

Agaricus blazei Murill - immunomodulatory properties and health benefits

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Submission date: August 27, 2012, Acceptance date: November 15, 2012; Publication date: November 17, 2012

Abstract

The *Agaricus blazei* Murill (AbM), also known as *Agaricus brasiliensis* L. due to its origin in Brazilian rain forest, is an edible mushroom of the *Basidiomycetes* family, which also comprises medicinal mushrooms such as *Hericium erinaceus* and *Grifola frondosa*. AbM has been used in traditional medicine locally and also recently as a health food worldwide. Since it has been found to possess immunomodulatory properties, its biological and health-related effects, as well as its isolated active ingredients e.g. beta-glucans, have been examined by scientists. Other investigations have been performed with mixed mushroom products, such as AndoSanTM, which contains mostly AbM, but also the two other mushrooms above. AbM-related benefits reviewed here include effects against cancer, infections, inflammation, allergy/ asthma and diabetes. Effects of AndoSanTM and other AbM-based extracts have been compared in a bacterial sepsis model.

Keywords: *Agaricus blazei*, AndoSanTM, allergy, asthma, cancer, infection, inflammation, immunomodulation.

Introduction:

From the very beginning of our civilization, man has used mushrooms to produce fermented foods and medicines. In ancient Egypt, fermentation was considered a gift from the gods, just like in ancient Rome. Asians have attributed curative properties to mushrooms, and there are Chinese reports from around 500 BC on the general medicinal and anti-cancer properties of *Ganoderma* extracts which have been passed on from generation to generation. In the late twentieth century, researchers in Japan demonstrated the beneficial effects of a Brazilian mushroom, later identified as *Agaricus blazei* Murill (AbM), which quickly gained attention in the scientific world [1, 2].

Agaricus blazei Murill (Cogumelo de Deus), which means "fungus from God", is an edible, medicinal mushroom originally found in a small village of Piedade, in the mountain region near São Paulo, Brazil. The absence of serious disease in the elderly, and the longevity in the population surprised researchers who found that this was due to AbM being a part of the diet of this healthy local population.

AbM has traditionally been used for the prevention of cancer. Its putative anti-tumorigenic effects have sparked the interest of scientists who decided to put AbM through rigorous scientific scrutiny. A series of laboratory studies gathered from all over of the world have demonstrated anti-cancer properties of AbM and its impact on the immune system [3-10]. AbM is also used to treat a wide variety of diseases including chronic hepatitis [11, 12], allergies [13], and asthma [14].

AbM contains large quantities of polysaccharide compounds like β (beta) -1,6-glucan [7], alpha-1,6- and alpha-1,4-glucan [15], glucomannan [16], β -1,3-glucan [8] and more. Beta-glucans are quite diverse in size and structure and thus possess varied immunomodulating abilities. Data from in vitro studies and animal models indicate that AbM extracts bring about positive effects in different disease models. They boost the immune system by activating white blood cells, including "immune directors", and thus enhancing its action against cancer [3, 4, 7, 16-19] and infection [12, 20-23]. These immune-enhancing effects on the activity of macrophages and natural killer (NK) cells, lead to the destruction of microbes and tumor cells much more efficiently, not only through innate immunity, but also adaptive immunity by the activation of dendritic cells, and in consequence, engagement of specialized lymphocytes [24, 25].

Recently, AbM extracts were used in conjunction with traditional classical cancer treatments, such as chemotherapy [26], which similar to X-irradiation of tumors, have many undesirable side effects that degrade the quality of life. AbM could prove valuable in easing side effects and thus improving the quality of life for cancer patients [26]. Careful clinical studies comparing the activity of isolated compounds from whole mushroom extracts and epidemiological data are still necessary to determine whether AbM offers real clinical benefits. Besides beta-glucans and proteoglucans, there are undoubtedly other substances in this mushroom with direct health-promoting properties. As time and research go on, it has become more and more certain that AbM can serve as a support for patients of today with different diseases as a supplemental treatment to Western medicine, and not only be regarded as a remedy in traditional medicine.

