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Takashi Mizuno (1931-2000)
Professor Emeritus of Shizuoka University

This article is written in memory of Professor Takashi Mizuno who died in May 2000. He had focused a lifetime of research upon development of various antitumor substances from medicinal mushrooms, and is considered one of the 20th century greatest scientist in his field. Mizuno proved that many polysaccharides with antitumor and immunopotentiating qualities were synthesised in cultured mycelia no less and in fact often better than in fruiting bodies. This result virtually revolutionised mushroom producing and processing business.

"Medicine and food both originate from the same root" is a Japanese proverb Mizuno often quoted.

Minireview

Anti-cancer effect of polysaccharides isolated from higher basidiomycetes mushrooms

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Anti-tumor activity of mushroom fruit bodies and mycelial extracts evaluated using different cancer cell lines. These polysaccharide extracts showed potent antitumor activity against sarcoma 180, mammary adenocarcinoma 755, leukemia L-1210 and a host of other tumors. The antitumor activity was mainly due to indirect host mediated immunotherapeutic effect. These studies are still in progress in many laboratories and the role of the polysaccharides as immunopotentiators is especially under intense debate. The purpose of the present review is to summarize the available information in this area and to indicate the present status of the research.

Key words: Mushroom, polysaccharide, antitumor, lentinan, schizophyllan, maitake, apoptosis, and metastasis.

INTRODUCTION

The global awareness of cancer as the second largest cause of death in people of various ages and racial background has lead to so much research efforts and clinical studies in the fight against the disease. The prevention of cancer by the ingestion of chemical agents aimed at minimizing the risk of carcinogenesis has greatly reduced the morbidity and mortality rates. These agents include non-steroid anti-inflammatory drugs (NSAID) such as aspirin, sulindac, piroxican and indomethacin, which inhibit cyclooxygenase (COX). Cyclooxygenase inhibition is necessary because it catalyzes the conversion of arachidonic acid to pro-inflammatory substances such as prostaglandin, which can stimulate growth of tumor cells and suppress immune surveillance. It also activates carcinogens to take up forms that damage the genetic material ((Jang et al., 1997; Wasser and Weis, 1999).

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The curious question, which everyone asks, is " what really causes cancer." The immediate response from most people will be tobacco smoking, alcohol consumption, excess use of caffeine and other drugs, sunshine, infection from such oncogenic virus like cervical papillomaviruses, adenoviruses Karposis sarcoma (HSV) or exposure to asbestos. These obviously are implicated as causal agents of mammalian cancers. However a large population of people are often exposed to these agents with only very few being affected which tell us that these may not really be the root cause of cancer.

Gibbs (2003) explained that the immediate cause of cancer must be some combination of insults and accidents that induce normal cells in a healthy human body to turn malignant, growing uncontrollably in unnatural places. The elucidation of DNA as the genetic material ushered in the era of molecular genetics and cancer is now seen as a result of cumulative mutations that alter specific locations in a cell's DNA and thus change the particular protein encoded by cancer–related genes at those locations. These mutations affect two kinds of cancer genes namely: tumor suppressors and oncogenes. Tumor suppressors normally restrain the cell's ability to divide and mutations on this gene render it inactive. Oncogenes on the other hand stimulate cell growth and cell division. Mutations on the oncogenes make them permanently active thus promoting cell growth and progressive changes leading to the increase of the cancer genes (Gibbs, 2003). Consequently cancer cells continue to divide even in situations in which normal cells will usually wait for a special chemical transduction signal. The tumor cells would ignore such stop signals that are sent out by adjacent tissues. Cancer cells also have the character of immortality even *in vitro* whereas normal cells stop dividing after 50-70 generations and undergoes a programmed cell death (Apoptosis). Cancer cells continue to grow invading nearby tissues and metastasizing to distant parts of the body. Metastasis is the most lethal aspect of carcinogenesis. The phenomena is such that before a tumor is discovered and removed by surgery a few cells of the tumor would detach from the initial mass, float through the circulation system and start a new colony in a different organ from the one that gave birth to it.

