1. Every clinical study on the medicinal mushroom “Agaricus Blazei Murill” (ABM) that you can find.

## Results of Research

**TITLE:** Tumor-specific cytocidal and immunopotentiating effects of relatively low molecular weight products derived from the basidiomycete, Agaricus blazei Murill.

**AUTHORS:** Fujimiya Y; Suzuki Y; Katakura R; Ebina T

**AUTHOR AFFILIATION:** Division of Immunology, Miyagi Cancer Center Research Institute, Natori, Japan.

**SOURCE:** Anticancer Res 1999 Jan-Feb;19(1A):113-8

**CITATION IDS:** PMID: 10226531 UI: 99243118

**ABSTRACT:** Currently, some natural herbal extracts are believed to have a marked tumoricidal effect and low toxicity for normal tissues. We investigated the effect of relatively low molecular weight products extracted from the basidiomycete, Agaricus blazei Murill, on MethA tumor cell growth with the aim of producing synthetic derivatives based on these products. Inoculation of the low molecule fraction (LM) into the primary tumor of a two-tumor model resulted in the marked inhibition of the tumor, not only in the right flank, but also in the non-injected left flank. Chromatographic purification and physicochemical characterization showed the main tumoricidal activity to be located in a low molecule fraction-3 (LM-3), containing alpha-1,4-glucan-beta-1,6-glucan complex with an average molecular weight of 20 kDa. A11 LM fractions and crude ATF showed in vitro selective cytotoxicity for MethA tumor cells, having no effect on normal cells. Serum levels of immunosuppressive acidic protein (IAP) in mice receiving LM fractions, particularly LM-3, significantly increased, indicating the possible activation of granulocytes. We speculate that the inhibition of the distant tumor might be due to the increased migration of granulocytes, enhanced by the effect of extract injections at the primary tumor site.

**MAIN MESH HEADINGS:** Adjuvants, Immunologic/pharmacology

**ADDITIONAL MESH HEADINGS:** Animal

**PUBLICATION TYPES:** JOURNAL ARTICLE

**CAS REGISTRY:** 0 (Adjuvants, Immunologic)
Studies in the Japan further showed that:

- Agaricus mushroom can be used effectively with orthodox anticancer treatments including chemotherapy and radiation and that there is a synergistic effect between the ABM and the orthodox treatments.
- Agaricus mushroom can reduce some of the side-effects of chemotherapy and radiation.
- Agaricus mushroom can be effective in the prevention of cancer.
- Agaricus mushroom is effective against menstrual pain.
- Agaricus mushroom can be effective against arthritis, rheumatism and gout.
- Agaricus Blazei has a positive therapeutic effect on chronic hepatitis.
- Agaricus mushroom can be effective in the control of various allergies and autoimmune diseases like rheumatoid arthritis, dermatitis, diabetes, lupus, multiple sclerosis and fibromyalgia.
- Agaricus mushroom can reduce blood sugar and can be effective in fighting diabetes.
- Agaricus mushroom can be effective in controlling blood pressure (high and low blood pressure).
- Agaricus mushroom can lower cholesterol levels and can ease arteriosclerosis.
- Agaricus mushroom can be effective in the control of migraine headaches.

http://agaricus.net/research/

Research with Agaricus blazei Muril
J Nutr 2001 May;131(5):1409-13
Isolation of an antitumor compound
The Basidiomycete fungus Agaricus blazei Murill has traditionally been used as a health food for the prevention of cancer, diabetes, hyperlipidemia, arteriosclerosis and chronic hepatitis.

In the present study, we examined the antitumor activities of various substances isolated from the lipid fraction of A. blazei. Tumor growth was retarded by the oral administration of the lipid fraction extracted from A. blazei with a chloroform/methanol mixture in sarcoma 180-bearing mice.

The substance with the antitumor activity in the lipid fraction was isolated via silica gel column chromatography, eluted with an acetonitrile/methanol (3:2) mixture and identified as ergosterol by direct comparison of the (1)H NMR and mass spectrometry spectral data of an authentic sample.

The oral administration of ergosterol to sarcoma 180-bearing mice significantly reduced tumor growth at doses of 400 and 800 mg/kg administered for 20 d without side effects, such as the decreases in body, epididymal adipose tissue, thymus, and spleen weights and leukocyte numbers induced by cancer chemotherapy drugs.

Ergosterol had no cytotoxicity against tumor cells. To clarify the antitumor activity of ergosterol, we examined the effects of ergosterol on tumor-induced angiogenesis using two in vivo models. Intraperitoneal administration of ergosterol at doses of 5, 10 and 20 mg/kg for 5 consecutive d inhibited the neovascularization induced by Lewis lung carcinoma cell-packed chambers, suggesting that either ergosterol or its metabolites may be involved in the inhibition of tumor-induced neovascularization.

Therefore, we further examined the inhibitory effects of ergosterol on Matrigel-induced neovascularization. Female C57BL/6 mice were subcutaneously inoculated with Matrigel containing acidic fibroblast growth factor and heparin with or without ergosterol. Ergosterol inhibited the Matrigel-induced neovascularization, suggesting that ergosterol directly inhibits Matrigel-induced neovascularization.

From these results, it seems likely that the antitumor activity of ergosterol might be due to direct inhibition of angiogenesis induced by solid tumors. This is the first report of ergosterol as an antiangiogenic substance.

http://www.alternative-health-4u.com/agaricus.html
Agaricus Blazei Murill (ABM - though some have suggested that the correct species name for this new species is, Agaricus brasiliensis) is another botanical that has remarkable properties, some of which have been excessively touted or exaggerated by promoters, others which have been overlooked entirely. Known as "Cogmelo de Deus" ("Mushroom of God"), ABM is a regular part of the diet of the indigenous peoples outside San Paulo, Brazil, where researchers found very low incidence of a variety of adult illnesses. They believe that ADM is a contributing factor.

It is well-established that various mushrooms have important immunological effects - and their effects have been known for centuries. The ABM mushroom was tested at the Tokyo University National Center Center Laboratory and the Tokyo College of Pharmacy. Compared to the other commonly used mushrooms - including maitake, reishi, and shiitake - ABM exhibited far superior Immune Enhancing Effect. Compositional studies attribute this effect to the much higher levels of key polysaccharides and other components unique to ABM.

Again, we are careful to distance ourselves from those who would label ABM a "cancer cure." Other than a handful of positive claims from leukemia patients, we have not witnessed a stampede of mushroom users who claim that Agaricus has cured their cancer. On the other hand, this is another "herbal" that orthodoxy "pooh-poohs" out of hand, with no real appreciation for its medicinal history, tradition, and the protocols for its use for which there is substantial evidence of healing ability. (Dr. Alexander Yuan in Hong Kong has produced a very good summary of its history and usage. Read Part I, and Part II - both are sizeable Adobe Acrobat files and can a good while to download, so be patient.)

Agaricus (ABM): Known Medicinal Effects

**Anti tumor effect:** ABM contains high level of effective elements such as polysaccarides, both Beta(1-3)D-glucan & Beta(1-6)D-glucan protein compounds, ribonucleic acid protein compounds, acid heteroglucan, xyloglucan, lectin, etc. When these elements are introduced into our white blood cells, it enhances the activity of macrophage, an antibody cell that destroys or delays the proliferation of cancer cells.

**Anti cancer effect:** ABM contains natural steroids, known for it's anti cancer effect. (It is different from the chemically produced steroids that enhance the body that is often said to be the cause of cancer). It is particularly effective in prevention of uteran cancer.

**Preventative effect:** ABM contains large amounts of non digestive dietary fibers that absorb cancerous materials in our body and discharge away from our system.

**Blood glucose reducing effect:** Effective elements mentioned above such as Beta(1-3)D-glucan & Beta(1-6)D-glucan protein compounds, ribonucleic acid protein compounds also has positive effects to reduce the blood glucose(sugar).

**Blood pressure, Cholesterol & Arteriosclerosis reducing effect:** The above mentioned dietary fiber and unsaturated fatty acid, such as linolin, contained in ABM,
have effects to reduce blood pressure, cholesterol and arteriosclerosis.

**Vitamin D2 effect:** ABM contains vitamins B1 and B2 but also contains large amounts of ergosterol, which would be converted to vitamin D2 when it is dried in the sun or heated in a mechanical drying process. Vitamin D2 has positive effects to lessen the danger of osteoclasis. [Top of Page]

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**Agaricus (ABM): General Info**

*This section written by Osmar Borzacchini and found in several places on the net -- provided for background reading only*

ABM originates from Piedade, located in the suburbs of Sao Paulo, Brazil. The climatic conditions in Piedade include temperatures that soar to 35-38 degrees C. during the day and dip to between 20-25 C. at night, with the humidity that averages 80%. The place also experiences regular squall toward evening. ABM thrives only under these conditions, suggesting that its survival is significantly affected by these climatic conditions.

Around the same time, ABM was introduced to Japan. Dr. Shoji Shibata, who at the time was a professor in the pharmacological Department of Tokyo University, and Dr. Tetuo Ikegawa of the National Cancer Center, jointly researched the pharmacological effects of 4 Agaricus. The research results were released at the general convention of the Japan Pharmacological Association and the Japan Cancer Association. The experience with mice verified that the polysaccharide beta-D-glucan contained in 4 Agaricus significantly activated the immune system.

The polysaccharide contained in ABM vitalizes production of interferon and interleukin in small animals (guinea pigs). This effect indirectly functions to destroy or prevent the proliferation of cancer cells. This refers to a cytokine inducing effect. Moreover, the experiments conducted by the researchers named above proved that this effect can also prevent viruses and other external factors from entering the tissue.

Clinical results obtained in collaboration with university researchers and hospital since the report on the anti-cancer effect of ABM was released at the general convention of the Japan Cancer Association in 1980 proved that although many fungi polysaccharide only effect solid cancer and polysaccharide of ABM is effective against Ehrlich's ascites carcinoma sigmoid colonic cancer, ovarian cancer, breast cancer, lung cancer, and liver cancer as well as against solid cancer.

This trial showed ABM to be 80% more effective than the world's number one cancer drug PSK, an extract of Coriolus Versicolor. ABM was found to contain far higher levels of beta glucans than Maitake, Shiitake, and Reishi. The renown Dr. M Ghoneun (MGN-3) developed studies about the NK cells activities and presented them at the International Congress on Immunology held in San Francisco. One theme was the explanation of ABM's power in intensifying the NK cells activities and it's importance in immunological recording and in cancer treatments. He also related a study with ABM on the effect in 57 mice with active NK cells. "IT INCURRED A DEFENSE RATE UP TO 9 TIMES IN ABM INJECTED MICE". At present ABM is considered the most potent mushroom in supporting the immune system and in controlling various diseases such as
Cancer and Aids. So successful are the studies in Japan that they are now buying over 90% of the available Agaricus from Brazil.

The results of the experiments suggest that it also activates metabolism by revitalizing normal biological tissue. Digestive enzymes such as amylase trypsin martase and the protease contained in fungus also enhances digestion. Furthermore, tyrosinase, an enzyme which oxidizes tyrosine and produces melanin, has a hypotensive effect.

Living organisms are equipped with immunity system which expels pathogens, toxic chemicals and tumorous cells generated through mutation. When tumorous cells attach to form a simple protein lump, it is Decomposed by microorganisms such as bacteria. Thanks to this function, which is called immunity, bodies can maintain their health by fighting off harmful microorganisms, stopping them from entering tissue or discharging them from tissue.

The human immune system is comprised of more than 130 subsets of white blood cells. About 15% of them are called Natural Killer (NK) cells, These provide the first line of defense for dealing with any form of invasion to the body. Each cell contains several small granules which act as ‘ammunition.’ When an NI cell recognizes a cancer cell, for instance, it attaches itself to the cells outer membrane and inject these granules directly into the interior of the cell.

More specifically, natural killer cells or NK cells play a critical role neutralizing outside organisms such as viruses and bacteria that enter the body. Through a process called phagocytosis, the NK cells “kiss” and engulf foreign agents and release toxic chemicals that destroy them.

In cancer patients, the NK cells and cancer cells constantly baffle one another. The NK cells attempt to destroy cancer cells while at the same time cancer cells try to neutralize NK cells before they can harm them. If the NK cells are weak or if there are not enough NK cells in the body, the cancer spreads throughout the body resulting in death. Several factors contribute to this weakening of the immune system: most notably stress, age, pollution, and smoking.

When their immune systems work properly, humans remain healthy. However, the immune systems in many people are weakened by stress, poor eating habits and pollution, such as air pollution. These people can suffer from a number of diseases.

It is widely believed that cancer is caused by a decline in the immune system caused by aging and other factors. It is also known that atopic dermatitis asthma, pollinosis and rheumatism result from an excessive immuno reaction. The AIDS virus destroys immune cells and triggers diseases.

ABM, which is enjoying growing attention, contains a large amount of polysaccharide, which is believed to enhance immunity. It does not only bolster immunity but reduces excessive immuno reactions to maintain a balance. Of all fungi, ABM Mushroom is particularly rich in polysaccharide, and has shown particularly strong results in treating and preventing cancer. We believe that ABM is the ideal food for the people of today, who are exposed to a difficult living environment.  

The story of the life-enhancing ABM by Osmar Borzacchini

The ABM mushroom was discovered by Takatoshi Furumoto in the district of
Piedade near São Paulo in Brazil. The scientist discovered that very few people suffered from geriatric illnesses in this community...

Furumoto's research revealed that the healthy state of the people of Piedade was due to their frequently eating the ABM mushroom. In order to examine the mushroom species and establish its effect on cancer, samples were sent to the University of Buenos Aires and the University of the Province of Mie in Japan. Finally, Dr. Shobo Shibata, who was then working at the Department of Pharmacology as a professor, and Dr. Tetsuo Ikegawa of Japan's National Cancer Research Centre managed to detect the pharmacological action of ABM. That was about 30 years ago. Their research findings were published on the occasion of the General Assembly of the Japanese Pharmacology Association and the Japanese Cancer Research Society.