The immune system – our defense mechanism system

The immune system is comprised of a collection of molecules, cells, and organs whose complex interactions form an efficient system that is usually able to protect an individual from both outside invaders and its own altered internal cells, e.g. cancer cells. When the immune system works properly, humans remain healthy. However, the immune system in many people is weakened by stress, poor eating habits and a multiplicity of pollutions in air, water and food. With an immune system in disarray, affected people can suffer from a number of diseases.

The immune system is supported by signal molecules such as cytokines. including the interferons and chemokines among others, that are essential to the immune system as they induce

an antimicrobial state in other cells. These signaling chemicals are secreted by various leukocytes responsible for activating other cells, coordinating, and regulating important biological processes such as inflammation [27]. Inflammation is an immune defense response; it results in quicker blood flow, increased temperature and finally, attraction of immune cells to the threatened area [27]. Inflammation can be caused by microbial infections and physical agents causing tissue injury. The function is to destroy invaders and induce tissue repair and healing.

The elements of the immune system are divided into two functionally distinct parts: the innate and the adaptive (acquired) immune systems. It is apparent that these two groups are in no way isolated; a complex dialog is constantly going on between them [28]. The numerous cytokines that are produced, and the array of surface molecules that are expressed in response to external or internal threats, are critical for the control of all other immune elements and their concerted action [29]. The dendritic cells, which can be of myeloid or lymphocytic origin, are directors of the entire immune system.

T-cells are one of the most important elements in the human adaptive immune system. Their role is in directly destroying infected or cancerous cells, giving help to develop antibody-producing cells and in maintaining memory in so-called memory cells that live for years and can recognize former microbial attackers. Whereas dendritic cells are the directors of the entire immune system, T helper cells represent the local control center for the adaptive immune system [30, 31].

Many other cell types, including macrophages, NK cells, and B-cells are important in the “effector phase” of the immune response [30, 32].

The innate immune system

The innate immune response functions as a first line of defense against infections. It consists of soluble factors, such as complement proteins, and diverse cellular components of the myeloid lineage; granulocytes, monocytes/macrophages, dendritic cells and mast cells, as well as natural killer (NK) cells [27, 28, 30, 33]. Macrophages (“grand eaters”) and dendritic cells are important regulators of immune responses through their uptake of foreign elements, digestion of them, and presentation of smaller bits of these so-called antigens to T cells. This antigen presentation is a critical determinant of the degree of T-cell activation, and contributes to the T-cell’s ability to discriminate between self-cells and foreign antigens [33]. Granulocytes, monocytes/macrophages, and NK cells are responsible for killing invaders, function as key innate effector cells, and facilitate communication by releasing important chemo-attractants [27]. In contrast to the above non-specific effectors, NK cells have a specific function in their killing of infected and cancerous cells. Unfortunately, their mode of action is restricted to attacking only cells to which they have been previously exposed. They play a significant role because they are an important first line of defense against malignant cells and cells infected with viruses, bacteria, and protozoa. Like monocytes and granulocytes, NK cells produce signal substances, cytokines that activate and regulate other immune elements with specific receptors on their surfaces, for such cytokines. In addition, they independently handle the destruction of tumor cells [10, 34].

The adaptive immune system

The adaptive immune system is composed of highly specialized, systemic cells and processes

that eliminate or prevent pathogenic challenges. It is also able to distinguish foreign cells from self, and can distinguish one foreign antigen from another. While a macrophage will engulf any foreign cell (and many destructed self-cells), adaptive immune cells have mechanisms for selecting only a precisely defined target. A specific feature of the adaptive immune system enables immunization and resistance to reinfection, from the same microorganism [30].