The theory that cancer results from cumulative mutations that alter specific genes in the DNA has come under great challenge recently. Biologists now trace the cause of tumor to include other abnormalities at work inside the cell nucleus that though not yet malignant is prone to become so. Recent hypotheses advanced to challenge the earlier one include the hypothesis that a breakdown in DNA duplication or repair leads to many thousands of random mutations in cells. Secondly that damage to a few "master" genes mangles the chromosomes, which then becomes dangerous, plunging the cells into a ploidal confusion and chaos. Finally that the abnormal number of chromosomes in a cell may be the milestone on the road to cancer (Gibbs, 2003). However, there is great expectation that one day science will produce a definitive answer to the root cause of cancer. It may probably be a very complicated answer or a simple one, which may force us to shift our hope from curative drugs to chemopreventives.

It has been difficult to develop specific remedies against cancer as with the development of vaccine against viral infections and antibiotics. Cancer cells originate from normal cells and a novel drug able to select and destroy only affected cells preventing carcinogenesis without injury to normal cell would be an ideal chemotherapy against cancer. This is a very difficult task facing cancer researchers. Recent investigations has been channeled on the development of immunotherapy to target and remove cancer cells as well as on substances such as immunopotentiators, immunoinitiators and biological response modulators (BRM) that act to prevent carcinogenesis and induce carcinostasis (Wasser and Weis, 1999).

Mushrooms belong to this group of immunoceuticals by their mode of action. The use of medicinal mushrooms in the fight against cancer is known for a very long time in Korea, China, Japan, Russia, USA and Canada. An old Japanese legend reports that wild monkeys rarely experience cancer, high blood pressure, or diabetes. The legend suggests that perhaps it is due to some extent their consumption of wild mushrooms. This legend in Japan may have help spur research into the role of mushroom in medicine. These mushrooms belonging to the family Polyporaceae have been effective against esophageal, stomach, prostate and lung cancers. Lucas et al. (1957) demonstrated the anti-tumor effect of higher basidiomyces (specifically extracts of fruiting bodies of *Boletus edulis*). Yohida et al. in 1962 isolated from *Lampteromyces japonicus* (kowamura) Sing, an agent active against Ehrlich carcinoma of the mouse. Gregory (1966) experimented on more than 7000 cultures of higher basidiomyces for anti-tumor activity against rodent tumor systems. Positive inhibitory effects were obtained using fermentation media materials against sarcoma 180, mammary adenocarcinoma 755, and leukemia L-1210. Ikegawa et al. (1968) reported that an essence obtained from the fruit body of edible mushrooms exhibited remarkable host-mediatory anti-tumor activity against grafted cancer in animals such as sarcoma 180. Daba (1998) and Daba et al. (2002) reported that *Pleurotus ostreatus* mushrooms cultivated on date waste posses a potent antitumor activity against Ehrlich ascites carcinoma. Further biochemical studies were carried out by the authors on the effect of mushrooms and isolated polysaccharides on the tumors transplanted in mice. The anti-tumor essence was later discovered to be a type of β-D-glucan, a polysaccharide yielding D-glucose only by acid hydrolysis (Mizuno, 1999). β-D-glucan has a different mode of action from the conventional

chemotherapeutic agents in that it is immunotherapeutic. Inhibiting the growth of cancer cells by activating and reinforcing the host immune system. This present review elucidates the anti-tumor polysaccharides from mushrooms, their chemical structures, results obtained from experimental and clinical trails, and their mode of action.

