, PSK and other mushroom polysaccharides appear to mediate their anti-tumour activity by activation or augmentation of the host's immune system (via stimulated cytotoxic macrophages, cytotoxic T-cells and antibody-mediated cytoxicity of targeted cancer cells), rather than direct cytotoxicity. Thus, both cell-mediated immune responses against the target T-cells initiated by macrophage-lymphocyte interactions and cytoxicity induced by antibodies to target T-cells are believed to contribute to the elimination of targetted tumour cells. Recent evidence suggests that several mushroom polysaccharides may also possess cytotoxic properties. Grifron-D from *G. fondosa* mushroom was reported to induce apoptosis (programmed cell death) in human prostate cancer cell-lines.

The likely mode of immunopotentiation by mushroom macromolecules involves activation of cytotoxic macrophages, helper T-cells and NK cells, and the promotion of T-cell differentiation. Macrophages are one of the many critical components in the immune system, necessary for tumour rejection. Macrophages have a highly selective cytotoxicity towards cancer cells *in vitro*; and there is evidence that they may also destroy malignant T-cells *in vivo*. T-cell competence appears necessary for selection of macrophage resistance, which suggests that these two cell types interact in the intact host in response to a tumour challenge. Neither Schizophyllan nor Lentinan demonstrated any anti-tumour activity against Sarcoma 180 in *in vivo* experiments with cyclosporin A as a T-cell suppressor, which suggests that an immunocompetent T-cell component is necessary for developing anti-tumour activity. These results indicate that Schizophyllan and Lentinan are T-cell oriented immunopotentiators and, therefore, require a functional T-cell component for their biological activity and that the action of (1-3)-β-D-glucans on the host's

immune system might increase helper T-cell production, increase macrophage production, and bring about stimulation of acute phase proteins and colony stimulating factors, which in turn effects proliferation of macrophages, neutrophils, and lymphocytes and activation of the complement system. The immunopotentiating activity of β (1-3)-D-glucans would appear to depend on the presence of a helical conformation with hydrophilic groups located on the outside surface of the helix.

By way of further examples, both PSK and PSP are potent immunostimulators with specific activity for T-cells and for antigen-presenting cells such as monocytes and macrophages. The biological activity is characterized by their ability to increase white blood cell counts, interferon-γ and interleukin-2 production and delayed type hypersensitivity reactions. Numerous reports have documented the ability of PSK and PSP to activate cellular (helper and cytotoxic T-cells) and humoral (antibody) components of the host immune system. In addition, these polysaccharides have been shown to inhibit the growth of tumour cell lines and to have *in vivo* anti-tumour activity. Recently, the anti-tumour activity of medicinal mushrooms has been evaluated in Japan for the prevention of oesophageal, gastric, and lung cancer in humans with promising results. In Phase II and Phase III trials in China, PSP significantly enhanced immune status in 70 to 97% of patients with these cancers. In these studies, PSK and PSP increased the number of immune cells and facilitated dendritic cell (antigen-presenting cell) and cytotoxic T-cell infiltration of tumours.

The proprietary mushroom polysaccharides such as Lentinan, Schizophyllan, PSK and PSP, and Grifon-D® are not miracle drugs but can increase the quality of life of cancer patients and may offer increased survival rates for some types of cancer, especially when they are used as adjuncts to conventional forms of treatment. The enhanced survival rates with such compounds appear very promising and it is to be

hoped that Western oncologists will now look more carefully and appreciatively at the wealth of information set out in this document, and assess the potential for incorporating some aspects into current cancer research. The Maitake compound, Grifron-D®, is now undergoing extensive clinical trials in the US. Many thousands of US physicians now use proprietary mushroom polysaccharides in treatment programmes, possibly aided by a much higher Asian-derived populace.

These compounds have been shown to be safe when taken over long periods of treatment; and significantly, these compounds appear to reduce the adverse effects of radiotherapy and chemotherapy. These results are in contrast to the well-documented adverse side-effects associated with most chemotherapeutic compounds and also, but to a lesser extent, certain immunotherapeutics. Such compounds have been shown to be capable of causing fevers, chills, rash, eodema, arthralgia, hypotension, congestive heart failure or CNS toxicities.

Almost all of the current cancer rearch studies utilising mushroom polysaccharides have been performed using only individual components. Only a few studies have applied mixtures of proprietary compounds. This is to be expected since the purified compounds such as Lentinan, PSK and Grifron-D® are produced by different companies. It could be expected that by utilising mixtures of these proven compounds the immune system would receive multiple stimuli possibly leading to stronger anti-cancer effects. At the recent Kiev Conference on Medicinal Mushrooms, Professor Ikekawa strongly advocated the use of mixtures of the accepted anti-cancer mushroom-derived compounds for clinical cancer treatments (personal communication). Some recent clinical studies have been mentioned in Chapter 7.

As stated by Kidd (2000), “glucan and proteoglycan mushroom immunoceuticals offer hope for cancer patients. These substances are pro-homeostatic, uniquely effective immune boosters, which pose no threat of autoimmune backlash. As dietary supplements, they are safe, and exhibit near-perfect benefit-risk profiles. Mushroom immunoceuticals are a potential boon to individuals afflicted with cancer living with impaired immunity, or merely descending into ill-health with the passing of time.”

The effect of whole medical mushrooms and concentrated extracts (dietary supplements) as functional foods

Scientific and medical studies with the medicinal mushrooms have mainly concentrated on the application of purified polysaccharide compounds such a Lentinan, Schizophyllan, Grifron-D, PSK and PSP on animal and human systems. What then can be surmised on potential health benefits from consuming fresh mushrooms or crude concentrates derived from them?

As a source of nutrients, mushrooms continue to be under-valued in the UK where many believe that mushrooms are low in nutrients and are of little or no value to health. In truth, most edible and culinary mushrooms (e.g. *Agaricus bisporus* – the button mushroom, and *Lentinus edodes* – the Shiitake), are very rich in minerals (e.g. potassium, calcium and magnesium), various vitamins (D_2, B_2, C and niacin), dietary fibres, proteins and all the essential amino acids and yet are extremely low in calories, fat and cholesterol. The potential nutritional value of mushrooms ranked by internationally-accepted tests compares well with meat and milk and more so than some common vegetables. Many wild edible mushrooms are often more flavoursome than their cultivated relatives (e.g. the Chanterelle). In Japan, mushrooms are highly rated and recommended by doctors and nutritionists, together

with green vegetables, to promote good health. Edible mushrooms must, therefore, be considered foremost as 'healthy' foods and their consumption should be encouraged in healthy eating programmes especially where the levels of fats and cholesterol are to be minimilised, e.g. with respect to cardiovascular diseases.

In Traditional Chinese Medicine, many mushrooms have long been part of a wide range of treatment regimes mostly used in the form of dried powders of the mushroom fruit-bodies or as hot water extracts of the same. While many of these medicinal mushrooms were both medicinal and nutritious, others were inedible and only used for their medicinal qualities (how these qualities were first identified will forever be a mystery). When used for medicinal purposes the medicinal properties are derived from a relatively large quantity of fresh mushrooms, not normally consumed in the average eating portion. The question, then, arises on how valuable to the individual is the consumption of fresh medicinal mushrooms? In murine studies, regular feeding of certain whole medicinal mushrooms stimulated aspects of the immune system and did inhibit growth of existing tumours. There has been no research on the prophylactic effects of medicinal mushroom intake on, for instance, the development of spontaneous tumours in humans. However, in this respect, a survey conducted among Japanese mushroom workers in the Nagano Prefecture in Japan, implied that the regular eating of mushrooms (mostly the edible, medicinal variety *Flammulina velutipes*) was associated with a lower death rate from cancer than of other people in the Prefecture (Table 1). This study strongly suggests that quantity and frequency of intake of the mushrooms were related to the lowered cancer death rate. A detailed large-scale epidemiological study is ongoing conducted by the NCC Research Institute of Japan, Nagamo Agricultural Technology

Institute, and Hospitals as a cohort study of the Japanese Ministry of Health and Welfare.