The cells which make up the immune system are the lymphocytes, of which there are two main sub-populations: B cells and T cells. The lymphocytes originate in the bone marrow. The T lymphocytes are “educated” in the thymus to distinguish between own and foreign antigens (processed bits of bacteria, cells etc).

Lymphocytes are developed from stem cells in the bone marrow. Some migrate to the thymus and develop into T lymphocytes (or T cells), while others remain in the bone marrow and develop into B lymphocytes (or B cells). Both B- and T-cells then migrate to lymphoid tissue such as lymph nodes. B cells are most effective against invading bacteria and their toxins, while T cells recognize and destroy body cells gone awry, including virus-infected cells and cancer cells [27, 32]. T cells also give help to develop the B cell-derived antibody-producing plasma cells.

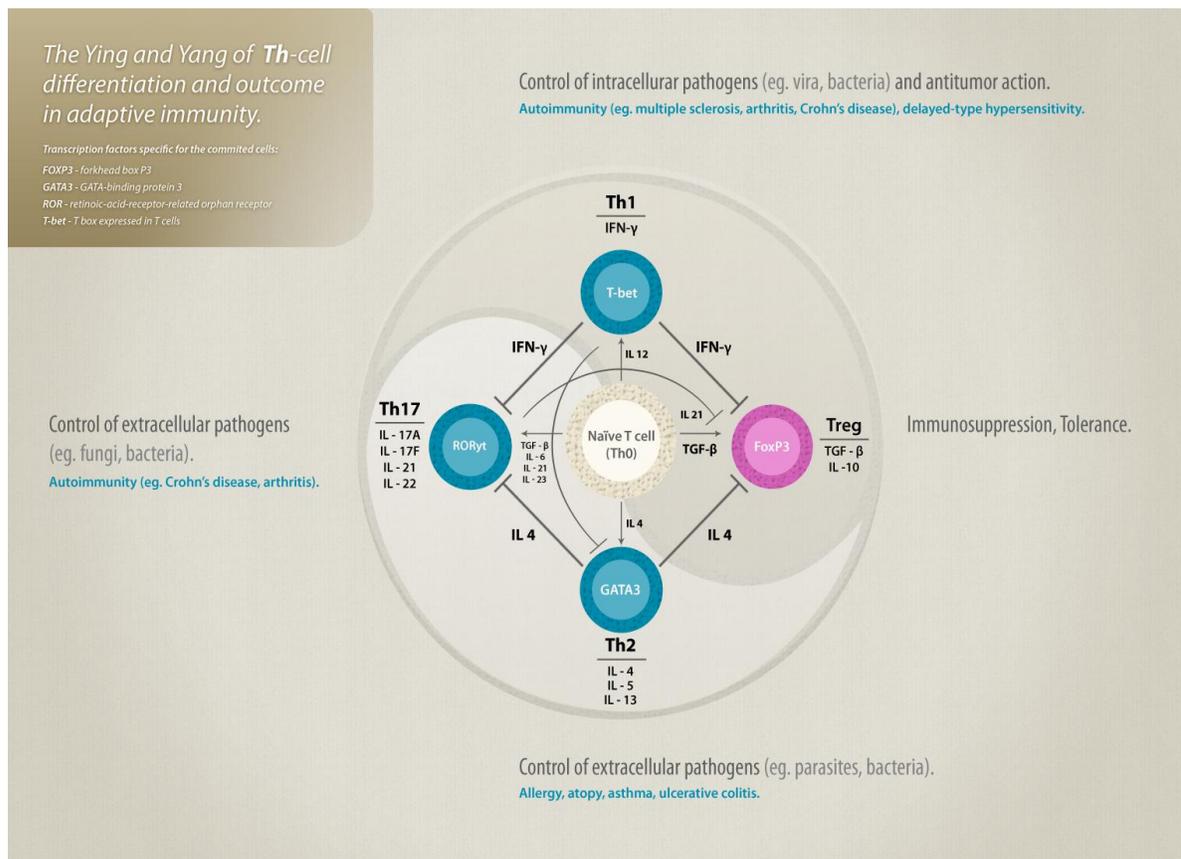


Figure 1. T helper (Th) cells are divided into Th1 cells that normally have anti-infection and anti-tumor effects, and Th2 cells that normally have anti-parasitic and anti-rejection (e.g. of fetus during pregnancy) effects. There are also interacting T-regulatory cells and Th17 cells, which control the Th1 and Th2 responses. The cellular interaction is done by cytokines, which promote (→) or inhibit (---I) T cell differentiation and activation.