ABM works like an "abridged version of the life cycle". Setting out from the assumption that, in nature, plants behave like producers and animals like consumers, scientists found that, in the case of this mushroom, it turns the animal and plant remains into the basic substances from which it makes new organic matter.

Currently, mushrooms like ABM are a focal point of attention because they are regarded as the new health boosters. Oriental medicine has used various species of mushroom for over a thousand years. Chinese medicine, which has now adopted some western methods of treatment, has developed processes for extracting the active ingredients from the mushroom. These substances are used as immunological activators, since the polysacharide β-glucan that is contained in ABM enhances the immune system.

Characteristics of ABM

The ABM mushroom grows in temperate regions with ambient temperatures ranging from 23° to 28°C. On account of the species having been discovered in the Piedade region, it is now referred to world-wide as the "Piedade mushroom". In some regions it also goes by the term "Royal Agaricus", while in Japan it is called "himmematsutake".

Depending on where they are grown, the ABM mushrooms attain a height of ten to 15 centimetres. Unlike other species, this mushroom does not thrive in the shade of trees but in open spaces. Its cultures can be found in pastures, by the roadside and in open fields, especially where mules are in use.

Since the ABM only grows in very special conditions, attempts to achieve constant yields proved unsuccessful until the early 1990s. For years, breeding ABM in artificial conditions proved difficult, even with considerable Japanese support. It was not possible to achieve a stable yield for marketing purposes.

Immunological Significance of ABM

Living organisms have an immune system whose function is to eliminate pathogens, chemical toxic substances and mutation-induced cell proliferation. Cell proliferation causes the formation of a layer containing proteins that is decomposed by microorganisms, e.g. bacteria. It is thanks to this function, which we know as immunity,
the body stays healthy. It combats dangerous organisms by preventing them from penetrating the tissue, and therefore the body as a whole.

It is widely held that cancer develops through a weakening of the immune system through aging or other factors. This is one of the reasons why attention is focusing more and more on ABM. The mushroom contains large quantities of polysaccharides, which have been attributed an immunity enhancing effect. In addition, it reduces hyper-reaction of the immune system and thus contributes to establishing an equilibrium in the system as a whole.

Residue of pressed sugar cane is used for breeding compost used in breeding Agaricus bisporus, the garden champignon, is also ideally suited to breeding ABM. In Brazil, raw material such as horse dung and the residue of pressed sugar cane is used normally for this purpose. However, great care has to be taken that the compost material is not infested with fungi, insects, mites, and other undesirable intruders during the composting process. Pasteurisation is achieved through heating the compost material to temperatures of 600 to 650 Celsius in a chamber or a plastic tunnel with steam coming from a boiler. Once it has been pasteurised, the compost material is filled into plastic sacks with a capacity of four to five kilograms and an opening of 50 to 60 centimetres.

Immediately afterwards, the ABM hyphae are injected. Following inoculation, the plastic sacks are closed simply by folding the sack opening in order to prevent contamination.

Then, together with the fully developed mycelia, the compost material is brought to the final location, which may be greenhouses, sheds or open land. To grow the mushrooms in greenhouses or sheds, the inoculated compost material that already contains mushrooms is taken out of the plastic sacks and distributed on shelves in framed beds set up inside the structure. These beds have to be covered with treated earth and must be constantly kept moist. If the mushrooms are to be bred in the open, a gentle slope that has been weeded is preferable. The compost material should be put into 40 cm-long, ten cm-wide furrows and covered with a 2-3 cm-thick layer of treated soil. It must be constantly kept moist. The ABM mushroom requires a constantly moist layer of soil, for it is only in these conditions that fruit formation will occur. Using a spraying device with a large opening is recommendable for watering in sheds or greenhouses. Out in the open, a watering can may be used. [Top of Page]

**Harvesting ABM**

The mushrooms are best harvested when the cap, or pileus, is still covered by the membrane but is already slightly opened. The mushroom is held at the head and turned out of the soil at the roots while a slight downward pressure is applied. Immediately after harvesting and cleaning, the mushrooms are cut down the middle for drying and laid on bamboo or taquara sieves. Drying can be carried out in the open or in greenhouses, but should progress slowly. A temperature of 400 to 420 Celsius is required. The dried mushrooms are packaged in plastic bags with a net weight of one kilogram or less. These are distributed in response to individual orders from direct customers or exporters for Japan or the USA. On the Brazilian market, the prices fluctuate considerably depending on the quality of the product and the producer. In Brazil, the mushroom is sold at kilo
prices of 250 Cruzeiro Real (US$ 275). Elsewhere a kilogram costs US$ 350. [Top of Page]

Osmar Borzacchini is agricultural director of Piedade District, São Paulo, Brazil.

Agaricus (ABM) Mushroom Research

AGARICUS RESEARCH - Item #1

TITLE: Anti-tumor Activity and Some Properties of Water-insoluble Heteroglycans from "Himematsutake," the Fruiting Body of Agaricus blazei Murill.
JOURNAL: Agricultural and Biological Chemistry; 54(11):2889-2896 1990
ADDRESS: Faculty of Agriculture, Shizuoku Univ., Ohya, Shizuoku 422, Japan
ABSTRACT: After extraction of a hot-water-soluble polysaccharide (FI) from the fruiting bodies of Himematsutake (Agaricus blazei Murill), water-insoluble polysaccharides were obtained by successive extraction with 1% ammonium oxalate solution (FII), 5% sodium hydroxide solution (FIII and FIV), 20% sodium hydroxide solution (FV), and 5% lithium chloride-dimethylacetamide solution (FVI) in that order. These water-insoluble fractions were further fractionated by ethanol precipitation, gel-filtration, etc. Polysaccharides, polysaccharide-protein complexes, and chitin substances thus obtained were assayed for their antitumor activities using the Sarcome 180/mice i.p. o.p. method.

The heteroglycan-protein complexes, FII-a,-b,-c, obtained from FII had weak antitumor activities. A remarkable antitumor activity was found in a lycoprotein, FIII-2-b, fractionated and purified from FIII. The polysaccharide portion of this polysaccharide-protein complex (polysaccharide, 50.2% and protein, 43.3% each on a weight basis) consisted of (1→6)-beta-D-glucan, and its protein portion was rich in Asx, Glx, Ala, Leu, and Pro.

A high antitumor activity was found in a xyloglucan-protein complex, FIV-2-b, fractionated and purified from FIV. Antitumor activity was found also in a glucoxyylan, FV-2-a, obtained from FV. No significant antitumor activity was found in a chitin substance, FVI.

AGARICUS RESEARCH - Item #2

TITLE: Antitumor Activity and Some Properties of Water-soluble Polysaccharides from "Himematsutake," the Fruiting Body of Agaricus blazei Murill.
AUTHORS: Mizuno T, Hagiwara T, Nakamura T, Ito H, Shimura K, Sumiya T, Asakura A
JOURNAL: Agricultural and Biological Chemistry; 54(11):2897-2906 1990
ADDRESS: Faculty of Agriculture, Shizuoku Univ., Ohya, Shizuoku 422, Japan

ABSTRACT: Polysaccharides extracted from Himematsutake, the fruiting body of Agaricus blazei Murill with hot water were fractionated and purified by ethanol precipitation, ion-exchange chromatography, gel-filtration, affinity chromatography, etc. A total of 17 polysaccharide samples thus obtained were given an antitumor activity test (Sarcoma 180/mice i.p. p.o. method) and traces of their activities through the fractionation and purification processes were found. F10-a-beta, FA-1-a-alpha, FA-1-a-beta, and FA-2-b-beta, were obtained as water soluble polysaccharides fractions having great antitumor activities. Analyses of physico-chemical properties and IR- and NMR-spectra of the these active fractions showed that their main components were: F10-a-beta, (1-->6)-; (1-->3)-beta-D-glucan; FA-1-a-alpha, acidic (1-->6)-; (1-->4)-alpha-D-glucan; FA-1-a-beta, acidic (1-->6)-; (1-->3)-alpha-D-glucan; and FA-2-b-beta, acidic RNA-protein complex.

AGARICUS RESEARCH - Item #3

TITLE: Selective Tumoricidal Effect of Soluble Proteoglucon Extracted from the Basidiomycete, Agaricus blazei Murill, Mediated Via Natural Killer Cell Activation and Apoptosis.


ADDRESS: Division of Immunology, Miyagi Cancer Center Research Institute, Natori, Japan.

ABSTRACT: We have isolated a novel type of natural tumoricidal product from the basidiomycete strain, Agaricus blazei Murill. Using the double-grafted tumor system in Balb/c mice, treatment of the primary tumor with an acid-treated fraction (ATF) obtained from the fruit bodies resulted in infiltration of the distant tumor by natural killer (NK) cells with marked tumoricidal activity. As shown by electrophoresis and DNA fragmentation assay, the ATF also directly inhibited tumor cell growth in vitro by inducing apoptotic processing; this apoptotic effect was also demonstrated by increased expression of the Apo2.7 antigen on the mitochondrial membranes of tumor cells, as shown by flow-cytometric analysis. The ATF had no effect on normal mouse splenic or interleukin-2-treated splenic mononuclear cells, indicating that it is selectively cytotoxic for the tumor cells. Cell-cycle analysis demonstrated that ATF induced the loss of S phase in MethA tumor cells, but did not affect normal splenic mononuclear cells, which were mainly in the G0G1 phase. Various chromatofocussing purification steps and NMR analysis showed the tumoricidal activity to be chiefly present in fractions containing (1-->4)-alpha-D-glucan and (1-->6)-beta-D-glucan, present in a ratio of approximately 1:2 in the ATF (molecular mass 170 kDa), while the final purified fraction, HM3-G (molecular mass 380 kDa), with the highest tumoricidal activity, consisted of more than 90% glucose, the main component being (1-->4)-
alpha-D-glucan with (1-->6)-beta branching, in the ratio of approximately 4:1.

http://www.altcancer.com/agaricus.htm

Articles

Isolation of an Antitumor Compound from Agaricus Blazei Murill and Its Mechanism of Action

Takeshi Takaku*, Yoshiyuki Kimura^2 and Hiromichi Okuda^1

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The Basidiomycete fungus Agaricus Blazei Murill has traditionally been used as a health food for the prevention of cancer, diabetes, hyperlipidemia, arteriosclerosis and chronic hepatitis. In the present study, we examined the antitumor activities of various substances isolated from the lipid fraction of A. blazei. Tumor growth was retarded by the oral administration of the lipid fraction extracted from A. blazei with a chloroform/methanol mixture in sarcoma 180–bearing mice. The substance with the antitumor activity in the lipid fraction was isolated via silica gel column chromatography, eluted with an acetonitrile/methanol (3:2) mixture and identified as ergosterol by direct comparison of the 1H NMR and mass spectrometry spectral data of an authentic sample. The oral administration of ergosterol to sarcoma 180–bearing mice significantly reduced tumor growth at doses of 400 and 800 mg/kg administered for 20 d without side effects, such as the decreases in body, epididymal adipose tissue, thymus, and spleen weights and leukocyte numbers induced by cancer chemotherapy drugs. Ergosterol had no cytotoxicity against tumor cells. To clarify the antitumor activity of ergosterol, we examined the effects of ergosterol on tumor-induced angiogenesis using two in vivo models. Intraperitoneal administration of ergosterol at doses of 5, 10 and 20 mg/kg for 5 consecutive d inhibited the neovascularization induced by Lewis lung carcinoma cell–packed chambers, suggesting that either ergosterol or its metabolites may be involved in the inhibition of tumor-induced neovascularization. Therefore, we further examined the inhibitory effects of ergosterol on Matrigel-induced neovascularization. Female C57BL/6 mice were subcutaneously inoculated with Matrigel containing acidic fibroblast growth factor and heparin with or without ergosterol. Ergosterol inhibited the Matrigel-induced neovascularization, suggesting that ergosterol directly inhibits Matrigel-induced
neovascularization. From these results, it seems likely that the antitumor activity of ergosterol might be due to direct inhibition of angiogenesis induced by solid tumors. This is the first report of ergosterol as an antiangiogenic substance.

http://jn.nutrition.org/cgi/content/abstract/131/5/1409

The Journal of Alternative and Complementary Medicine
The Mushroom Agaricus blazei Murill Extract Normalizes Liver Function in Patients with Chronic Hepatitis B

To cite this paper:

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ABSTRACT

Background: Hepatitis B is a global health problem. Use of complementary and alternative medicine has been popular among patients with hepatitis B. This 1-year open-label pilot study aims to observe whether Agaricus blazei Murill extract improves liver function in patients with hepatitis B.
Methods: This study involved 12 months of clinical observation. Four (4) patients with hepatitis B who met the criteria (1) aged between 20 and 65 years; (2) being Chinese; (3) having been a hepatic B carrier (HBAg(+)) for more than 3 years; (4) alanine aminotransferase > 100 IU/L; and (5) not taking lamivudine, α-interferon, or other drugs for hepatitis participated in the study with informed consent. The enrolled patients were given Agaricus blazei Murill (ABM) extract of 1500 mg daily for 12 months. The level of alanine aminotransferase was taken as the major outcome measurement.

Results: At the end of the study, the mean level of aspartate aminotransferase and alanine aminotransferase decreased from 246.0 (± standard deviation [SD] 138.9) to 61.3 (± SD 32.6) IU/L and 151.0 (± SD 86.9) to 46.1 (± SD 22.5) IU/L, respectively.

Conclusions: Our initial observation seems to indicate the potential benefit of ABM extract in normalizing liver function of patients with hepatitis B. Controlled studies with larger samples should be conducted in the future.


Lack of carcinogenicity of lyophilized Agaricus blazei Murill in a F344 rat two year bioassay.