Table 1 Comparison of Cancer Death Rate (Ikekawa, 2001)

Average cancer death rate in Nagano Prefecture	
Total	160.1
Man	90.8
Woman	69.3

Average cancer death rate of farmers producing an edible mushroom in Nagano Prefecture	
Total	97.1
Man	57.5
Woman	39.7

Cancer death rate: rate per 100,000 age-adjusted rates.
Total population: 174,505. Years investigated 1972-1986.
The cancer date rate of the farmers producing *Flammulina velutipes* as a main occupation was remarkably lower than that of the total Nagano Prefecture, Japan ($p<0.01$).

Agaricus blazei has become an important source of antitumour polysaccharides and is now being artificially grown in Japan and studied pharmacologically. This fungus is native to a small mountainous area near Sao Paulo, Brazil. Epidemiologists when studying the native population of this small area found that such people had a very low incidence of several illnesses, including cancer, viral and bacterial diseases, together with a disproportionally higher number enjoying longevity. This has been correlated with the constant consumption of this mushroom in their normal diet. In recent years there has been extensive research on the medicinal properties of this mushroom, mostly in Japan, clearly demonstrating immunostimulatory activity and antitumour action (Reshetnikov *et al*., 2001).

In Asia, several medicinal/edible mushrooms are regularly (often daily) consumed in the average diet. Whether the amount consumed by this route can be

expected to have a limited or a significant effect on the immune system in sick or healthy people must be the subject of future research. In this respect the final outcome of the Nagano epidemiological study could have tremendous significance.

As previously discussed, oral ingestion of concentrates of *L. edodes* and *G. frondosa* can modulate certain murine immune functions. The consumption of *L. edodes* concentrates as part of a regular diet had immune stimulatory as well as immunosuppressive effects in mice depending on the strain and the specific immune function studied. Could regular ingestion of medicinal mushroom concentrates as dietary supplements be of significance to human health? The huge, world-wide sale of such products, can testify to the beliefs of many, of their efficacy. Proof of efficacy must surely be the defining factor for those who remain sceptical. Again, the previous statement of Kidd (2000) extolling the value of partially purified mushroom polysaccharides as immune system modulators must also be relevant to some extent with whole medicinal mushrooms and, more especially, with crude concentrates.

There is now increasing evidence with experimental animals that regular feeding of powdered medicinal mushrooms can have a cancer prevention effect, demonstrating both high antitumour activity and restriction of tumour metastasis. A recent study by Professor Ikekawa (2001) demonstrated the preventive effects of the edible mushroom *Hypsizygus marmoneus*. Control mice were bred on an ordinary feed and the treated mice on the feed also containing 5% dried fruit-bodies of the mushroom. All mice were injected i.p. with a strong carcinogen, methylcholanthrene, and carcinogenesis of the mice recorded. After 76 weeks, 21 of 36 mice developed tumours in the control mice but only 3 of 36 mice in the treated group had tumours. Thus, the intake of this medicinal mushroom proved to be effective in cancer

prevention and tumour growth inhibition. This study confirms previous studies with *Lentinus edodes* and *Pleurotus ostreatus*.

Yet another interesting study on cancer chemoprevention using mushroom polysaccharides is the yet unpublished paper by Shon and Nam (2002) using the mouse skin carcinogenesis model (Holden *et al*., 1997) to study changes involved in tumour promotion. In these studies 12-0-tetradicanoylphorbol-13-acetate (TPA)-induced tumour promotion in mouse epidermis previously initiated with 7, 12-dimethylbenz[a] anthracene (DMBA) was used. The effects of polysaccharides derived from the medicinal mushrooms *Phellinus igniarius* and *Agrocybe cylindracea* applied topically were then studied. Topical application of polysaccharides from either *P. igniarius* or *A. cylindracea* together with TPA twice weekly for 12 weeks inhibited the number of skin tumours per mouse by 69.7 or 88.2% respectively and the percentage of mice with tumours was lowered by 70.0 or 30.0% respectively.

It is tempting to draw an analogy between the medicinal/edible mushrooms, with their proven medicinal properties when applied in purified and crude concentrate forms, and the anticancer phytochemicals of fruit and vegetables. However, it is perhaps unlikely that there will be a major increase in fresh or processed medicinal mushroom consumption in the UK in line with Asian culinary practice. The imbalance could be reduced by the consumption of medicinal mushrooms as dietary supplements in the form of capsules or elixirs. Notwithstanding, the value of the medicinal mushrooms as true functional foods/dietary supplements can no longer be in doubt. Indeed, the abundance of scientific evidence confirms the role of certain mushrooms as valuable functional foods/dietary supplements in the human diet. The very considerable historical evidence with edible medicinal mushrooms would strongly suggest that they are the very first truly functional foods.

When the British public become more aware of the significant medical and flavour attributes of the edible medicinal mushrooms, perhaps there will be a positive change in consumer acceptance and consumption. Such a change has already occurred in the US with the recognition of certain medical mushrooms, with 'healthy' food and 'healthy' medicine. Perhaps the major supermarkets will take a lead and promote mushrooms in general and medicinal mushrooms in particular as novel, scientifically and medically proven functional foods. Sadly, so many products now being sold as functional foods, are mere marketing ploys and have dubious and, indeed, unproven efficacy – not so the medicinal mushrooms!

The vast majority of medicinal mushrooms are cultivated in the Far East though production of the Shiitake (*L. edodes*) and the Oyster mushroom (*P. ostreatus*) are now increasing in the UK and Europe. Recent studies in Japan have shown that the concentration of the polysaccharide Lentinan (*L. edodes*) and Grifron-D (*G. frondosa*) remain relatively stable in the fruit-bodies when the harvested mushrooms are stored at $4^{o}C$ for up to one week. In contrast, these same polysaccharides are greatly reduced by endogenous enzyme activity at $20^{o}C$ over the same time period as measured by reduced tumour necrosis factor (TNF)-α and nitric oxide production from macrophages. Clearly, time from harvesting to consumption is of critical importance to retain the important medicinal properties of these mushrooms and should certainly be a significant factor for fresh mushroom production and also for the production of concentrates or dietary supplements.

In final summary this Report has demonstrated that many mushroom species (the medicinal mushrooms) contain some unique and intriguing biochemical compounds that have undergone controlled clinical studies in Asian and some Western research institutions and hospitals demonstrating considerable

effectiveness in the treatment of many diseases, especially cancer. In cancer treatment they have largely been used as adjuncts to traditional chemotherapy and radiotherapy. In addition, the remarkable ability of many of these non-toxic and compatible compounds to reduce the debilitating effects of traditional chemotheropeutic drugs is notable. Indeed, there are also many examples where the use of these compounds allows the reduction in dose level of the toxic chemotherapeutic compound without reduced efficacy. Chemotherapy can severely impair immune function. By limiting this 'iatrogenic' or 'physician-induced' immunosuppression by means of mushroom polysaccharides could be the great benefit to physicians.