COMPOSITION OF MUSHROOM ANTITUMOR POLYSACCHARIDES

Polysaccharides are polymers of sugars (monosaccharides) joined to each other by glycosidic linkages. These are very complex molecules because sometimes covalent bonds occur between many pairs of carbon atoms. Consequently one sugar unit can be joined to more than two other sugars, which results in the formation of highly branched enormous macromolecules. Polysaccharides are a structurally diverse class of macromolecules able to offer the highest capacity for carrying biological information due to a high potential for structural variability (Wasser, 2002). Whereas the nucleotides and amino acids in nucleic acids and proteins effectively, interconnect in only one way, the monosaccharide units in polysaccharides can interconnect at several points to form a wide variety of branched or linear structures (Sharon and Lis, 1993). This high potential for structural variability in polysaccharides gives the necessary flexibility to the precise regulatory mechanisms of various cell-cell interactions in higher organisms. The polysaccharides of mushrooms occur mostly as glucans. Some of which are linked by β-(1-3), (1-6) glycosidic bonds and α-(1-3) glycosidic bonds but many are true heteroglycans.

Most often there is a main chain, which is either β(1-3), β(1-4) or mixed β(1-3), β(1-4) with β(1-6) side chains. Hetero-β-D-glucans, which are linear polymers of glucose with other D-monosaccharides, can have anticancer activity but α-D-glucans from mushroom usually lack anticancer activity (Wasser, 2002). Heteroglucan side chains contain glucuronic acid, galactose, mannose, arabinose or xylose as a main component or in different combinations. Glycans are polysaccharides containing units other than glucose in their backbone. A wide range of antitumor or immunostimulating polysaccharides of different chemical structure from higher Basidiomyces mushrooms has been investigated (Wasser, 2002). Some correlation has been drawn between the chemical structure and antitumor activities of mushroom polysaccharides. A wide range of glycans extending from homopolymers to highly complex heteropolymers (Ooi and Liu, 1999) exhibits antitumor activity. Differences in activity can all be correlated with ability of the polysaccharide molecule to solubilize in water, size of the molecules, branching rate and form. Such structural features as β-(1-3) linkages in the backbone (main chain) of the glucan and additional β-(1-6)-branch points are needed for antitumor activity (Wasser, 2002). β-glucans with only (1-6) glycosidic linkages have little or no activity. Higher molecular weight glucans have been reported by Mizuno et al. (1996) and Mizuno (1999) to be more effective than those of low molecular weight against tumors.

There is a broad similarity in the various methods that have been developed for extraction of anti-cancer polysaccharides from mushrooms. Usually dried mushroom in powdered form is heated in 80% ethanol to eliminate low molecular weight substances. Crude fractions are obtained from the remaining ethanol extracts by further extraction with water, 1% ammonium oxalate and 5% sodium hydroxide. The polysaccharides are then fractionally purified by a combination of techniques, including ethanol concentration, fractional precipitation, ion-exchange chromatography, gel filtration and affinity chromatography. A recent study by Yap and Ng (2001) has established a more efficient procedure for the extraction of β-glucan from mushroom. The β-glucan is isolated through ethanol precipitation and freeze-dried in liquid nitrogen. Purity testing using a carbohydrate analysis column produced 87.65% purity.

MUSHROOMS POLYSACCHARIDES IN THE TREATMENT OF CANCER

Immunoceuticals are substances having immunotherapeutic efficacy when administered orally. More than 50 mushrooms species have yielded potential immunoceuticals that exhibit anticancer activity invitro or in animal models. Six of these polysaccharides that have been investigated in human cancers include Lentinan, Schizophyllan, Active hexose correlated compounds (AHCC), Maitake D-fraction, polysaccharide-K and Polysaccharide-P.

Lentinan

Lentinan, produced from Shiitake mushroom, *Lentinus edodes,* is a β(1-3), β(1-6) glucan. There is an immense literature related to the anticancer effect of lentinan on animals and human carcinomas. It was first isolated and studied by Chihara et al. (1970) who demonstrated that its antitumor effects were greater than other mushroom polysaccharides. Maeda et al. (1974) however reported that lentinan was active for some but not all types of tumors. There have been numerous clinical trials of lentinan in Japan, and the drug is now manufactured and sold by several pharmaceutical companies.