Lee IP, Kang BH, Roh JK, Kim JR.

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The Brazilian mushroom Agaricus blazei Murill has antimutagenic, antioxidant, immunostimulatory and antitumorigenic activities, and is increasingly consumed as a health food worldwide. We undertook the present study to evaluate the chronic toxicity and oncogenicity of A. blazei Murill in F344 rats. To establish a no-observed-adverse-effect level (NOAEL), four treatment groups of 100 rats each (50 males and 50 females) were fed a powder diet containing lyophilized A. blazei aqueous extract at 0, 6250, 12,500, and 25,000 ppm for up to 2 years. During this period, there was no remarkable change in mean body weight, body weight gain, hematologic or serum chemistry parameters, or absolute or relative organ weights in control or treatment groups. Mortality in male treatment groups (26%, 16%, and 30%), however, was significantly lower than in controls (48%). Histopathological studies showed no increased incidence of tumors in any treatment group, and total tumor incidence across all groups was comparable to historical data. In conclusion, an A. blazei Murill lyophilized powder diet even at
25,000 ppm (1176 mg/kgb x w x /day for male rats and 1518 mg/kgb.w./day for female rats) resulted in no remarkable carcinogenic effects in F344 rats over a 2-year period. Therefore, the dietary NOAEL is 25,000 ppm.

PMID: 17707568 [PubMed - indexed for MEDLINE]


Health Benefits of Agaricus blazei Murill

- Agaricus blazei Murill and Cancer (Research)

Agaricus blazei Murill may help to prevent and treat various types of Cancer:

Peer-Reviewed Professional Journals


Second Department of Medical Biochemistry, School of Medicine, Shigenobu Station, Ehime University, Shigenobu-cho, Onsen-gun, Ehime, Japan.

The authors previously found that ergosterol isolated from Agaricus blazei inhibited tumor growth through the inhibition of tumor-induced neovascularization. In the present study, the authors isolated further anti-angiogenic substances (A-1 and A-2) from this fungus using an assay system of angiogenesis induced by Matrigel supplemented with vascular endothelial growth factor, and A-1 was identified as sodium pyroglutamate.

. Next, they examined the antitumor and antimetastatic actions of A-1 using Lewis lung carcinoma (LLC)-bearing mice. A-1 (30, 100 and 300 mg/kg) inhibited tumor growth and metastasis to the lung. The reduction of the numbers of splenic lymphocytes, CD4+ and CD8+ T cells in LLC-bearing mice was inhibited by the oral administration of A-1 (30, 100 and 300 mg/kg). Further, A-1 increased the number of apoptotic cells of tumors and the numbers of CD8+ T and natural killer cells invading the tumors, and inhibited the increase of von Willebrand factor expression (a measure of angiogenesis) in the tumors. These results suggest that the antitumor and antimetastatic actions of A-1 (sodium pyroglutamate) may be associated with inhibition of the reduction of immune response caused by the tumor growth and tumor-induced neovascularization. This is the first report showing that sodium pyroglutamate isolated from A. blazei as
an anti-angiogenic substance has potent antitumor and antimetastatic actions, as well as immune-modulatory activity, in tumor-bearing mice.


Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine, Shizuoka, Japan.

The Basidiomycete fungus Agaricus blazei Murill has traditionally been used as a health food for the prevention of cancer. The authors examined whether beta-(1-6)-D-glucan extracted from A. blazei is a potential anticancer agent in an in vitro and in vivo animal model. (1) beta-glucan had cytotoxic effect against human ovarian cancer HRA cells, but not against murine Lewis lung cancer 3LL cells, in vitro; (2) beta-glucan promotes p38 MAPK activity for suppressing HRA cell proliferation and amplifying the apoptosis cascade; (3) beta-glucan stimulates translocation of the proapoptotic protein, Bax, from the cytosol to mitochondria, cytochrome c release, and subsequent caspase-9 activation; (4) treatment with SB203580, a p38 MAPK-specific inhibitor, suppresses beta-glucan-induced effects, indicating that activation of p38 MAPK is involved in the suppression of cell proliferation and mitochondrial activation-mediated cell death pathway; (5) in mice, oral supplementation with beta-glucan reduces pulmonary metastasis of 3LL cells and peritoneal disseminated metastasis of HRA cells and inhibits the growth of these metastatic tumors in lung or peritoneal cavity, in part, by suppressing uPA expression; and (6) in an in vivo experimental metastasis assay, however, the oral supplementation with beta-glucan after i.v. tumor cell inoculation did not reduce the number of lung tumor colonies. Treatment with beta-glucan may be beneficial for cancer patients with or at risk for metastasis. The beta-glucan-dependent signaling pathways are critical for our understanding of anticancer events and development of cancer therapeutic agents.

Division of Science of Biological Resources, Graduate School of Science and Technology, Kobe University, Japan.

Subset analysis of splenic lymphocytes using flow cytometry showed that the percentages of Thy1.2-(pan T-cells), L3T4-(CD4, helper T-cells), and Lyt2-(CD8, cytotoxic T-cells) positive cell populations were significantly increased in mice orally administered a hot water-soluble fraction from Agaricus blazei as compared with mice treated only with saline. 13C-NMR data indicates that the main component in the active polysaccharide is the complex of alpha-1,6- and alpha-1,4-glucan, which had already been shown to have anti-tumor activity against Sarcoma 180. It seems that the polysaccharide from Agaricus blazei may be an effective prophylactic, protecting humans against cancer by stimulating lymphocytes such as cytotoxic T-cells.


Division of Biotechnology, Sumitomo Forestry Tsukuba Research Institute, Tsukuba, Ibaraki, Japan.

There is an increasing demand from both patients and practicing oncologists for orally formulated chemotherapy. The present study focused on the oral formulation for natural products that may be effectively used in oncologic treatment regimens. Tumor-bearing mice treated with intratumoral administration of aqueous ammonium oxalate-soluble and ethanol-insoluble derivatives of Agaricus blazei showed marked tumor regression at doses ranging from 0.1 to 2.5 mg (p < 0.05 vs. saline control; n = 7). However, oral administration of this same fraction, either prior to, simultaneously with, or after, tumor cell inoculation did not result in tumor regression (p > 0.05 vs. control). When this fraction was treated with hydrochloric acid (acid-treated fraction; ATF), intratumoral administration resulted in a marked regression of tumor growth comparable to that of the acid-untreated fraction. More importantly, parenteral administration of ATF resulted in a significantly greater regression of tumor growth than that produced by the untreated fraction (p < 0.05 vs. untreated; n = 7). When a total of 4.5 mg of ATF was given orally at varying schedules prior to, simultaneously with, or after, tumor inoculation, a significant regression was seen using a schedule starting 4 days prior to inoculation (p < 0.05 vs. all other treatments; n = 7). NMR and molecular analyses showed that the ATF
fraction had a molecular weight of approximately 10 kDa and consisted mainly of only (1,6)-beta- D-polyglucose. These results suggest that the oral administration of simple acid-treated ATF results in a remarkable tumor regression.

Thus, simple acid hydrolysis of natural products may not only bring measurable benefits in oncological practice, but may also be a useful general formulation for natural products for oral chemotherapy.

- **Agaricus blazei Murill may help to prevent Liver Cancer.**

**Agaricus blazei Murill and Liver Cancer (Research)**

**Peer-Reviewed Professional Journals**


The modifying potential of prior administration of an aqueous extract of the mushroom Agaricus blazei Murrill (Agaricaceae) (Ab) on hepatotoxicity induced by different doses of diethylnitrosamine (DEN) in male Wistar rats was evaluated. During 2 weeks, animals of groups G3 (Ab+DEN(50)), G5 (Ab+DEN(100)), G7 (Ab+DEN(200)), and G8 (Ab-treated) were treated with the A. blazei through drinking water. After this period, groups G2 (DEN(50)), G3 (Ab+DEN(50)), G4 (DEN(100)) G5 (Ab+DEN(100)), G6 (DEN(200)), and G7 (Ab+DEN(200)) were given a single i.p. injection of 50, 100 and 200 mg/kg of DEN, respectively, while groups G1 (non-treated) and G8 (Ab-treated) were treated with 0.9% NaCl only. All animals were killed 48 h after DEN or NaCl treatments. The hepatocyte replication rate was estimated by the index of the proliferating cell nuclear antigen (PCNA) positive hepatocytes and the appearance of putative preneoplastic hepatocytes through expression of the enzyme glutathione S-transferase placental form (GST-P). After DEN-treatment, ALT levels, PCNA labeling index, and the number of GST-P positive hepatocytes were lower in rats that received A. blazei treatment and were exposed to 100 mg/kg of DEN. These findings suggest that previous treatment with A. blazei exerts a hepatoprotective
effect on both liver toxicity and hepatocarcinogenesis process induced by a moderately toxic dose of DEN.


Departamento de Morfologia, Instituto de Biologia, UNESP, Botucatu, SP, Brazil.

The effects of crude extracts of the mushroom Agaricus blazei Murrill (Agaricaceae) on both DNA damage and placental form glutathione S-transferase (GST-P)-positive liver foci induced by diethylnitrosamine (DEN) were investigated. Six groups of adult male Wistar rats were used. For two weeks, animals of groups 3 to 6 were treated with three aqueous solutions of A. blazei (mean dry weight of solids being 1.2, 5.6, 11.5 and 11.5 mg/ml, respectively). After this period, groups 2 to 5 were given a single ip injection 200 mg/kg DEN and groups 1 and 6 were treated with 0.9% NaCl. All animals were subjected to 70% partial hepatectomy at week five and sacrificed 4, 24 and 48 h or 8 weeks after DEN or 0.9% NaCl treatments (10th week after the beginning of the experiment). The alkaline comet assay and GST-P-positive liver foci development were used to evaluate the influence of the mushroom extracts on liver cell DNA damage and on the initiation of liver carcinogenesis, respectively. Previous treatment with the highest concentration of A. blazei (11.5 mg/ml) significantly reduced DNA damage, indicating a protective effect against DEN-induced liver cytotoxicity/genotoxicity. However, the same dose of mushroom extract significantly increased the number of GST-P-positive liver foci.


The chemopreventive potential of an Agaricus blazei (Ab) Murrill mushroom meal was investigated in a medium-term rat liver carcinogenesis assay. Male Wistar rats initiated for hepatocarcinogenesis with diethylnitrosamine (DEN, 200 mg/kg i.p.) were fed during a 6-week period with the dry powdered mushroom strains Ab 29 or 26, each one with opened (OB) or closed basidiocarp (CB), mixed at 10% level in a basal diet. All experimental animals and controls were subjected to partial hepatectomy at week 3 and killed at week 8. Chemopreventive activity of the mushroom meal was observed for the Ab 29 (OB and CB) and Ab 26 (CB) strains in terms of the
number of putative preneoplastic altered foci of hepatocytes which express either the enzyme glutathione S-transferase, placental form (GST-P+) or the transforming growth factor-alpha, and for the Ab 29 (OB) and Ab 26 (CB) strains on the size of GST-P+ foci. This was associated with inhibition of foci cell proliferation in the animals fed the Ab 29 (OB) and Ab 26 (CB) strains. The results suggest that the protective influence of the Ab meal against the DEN potential for rat liver carcinogenicity depends on both the strain and period of mushroom harvest.

- Agaricus blazei Murill may enhance the function of NK Lymphocytes and may improve the ability of NK Lymphocytes to destroy Cancer cells.

Agaricus blazei Murill and NK Lymphocytes (Research)

Peer-Reviewed Professional Journals


Department of Obstetrics and Gynecology, College of Medicine, The Catholic University of Korea, Seoul, South Korea.

A mushroom extract, Agaricus blazei Murill Kyowa (ABMK), has been reported to possess antimutagenic and antitumor effects. The authors investigated the beneficial effects of ABMK consumption on immunological status and qualities of life in cancer patients undergoing chemotherapy. One hundred cervical, ovarian, and endometrial cancer patients were treated either with carboplatin (300 mg / m(2)) plus VP16 (etoposide, 100 mg / m(2)) or with carboplatin (300 mg / m(2)) plus taxol (175 mg / m(2)) every 3 weeks for at least three cycles with or without oral consumption of ABMK. We observed that natural killer cell activity was significantly higher in ABMK-treated group (ANOVA, n = 39, P < 0.002) as compared with nontreated placebo group (n = 61). However, no significant difference in lymphokine-activated killer and monocyte activities was observed in a manner similar to the count of specific
immune cell populations between ABMK-treated and nontreated groups. However, chemotherapy-associated side effects such as appetite, alopecia, emotional stability, and general weakness were all improved by ABMK treatment. Taken together, this suggests that ABMK treatment might be beneficial for gynecological cancer patients undergoing chemotherapy.


Agaricus blazei Murill is an edible fungus used in traditional medicine, which has various well-documented medicinal properties. The authors investigated the effects of hemicellulase-derived mycelia extract (Agaricus blazei fraction H: ABH) on the immune system. First, we examined the cytokine-inducing activity of ABH on human peripheral mononuclear cells (PBMC). The results indicated that ABH induced expression of IL-12, a cytokine known to be a critical regulator of cellular immune responses. Flow cytometric analysis demonstrated the induction of IL-12 production by the CD14-positive cell population, consisting of monocytes/macrophages (Mo/Mphi). Furthermore, the elimination of Mo/Mphi attenuated IL-12 production in PBMC. ABH-induced IL-12 production was inhibited by anti-CD14 and anti-TLR4 antibodies but not by anti-TLR2 antibody. The activity of ABH was not inhibited by polymyxin B, while the activity of lipopolysaccharide used as a reference was inhibited. Oral administration of ABH enhanced natural killer (NK) activity in the spleen. These findings suggest that ABH activated Mo/Mphi in a manner dependent on CD14/TLR4 and NK activity.

- Agaricus blazei Murill may stimulate the production of Interleukin 12 (IL-12).