There is now increasing evidence that whole mushroom powders and extracts can exert cancer chemoprevention when incorporated into the diet or applied topically to experimental animals. Human epidemiological studies in Japan and Brazil strongly suggest that regular consumption of certain medicinal mushrooms over prolonged periods of time significantly reduce the levels of cancer incidence.

In Asia, mushrooms occupy an important and regular part of the daily diet whereas in the West this does not happen to the same extent. Perhaps as the edible medicinal mushrooms such as the Shiitake and Enoke become more readily available to the Western customer and their culinary and medicinal values become more appreciated then the level of uptake will increase. In the meantime, dietary supplements consisting of whole mushroom extracts are becoming increasingly popular. Unlike most dietary supplements mushroom dietary supplements contain a veritable Pandora's box of compounds with variable medical claims. What level of consumption or intake can exert healthy benefits is still a matter of debate and warrants some level of clinical study.

The edible medicinal mushroom must be considered as the ultimate functional food when consumed in the whole fresh form or as concentrates or dietary supplements. Non-edible medicinal mushrooms will be consumed only as dietary supplements.

As Mark Twain said:

"*Good health is not just the absence of disease"*

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Overview of the human immune system

A general overview of the immune system is provided so that an appreciation can be gained of how biological molecules from certain mushrooms may modulate the immune response and tackle cancer cells. Immunology is the study of the methods by which the body defends itself from infectious agents and other foreign substances in its environment. The immune system is a remarkably adaptive defence system that has evolved in humans to protect against invading pathogenic microorganisms and evidence is accumulating that the immune system can provide protection against some tumours (Wood, 2001). An infectious organism that causes a disease is called a pathogen and the individual (person or animal) that is infected by a pathogen is called the host. There are thousands of components to the immune system and it would appear that the immune system is far more complicated than necessary for achieving what is, on the surface, a simple task of eliminating a pathogenic organism or abnormal 'self' cells. However there are a number of reasons for this complexity, including the desirability of eliminating pathogens without causing damage to the host.

Getting rid of a pathogen or dead host cells is theoretically easy, but eliminating these without damaging the host is much more complicated. The immune system must be able to distinguish between pathogens or abnormal cells and healthy host cells so that it can direct its destructive powers towards their elimination. As a consequence of this dynamic complexity, the immune system is able to generate a tremendous variety of cells and molecules capable of specifically recognising and eliminating an apparently limitless variety of foreign invaders, in addition to the recognition and destruction of abnormal cells. Furthermore, these host cells and molecules act together in an exquisitely adaptable dynamic manner.

Functionally, an immune response can be divided into the interrelated activities of recognition and response. The immune system is remarkably specific as it is able to recognise subtle chemical differences that distinguish foreign or 'non-self' cells from healthy self-cells. At the same time, the system is able to discriminate between foreign

molecules and the body's own cells and proteins. Once a foreign protein, microorganism (e.g., bacterium, fungus or virus) or abnormal cell is recognised, the immune system enlists the participation of a variety of cells and molecules to mount an appropriate effector response to eliminate or neutralise them. Later exposure to the same foreign organism (e.g., a virus that may have the potential to transform normal healthy cells into tumour cells) induces a memory response, characterised by a heightened immune reactivity, which serves to eliminate the microbial pathogen, prevent disease and protect against the development of some tumour cells (Wood, 2001).

Immunity - the state of protection from infectious disease, has both non-specific and specific components. Innate, or non-specific immunity refers to the basic resistance to disease that an individual is born with. Acquired or specific immunity requires activity of a functional immune system, involving cells called lymphocytes and their products. Innate defence mechanisms provide the first line of host defence against invading microbial pathogens and also provides protection against some tumour cells until an acquired immune response develops. In general, most of the foreign molecules or microbial cells encountered by a healthy individual are readily cleared within a few days by non-specific defence mechanisms without enlisting a specific immune response. When the non-specific defences fail to eliminate foreign invaders or abnormal cells, a specific or humoral immune response is then enlisted. Because immunity was shown to be mediated by molecules known as antibodies that were contained in body fluids (known in earlier times as *humors*), it was known as humoral immunity (Wood, 2001). An antibody is a protein or immunoglobulin that recognises a particular epitope or site on an antigen, which is any substance that binds specifically to an antibody or T-lymphocyte receptor, and facilitates clearance of that antigen. The other arm of the specific immune response is cell-mediated immunity or CMI. CMI response refers to host defences that are mediated by antigen-specific T lymphocyte cells (i.e., leukocytes) and various non-specific cells of the immune system. It protects against intracellular bacteria, viruses and cancer and is responsible for graft rejection. Acquired immunity does not operate independently of innate immunity; rather, the

specific immune response supplements and augments the non-specific defence mechanisms, producing a more effective total response (Wood, 2001).

Innate (non-specific) immunity

Innate immunity can be envisioned as comprising four types of defensive barriers: anatomic, physiologic, endocytic and phagocytic, and inflammatory. Tissue damage and infection induce leakage of vascular fluid, containing serum with antimicrobial activity, and influx of phagocytic cells into the affected area. While physical and anatomic barriers, such as skin and the surface of mucous membranes, prevent the entry of pathogenic microorganisms and are the body's first line of defence, this component of innate immunity will not be developed any further as it has no bearing on immuno-modulation or anti-tumour responses. The physiologic barriers that contribute to innate immunity include elevated temperature (e.g., fever), pH (e.g., acidity produced in stomach and within macrophages), oxygen tension, and various soluble factors (Kuby, 1997). Among these soluble proteins are lysozyme (a hydrolytic enzyme found in mucous secretions that kills bacteria), interferons (INF) and other cytokines (chemical messengers), and complement (plasma proteins that participate in a controlled enzymatic cascade which results in damage to the membranes of pathogenic organisms or abnormal cells, either destroying or facilitating their clearance), markedly influence immunomodulation and regulation, in addition to the prevention of some tumour cells (Kuby, 1997).

Cytokines: the chemical messengers

The term cytokine covers a variety of small proteins less than 20 kDa (usually) that serve a hormone-like function in enabling cells to communicate with each other (Wood, 2001). There are many cytokines and they can be divided into families (Table 2). The main families of cytokines are the interleukins (ILs), colony-stimulating factors (CSF), interferons (INFs), tumour necrosis factors (TNFs), chemokines and growth factors. The functions of cytokines will be described in detail at the appropriate times

when particular immunological mechanisms are being explained. Cells in the body are never exposed to single cytokines – they will be exposed to a number of different cytokines, probably produced by a number of different cell types (Wood, 2001). Different cytokines can either act cooperatively in promoting a response, or act antagonistically in inhibiting each other's actions (Kuby, 1997, Wood, 2001).