Lentinan has proved successful in prolonging the overall survival of cancer patients, especially those with gastric and colorectal carcinomas (Furue et al., 1981 Taguchi et al., 1985 a,b). In patients with recurrent gastric

cancer, tumor responses and prolonged median survival were also noted. In a randomized controlled study of patients treated with tegafur or a combination of lentinan and tegafur overall survival was significantly prolonged in the lentinan plus tegafur group. 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 tumours, patients treated with lentinan had a significantly higher response rate (14.9% than patients in the control arm.

Few adverse reactions to lentinan have been noted. In a detailed study of 469 patients, 32 (6.8%) experienced an adverse reaction, only 2 patients required discontinuation of treatment due to unacceptable tolerance. Perhaps the most intriguing aspects of lentinan use in conjunction with other chemotherapeutic agents is its apparent ability to greatly reduce the debilitating effects of the chemotherapy, e.g. nausea, pain, hair loss and lowered immune status.

Schizophyllan

This polysaccharide derived from the mushroom *Schizophyllum commune* has been shown to be cytostatic in sarcoma 180 tumor xenographs. The survival of these xenographs was not affected by pretreatment with schizophyllan. Schizophyllan had no effect on the survival of sarcoma 37, Ehrlich carcinoma or Yoshida sarcoma ascites tumors (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 randomized controlled study of 367 patients with recurrent and inoperable gastric cancer resulted in a significant increase in median survival (Furne, 1985). However, a similar study was unable to confirm this apparent success with schizophyllan (Fuginoto 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 randomized controlled study of schizophyllan in combination with radiotherapy, schizophyllan significantly prolonged the overall survival of stage II cervical cancer patients but not stage III (Okamura et al., 1986, 1989). In a prospective, randomized clinical trial involving 312 patients treated with surgery, radiotherapy, chemotherapy (fluorouracil) and schizophyllan in various combinations, patients treated with schizophyllan had a better overall survival than patients who had not received the polysaccharides (Miyazaki et al., 1995). Schizophyllan is currently produced commercially by several Japanese pharmaceutical companies.

Active Hexose correlated compound (AHCC)

AHCC is a proprietary extract prepared from co-cultured mycelia of several species of basidiomycete mushrooms, including Shiitake *(Lentinus edodes).* Animal research and preliminary human studies indicated AHCC has anticancer efficacy. Beginning in 1992, Kamiyama conducted a trial in Japan to evaluate the preventive effect of AHCC against recurrence of hepatocellular carcinoma following surgical resection. The investigators reported that after one year the AHCC group showed a significant higher survival rate than the control group, as well as significant lowering of certain tumor markers in the serum. The AHCC Research Association was formed in Japan in 1996 to foster the development of AHCC as an anticancer therapy. In their circulating abstracts they report that of 300 cancer patients administered AHCC, 58 were effectively treated, with 46 showing complete or partial regression and 12 experiencing no change of tumor size.

Maitake extracts

Several studies have shown that B-D-glucan derived from *Grifola frondosa* (also known as Maitake) have strong antitumor 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 (B-1,6 glucan branched with a β 1-3-linkage) (Grifron-D, GD) has become available. GD has considerable immunomodulating and antitumor activities in animal models, and is orally bioavailable (Nishida et al., 1988). Maitake D-fraction and crude Maitake powder have demonstrated remarkable inhibition of metastasis in a mouse model, especially in the prevention of hepatic metastases, which in one series of experiments was reduced by 81% (Maitake powder) to 91% (D-fraction) (Nanba, 1995). GD has been shown to have a cytotoxic affect on human prostate cancer cells (PC9) in vitro, possibly acting through oxidative stress, and causing 95% cell death by an apoptosis (Fullerton et al., 2000). Vitamin C addition reduced the effective level of GD required.

Jones (1998) reported an early pilot study from China involving 63 cancer patients which showed a response rate (partial and complete) against solid tumour at 95% and for leukaemia 90%. Nanba (1997) observed tumour regression or significant symptomatic improvement in 11 out of 15 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 cancer types were combined. The Food and Drug Administrarion (USA) has approved Grifon-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).