Agaricus blazei Murill and Interleukin 12 (Research)

Peer-Reviewed Professional Journals
Agaricus Blazei Murill is an edible fungus used in traditional medicine, which has various well-documented medicinal properties. The authors investigated the effects of hemicellulase-derived mycelia extract (Agaricus Blazei fraction H: ABH) on the immune system. First, we examined the cytokine-inducing activity of ABH on human peripheral mononuclear cells (PBMC). The results indicated that ABH induced expression of IL-12, a cytokine known to be a critical regulator of cellular immune responses. Flow cytometric analysis demonstrated the induction of IL-12 production by the CD14-positive cell population, consisting of monocytes/macrophages (Mo/Mphi). Furthermore, the elimination of Mo/Mphi attenuated IL-12 production in PBMC. ABH-induced IL-12 production was inhibited by anti-CD14 and anti-TLR4 antibodies but not by anti-TLR2 antibody. The activity of ABH was not inhibited by polymyxin B, while the activity of lipopolysaccharide used as a reference was inhibited. Oral administration of ABH enhanced natural killer (NK) activity in the spleen. These findings suggest that ABH activated Mo/Mphi in a manner dependent on CD14/TLR4 and NK activity.
Beta glucan is a scientifically proven biological defense modifier (BDM) that nutritionally potentiates and modulates the immune response. As a supplement, after swallowing orally, Beta glucan is ingested primarily through macrophage and dendritic immune cells, to nutritionally and safely yield, through immune response potentiation and modulation, in many instances various therapeutic healing effects generated by the immune cells. For many years Glucans have been investigated (History) for these immune enhancing properties, particularly their ability to activate macrophage immune cells and NK-Cells, plus in turn, the T-Cells, and B-Cells including selected cytokines and complement.

Poly-branched B-1,3-(D)-Glucans are naturally occurring polysaccharides, with or without B-1,6-(D)-glucose side chains, that are integral cell wall constituents in a variety of bacteria, plants and fungi. Glucan receptors to deliver non-self derived glucan to the immune response have been identified on macrophages, dendritic cells and other cells. The Beta-1,3-(D)-glucan with Beta-1,6-glucan linkage extracted from yeast cell wall (Saccharomyces cerevisiae) has been shown to act as a potent non-specific immune-activator.

The scientific literature on glucans is voluminous over many decades, and there is also a considerable body of patent literature. This Index is not intended to be a complete compilation of all beta glucan research, but rather is keyed by health condition and targeted to research on yeast-cell-wall-derived Beta-1/3,1/6-glucan. This indexing format varies from standard research classified by "researcher(s)" to make finding applicable research to a specific health condition easier for both the scientific and nonscientific user. As a note, particulate insoluble beta glucan is the glucan form in nutritional dietary supplements, while soluble glucans are primarily utilized in pharmaceutical applications. Particulates are not generally safe as injectables.

**The Key Determinants in Beta Glucan Effectiveness**

Neither the volume nor weight of beta glucan determines effectiveness or optimum dosage, but rather the key determinant is the degree of activation of the immune response by any given glucan in a specific amount, determined by an acceptable scientific method. In other words, more is not always better - judge by the extent to which the amount of beta glucan in a product enhances the immune response and not by the quantity of any given beta glucan in a capsule. High milligram dosages and high percentages of beta glucan in a capsule are not determinants of immune response or ingredient purity and cell activation. The true determinants of immune response activation and effectiveness are beta glucan source, processing (including avoidance of reaggregation during digestion), sizing and uniformity of beta glucan particles ingested.
Several prior beta glucan studies used larger volumes of beta glucan than found in current effective oral beta glucan nutritional supplements, but these were not experiments designed to determine optimum dosage relative to the immune potentiation capabilities of a specific glucan and most were performed in the 1970's and 1980's. Current science has demonstrated 10 mg or less of a properly processed and high quality Beta- 1,3/1,6-glucan or Beta-1,3-(D)-glucan with Beta- 1,6-glucan linkage extracted from yeast cell wall is a proper dosage amount (see "Dosage").

Lower quality, or glucans that reaggregate in the digestive process to non-uniform globular size, usually are sold in high milligram amounts that, without adequate immunopotentiation capability proven by independent laboratory testing, are minimally effective in any amount. While generally inexpensive, these high milligram content capsules are inexpensive due to minimal processing to minimize detriments to beta glucan immunopotentiation and no efforts to reduce particle size to uniform microparticulate sizes of 1-2 microns.

Bigger and heavier are not a positive characteristic of Beta glucans and can indicate lower grade Beta glucans only minimally effective in immune response activation. Lower grade Beta glucans must be provided in higher milligram amounts to be even minimally effective in immune response activation. Marketing often hypes high milligram content or percentage of a capsule containing Beta glucan, but science measures only immune potentiation by the amount of Beta glucan actually contained; i.e. a small amount of high grade Beta glucan in a capsule is many times as effective in immune potentiation as a high volume of inferior Beta glucan.

Additionally, current research has conclusively demonstrated particulate beta glucan from yeast cell wall even when micronized to 1-2 microns prior to oral ingestion, reaggregates or clumps back together when subjected to water in the digestive process, yielding a much larger size glucan effective particle size for ingestion by the immune cells, similar to grapes in a bunch. Ingestion is optimized by beta 1,3/1,6 glucans that are specially processed to prevent reaggregation that in turn yields maximum ingestion and nutritional potentiation of the targeted immune cells, normally the macrophages. The immune response with non-reaggregated glucan particles ingested by macrophage or dendritic cells responds faster and in greater numbers to attack non-self in the form of pathogens.

**Valid Test for Effectiveness and Digestion Impact on Glucan Potentiation**

A common test to determine a glucan's immune response potentiation effectiveness is the measure of the degree to which a glucan increases the nitric oxide burst in the macrophage immune cell. Particle size and uniformity of same
have additionally been scientifically proven to be important factors in nutritional potentiation of the immune response with microparticulates of 1-2 microns optimum, and for this reason "particle size" has been included as a research category below for review.

Be sure in evaluating comparisons of glucans to confirm the compared glucans are both in the form delivered to the immune cells (macrophages) after being subjected to the digestive process, including acid, enzymes and water. Most glucans, even if processed to reduce particle size, reaggregate or clump like grape clusters after being subjected to water in the digestive process and again become globular in characteristics, which minimizes ingestion and internalization by immune cells and immune response potentiation. (The Presentation Cover photo shows internalized MG Glucan as orange in macrophage cells)

Being a small particle glucan when swallowed is a positive step, but the critical determinant is being an extremely small microparticulate of 1-2 microns after going through the digestive process and then being delivered to the Peyer's patch in the lymphatic system to effectively activate a beta glucan receptor on a macrophage or dendritic cell to potentiate and enhance the immune response in a superior manner.

**Certificates of Analysis, Labeling**

A certificate of analysis for a specific beta glucan or beta glucan product should be from a recognized independent laboratory and not the manufacturer or distributor (conflict of interest), with clear delineation of specific tests and/or specifications for each category accepted and approved as industry standards. Confirm the date of the testing and the actual ingredient tested, as some unscrupulous marketers use old test unrelated to the specific product being sold and irrelevant graphs and factors having no relationship to actual effectiveness of the specific beta glucan involved in potentiating the immune response.

Is the product a private branded product provided the reseller by another manufacturer, or is the product from the original manufacturer? Is the entity selling the product actively involved in any medical school research related to the product in the bottle? "No" to the first question and "yes" to the second indicate a preferable beta glucan product from a primary source involved in scientific studies to study and improve glucans. Acquiring intellectual property can be
beneficial, but actively creating science in on-going programs to produce intellectual property and glucan improvements with new understanding is far preferable in product manufacturer activities.

Be on alert if tests, graphs and results do not include immune potentiation capabilities, particle size and ability to negate reaggregation in the digestive process, but rather disclose or compare only glucan volume in milligrams in the product and capsule ingredients. Product dollar cost per milligram of glucan also has nothing to do with immune potentiation capabilities of a specific product or glucan and may represent only an inexpensive, poorly manufactured or processed glucan, compared to a justifiably more expensive premium beta glucan product processed to higher standards. While everyone seeks a bargain, health is not an area to seek a minimal price at the cost of quality and effectiveness.

Be sure labeling is also in compliance with FTC and other government regulations, which are specific in format and content. Dietary supplement and food labeling requirements differ, although some manufacturers seem to confuse the two by placing, as an example, fat, caloric and carbohydrate information unnecessarily on supplements. There is no detriment to inclusion of such information; however, same is not required nor is associated with labeling of major supplement manufacturers.

**Validate Health Claims, Product Compositions and Credentials of Researchers and Spokespersons - Know the Quality of the Product and those Producing and Marketing a Beta Glucan Product**

Be aware and suspicious of broad health claims in supplements unsubstantiated by science and cautiously evaluate scientific research on glucan compositions that differ substantially from the product being sold (examples: insoluble-particulate glucans research to support soluble glucan, or yeast beta glucan (Beta-1,3-(D) or Beta-1,3/1,6) studies to support oat and barley derived glucan (beta 1,4) immune potentiation capabilities and vice versa). Obviously, avoid resellers who are not aware there are differences in glucans or those who do which only seek to sensationalize by inappropriate marketing.

Check credentials and background of researchers and spokespersons. Amazingly, one hawker selling beta glucan supplements under multiple product
names, some in multilevel marketing schemes, promises to tell the "truth" and "facts" about beta glucan, while prominently displaying the BBB (Better Business Bureau) symbol in many of his related web sites. The "truth" and "facts" are this man has no significant educational credentials, no peer-reviewed publications or intellectual property involvement, constant litigation and an extensive criminal record with time served in federal prison!

Do not be shy when your health or the health of your patients or customers is involved and demand complete information on the product and the parties involved to make informed decisions. Be informed to determine appropriate applications, premium beta glucan products and how to differentiate the effective from the promoted. Remember individual products containing Beta glucan can (1) range widely in forms of Beta glucan chemically and structurally; (2) range widely in processing procedures from unique U.S. Patents to none when private labeling; (3) range widely in quality of the processed Beta glucan due to source, processing and microbial testing during preparation and (4) be subject to various levels of honesty in immune potentiation capabilities of an individual product, including outright and purposeful deviousness in marketing utilizing performance criteria such as weight and volume in a capsule, known to have no meaningful relationship to an ingredients ability to potentiate or modulate the immune response nutritionally.

Beta Glucan, particularly Beta- 1,3/1,6 Glucan extracted from yeast cell wall, is a potent and proven immune response potentiator and modulator when processed and distributed in a reputable and scientifically proven composition in the proper dosage. The research recorded on this non-commercial website, with constant additions and updates on publication, is a first step in understanding this complex and nutritionally beneficial natural substance - Beta glucan.

Research Overview


Quote: “…the presence of a particulate activator can rapidly initiate assembly and amplification of a host defense system involving humoral and cellular interactions with B-glucans. …Animals pretreated with purified glucan particles are subsequently more resistant to bacterial, viral, fungal, and protozoan challenge, reject antigenically incompatible grafts more rapidly and produce higher titers of serum antibodies to specific antigens.

Administration of glucan particles …stimulates…proliferation of macrophages and increases in phagocytic and secretory activities of macrophages. …A cascade of interactions and reactions initiated by macrophage regulatory factors can be envisioned to occur and to
eventuate in conversion of the glucan-treated host to an arsenal of defense.”


Quote: “MG Beta Glucan has been shown to enhance the envelopment and digestion (phagocytosis) of pathogenic microorganisms that cause infectious disease…The Beta-1,3/1,6 glucans additionally enhance the ability of macrophages, one of the most important cells in the immune system, to kill tumor cells. Laboratory studies have revealed the new MG Glucan is significantly effective at activating macrophages, and via the macrophages, the entire immune cascade including T-Cells and B-Cells.”


Quote: “…glucan enhances the immune response through stimulation of macrophages by increasing their number, size, and function, stimulates secretion of lysozyme and TNF by activated macrophages, increases the phagocytosis of antigens, activates the formation of granulocyte and monocyte colonies, and factors increased activity of T and B lymphocytes, as well as complement activation.”

The Beta Glucan Research Organization is a "not-for-profit" entity. References and quotes contained herein are for information, education and research purposes only and should not be construed as express or implied representations, endorsements or warranties of The Beta Glucan Research Organization nor Nutritional Scientific Corporation, the latter having supported compilation of this non-commercial Research Index through a donation to The Beta Glucan Research Organization.

Note on various Glucan forms: No commercial brand names of products are presented or endorsed on this research website. Beta 1,3/1,6-D glucan is a baker’s yeast-derived beta glucan with a Beta 1,6 linkage (4-8%) and the molecule skewed to the right. MG Glucan is a specially processed proprietary form of microparticulate Beta 1,3/1,6 glucan that is uniform homogeneous and non-aggregated purified Beta 1,3-D glucan that does not reaggregate after the digestive process. “PGG-glucan” is poly-[1,6]-B-D-glucopyranosyl-[1-3]-B-D-glucopyranose (β-1,6/1,3-glucan).

“Beta glucans” refers generally, but not always, to Beta- 1,3/1,6-glucan. “Scleroglucan” and “PSAT” are two Beta-1,3/1,6-polysaccharides. Beta glucans are derived primarily from yeast cell wall, various fungi, grains, and mushrooms. Beta 1,4 glucan is derived from oats and barley, minimally effective in immune potentiation and not included in this research summary of forms of Beta 1,3/1,6 glucan. Many beta glucans are marketed under various trademark names that are not unique ingredient formulations. Letters such as NSC, WGP and others are associated with brand names
and are not specific forms of Beta glucan, although the individual products often contain Beta glucan.

The Journal of the American Nutraceutical Association and Transfer Point Beta Glucan.

In an independent study released from the University of Louisville, Transfer Point beta glucan has been shown again to be America's number one immune system support product.