Table 2. **Cytokine families***

Family	***Members***	***Comments***
Interleukin (IL)	IL-1 to IL-22	Different IL have different functions and are secreted by different cells
Interferon (IFN)	IFNα	Leucocyte IFN. Inhibits viral replication
	IFNβ	Fibroblast IFN. Inhibits viral replication
	IFNγ	Secreted by T lymphocytes and NK cells. Many immunoregulatory functions
Tumour necrosis Factor (TNF)	TNFα	Secreted by monocytes and other cells. Factor activates macrophages and endothelial cells
	TNFβ	Secreted by T cells. Similar activity to TNFα
Colony-stimulating Factors (CSF)	G-CSF, M-CSF, GM-CSF and others	Originally identified by ability to make bone-marrow cells differentiate into particular cell type, e.g. neutrophil. Also have effects on mature cells of same lineage, e.g. monocytes, macrophages and neutrophils
Chemokine	MCP, Ecotaxin and others	Very important in controlling the migration of cells between and within tissues. Also influence function of many cells

** Source: Wood, 2001*

Macrophages and phagocytosis

Other important components of innate immunity are phagocytic cells (macrophages, neutrophils) and other lymphocytes such as natural killer (NK) cells that do not require activation but can lyse certain infected or abnormal cells. Macrophages are large leukocytes (any blood cell that is not an erythrocyte; white blood cell) derived from monocytes that functions in phagocytosis, antigen processing and presentation, secretion of cytokines, and antibody-dependent cell-mediated cytotoxicity (ADCC). While explained in more detail later, ADCC is a cell-mediated reaction in which non-specific cytotoxic cells that express Fc receptors, such as neutrophils, macrophages, NK cells, recognise bound antibody on a target cell and subsequently causes lysis (destruction) of the target cell. Phagocytosis is a process by which certain cells (phagocytes) engulf microorganisms, other cells, and foreign particles (Figure 1).

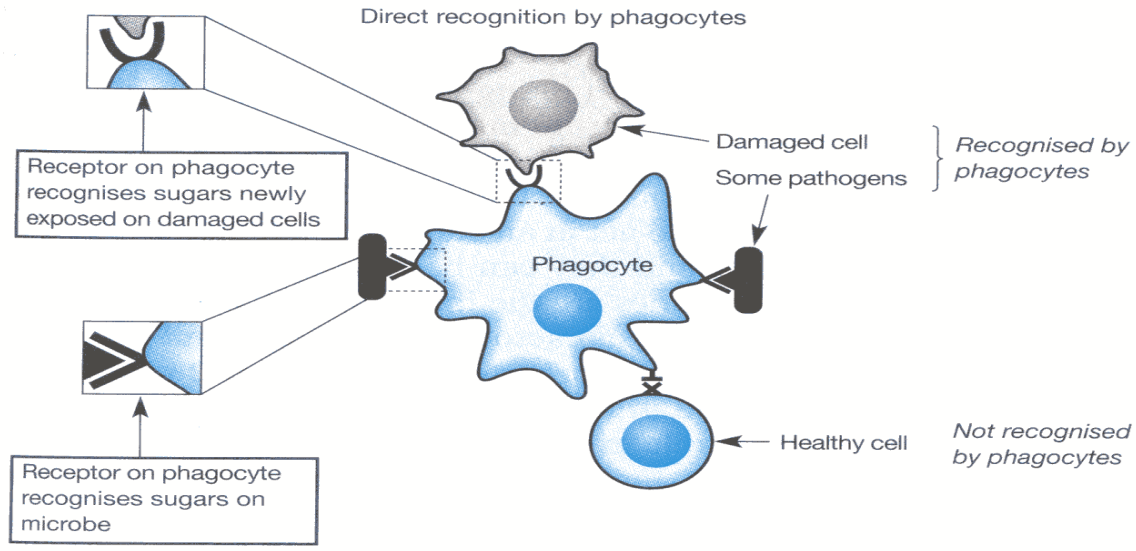

Figure 1. Recognition by phagocytes. *Phagocytes must distinguish microbes and dead host cells from healthy host cells so that healthy host cells are not phagocytosed. Phagocytes have receptors on their surface that recognise sugars present on microbes or sugars that are newly expressed on dead or damaged host cells. These sugars are not present on healthy host cells and therefore the host cells are not phagocytosed (Source: Wood, 2001).*

Natural Killer (NK) cells

Another population of cells that form part of the innate immune system are natural killer cells (NK) cells. NK cells are large, granular lymphocytes that are capable of lysing or killing infected or tumour cells without overt antigenic stimulation (recruiting specific immune response). NK cells osmotically lyse target cells and induce apoptotic cell death. Apoptosis is known as programmed cell death that is characterised by morphologic changes including nuclear fragmentation, blebbing, and release of apoptotic bodies, which are phagocytosed. In contrast to necrosis, it does not result in damage to surrounding cells (Kuby, 1997). NK cells lack the T lymphocyte receptor for antigen recognition. Another important role for NK cells is in the inflammatory response (discussed in more detail later). NK cells enter sites of inflammation where they can be stimulated by a cytokine called IL-12 that is produced by activated macrophages. The NK cells are stimulated by IL-12 to produce IFN-γ that is a powerful activator of macrophages (Wood, 2001). The cellular origin of natural killer cells is unknown.

Complement system

Target cells can also be destroyed through the activation of complement which is a complex series of interrelated proteins present in normal serum. Components of the complement system (i.e., activated components C3a, C3b through to C9) mediate and amplify immune reactions. Following the release of chemotactic factors and histamine C3a this induces considerable inflammation and tissue damage at the sites of reactions with antibodies. Residual C3b component bound to the antigen-antibody complexes attaches to C3b receptors present on macrophages and thus acts as an opsonin, promoting enhanced phagocytosis. Where antibody has reacted with the surface of virus-infected or transformed cells, the complement system is activated to form a membrane attack complex resulting in cell lysis. The latter processes are known as antibody-dependent cellular cytotoxicity (ADCC). As with the effector response to unwanted or ‘non-self’ antigen-presenting cells, a well-orchestrated *in vivo* system regulates the overproduction of specialised B and T lymphocytes (discussed in more

depth later). For example, transforming growth factor (TGF)-β inhibits B and T cell proliferation; INF-γ inhibits IL-4 activation of B cells; and IL-4 / IL-10 inhibit INF-γ activation of macrophages.

Inflammatory and acute phase responses

Usually there are not enough macrophages or monocytes present in tissue to phagocytose and remove all invading pathogens and therefore the tissue macrophages must initiate a response that will bring additional phagocytes, together with a variety of host proteins (cytokines) and cells (lymphocytes), to the site of infection from the bloodstream (Kuby, 1997). This response is known as the inflammatory response and in addition to removing pathogens it also eliminates dead or abnormal host cells. Figure 2 illustrates the four main events occurring in an inflammatory response that are:

1. **Vasodilation** – causes increased blood flow to the area, increasing the supply of cells and factors
2. **Activation of endothelial cells** – lining the blood vessels makes them more 'sticky' to white blood cells so that the blood cells can adhere more strongly to the endothelium
3. **Increased vascular permeability** – makes it easier for cells and proteins to pass through the blood vessel walls and enter the tissue
4. **Chemotactic factors** are produced – these are molecules that attract cells into the tissue from the blood (Wood, 2001).

The first stage of the inflammatory response is recognition of the pathogen and activation of tissue macrophages that on stimulation, produce a number of factors including prostaglandins (small biologically active lipid molecules), platelet-activating factor (PAF) and cytokines (of particular importance are interleukin–2 and IL-8, and tumour necrosis factor-α or TNF-α).