Development of carcinostatic drugs

Three polysaccharide based carcinostatic (immunotherap eutic) agents, Krestin, Lentinan and Sonifilan, have already been developed from mushroom. These are used currently in the treatment of cancer of the digestive organs, lung and breast, as well as cancer of the stomach and cervical cancer respectively. Several mushroom species belonging to the polyporaceae family are now regarded as the next drug producers. Mushroom polysaccharides are also expected to be developed into multipurpose medicines that are not only carcinostatic but also anti-inflammatory, antiviral (against AIDS), hypoglycaemic and antithrombotic.

MECHANISM OF ACTION OF MUSHROOM POLYSACCHARIDES

The Egyptians as far back as 3000 BC believed that mushroom were a sacred food that prolonged life. A mummified 5000-year old “Ice-man” found in the mountains of Europe carried a medicine kit of dried mushrooms. Indeed the oldest written record of mushrooms as medicines is in Indian medical treatise from 3000BC (Kual, 1997). Of significant relevance and importance is the ability of particular mushrooms-derived compounds to modulate the human immune response and to inhibit certain tumour growths (Wesser and Weis, 1999a, 1999b). Medicinal mushroom research has focused on discovery of 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 disease or for generalised immunosuppression following drug treatment. They are also sought for combination therapy with antibiotics and as adjuncts for vaccines (Jang et al., 1997). Wasser (2002) reported that mushroom polysaccharides are regarded as biological response modifiers (BRM). This basically means that they cause no harm and place no additional stress on the body, but help the body to adapt to various environmental and biological stresses. Mushroom polysaccharides support some or all of the major systems of the body, including nervous, hormonal and immune systems as well as regulatory functions. The polysaccharides from mushrooms do not attack cancer cells directly, but produce their antitumour effects by activating different immune response in the host (Wasser, 2002).

Hobbs (2000) reported that *L. edodes* produces two bioactive preparations, which are efficient immune modulators, mycelium extract and Lentinan. These two bioactive polymers appear to act as host defence potentiators restoring and enhancing the responsiveness of host cells to lymphocytokines, hormone and other biologically active substances. The immunopotentiation has been shown to occur by stimulating the maturation, differentiation or proliferation of cells involved in host defence mechanism. Chihara et al. (1998, 1992) reported that Lentinan increase host’s resistance against various kinds of cancer and has the potential to restore the immune function of affected subjects. Many of these pathways stimulated by Lentinan are illustrated by Chihara et al. (1999) (Figure 1). The interaction of Lentinan with many kinds of immune cells was not known until recently. Ross et al. (1999) provided an insight into receptor binding in immune cells by β-glucan from fungi and further showed that β-glucan from yeast bind to iC3b-receptors (CR3, CD11b/CD18) of phagocytic and natural killer (NK) cells. When this happens, it will stimulate phagocytosis and/or cytotoxic degranulation. Lentinan has also 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. This observation was made using the blood of healthy donors and cancer patients. 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 including 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).

CONCLUSION

Mushroom polysaccharides offer a lot of hope for cancer patients and sufferers of many devastating diseases. A fundamental principle in oriental medicine is to regulate homeostasis of the whole body and to bring the disease person to his or her normal state (Chihara et al., 1992). Potentiation of the physiological constitution in favour of host defence results in the activation of many vitally important cells for the maintenance of homeostasis. A variety of polysaccharides from a number of mushroom varieties have been demonstrated to enhance the immune system. All of these have shown significant antitumour activity as a result of their ability to activate the host immune system rather than direct cytotoxicity. The mushroom polysaccharides appear to be well tolerated and compatible with chemotherapy and radiation therapy. However, studies that identifies the molecular mechanisms that occur in specific immune