This study has been published in The Journal of the American Nutraceutical Association. It is the leading peer-reviewed journal on nutraceuticals and nutrition.

JANA is published quarterly. Health care professionals, researchers, naturopaths and many others consider it the bible for identifying safe and effective dietary supplements for their practice and/or personal use.

JANA contains original research articles not found anywhere else, comprehensive review articles, timely editorials, opinion articles, book reviews and interviews with leaders in the fields of nutraceuticals, MD's, researchers and others that are involved in the clinical use of nutraceuticals.

The study, titled "An Evaluation of the Immunological Activities of Commercially Available Beta 1, 3 Glucans" authored by Doctor Vaclav Vetvicka, PhD, Professor at the Department of Pathology and Laboratory Medicine. Doctor Vetvicka tested a large variety of immune support products. The study also included a large variety of other products claimed to support proper immune system support function.

Doctor Vetvicka used a wide variety of biological reactions to identify the immune enhancing effects of the various products tested. These tests were:

- Phagocytosis
- Surface markers on splenocytes
- Cytokine synthesis
- Stimulation of antibody response

To review the results of this independent test on beta glucan and other products click here.
Phagocytosis[1] is the engulfing and ingestion of bacteria or other foreign body invaders by phagocytes. Phagocytes are the white blood cells of the immune system who's primary responsibility is to destroy diseased cells or invaders in our body.

**Phagocytosis is a primary response of the innate immune system of the body.**
Without proper and effective Phagocytosis we would succumb to disease and/or infection.

Splenocytes are spleen cells (e.g. lymphocytes, granulocytes, other immune cells). These cells play an important role in regulation of immune responses, including CD4 and CD8. The amount of surface markers helps determine immune response characteristics.

Cytokines are a non-antibody proteins produced by leukocytes and non-leukocytic cells that can be a "communication line" between cells and can influence the behavior of cells in the generation of an immune response. Cytokines include interleukins, lymphokines and cell signal molecules, such as tumor necrosis factor and the interferons. They can trigger inflammation and respond to infections. They help regulate the immune system. The quicker Cytokines are synthesized the more effective the response rate of the immune system to a threat upon it.

A antibody is a product of the immune system that helps the immune system recognize and fight infections and other invaders in the body. The faster the response of antibodies to an infection or invader the more effective the immune system response will be against it.

To review the results of this independent test on beta glucan and other products click here.

Many products that claim to support proper immune system function do not even provide proof of their actual claim. Others use a single test result to make the claim, usually the rate of increase of Natural Killer Cells, which is a single component of the immune system. This theory is flawed because it is only looking at one response characteristic. The immune system is a complex system comprising of many components.

A proper test to establish effective immune support would be to compare more then one component for immune system response. These are the tests that were performed at the University of Louisville and a wide variety of immune system support products.

The independent test results showed that Transfer Point Beta glucan products out performed all the competitors. To review the results of this independent test on beta glucan and other products click here.

References:

Research - Medline Quotes

This page is for educational purposes only. The information contained on this page is intended to provide you with a balanced overview of information relating to health and well-being. The articles are intended for general education purposes and not to sell any specific product. This page does not constitute labeling, endorsements or advertisements for any particular products and should not be interpreted as recommending how to treat any particular diseases or health-related conditions.

The following are a series of one line quotes from the scientific literature. Cites may be obtained off Medline. Medline is one of the largest medical research archives in the world.

The emergence of multiple antibiotic-resistant microorganisms has led to a search for alternatives to traditional therapeutic regimens. Beta Glucan, an immunomodulator, can selectively enhance the microbicidal activities of neutrophils and macrophages without stimulating proinflammatory cytokine production.

Glucan is a potent reticuloendothelial-modulating agent whose immunobiological activity is mediated, in part, by an increase in the number and function of macrophages. Lysozyme concentrations were increased approximately sevenfold in some studies. Biologic response modifiers, like Beta Glucan, will modulate immunity, modify neoplastic (cancer) disease and increase resistance to microbial challenge. Glucan can enhance some elements of the immune system against staphylococcal infections.

Beta Glucan has been found to protect against infection with Babesia microti, an intra-erythrocytic protozoan parasite, nonspecific protection. Glucan has been shown to significantly inhibit renal necrosis associated with systemic staphylococcal diseases and showed enhanced survival. Glucan has been shown to increase peripheral leukocyte counts. Beta Glucan induced increase in phagocytosis and induced hyperplasia of macrophages. Glucan significantly enhanced survival when challenged systemically with Staphylococcus aureus.

These studies indicate that glucan confers an enhanced state of host defense against bacterial infections. With orally administered glucan, interleukin-2 was evaluated in treated animals and showed an increase. There was significant increase in polymorphonuclear leukocytes and peripheral monocyte number. A significant increase in number and in vitro candidacidal activity was also observed for alveolar (lung) macrophages. The resistance towards systemic infection with Candida albicans of Staphylococcus aureus increased, significantly reducing the growth of microorganisms in the kidneys of infected animals. Toxicological studies showed that glucan is highly tolerated. In the area of protective capacity in respiratory infection, Beta Glucan significantly increased rated of phagocytosis and killing of Staphylococcus aureus.
Beta Glucan greatly increased numbers of macrophages in the lungs of glucan-treated rats; the lungs of glucan-treated mice appeared normal and that glucan can enhance intrapulmonary bacterial killing. The ability of glucan was shown to increase the number of lung macrophages resulting in increased bacterial ingestion. Particulate glucan resulted in significant reductions in the growth of a syngeneic anaplastic mammary carcinoma and melanoma and showed enhanced survival. Studies demonstrate that prophylaxis with Beta Glucan in combination with antibiotics provided enhanced protection against lethal challenge with Escherichia coli or Staphylococcus aureus as compares with the use of antibiotics alone.

Dr. J. K. Czop, Department of Medicine, Harvard Medical School, Boston, MA. "The cell wall glucans of Saccharomyces cerevisiae [yeast cell wall] consist of two structurally distinct Beta-glucans: major components comprised of consecutively, 1,3-linked glucopyranosyl residues with small numbers of 1,6-linked branches, and minor components with consecutive 1,6-linkages and 1,3-branches." Browder W., et al.,

"Modification of Post-Operative C. albicans Sepsis by Glucan Immunostimulation," Int. J. Immunopharmac.; 6:19-26. 1984. Dept of Surg and Physiol, Tulane U Sch of Med, LA Quote: "These observations suggest that Biologic Response Modifiers such as glucan may be effectively employed in patients who are at risk for post-operative infections."*


This is just a sampling of the data that exists in abundance out of peer reviewed volumes. Anyone who takes the time to examine this data would be prudent to explore Beta Glucan as a source to help your immune system be all that it could be without the misuse, overuse, or abuse of antibiotics.

**Summarized Studies**

Studies Conducted at: HARVARD | NATIONAL CANCER INST | MAYO CLINIC | DEPARTMENT OF AGRICULTURE | TULANE UNIVERSITY | BAYLOR COLLEGE OF MEDICINE | ARMY RADIATION RESEARCH INSTITUTE | VANDERBILT

Studies conducted on: ABDOMINAL OR THORACIC SURGERY | CANCER | CANDIDA ALBICANS | CHOLESTEROL | DIABETES | E. COLI | FUNGAL INFECTION | HEPATITIS | HERPES SIMPLEX | HIGH RISK SURGICAL PATIENTS | INFECTION PREVENTION | INTERLEUKIN | PARASITES | PNEUMONIA | RADIATION | STAPHYLOCCAL | STAPHYLOCOCCUS | STRESS | TRAUMA | WOUNDS
ABDOMINAL OR THORACIC SURGERY - HARVARD MEDICAL SCHOOL (USA) "There were no adverse drug experiences....is safe and appears to be effective in the further reduction of the morbidity and cost of major surgery."

BACTERIAL INFECTIONS - BAYLOR COLLEGE OF MEDICINE; Wyde, P., "Beta-1,3-glucan activity in mice: intraperitoneal and oral applications." " Beta glucan, through the stimulation of host defense systems, creates a more supportive environment within the body to assist the primary killing action of the conventional agent."

CANCER, LUNG AND BREAST - NATIONAL CANCER INST (USA); "The initial 9 patients studied had malignant melanoma, adenosquamous carcinoma of the lung, or carcinoma of the breast. Control and experimental lesions were injected: subsequently biopsies were performed at varying intervals for histologic evaluation. Always when glucan or glucan and RF fraction were administered intralesionally, the size of the lesion was strikingly reduced in as short a period as 5 days. This reduction was associated with necrosis of the tumor and a monocytic infiltrate. In small lesions, resolution was complete, whereas in large lesions, resolution was partial.

CANCER - MAYO CLINIC (USA); "..... beta-glucan interacts with vitronectin and stimulates tumor necrosis factor alpha release from macrophage's."

CANCER - CHEM PHARM BULL (Japan); "Antitumor and immunomodulating activities of a beta-glucan...."

CANCER - UNIVERSITY OF TROMSO (Norway); "Macrophages stimulated by an insoluble beta 1-3-D-glucan from yeast cell walls were able to destroy tumour cells as measured by the release of radioactive label from prelabelled 14C-thymidine cells. Target cells were B-16 melanoma, P-815 mastocytoma, and the L-929 cell line. A significant target cell killing by macrophages stimulated by glucan was observed after 72-96 h."

CANDIDA ALBICANS - DEPARTMENT OF SURGERY, TULANE UNIVERSITY; "Protection against C. albicans was observed in the glucan-treated groups. ...These observations suggest that Biologic Response Modifiers such as glucan may be effectively employed in patients who are at risk for post-operative infections."

CHOLESTEROL (LDL) - OTTAWA CIVIC HOSPITAL (Canada); "CONCLUSIONS: The main component of the soluble fiber of oats, beta-glucan, significantly reduced the total and LDL cholesterol levels of hypercholesterolemic adults without changing HDL cholesterol."

CHOLESTEROL - DEPARTMENT OF AGRICULTURE (USA); "Beneficial reduction of cholesterol was obtained with modest amounts....."
DIABETES - OTTAWA CIVIC HOSPITAL (Canada); "A diet rich in beta-glucan may therefore be of benefit in the regulation of plasma glucose levels in subjects with Type 2 diabetes."

DIABETES - NESTLÆ RESEARCH CENTER (Switzerland); "Diabetic individuals can benefit from diets that are high in beta-glucan, .."

E. COLI - TULANE UNIVERSITY (USA, La); "Glucan therapy also increased bone marrow proliferation. We conclude that (1) glucan enhances peritoneal neutrophil levels, (2) Peripheral blood neutrophils are increased following glucan and E. coli, (3) ip glucan increase bone marrow proliferation, .... Thus, the beneficial effect of glucan is mediated not only by activated macrophages, but also by the neutrophilic leukocyte."

E. COLI, STAPHYLOCOCCUS - DEPARTMENT OF PATHOLOGY, BRIGHAM AND WOMEN'S HOSPITAL (USA, Mass) "Mice challenged with Escherichia coli or Staphylococcus aureus were protected against lethal peritonitis by the intravenous administration of 10 micrograms of poly-beta 1-6-glucotriosyl-beta 1-3-glucopyranose (PGG) glucan per animal 4 to 6 h prior to bacterial challenge."

FUNGAL INFECTION - TULANE UNIVERSITY; "The broad spectrum of immunopharmacological activities of glucan includes not only the modification of certain bacterial, fungal, viral and parasitic infections, but also inhibition of tumor growth."

HEPATITIS, VIRAL - SCIENCE (1980);"Thus glucan is capable of increasing survival, inhibiting hepatic necrosis, and maintaining an activated state of phagocytic activity in mice challenged with [mouse hepatitis virus strain] MHV-A59."

HERPES SIMPLEX 1 - PLANTA MED., 62:4, 301-7. (1996); "The antiviral effect of scleroglucan seems to be related to its binding with membrane glycoproteins of HSV-1 particles which impedes the complex interactions of the virus with the cell plasma membrane."

HIGH RISK SURGICAL PATIENTS - HARVARD MEDICAL SCHOOL (USA); "Patients who received PGG-glucan had significantly fewer infectious complications (3.4 infections per infected patient vs. 1.4 infections per infected patient, p = 0.05), decreased intravenous antibiotic requirement (10.3 days vs. 0.4 days, p = 0.04) and shorter intensive care unit length of stay (3.3 days vs. 0.1 days, p = 0.03). CONCLUSIONS: PGG-glucan is safe and appears to be effective in the further reduction of the morbidity and cost of major surgery."

INFECTION PREVENTION - GYNECOLOGY & OBSTETRICS, 177:383-388. (1993); "The incidence of hospital pneumonia of 55% and sepsis of 35% confirms results of previous studies of patients with multitrauma. Glucan decreased pneumonia and sepsis to a significantly lower level of 9.5%....The mortality rate related to infection decreased from 30.0 to 4.8%. The lower number of instances of pneumonia and
sepsis...decreased the period of time in the intensive care and the hospital, with a global reduction of 40% on hospital cost."

**INTERLEUKIN - INT J IMMUNOPHARMACOL, 1987, 9:3, 261-7;** "The study demonstrates that: (1) glucan will enhance IL-1 and IL-2 production and (2) elevations in lymphokine production can be maintained up to 12 days post-glucan"

**PARASITES - TULANE UNIVERSITY (USA);** "Trypanosoma cruzi, the causative agent of Chagas' disease, infects humans and animals..... Glucan significantly (P less than 0.05) increased survival rate as denoted by 60%...."

**PNEUMONIA - HOSPITAL ARTHUR RIBEIRO DE SABOYA (Brazil);** "The mortality rate related to infection was 30.0 percent in patients in the control group and 4.8 percent in the group treated with glucan...."

**RADIATION SURVIVABILITY- ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE (USA);** "Immunomodulators, either microbial agents (e.g. glucan, TDM) or recombinant cytokines (e.g. Interleukin-1, colony-stimulating factor), can enhance hematopoietic and functional cell recovery after irradiation."