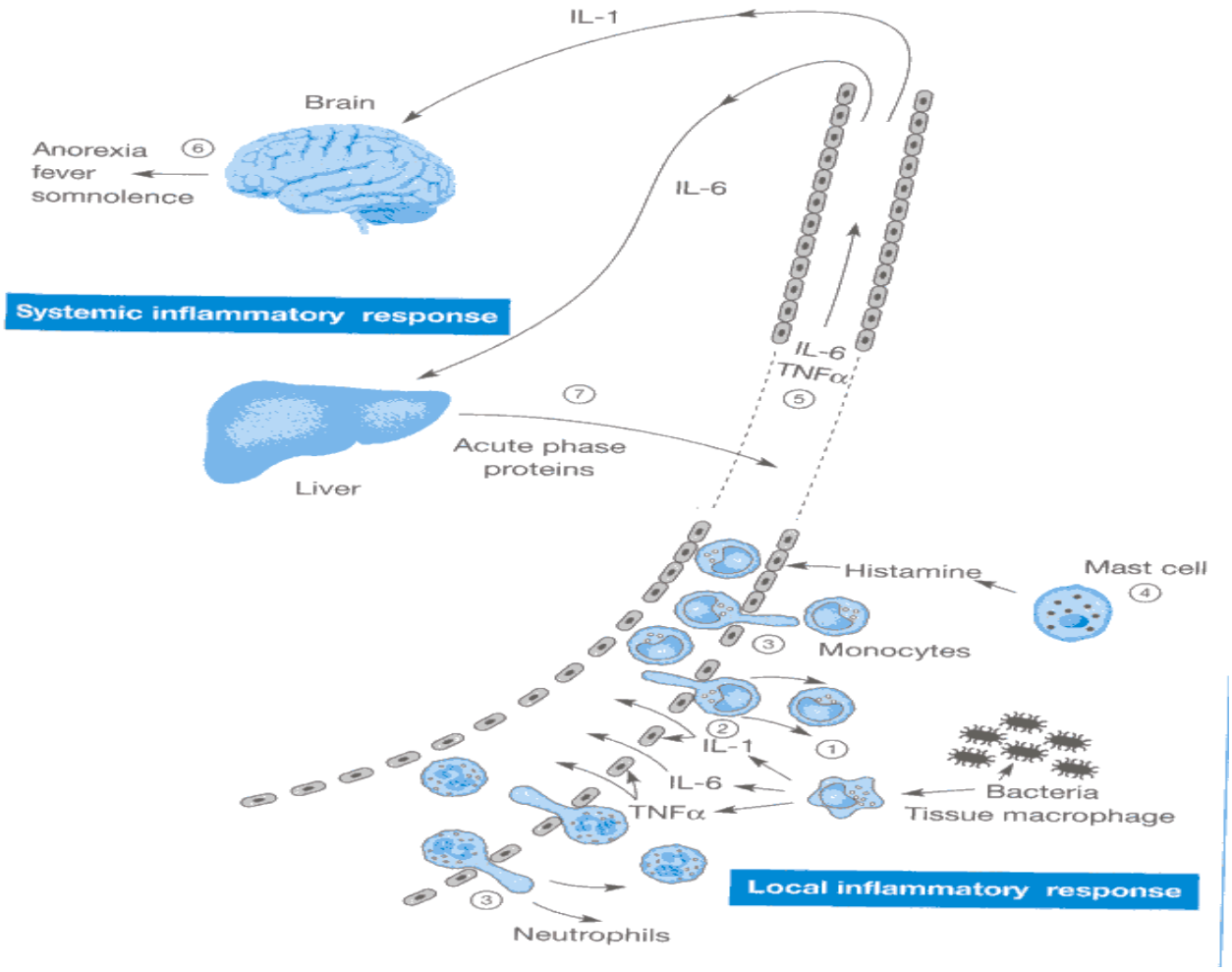

Figure 2. Inflammatory responses. *Inflammatory responses can be local or systemic. 1, tissue macrophages recognise microbial products. 2, macrophages release cytokines and other inflammatory mediators (IL-1, TNF-α) that cause vasodilation, increased vascular permeability and have chemotactic effects on monocytes and neutrophils. 3, Monocytes and neutrophils are recruited to the site and there is accumulation of plasma fluid and proteins at the site, causing oedema or swelling. 4, Inflammatory mediators can activate mast cells to release further mediators that amplify the response. 5, if the local production of cytokines is high enough, the cytokines travel in the blood and affect other organs. 6, IL-1 affects the brain causing fever. 7, IL-6 stimulates hepatocytes to produce acute phase proteins (source: Wood, 2001).*

These cytokines act directly on the endothelium to increase vascular permeability and PAF also causes platelets to release histamine (another agent that increases vascular permeability). IL-1 and TNF-α activate endothelial cells lining the blood vessels

at the site of infection that causes these cells to express surface molecules that neutrophils in the bloodstream can bind to, enabling the neutrophils to leave the bloodstream and enter the tissue. Neutrophils (also promoted by IL-8) and macrophages eliminate pathogens by the process of phagocytosis (Wood, 2001).

Other cell types and biochemical pathways can also be activated during an inflammatory response that can result in the accumulation and activation of granulocytes and monocytes resulting in the removal of pathogenic microorganisms by phagocytosis. Activation of the complement and clotting systems is also important for the inflammatory response. The complement system is made up of a number of different plasma proteins that participate in a controlled enzymatic cascade that results in damage to membranes of pathogenic organisms or abnormal cells, either destroying or facilitating their clearance. The roles of activated complement components in eliminating pathogens and abnormal cells will be addressed later. The clotting system leads to the cleavage of fibrinogen to generate fibrin threads that form blood clots and fibrinopeptides with are chemotactic for phagocytes (Wood, 2001).

If the pathogen is not eliminated the continued recruitment and stimulation of macrophages will lead to a rise in the concentration of macrophage-derived cytokines in the plasma (Wood, 2001). These cytokines can affect organs such as the brain and liver, that causes a systemic response known as an acute phase response. Of particular importance is the production of a series of proteins called acute phase proteins (APPs) such as, fribrinogen (involved in the clotting and generation of fibrinopeptides), heptoglobulin (binds iron whereby limiting bacterial growth), complement component C3 (its cleavage to C3a - activates mast cells that contain large granules of histamine, heparin and proteolytic enzymes (protein attacking), and C3b - helps phagocytes recognise pathogens), and proteins such as C-reactive and mannose binding proteins that target specific receptors on invading microorganisms facilitating their elimination by phagocytosis. Because the cells and proteins of the inflammatory and acute phase responses are pre-existing, they provide an immediate response to tissue damage and infection (Wood, 2001).

Acquired (non-specific) immunity

Acquired, or specific, immunity reflects the presence of a functional immune system that is capable of specifically recognising and selectively eliminating foreign microorganisms and molecules (i.e. foreign antigens). Unlike innate immune responses, acquired immune responses are adaptive and display the following characteristics:

1. **Antigenic specificity** – permits the immune response to distinguish subtle differences among antigens. Antibodies can differentiate between two molecules that differ by a single amino acid (building block of proteins).
2. **Diversity** – it is capable of generating tremendous diversity in its recognition molecules, allow it to specifically recognise billions of uniquely different structures on foreign antigens.
3. **Immunologic memory** – once the immune system has recognised and responded to an antigen, a second encounter with the same antigen induces a heightened state of immune reactivity.
4. **Self/nonself recognition** - the immune system normally responds only to foreign antigens indicating that it is capable of self/nonself recognition. The ability of the immune system to distinguish self from nonself and respond only to nonself-molecules is essential, for the outcome of an appropriate response to self-molecules can be a fatal autoimmune disease (Kuby, 1997).