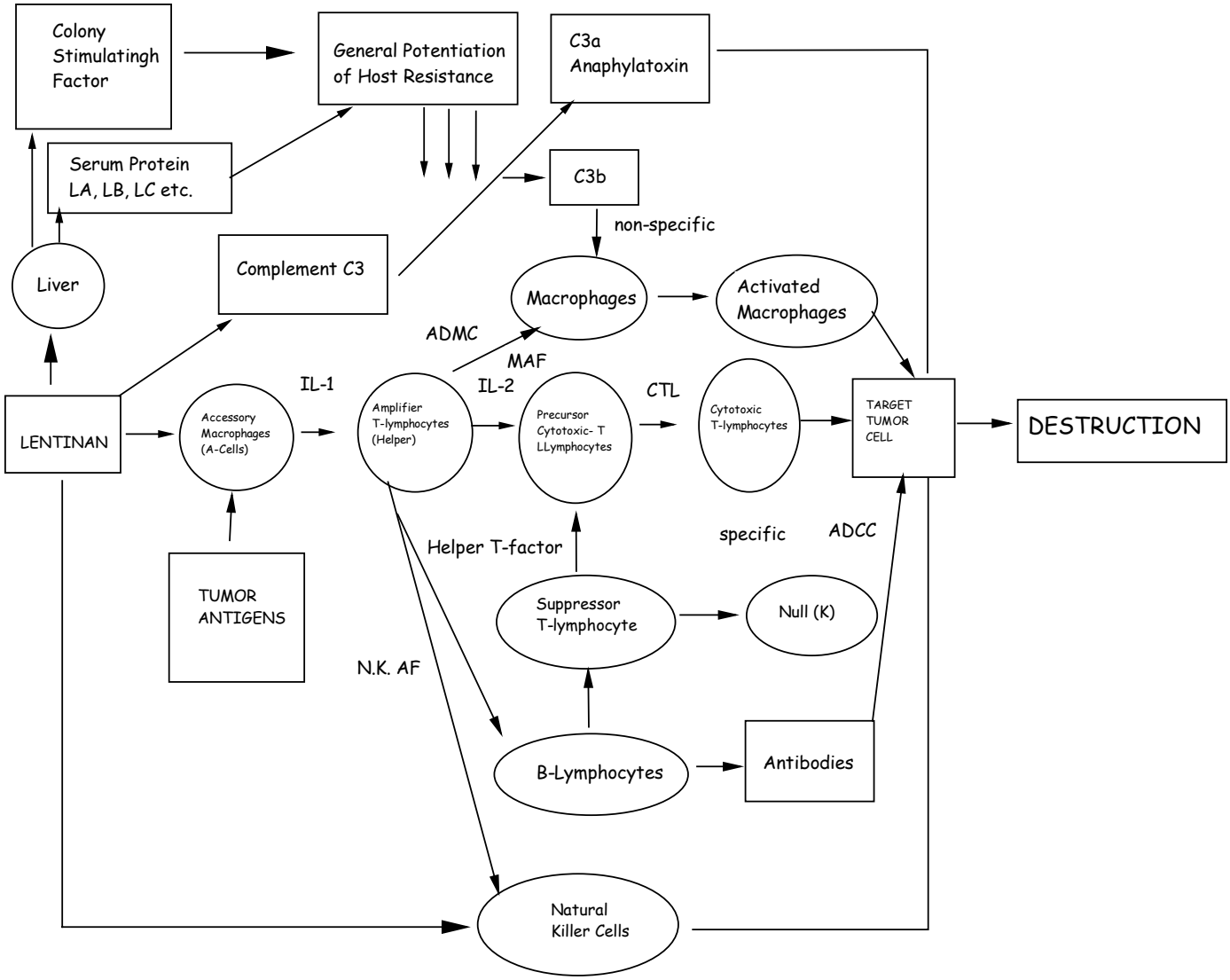

Figure 1. Host immune response involved in Lentinan-mediated destruction of cancer cells (Chihara et al., 1992).

modulation by mushroom polysaccharides such as receptors and what downstream events are triggered by the binding of these polymers to their target cells are urgently needed.

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