**RADIATION - RADIOPROTECTIVE EFFECT;** "These results suggest that early after irradiation glucan may mediate its radioprotection by enhancing resistance to microbial invasion via mechanisms not necessarily predicated on hemopoietic recovery. In addition, preliminary evidence suggests that glucan can also function as an effective free-radical scavenger."

**RADIATION SURVIVABILITY: Abstract: **"Glucan, a beta-1, 3 polyglucose, was administered to mice either 1h before or 1h after a 650 rad exposure to cobalt-60 radiation. Compared to radiation controls, glucan-treated mice consistently exhibited a more rapid recovery of pluripotent stem cells and committed granulocyte, macrophage and erythroid progenitor cells. This may partially explain the mechanism by which glucan also enhances survival in otherwise lethally irradiated mice."

**STAPHYLOCOCCAL WOUND INFECTION - VANDERBILT UNIVERSITY SCHOOL OF MEDICINE (USA);** "We conclude that PGG Glucan reduces the risk of staphylococcal abscess formation."

**STRESS, PHYSICAL, OR EMOTIONAL - TOWNSEND LETTER FOR DOCTORS, (1996);"The following list includes benefits from the use of Beta 1,3-glucan supplementation: Professional and amateur athletes as well as people who work outdoors intensively. People under physical or emotional stress"**

**TRAUMA PATIENTS - TULANE UNIVERSITY (USA);** "total mortality rate was significantly less in the glucan group (0% versus 29%) (p less then 0.05), the mortality rate from sepsis was not statistically different (0% versus 17.6%). Glucan therapy significantly decreased septic morbidity (9.5% versus 49%; p less than 0.05). Serum IL-1
had a greater increase in glucan patients on day 3 after trauma (143.4 +/- 19.3% versus 78.6 +/- 11.7%; p less than 0.05)."

WOUND HEALING - EAST TENNESSEE STATE UNIVERSITY (USA); "These data indicate that macrophage modulation with glucan phosphate will increase tensile strength in experimental colon and skin wounds. These are just a few of the many studies on Beta Glucan, further, these studies do not reflect the results of any one product, or product line(s) shown to contain Beta Glucan. Also, the delivery methods of the Beta Glucan to the test subjects may have been of different types, two of which are oral and intravenous delivery.

http://www.beta-glucan-info.com/general_research.htm#HARVARD

Beta Glucan Research

Beta glucan is a polysaccharide found in oats, barley, yeast and mushrooms. The miraculous powers of beta glucan to lower cholesterol and triglycerides and strengthen our immune systems have been known about for more than a decade now but until recently, it has been all but impossible to economically extract it from even inexpensive oats and leftover beer brewing yeast. Finally, in the year 2000 technology had advanced and you could get inexpensive beta glucan in 100 and 200 mg capsules very inexpensively. At the University of Hamburg in Germany it was shown that all 1,3 configuration beta glucans have the same biological potency whether they are derived from oats or yeast - the two major sources (Carbohydrate Research 297, 1997, p. 135-43). They said, "All glucans investigated, regardless of molar mass and solution structure, stimulate the investigated immunological measures." Just so you will know, yeast and mushrooms are 1,3/1,6 arrangements and oat and barley are 1,3/1,4 structural arrangements, but they are all basically true 1,3 beta glucans. Some companies will tell you one is better than the other in order to sell their product so please don't listen to advertising pitches. Read the label and compare products carefully as you need at least 100 mg a day to be effective.

Actually beta glucan is probably the most powerful immunity enhancer known to science regardless of cost. There are many studies on animals and humans, showing the great value it has to strengthen our immune systems and even the potential to help against tumors and cancer growth. At the University of Saskatchewan in Canada (Microbiol. Immun. 41, 1997, p. 991-8) researchers showed the power to stimulate the immune system. Other studies have found such potential uses as fighting infections, improving intestinal flora, irritable bowel syndrome, diabetic conditions, ulcers and digestion. This, however, is a book on cholesterol so that is what we'll stress. There are many, many studies on blood lipids so we'll just talk about some of the more interesting of the human studies.

At Harvard Medical School in Massachusetts (Crti. Rev. Food Sci. Nutr. 39, 1999, p. 189-202) doctors found that both oat and yeast beta glucans lowered serum cholesterol levels. They did this by simply adding beta glucan to the diets of the people they studied. Notice that there is no use of drugs here and this comes from Harvard Medical School where they are traditionally concerned with prescription drugs and not natural plant supplements. In their words, "In addition to decreasing
the intake of total fat, saturated fat and dietary cholesterol, blood serum cholesterol can be further decreased by dietary fiber, especially from sources rich in beta glucan such as oats and yeast.

At the University of Syracuse in New York seventy-one men and women with high cholesterol were given various combinations of low fat diets or regular diets with and without oat beta glucan. In a matter of weeks total cholesterol levels were reduced as much as 17% (Journal of the American Dietary Association 90, 1990, p. 223-9). Their high-density cholesterol levels were also increased significantly. This shows the benefit of making better food choices along with taking effective supplements since the people who ate the low fat diet while taking the oat supplement got the best results.

At the University of Massachusetts (Am. J. Clin. Nutr. 70, 1999, p. 208-12) researchers found that giving obese men with high cholesterol levels yeast derived beta glucan lowered both their total and LDL levels by a full 8% with no change in diet. They summarized the study, "Thus, the yeast derived beta glucan fiber lowered the total cholesterol concentrations and was well tolerated." As usual any side effects were positive in nature.

At the U. S. Human Nutrition Research Center in Maryland (J. Nutr. Biochem. 8, 1997, p. 497-501) people were given oat extracts high in beta glucan content and lowered their cholesterol levels with no changes in diet or exercise. The also found out that other metabolic conditions improved, so new benefits of beta glucan are always being discovered.

Again at the Human Nutrition Research Center (J. Am. Coll. Nutr. 16, 1997, p. 46-51) men and women with high blood lipid levels were given oat extracts high in beta glucan. After only five weeks the groups were switched and those getting the beta glucan received only the usual American diet. Both total cholesterol and LDL levels decreased significantly. In their words, "A significant dose response due to beta glucan concentration in the oat extract was observed in the total cholesterol levels." Thorough studies like this in real people at the most prestigious research centers in the world leave no doubt about the power of beta glucan to lower blood fats.

At Industrial Research Limited in New Zealand (Carbo. Polymers 29, 1996, p. 7-10) researchers used barley derived beta glucan to try and understand the actual metabolic mechanisms by which it lowered blood fats. They first discovered that it increased the secretion of bile acids from the gall bladder. By using highly sophisticated NMR spectroscopy techniques they found the situation to be more complicated than the mere enhanced gall bladder activity. We are more concerned with the practical matters that beta glucan actually works rather than the how and why of it all.

At the University of Lund in Sweden (Ann. Nutr. Metab. 43, 1999, p. 301-9) 66 mildly hypercholesterolemic men were given oat milk in their diet high in beta glucan content for five weeks. This was a classic double blind study where half the men received rice milk with no beta glucan. Of course the men getting the oat milk lowered their total cholesterol and they said, "It is concluded that oat milk has cholesterol reducing properties"

You can see from studies like these there is no doubt that beta glucan is a safe, effective, powerful, proven and inexpensive way to lower your cholesterol levels, yet
most people have never even heard of it. Most vitamin companies don't even sell it and it can be difficult to find a reliable, strong, inexpensive brand in your drug store or even in your health food store. People keep taking dangerous, expensive, prescription cholesterol-lowering drugs when then can use natural remedies like beta glucan. Now that you have this book in your hands you know better.

In addition to the benefits we've just covered, it is important to take this because it may well be the most important immune enhancer known to science. Beta glucan strengthens our immune systems so we have optimum healing power in our body and fight off infections of all kinds whether bacteria, fungi or viruses.

You should understand that it is very difficult to study human beings for immune function. Animal studies are used because obviously you can't infect humans with deadly microorganisms and then give half of them beta glucan and see who lives and who doesn't. Animal studies have shown results for such conditions infections of many types, tumors, diabetes, intestinal function and ulcers.

The animal studies have been done for the last ten years in clinics worldwide and published in the major medical journals but beta glucan has only become economically available to consumers in the last few years. In fact only in the year 2000 did the price fall enough to allow you to buy 100 mg and higher capsules reasonably.

At the University of Saskatchewan beta glucan protected mice from deadly injections of Staphalococcus aureus. In another study nice were injected with equally deadly Eimeria vermiformis but beta glucan protected them. And yet in a third study mice were given the toxic drug dexamethasone and then injected with the deadly Eimeria virus. Even after their immune systems were impaired by the drug the beta glucan protected them. At SRI International the Euglena gracilis virus was injected into various test animals but beta glucan stopped them from dying. At the University of Kansas pigs were given deadly Staphalococcus suis and beta glucan saved them. The doctors there did an in-depth study of various immune system markers to see how it worked.

At the Mayo Clinic lung cancer in mice was reduced by beta glucan. At Tokyo College doctors found strong anti-tumor properties for beta glucan. At Tokyo University doctors found anticancer activity in mice when given beta glucan and suggested they be used as, "biological response modifiers in cancer patients." At Wuhan University they found powerful anti-tumor activity in mice given beta glucan. At the University of Louisville they found the anti-tumor effect of beta glucan was largely due to enhancing beneficial natural killer (NK) cells. Please read my book "What Is Beta Glucan" for more information. This is a basic supplement you should be taking daily for many reasons.

http://www.healthsupply.ws/cholesterol/beta-glucan.html
3. Best website explaining how the human immune system works.

6. List of companies that sell an “ABM” product of any kind.
   http://www.americannutrition.com/store/Mushrooms.html
   http://www.iherb.com/ProductDetails.aspx?c=1&pid=30&at=0
   http://www.agaricusfarm.com/
   http://www.atlasworldusa.com/products.html
   http://www.hplus.com/a36.html

SASA BAMBOO LEAF
Immunostimulation-Mediated Anti-tumor Activity of Bamboo (*Sasa senanensis*) Leaf Extracts Obtained Under ‘Vigorous’ Condition

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Abstract

Traditional Japanese medicine uses the leaves of Kumaizasa bamboo extracted in hot water at 100°C. For this study, we developed a new, ‘vigorous’ extraction method involving steps at 100, 121 and 196°C. This procedure not only yielded greater amounts of extract but also with significant increase in immunostimulating activity, which induces activation of human natural killer (NK) cells, macrophages and potent induction of IL-2, IL-12 and IFN-γ in tumor bearing mice. The efficacy of the extract to facilitate phagocytosis and nitric oxide production by mouse peritoneal macrophages was determined and compared with that of 1,3-β-glucan. Anti-tumor activity was evaluated in vivo in several mouse tumor models (S-180, C38 and Meth-A). Oral administration of the extracts was carried out when tumor reached size of approximately 6 mm at concentrations of 0.05% or higher. The extracts significantly suppressed tumor growth in S-180 and C38 tumor models. Overall survival was significantly prolonged in the treatment group than that of control. Activation of macrophages and NK cells by the extracts suggests that the anti-tumor efficacy of the extract is mediated by immunopotentiation. The extracts resolved into three major fractions (F-I, F-II and F-III) in Sephadex gel chromatography. Fraction F-I consists of 1,3-β-glucan and stimulated both macrophages and NK cells suggesting that it may be the primary immunopotentiating factor in suppressing cancer. Fraction F-III has potent free radical
scavenging effects and may play an important role in cancer prevention. These results warrant further translation and clinical investigations.

**Keywords:** high-temperature/high-pressure extraction – multistep extraction – NK cell activation – macrophage activation – 1,3-β-glucan

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## Introduction

One of the causal factors of tumor progression is suppression of immune functions. Restoring the immune competence can be one of the primary strategies of active immunotherapy. Polysaccharides from mushrooms or microbial cell-wall components were shown to be efficient immunostimulating agents. The extracts of the Kumaizasa bamboo leaves were shown to be anti-inflammatory (1). We hypothesized that the extracts may also possess immunopotentiating function. To test the hypothesis, we have undertaken this investigation. The first objective was to evaluate the anti-tumor activity and the immunopotentiating efficacy of the bamboo extracts under vigorous conditions. The second objective is to identify the active principle in the extracts that is associated with immunopotentiation. The results of this investigation encourage undertaking further translational and clinical investigation with the bamboo leaf extracts for treatment of cancer.

## Methods
Materials

Interleukin (IL)-2, IL-12 and interferon-γ (IFN-γ) were purchased from Pierce Biotechnology (Rockford, IL). 1,3-β-Glucan from Lentinula edodes (MW 10 000–40 000), 1,3-β-glucanase from Arthrobacter sp. and rabbit anti-asialo GM1 were obtained from Wako Pure Chemical Industries (Osaka, Japan). RPMI-1640 and fetal bovine serum (FBS) for culture medium was purchased from Invitrogen (Carlsbad, CA). All other reagents were obtained from commercial sources and were used without further purification.

High-Pressure, High-Temperature Extract of Kumaizasa Bamboo Leaves

Kumaizasa is a bush type of bamboo, with a wider leaf than that of the common bamboo found in southern Japan that grows predominantly in the Hokkaido region. The material used here, provided by the Cosmo Bios Research Institute (Akahira, Hokkaido, Japan), was extracted under extremely high temperature and high pressure (via two steps). First, it was extracted at 100°C for 60 min, and residues were subjected to extraction at 121°C, 2 atm, for 30 min. Second, the residues were further treated at 196°C, 15 atm, for 5 min, and then extracted at 120°C for 30 min. The total sugar concentration was determined by the phenol–sulfuric acid method with glucose as the standard (2). To identify the active principle in the extracts, they were subjected to a Sephadex G-50 Fine gel chromatography column (height 90 cm x diameter 1.2 cm; GE Healthcare, Uppsala, Sweden), with the elution buffer being 0.01 M phosphate-buffered 0.15 M NaCl (PBS). Fractions were collected by monitoring absorbance at 280 and 370 nm. The 1,3-β-glucan contents of these extracts, of three major fractions of different molecular size (F-I, F-II, F-III) from the extracts and of a conventionally prepared extract were determined by using a 1,3-β-glucan assay kit consisting of limulus lysate (Maruha, Tokyo, Japan), according to the manufacturer's instructions (3). Further amount of total phenolics in the bamboo leaf extracts and major fractions were also determined according to Folin–Ciocalteu method (4). Total phenolics content was expressed as caffeic-acid equivalents.