Acquired immunity does not occur independently of innate immunity. The phagocytic cells (NK cells, neutrophils, macrophages) crucial for non-specific immunity are intimately involved in the activation of the specific immune response. Conversely, various soluble factors produced during a specific immune response, have been shown to augment the activity of these phagocytic cells. Thus, through the carefully orchestrated interplay of acquired and innate immunity, the two systems work in tandum to eliminate a foreign invader or abnormal cells (Kuby, 1997). Generation of an effective immune response involves two major groups of cells: lymphocytes and antigen-presenting cells (APCs). Lymphocytes are one of the many types of white blood

cells produced in the bone marrow during the process known as hematopoiesis. There are three general classes of cells produced from hematopoietic stem cells, (1) red blood cells (erythrocytes) that are responsible for oxygen transport, (2) platelets that are responsible for the control of bleeding, and (3) white blood cells (lymphocytes), the vast majority of which are involved in host immunity. Lymphocytes leave the bone marrow, circulate in the blood and lymph system, and reside in various lymphoid organs (Kuby, 1997). Lymphocytes possess antigen-binding cell-surface receptors, mediate the defining immunologic attributes of specificity, diversity, memory, and self/nonself recognition. There are two major populations of lymphocytes – B lymphocytes (B cells) and T lymphocytes (T cells) (Kuby, 1997).

B lymphocytes

B lymphocytes mature within the bone marrow and leave the marrow expressing a unique antigen-binding receptor on their membrane. The B cell receptor is a membrane-bound antibody molecule. Upon activation, B cells specific for the antigen (usually foreign) proliferate and become antibody secreting or plasma cells. Antibodies are complex molecules (glycoproteins) that have the property of combining specifically to the antigen that induced its formation. The resulting antibodies bind to the invading pathogen, marking it for destruction by killer T-lymphocytes by a process called antibody dependent cell cytotoxicity (ADCC). Antibodies also mark cells for phagocytosis by neutrophils and other phagocytic cells by a process called opsonisation. Most of the daughter cells produced by B cell activation die within a few weeks but a proportion of them recirculate in the body for many years as memory cells. If they are reintroduced to the same antigen that elicited an initial response, they rapidly become reactivated and produce antigen-specific antibody. This function provides the basis for vaccination. It is estimated that a single antibody secreting or plasma cell can produce more that 2000 molecules of antibody per second (Kuby, 1997). Secreted antibodies are the major effector molecules of humoral immunity.

T lymphocytes

T-lymphocytes (T cells) also arise from hematopoietic stem cells in the bone marrow. Unlike B cells, which mature within bone marrow, T cells migrate to the thymus gland to mature. During its maturation within the thymus, the T cell comes to express a unique antigen-binding receptor on its membrane, called the T cell receptor (TCR). Unlike membrane bound antibodies on B cells, which can recognise antigen alone, TCRs can only recognise antigen that is associated with cell membrane proteins known as major histocompatability complex (MHC) molecules (Kuby, 1997). When a naïve T cell encounters antigen associated with a MHC molecule on a cell, the T cell proliferates (clones) and differentiates into memory T cells and various effector T cells.

There are two well-defined subpopulations of T cells: T helper (T_H) and T cytotoxic (T_C) cells. T helper and T cytotoxic cells can be distinguished from one another by the presence of either membrane glycoproteins CD4+ or CD8+ on their surfaces. T cells displaying CD4+ generally function as T_H cells, whereas those displaying CD8+ function as T_C cells. After a T_H cell recognises and interacts with an antigen-MHC II molecule complex, the cell is activated and becomes an effector cell that secretes various cytokines. These secreted cytokines play an important role in activating B cells, T_C cells, macrophages, and various other T cells, and initiate the delayed type hypersensitivity (DTH) response. The DTH reaction promotes local inflammation resulting in the recruitment of more lymphocytes and activated macrophages (i.e., converted monocytes from the bloodstream) to target cells. Under the influence of T_H-derived cytokines, a T_C cell that recognises an antigen-MHC I molecule complex proliferates and differentiates into an effector cell called a cytotoxic T lymphocyte (CTL). In contrast to the T_H cell, the CTL generally does not secrete many cytokines and instead exhibits cytotoxic activity (Kuby, 1997). The CTL has a vital function in monitoring the cells of the body and eliminating any that display antigen, such as infected or tumour cells. Figure 3 illustrates key cellular interactions involved in induction of acquired immune responses.

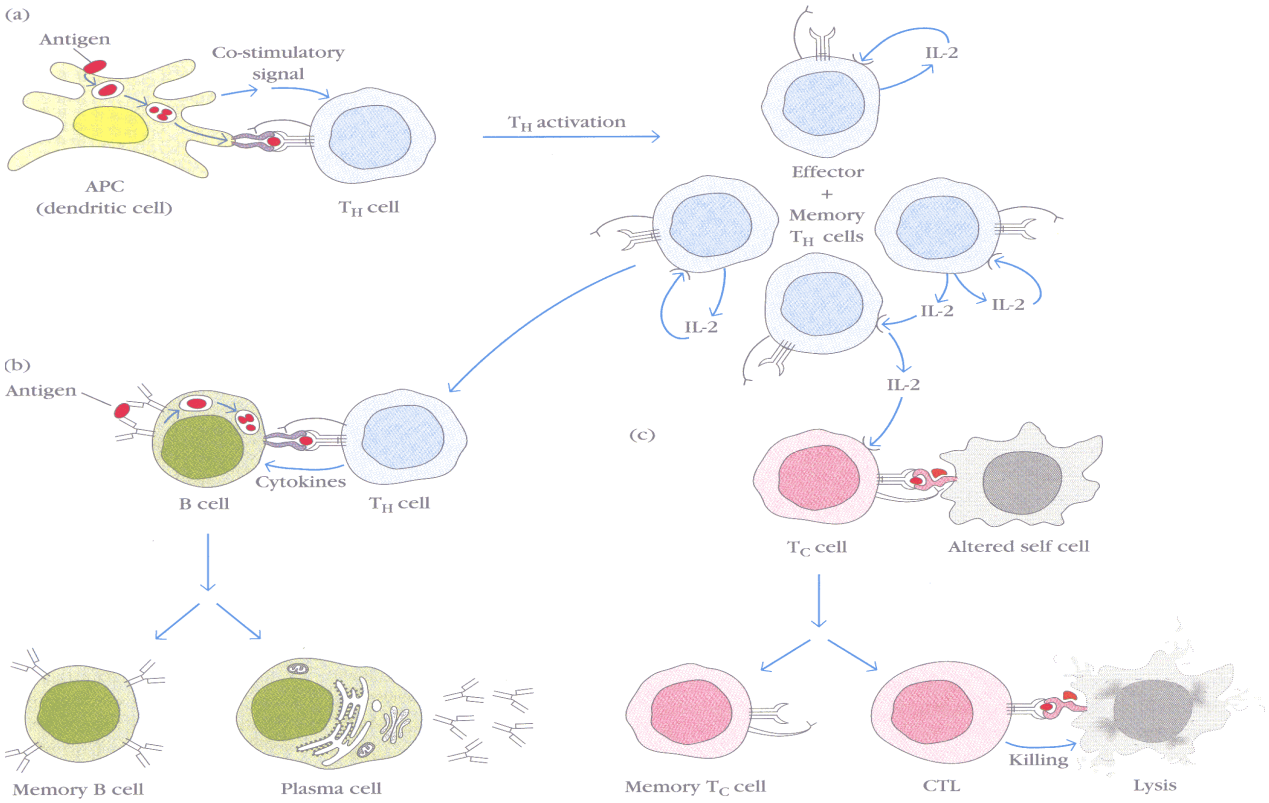

Figure 3. Cellular interactions involved in induction of the specific immune responses. *Activation and proliferation of T_H cells (a) is required for generation of a humoral response (b) and a cell-mediated response to altered self-cells (c). APC = antigen-presenting cell; Ag = antigen (Source: Kuby, 1997).*

Thus, acquired immunity is composed of activated CD4 (T_H) and CD8 (T_C) cellular responses. Furthermore, T_H cells recognise foreign proteins or antigens that have been processed through an exogenous pathway by antigen-presenting cells such as dendritic cells in lymph nodes, macrophages or B cells expressing major histocompatability complex (MHC) class II molecules (Fig. 4). This MHC II mediated-recognition of foreign antigens causes T_H cells to become activated, whereupon differentiation occurs into functional subsets termed T helper 1 or (T_H1)-type and T helper 2 or (T_H2)-type cells. Activation of T_H cells is central to cellular immunity and is facilitated through the action of IL-1 and INF-γ secreted by antigen-presenting cells.

