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"*

References

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