To further characterize F-I, 10 mg of F-I in 1 ml of PBS, pH 7.5, was added to 200 units ml⁻¹ of 1,3-β-glucanase, which was obtained from Arthrobacter sp., and was incubated at 25°C in a shaking water bath for 2 days. During the incubation, aliquots of the reaction mixture were taken at given time intervals, of which the enzyme reaction was stopped by heating at 100°C, and then amount of free glucose generated was quantified by using a high-performance liquid chromatography (HPLC) system (LC-VP column; Shim-pack ISA-07/S2504; Shimadzu, Kyoto, Japan) with post-column fluorescence derivatization, which reacted with arginine.

Animals

Male ddY (8 weeks of age), C57BL/6 Cr Slc mice (6 weeks of age) and female BALB/c mice were purchased from SLC, Inc. (Shizuoka, Japan). Mice were housed in groups of
four or five per cage and were maintained at 22 ± 1°C and 55 ± 5% relative humidity. Lighting was automatic on a 12-h light/dark cycle. All experiments were carried out according to the Laboratory Protocol of Animal Handling, Kumamoto University.

**Cell Lines**

K562 cells (human chronic myeloid leukemia) and YAC-1 cells (murine lymphoma) were obtained from Riken Bioresource Center Cell Bank (Tsukuba, Japan) and were maintained in RPMI-1640 containing 10% FBS, 100 units ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin.

**Diet**

The experimental diet was prepared by Funabashi Farm (F2; Funabashi, Chiba, Japan). Bamboo leaf extracts mixed with the diet at 0.05–0.5% (w/w) were vacuum packed followed by gamma ray sterilization and stored at 4°C until use.

**Effect of Bamboo Leaf Extracts and IL-2 on In Vitro Cytotoxicity of Human Natural Killer Cells against K562 Cells**

The Conray–Ficoll method was used to isolate human peripheral blood mononuclear cells from venous blood obtained from healthy volunteers. Human natural killer (NK) cells from heparinized peripheral blood from healthy volunteers were collected by using a StemSep kit (StemCell Technologies, Vancouver, Canada). Bamboo leaf extracts were dissolved in PBS. NK cells (2 x 10⁶ cells) were pre-treated with either IL-2 for comparison or the extracts at final concentrations of 1–100 µg ml⁻¹, or different fractions of the F-I, F-II, F-III and 1,3-β-glucanase-digested F–I at 37°C for 48 h in 5% CO₂/air. NK cells incubated with PBS were used as the control. After incubation, NK cells were washed with culture medium and then used for examination of cytotoxic activity against 2 x 10⁴ target K562 cells in the same culture medium at various ratios [effector/target (E/T) ratios] ranging from 5/1 to 50/1 in 96-well round-bottom microtiter plates. The microtiter plates were centrifuged at 200 g for 5 min to facilitate contact between effector with target cells followed by incubation at 37°C for 4 h in 5% CO₂/air. After incubation, the viable cell number was assayed with a CellTiter-Glo Luminescent Cell Viability Assay kit (Promega, Madison, WI). NK cytotoxic activity was calculated by using the following formula: % lysis = (1 – viable cells in tested group/viable cells in no-drug control group) x 100, where the positive no-drug control group consisted of K562 cells treated with NK cells activated by IL-2 at 0.1–1.0 µg ml⁻¹ in the same culture medium as the reference.

**In Vitro Phagocytic Activity and Nitric Oxide Production by Mouse Macrophages**

Peritoneal macrophages were obtained by intraperitoneal injection of 10% proteose peptone (Becton Dickinson) elicited in BALB/c mice. Peritoneal macrophages (1 x 10⁵ cells/well) were incubated in plastic plates (930 mm), with a cover glass placed in the bottom, at 37°C for 1 h in 5% CO₂/air. Macrophages were then treated for 24 h with the
extracts at concentrations of 0.1–100 µg ml⁻¹, or with different fractions (F-I, F-II, F-III or 1,3-β-glucanase-digested F-I) at concentrations of 10–1000 µg ml⁻¹. Then, 3 ml of 1.0% suspension of yeast (Saccharomyces cerevisiae) was added, and incubation continued for 30 min under the same conditions. The number of yeast cells phagocytosed were counted in evaluating more than 200 macrophages under a microscope.

Macrophages were similarly obtained from C57BL/6 mice, and 1 x 10⁵ cells/well) were then incubated in RPMI-1640 medium with 10% FBS at 37°C for 20 h with 1,3-β-glucan from L. edodes at 1000 µg ml⁻¹ as a reference, with bamboo leaf extract at 1000 µg ml⁻¹ or with and different fractions (F-I, F-II, F-III) from the extracts at 1000 µg ml⁻¹. After the incubation, the production of nitric oxide (NO) was determined by use of the Griess reagent.

**Activation of Mouse Macrophages by Lipopolysaccharide or Lipopolysaccharide Plus IFN-γ**

Peritoneal macrophages were obtained by intraperitoneal injection of 10% proteose peptone. These macrophages (1 x 10⁵ cells/well) were incubated with 1.0 µg ml⁻¹ lipopolysaccharide (LPS) or 1.0 µg ml⁻¹ LPS plus 0.2 µg ml⁻¹ IFN-γ at 37°C for 20 h in RPMI-1640 medium with 10% FBS. Then, production of IL-12 was determined by means of an enzyme-linked immunosorbent assay (ELISA) using mouse ELISA kits (Pierce Biotechnology).

**In Vivo Anti-tumor Activity of the Bamboo Leaf Extract Against S-180, C38 and Meth-A Solid Tumor Models**

S-180 tumors were maintained in ascitic form in ddY mice. S-180 cells (2 x 10⁶) obtained from ascitic tumor were implanted subcutaneously (s.c.) in the dorsal skin of ddY mice. Mice bearing tumors 5–6 mm in diameter were selected for study of anti-tumor activity, 7–10 days after tumor cell injection. For each experiment, control mice were fed a chemically defined basal diet without bamboo leaf extract. Experimental groups were fed a diet supplemented with 0.05–0.5% of the extract. During the experiments, tumor volume, body weight, food intake and survival rate of mice were measured daily. Tumor volume (V) was calculated by use of the following formula: \( V = (L \times W^2)/2 \), where L is the longitudinal cross-section and W is the transverse section of the solid tumor. Eight mice were used for each group. Similar experiments were carried out with the C38 solid tumor model, in which tumor fragments of about 30 mg were implanted s.c. in the dorsal skin of C57BL/6 mice by using a trocker needle.

In addition, anti-tumor activity of bamboo leaf extract was examined in the Meth-A solid tumor model. As with S-180 tumors and Meth-A cells were maintained by intraperitoneal passage in BALB/c mice. Meth-A cells (1 x 10⁶) were implanted s.c. in the dorsal skin of BALB/c mice, and 7 days later when the tumor diameter was ~5 mm, the anti-tumor experiment was conducted via the same protocol as that described above.
Effect of Bamboo Leaf Extracts on Cytokine Induction and NK Cell Activation in C38 Tumor-bearing Mice

As in the aforementioned protocol, C38 tumors were implanted in C57BL/6 mice, and when tumors became palpable (approx 4 mm), mice were fed a diet containing bamboo leaf extract. At 21 days after treatment, all mice were killed under ether anesthesia, and blood, spleen and tumor samples were obtained, for quantification of serum IL-2, IL-12 and IFN-γ by using mouse ELISA kits (Pierce Biotechnology). Splenic NK cells were prepared by using a StemSep kit, and cytotoxicity of NK cells against target YAC-1 cells cultured in vitro in RPMI-1640 medium with 10% FBS was examined. Each tumor sample was also weighed.

To investigate the role of NK cells in anti-tumor activity induced by the extracts, a similar set of in vivo experiments was carried out by eliminating NK cell function with anti-asialo GM1 antibody in C38 solid tumor. When C38 tumors were ~4 mm in diameter, one group of mice was fed a diet containing bamboo leaf extract at 0.1%, and then mice were injected intravenously with diluted (4 times) anti-asialo GM1 antibody on days 12, 15 and 19 (6). Another group fed with the same diet did not receive the antibody treatment.

Statistical Analyses

All data were expressed as means ± SD. Student's t-test was used to determine the significance of differences between each experimental group.

Results

Comparison of Efficiency of Vigorous High-temperature, High-pressure Extraction and Hot-water Extraction

To compare the efficiency of our high-temperature, high-pressure multi-step extraction method and the conventional hot-water (boiling water) extraction method, for each soluble extract we quantified the amount of total sugar (glucose equivalent), optical absorption and phenolic compound at every step. Figure 1 shows that the total sugar content of extracts obtained via the vigorous extraction method was about five times
higher than that obtained via hot-water extraction. Moreover, UV absorption of the extracts was also about six times higher both at 280 and 370 nm compared to that of hot-water extraction (Table 1). Further, total phenolics content from the vigorous extraction was two times higher than that from hot-water extraction (Table 1).

Figure 1. Comparison of the efficiency under conventional hot-water extraction (A) and under multi-step vigorous high-temperature, high-pressure extraction (B). Extraction of each step is shown in B.

Table 1. Increased extract ability: comparisons between conventional versus vigorous extraction, and each values of the Sephadex G50

To identify the active principle(s) in the extracts, we used Sephadex G-50 gel chromatography for purification. The extracts contained three major fractions: F-I (>5 kDa but <20 kDa); F-II (1–2 kDa) and F-III (<1 kDa) (Fig. 2A). F-I contained 1,3-β-glucan, as revealed by the modified limulus test, which is specific for 1,3-β-glucan (Table 1). About 65% (w/w) free glucose was recovered from F-I by 1,3-β-glucanase digestion (Fig. 2C), which indicates that the majority of F-I was 1,3-β-glucan; the remaining portion requires further chemical classification. In comparison, the elution profile of preparation by the conventional extraction method was completely different from that of vigorous extraction (Fig. 2A versus 2B).
Effect of Bamboo Leaf Extracts on *In Vitro* Cytotoxic Activity of Human NK Cells

To examine biological activities of unfractionated bamboo leaf extracts, we first evaluated their cytotoxic activity of NK cells *in vitro*. Cytotoxic NK activity against K562 cells increased in parallel with the increase in the E/T ratio (Fig. 3), with the 100 µg ml⁻¹ extract at the E/T of 50 showing the highest cytotoxicity. More important, the extracts showed that its immunopotentiating effect on NK cell cytotoxicity was comparable to that of pure IL-2 (Fig. 3A versus 3B). This NK cell activation of F-I was suppressed about 50% by 1,3-β-glucanase digestion, which indicates a role for 1,3-β-glucan (Fig. 3C).

**Figure 2.** (A) Sephadex G-50 gel chromatography of bamboo leaf extracts under vigorous condition and conventional condition (B). Absorbance at 280 nm (open square); absorbance at 370 nm (open diamond). Values of glucose equivalent obtained by the phenol–sulfuric acid method (closed square). Each tube contained 6.0 ml. (C) Time course of liberation of free glucose during digestion of 10 mg ml⁻¹ F-I with 200 units of 1,3-β-glucanase (see text for details).

**Figure 3.** Enhancement of cytotoxic activity of human NK cells at various concentrations of bamboo leaf extract at different E/T ratios (*n* = 8/group). Suspensions of effector NK cells were added to target K562 cells at E/T ratios of 5:1, 25:1 or 50:1. (A) Cytotoxic activity for control, PBS (closed circle); IL-2 at 1 µg ml⁻¹ (open square); IL-2 at 10 µg ml⁻¹ (closed square). (B) That for bamboo leaf extracts at 1 µg ml⁻¹ (closed triangle), 10 µg ml⁻¹ (open diamond) and 100 µg ml⁻¹ (closed diamond). (C) That for unfractionated bamboo leaf extract at 100 µg ml⁻¹ (closed diamond), F-I at 100 µg ml⁻¹ (closed triangle), F-II at 100 µg ml⁻¹ (closed square), F-III at 100 µg ml⁻¹ (open square), 1,3-β-glucanase-digested F-I at 100 µg ml⁻¹ (open triangle) and PBS (closed circle). Values represent means ± SD. **P < 0.01 and *P < 0.05 versus controls.**
The Effect of Bamboo Leaf Extracts on Phagocytic Activity and Production of NO by Mouse Macrophages

The extracts significantly increased macrophage phagocytic activity compared with the untreated group (Fig. 4A), with phagocytic activity induced by the extracts being about two to three times higher at 1.0, 10 and 100 µg ml⁻¹ than that of the control. Figure 4B illustrates phagocytic activity of macrophage induced by each fraction: F-I (1,3-β-glucan) significantly increased. This result was similarly seen in the unfractionated extract. However, this potentiation of phagocytotic activity of F-II was not so apparent, and also the 1,3-β-glucanase-digested F-I fraction had almost no activity (F-I versus digested F-I at 100 or 1000 µg ml⁻¹). F-III seemed to have <10% of the activity of F-I. Figure 4C shows that bamboo leaf extract and F-I significantly increased macrophage NO production, similar to 1,3-β-glucan from L. edodes. F-I induced higher NO production than the original unfractionated extract, whereas F-II and F-III caused almost no increased NO production.