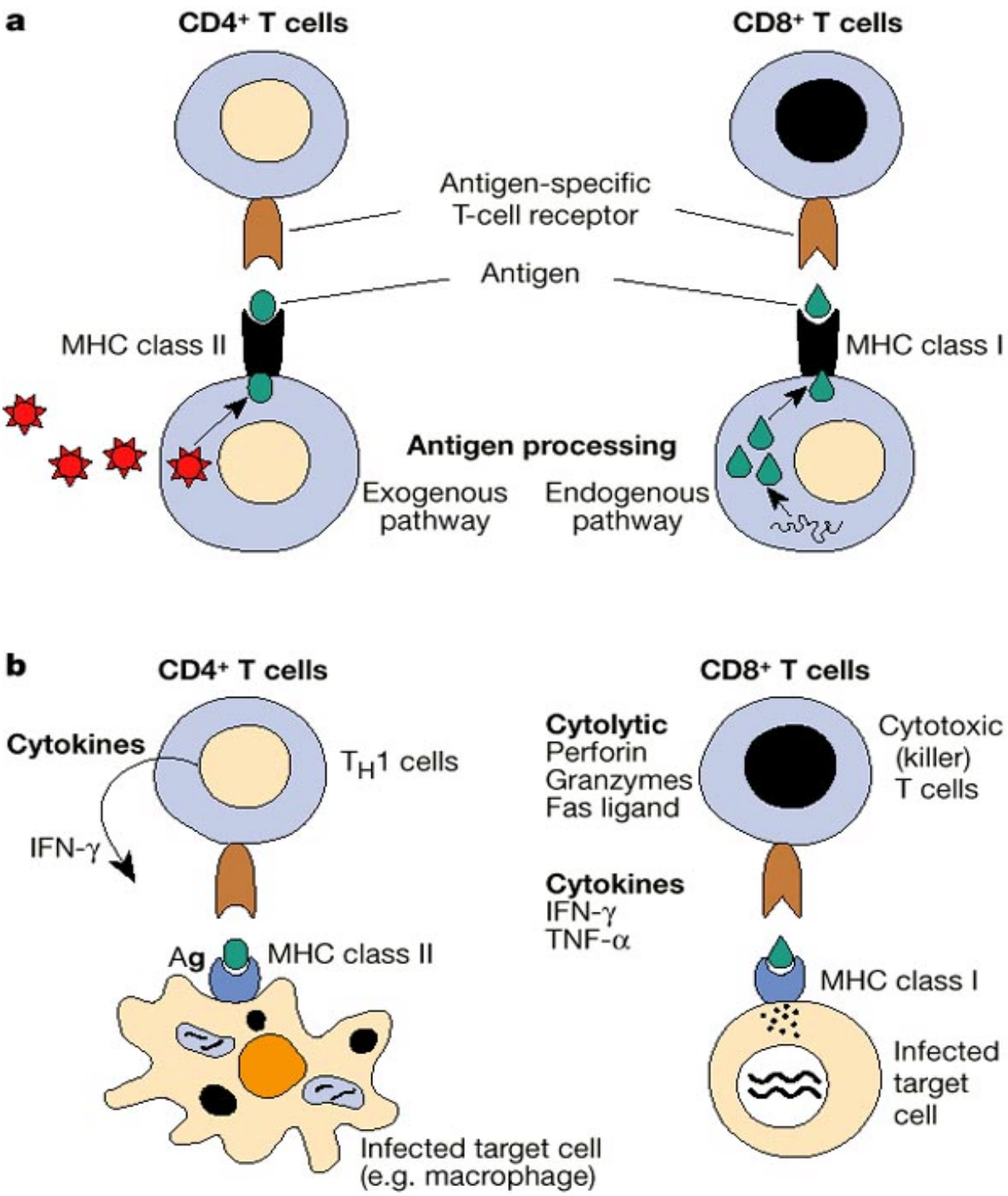

Figure 4. Mechanism of T-cell activation and effector function. **a** *Mechanism of antigen (Ag) processing and recognition by T cells.* ***b*** *Effector function of TH1 and CD8*$^{+}$ *T cells. (Source, Seder and Hill, 2000).*

Cytokines such as INF-γ and certain interleukins (including IL-2, IL-4, IL-5, IL-8, IL-10 and IL-12) assist T_H cells in the activation, proliferation and clonal expansion of effector lymphocytes such as NK cells, T_C cells and B cells. Additional factors produced by antigen-presenting cells, e.g., IL-1 and IL-6 act as co-stimulators of T cell activation.

Cytotoxic T lymphocytes (termed T_C cells) recognise antigens that are processed through an endogenous pathway and presented by infected or transformed cells expressing MHC I class molecules (Fig. 4). T_C cells also mediate their effector function through the production of cytokines such as INF-γ and tumour necrosis factor (TNF)-α and/or through a direct cytotoxic mechanism. The mechanism of cytotoxic killing can be mediated by the release of granule contents such as perforin and granzyme from T_C cells resulting in irreparable pore formation in the cell membrane and apoptosis (i.e., programmed cell death). In addition, T_C cells can destroy cells by a process of Fas-mediated lysis.

Antigen-presenting cells (APCs)

Activation of both humoral (antibody-generating) and cell-mediated (T-lymphocytes) branches of the immune system requires cytokines produced by T_H cells (Kuby, 1997). It is essential that activation of T_H cells be carefully regulated as an inappropriate T_H-cell response to self components can have fatal autoimmune consequences. To ensure carefully regulated activation of T_H cells, they only recognise antigen that is displayed together with class MHC II molecules on the surface of antigen-presenting cells (APCs). These specialised cells, which include macrophages, B lymphocytes, and dendritic cells, are distinguished by two properties: (1) they express class II MHC molecules on their membrane, and (2) they are able to deliver a co-stimulatory signal that is necessary for T_H-cell activation (Kuby, 1997). Dendritic cells are professional antigen-presenting cells that have long membrane processes. They are found in the lymph nodes, and thymus (follicular and interdigitating dendritic cells); skin (Langerhans cells); and other tissues (interstitial dendritic cells) (Kuby, 1997). Indeed, dendritic cell ability to prime naïve CD4+ or T_H cells is a unique and critical function both *in vitro* and *in vivo*.

In the presence of soluble antigen, T_H cells primed by dendritic cells can interact with B cells and stimulate antigen-specific antibody production. Dendritic cells are equally important in priming CD8+ or TC cells. Interestingly, dendritic cells can directly induce cytotoxic T_C cell proliferation with help from T_H cells. It remains to be determined if the unique ability of dendritic cells to prime T lymphocytes results from the expression of unique dendritic cells, or if it results from the high density of molecules involved in dentritic cell (DC)/Tcell interactions. However, a crucial factor for sustaining this DC/T cell interaction is the interaction of co-stimulatory molecules on dendritic cells (CD40, CD83, CD86) and their ligands (i.e., any molecule recognised by a receptor) on the T cells (Young and Steinman, 1990).

Therefore, as antigen-presenting cells (APC) they can also elicit a local rapid reaction or cascade of events that triggers the specific-immune responses. While APCs can be simply described as any cell that alters the immune system to respond to foreign invaders and cancer cells by presenting non-self molecules (or antigens) that are associated with these infected or abnormal cells. Specifically, APCs are any cells that can process and present antigenic peptides (usually foreign) in association with class II MHC molecules (heterodimeric membrane proteins that function in antigen presentation to T_H cells) on the surface of antigen-presenting cells or altered self-cells.

[for references, please see Chapter 6]

Standard antitumour activity test in Sarcoma 180/mice

The most commonly used method to assess the antitumour activity of the mushroom polysaccharides is with the Sarcoma 180/ICR-5 lc mouse model. (at a dose of 1-100 mg/kg body weight). The following method is taken from Mizuno (1999).

"A group of female mice (5-7 of JCB/Jcl strain, 7 weeks old) were used. Sarcoma 180 tumour cells (ca. $5x10^6$) were collected from a mouse on the seventh day after transplantation under the skin on the back of the right groin. Once a day for 10 days, a test sample (1-100 mg/kg) dissolved in a physiological saline solution, was injected intraperitoneally. After transplantation, the size of the tumour (diameter in millimetres or weight in grams) was recorded each week. During the fifth week, the tumour was enucleated and weighed and the inhibitory rate (%) was calculated and compared to that of the untreated group. After 45 days the tumour completely disappeared in some mice. In addition, a 50% inhibitory dose (ID_{50}) was calculated to compare the antitumour efficacies of the test samples. The dose (8 mg/kg) was plotted along the abscessa, and the tumour inhibitory ratio (%) was plotted along the ordinate on semilogarithmic paper in the logarithmic scale of base 10 (log 10) to show the inhibitory ratio (actual value) of 3 to 5 doses of each test sample. From the straight line obtained, a dose of test sample indicating 50% inhibition was determined as ID_{50}. Because the primary screening by intraperitoneal administration was shown to be effective, a secondary screening by the oral administration was performed as follows. Sarcoma 180 tumour cells (2×10^6, or a piece about 2 mm in diameter in the case of a solid cancer) were grafted. The test sample, dissolved in physiological saline solution, was administered orally after 3, 4, 6, 7, 8, 9, 10, 11, 13 and 14 days – 10 times in total. The diameter of the tumour (millimetres) was measured 25 days later to calculate the tumour growth inhibitory ratio (%). After 45 days, the mice showing complete disappearance of the tumour were counted. A reduction in body weight 3 to 15 days after grafting was examined together with the level of toxicity".

TABLE 1 Appraisal judgements in antitumour activity in Sacroma 180/mice by i.p. or p.o. administration.

Inhibition ratio (%)[a] of tumour proliferation	Judgement (symbol)	
0-25	Noneffective	(-)
26-50	Slightly effective	(±)
51-75	Effective	(+)
76-95	Considerably effective	(++)
96-100	Remarkably effective	(+++)

[a] Inhibition ratio (% = 100(1-B/A). A = average tumour size (diameter, cm or cm^3) in control group. B = average tumour size (diameter, cm or cm^3) in treatment group.

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Mizuno, T. 1999. The extraction and development of antitumour-active polysaccharides from medicinal mushrooms in Japan. *International Journal of Medicinal Mushrooms* **1**, 9-29.

Medicinal mushrooms and cancer chemoprevention

While the foregoing antitumour studies on animal models and human clinical trials (Chapters 6 and 7) have used relatively pure extracted polysaccharides it has also been possible to demonstrate antitumour effects when the animal diet has been enriched with either powdered fruit-bodies or liquid concentrates from various edible medicinal mushrooms.

Powdered fruit-bodies of *Lentinus edodes* were supplied orally in the diet (20%) of CDF_1 mice who had been inoculated with synergenic IMC carcinoma cells and C3H/He mice inoculated with MM-46 carcinoma cells (Nanba *et al*., 1987). The MM-46 carcinoma growth was strongly inhibited (79%) whereas the IMC carcinoma growth was much less affected (21% inhibition). A further series of experiments examined macrophage spreading and phagocytosis of latex beads in both normal and tumour-bearing mice fed on a diet supplemented with powdered *L. edodes* (Nanba and Kuroda, 1987). A decreased spreading rate of macrophage in CDF_1 mice but an increase in C_3H mice were observed. However, both the spreading rates and phagocytosis of macrophages in tumour-bearing mice of either strain were significantly depressed when compared to non-tumour bearing animals. Cytotoxicity activity of natural killer cells (NK) and/or lymphokine-activated killer cells (LAK) was significantly increased in mice on feed enhanced with powdered *L. edodes* when compared to mice on normal feed. Pre-treatment of the cells with anti-Thy 12 monoclonal antibodies and complement reduced the cytotoxicity activity by approximately 50% in both mushroom-fed mice and mice on normal feed strongly implying that cytotoxic T cells also participate in the tumour-inhibiting activity of *L. edodes*.

In a similar series of experiments using dietary supplementation (5%) with dried powdered fruit-bodies of either *L. edodes*, *Grifola frondosa* or *Pleurotus ostreatus* it was demonstrated that the incidence of urinary bladder carcinoma in female six-week old ICR mice previously treated with the carcinogen N-butyl-N-butanolnitrosamine (BBN) was decreased in all cases (Kuroshiga *et al*., 1997). While the chemostatic activity of macrophages and the mitogenic response of lymphocytes to conconavalin A were severely suppressed by BBN treatments all of the mushroom-enriched diets restored these activities to almost normal levels. The cytotoxic activity of LAK and NK cells depressed in tumour bearing mice was augmented significantly beyond even the level in non-tumour bearing mice not fed the mushroom supplemented diet.

Hypsizygus marmoreus is a relatively new edible medicinal mushroom now available in the Japanese food market and concentrated extracts have shown a strong inhibition ratio for the solid Sarcoma 180 cancer cells and also for tumour metastasis in mice (Ikekawa *et al*., 1992a; Saitho *et al*., 1997). Studies have shown that when mice were fed on a normal diet plus 5% dried fruit-bodies of *H. marmoreus* they displayed strong cancer prevention ability. Mice were injected with the strong carcinogen, methylcholanthrene and examined over a 76-week period. In the control group 21 of 36 mice developed tumours while only 3 of 36 mice in the mushroom-augmented group developed tumours (Ikekawa *et al*., 1992b; Ikekawa, 2001).

While it has been well noted in previous Chapters that the antitumour effect of medicinal mushrooms is largely due to immunomodulation effects several studies in particular involving animal diets supplemented with mushroom powder or extracts are now also implicating antioxidative activity, e.g. *H. marmoreus* (Matsuzawa *et al*., 1997, 1998), *F. velutipes* (Hiramatsu *et al*., 1989) and *L. edodes* (Kawagishi, 1996).

The antioxidant activity of the medicinal mushrooms has previously been outlined in Chapter 8. Thus while there is an abundance of peer-reviewed scientific evidence confirming that the antitumour effects of extracts of medicinal mushrooms are primarily by immunomodulaiton it would now appear that antioxidative effects are also contributing to the overall antitumour activity.

Epidemiological studies reported in Chapter 10 have highlighted some extremely interesting but as yet limited observations on cancer chemoprevention in humans through consumption of certain medicinal mushrooms. Whether regular and prolonged consumption of medicinal mushroom concentrates as dietary supplements could serve as an important approach for cancer chemoprevention in man must be subjected to critical controlled study.

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