**Figure 4.** *In vitro* phagocytic activity of peritoneal macrophages from the ddY mouse (A and B), and NO production by C57BL/6 mouse macrophages (C). ddY mouse macrophages were treated with different doses of bamboo leaf extracts; control macrophages did not receive the extracts (A). (B) Macrophages were treated with different doses of unfractionated bamboo leaf extracts or different fractions (F-I, F-II, F-III and 1,3-β-glucanase-digested F-I) of extract, or PBS (control). (C) Macrophage NO production after treatment with PBS (control), 1,3-β-glucan from L. edodes (reference), unfractionated bamboo leaf extract, F-I, F-II or F-III at 100 µg ml⁻¹. Values represent means ± SD (*n* = 3). **P < 0.01 and *P < 0.05 (extracts versus control).

Effect of Mouse Strain, C57BL/6 versus BALB/c, on Activation of Macrophages by LPS or LPS Plus IFN-γ

As shown in Fig. 5, macrophages from both mouse strains stimulated by LPS or LPS plus IFN-γ showed significantly increased IL-12 and NO induction compared with untreated groups. A notable finding was that C57BL/6 macrophages produced 10 times higher amounts of IL-12 and NO than BALB/c macrophages.
Figure 5. Difference in LPS response among different mouse strain—C57BL/6 (A) and BALB/c (B)—as related to macrophage response (IL-12 and NO induction) to activation by LPS or LPS plus IFN-γ. Macrophages were obtained as described in Methods. Note the great difference in the scale of the ordinates. Values are means ± SD. *P < 0.05 and **P < 0.01 versus the normal group.

Anti-tumor Activity of Bamboo Leaf Extracts in Mice

S-180 Solid Tumor in the ddY Mouse Model

All doses of bamboo leaf extracts significantly suppressed tumor growth compared with the control, with the extracts at the 0.1% concentration showing the strongest activity (Fig. 6A). This treatment also greatly improved the survival rate of tumor-bearing mice, again with the extracts at 0.1% producing the highest survival: i.e. the %T/C (treated/control) was 142.6% (Fig. 6B). Furthermore, a significant number of tumors in mice fed with bamboo leaf extracts regressed completely; the numbers of tumor-free mice after treatment/initial numbers of tumor-bearing mice were 1/8, 3/8 and 2/8 at extract concentrations of 0.05, 0.1 and 0.5%, respectively. Treated groups showed no significant difference in body weight (data not shown).

Figure 6. Anti-tumor activity of bamboo leaf extracts. Tumor growth (A), and survival curves (B), for S-180-bearing ddY mice fed with the whole extract. (C) A comparison of anti-tumor activity between vigorous extraction and conventional hot-water extraction in S-180-bearing ddY mice. ddY mice were inoculated with $2 \times 10^6$ S-180 cells. (A) Anti-tumor activity of the control die and of the diets containing bamboo leaf extracts added at 0.05% (open square), 0.1% (closed triangle) or 0.5% (open diamond). Values are
To compare anti-tumor activity of the conventional boiling water (100°C) extraction and the vigorous extraction methods, a similar set of in vivo experiments was performed in S-180 solid tumor model with the extracts at the 0.1% concentration. Tumor growth was not suppressed in the conventional extraction group (Fig. 6C).

**C38 Solid Tumor in the C57BL/6 Mouse Model**

As Fig. 7A shows, bamboo leaf extract significantly suppressed tumor growth compared with the control. This treatment also greatly improved the mean survival rate (%T/C was 158%) (Fig. 7B).

*Figure 7. Anti-tumor activity of bamboo leaf extract (A) and survival curves (B) in the C38 solid tumor model. Mouse colon C38 tissue specimens (about 30 mg) were implanted s.c. in C57BL/6 mice. (A) Anti-tumor activity of the control diet (closed circle) and of the diet containing bamboo leaf vigorous extract at 0.1% (closed triangle). *P < 0.05 versus the control. (B) Survival curves for the control diet and the diet containing bamboo leaf extract added at 0.1%.

**Change in Immunological Parameters in C38 Tumor-bearing Mice**

Bamboo leaf extract at 0.1% also significantly suppressed C38 tumor growth at 21 days after treatment compared with the control (Fig. 8). The average tumor weight of the treated group was 1.54 ± 0.46 g, whereas that of the untreated control group was 2.65 ± 0.51 g, significantly higher.
To further clarify the anti-tumor effect of the bamboo leaf extracts, we measured IL-2, IL-12 and IFN-γ levels in mice after feeding the extract in the diet for 21 days. Serum levels IL-12 and IFN-γ were significantly raised in C38 tumor-bearing mice fed with extracts at the 0.1% concentration compared with levels in the control group (Table 2). More important, a significantly higher ($P < 0.01$) concentration of IL-12 was induced by the extracts, which may stimulate NK cell activity, and, in fact, significantly elevated cytotoxic activity of splenic NK cells occurred in C38 tumor-bearing mice (Fig. 8B).

To clarify anti-tumor activity mediated by the NK cells, we used anti-asialo GM1 antibody in the C38 tumor model. Mice fed the bamboo leaf extract at 0.1% had significantly suppressed tumor growth, whereas a similar group of mice that received the extracts and anti-asialo GM1 antibody treatment showed no significant suppression of tumor growth, with tumor volume being similar to that of the control (Fig. 9).
**Discussion**

Alternative therapeutic tools obtained from plants to fight cancer have garnered great interest. In the present study, we demonstrated the remarkable anti-tumor activity of bamboo leaf extracts obtained under vigorous high-temperature, high-pressure extraction conditions. To our surprise, these extracts had far superior activity than extracts obtained via the conventional hot water method, as seen in Table 1, Fig. 1 and Fig. 6C. This new aqueous extraction method not only yielded a higher amount of extract from the leaves but also greatly increased the immunopotentiation effect of the extracts (Fig. 6C). Preliminary extract purification with Sephadex G-50 and G-100 chromatography produced three major fractions of different molecular size: F-I was large, between 5 and 20 kDa; F-II was of medium size, 1–2 kDa; and F-III was small, <1 kDa (Fig. 2A). F-II

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Figure 9. Involvement of NK cells anti-tumor activity of bamboo leaf extract in the C38 solid tumor model. Anti-tumor activity of the control diet (closed circle), the diet containing the extract at 0.1% (closed triangle), or the diet containing the extract at 0.1% plus treatment with anti-asialo GM1 antibody (open triangle). The open inverse triangle indicates injection of anti-asialo GM1 antibody. **P < 0.01 versus the control. The arrow at day 12 indicates the start of dietary extract feeding of the test group.

**Meth-A Solid Tumor in the BALB/c Mouse Model**

The bamboo leaf extract had no significant effect on Meth-A tumor growth and survival rate of mice compared with the control (data not shown). This finding is consistent with the fact that this strain had a much lower response than that of the C57BL/6 strain (about 10%) to LPS or LPS plus IFN-γ treatment, as shown in Fig. 5.
and F-III were not obtained by the conventional hot water extract (Table 1). F-I contained predominantly 1,3-β-glucan because 1,3-β-glucanase digested the majority of F-I free glucose (Fig. 2C).

With regard to biological activity, F-I significantly augmented the phagocytic activity of macrophages (Fig. 4B) to a degree similar to that achieved by unfractionated bamboo leaf extract (Fig. 4A). F-I digestion by 1,3-β-glucanase eliminated the enhancing potency of the phagocytic activity of F-I (Fig. 4B). F-II showed almost no activity and F-III, only very weak activity. Furthermore, F-I, similar to unfractionated bamboo leaf extract, significantly increased macrophage NO production (Fig. 4C). This F-I-stimulated NO production was comparable in potency to that of 1,3-β-glucan from L. edodes, an immunopotentiating agent approved for use in Japan. F-II and F-III did not increase NO production compared with controls.

As shown in Table 1 and other data in Results section, the present preparation (vigorous extraction) and conventional hot-water extraction product were greatly different in the quality and quantity, namely the chromatographic pattern upon Sephadex G-50 column, UV absorbing components as well as phenolic contents (Figs. 1A, B and 2A, B). The difference in biological activity was immense: practically no anti-tumor activity in the conventional extracts was seen, while the present extracts were clearly effective even at 0.05%, and more so at 0.1% in the diet (Fig. 6C). This again shows qualitative difference between the two extractions.

Cytotoxic activity of human NK cells obtained from peripheral venous blood of healthy volunteers was increased by bamboo leaf extracts at E/T ratios of 5, 25 and 50 (Fig. 3). Among the three major fractions, F-I induced the greatest increase in cytotoxic activity of these NK cells (Fig. 3C). Digestion of F-I by 1,3-β-glucanase resulted in little cytotoxic activity (Fig. 3C). Significant NK cell activation by the extract was also observed in tumor-bearing C57BL/6 mice (Fig. 8B). Depletion of NK cells with anti-asialo GM1 antibody in the C38 tumor model eliminated this anti-tumor activity (Fig. 9). Thus, NK cells appeared to serve as a major effector of tumor growth suppression (Figs 6A, 7A and 9).

Marked anti-tumor activity as evidenced by an improved survival was found for extracts in S-180 and C38 tumor-bearing mice (Figs 6B and 7B). However, improved survival was not the case for Meth-A tumor-bearing BALB/c mice. It is interesting to note that BALB/c mice are known to have a low response to LPS and IL-12. C57BL/6 macrophages produced 10 times more IL-12 and NO than did BALB/c macrophages (Fig. 5). This finding may indicate that a low immune response to bamboo leaf extract in BALB/c mice may result in the poor -tumor response against Meth-A tumor in this mouse strain.

We demonstrated that serum levels IL-12 and IFN-γ and splenic NK cell activity were significantly raised in C38 tumor-bearing mice fed with extract at the 0.1% concentration compared with levels in the control group (Table 2, Fig. 8). These results suggest that the
extracts magnified the immune response even in the host bearing tumor, thereby suppressing tumor growth.

In any case, the potency of these anti-tumor activities is comparable or similar to, published results with an advanced formulation technology (liposome formulation) of conventional anti-cancer agents, such as cisplatin (CDDP) or Adriamycin® (ADM) in the same mouse models (7). The tumor inhibition rates for these ADM and CDDP were 55.3 and 72.6% in S-180 solid tumor model (8, 9). In our study, bamboo leaf extracts at the concentration of 0.1% showed 70.1%, which is comparable to that of ADM and CDDP. Thus, these results indicate that the high-temperature and high-pressure extracts of bamboo leaves can offer a potential and alternative for anti-tumor strategy without any obvious toxicity.

We reported earlier that a hot-water extract of shiitake mushroom, KS-2, which contained water-soluble mannan and glucans, could be absorbed after oral administration, enter the blood circulation and accumulate in the kidney, liver and lymph nodes via intestinal uptake (10–12). Furthermore, some polysaccharide fragments were recovered from urine. This intestinal uptake and entry into circulation may also be valid for these bamboo leaf extracts, because of a similar chemical entity, \( \beta \)-glucan (12).

Several polysaccharide preparations such as lentinan, schizophyllan, krestin and KS-2 contain \( \beta \)-glucans (or 1,3-\( \beta \)-glucans) and that from various species of other mushrooms \( \text{Agaricus blazei} \) (10–19). Among these preparations, lentinan and krestin were approved by Japanese Government and are used as biological response modifiers for anti-cancer. Their immunostimulating effect results in activation of not only NK cells but also macrophages, which are known to produce IL-12 and induce production of IFNs, and thus anti-tumor effects as well (18). We (KS-2) and others \( \text{(A. blazei and other mushrooms)} \) discussed that these anti-tumor activity was mediated by multiple elements such as induction of IFN-\( \gamma \), IL-2 and IL-12 and activation of macrophages and NK cells (10–18).

A dietary supplementation of vegetable soup preparation given in clinical trials with patients having stage III and IV non-small cell lung cancer was reported (20, 21). The diets, which contain vegetables and fruits with multiple functional components have no side-effects, and believed to be effective for prevention of cancer and inflammation. On the basis of data presented here, we believe that an extract from leaves of Kumaizasa has a beneficial role in cancer treatment, via stimulating the immune response, and cancer prevention. Another candidate macromolecular components in plants, lignin is also known to have very potent radical-scavenging activity (22, 23), thus it may also warrant attention.

Treatment of tumors with conventional cytotoxic agents can reduce the number of cells to not less than \( 10^7 \)–\( 10^{10} \), but often simultaneously with suppression of the host's immune system. On the contrary, injection of tumor cells of about \( 5 \times 10^5 \) to \( 5 \times 10^6 \) cells into immunocompetent healthy mice in the experimental tumor model usually results in rapid tumor growth palpable within 2 weeks. Similarly, under clinical setting, rapid mass can be formed after chemotherapy, because formidably large numbers of surviving tumor
cells still remain unkillled and patients cannot tolerate any further dosing of chemotherapy. To make the situation worse, a patient's immune system is often greatly suppressed by such treatment that new solid tumor can recur with relative ease. Therefore, we still need potent immunostimulating agents that have no side effects like this bamboo extracts. Furthermore, the suppressed immune state is related to a higher incidence of cancer in the aged population, so immunostimulation may be useful for cancer prevention among aged and high-risk populations.

In conclusion, we report here that a dietary supplement of bamboo leaf extracts at a concentration of 0.1% suppressed solid tumor growth and prolonged the life span in S-180 and C38 models. Such oral administration of the extracts induced Th1-type cytokine production. Also, the extracts stimulated cytotoxic activity of NK cells and macrophages both in vitro and in vivo. These results strongly suggest the great potential of this bamboo leaf extract as an anti-cancer and cancer preventive agent.

Footnotes

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Acknowledgements

We thank Mr Shingo Kikuchi of Cosmobios Ltd Co, Hokkaido, Japan, for supplying samples of bamboo leaf extract, and to Ms Judith B. Gandy to English editing.


Received September 24, 2007; accepted March 20, 2008

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