Whenever T cells are activated, some of them become "memory" cells. Then, the next time that an individual encounters that same antigen, the immune system is ordered to destroy it quickly. The degree and duration of immunity depend on the kind of antigen, its amount, and how it enters the body. An immune response is also dictated by heredity; some individuals respond strongly to a given antigen, others weakly, and some not at all [30, 34].

T cells break down into two crucial sub-populations: T helper cells (Th) and cytotoxic (killer) T cells (CTL). Th cells are crucial because they are engaged in activating B cells and macrophages. This is a perfect example of immune system cooperation. CTL on the other hand, act by killing virus infected cells. A schematic illustration is shown in Fig. 1 of the balance between T cell responses in adaptive immunity and the resulting effect on health threats as well as the different T cell responses (blue text). The overall function of lymphocytes may be divided into i) B cells that are effectors of the humoral immune response through antibody production, and ii) T cells which are effectors of the direct cell-mediated immune response.

Autoimmunity

Autoimmunity is surprisingly common, and amazingly complex. This is an over-reaction of the immune system and is caused by cross-reactivity - the pathogen seems to look like self-cells, which are usually ignored because they are not seen in an inflammatory ("danger") context. However, the pathogen is seen in such a context and drives the activation and expansion of cross-reactive T cells that then return home and attack the self-antigen, causing autoimmune disease that can then expand and become more severe. This is a perfect example of how fragile the immune system is.

Autoimmunity may arise in several ways. Firstly, by a reduction in suppressor T cell (T regulatory cell) activity; secondly, due to the modification of normal self-antigens, by drugs, environmental chemicals, viruses, or mutations; and thirdly, due to an exposure in the direction of an antigen that is very similar to self-antigen ("molecular mimicry"). This is a good example of how important it is for the immune system to stay in balance with itself since any imbalance may cause a threat and might bring about unwanted repercussions [35-38].

Beta-glucans as an active component of *Agaricus blazei* Murill

Beta-glucans are naturally occurring polysaccharides, which are polymers of glucose with beta 1,3/1,6-linkages, and might be found in the cell walls of algae, bacteria and certain fungi and mushrooms. They differ in their structure, molecular mass and solubility.

AbM is well-known to contain a high level of beta-glucans in three different forms: β -(1-3)-D-glucan, β -(1-4)-D-glucan, and β -(1-6)-D-glucan [3, 7, 8, 16]. Research has shown that insoluble (1,3/1-6)- β -glucan, has greater biological activity than that of its soluble (1-3/1-4)- β -glucans counterparts [39]. The differences between β -glucan linkages and chemical structure are significant in regards to solubility, mode of action, and overall biological activity. Beta-glucans derived from AbM are known for stimulating immune cells like NK cells, macrophages, dendritic cells, and granulocytes (polymorphonuclear leukocytes) [3-5, 7, 16, 17].

In addition to beta-glucans, AbM's effect on the immune system is believed to be the result of other polysaccharides such as proteoglucans [10]. What is important, is the very high content of beta-glucans in AbM compared to other mushroom species, and which may be the antitumor

principle of this mushroom [8]. For instance, its relative - the champignon (*Agaricus bisporus*) - has much less beta-glucan and more mannan sugars. In general, the known active compounds of AbM include: beta-glucans, ergosterol (provitamin D2) derivatives, glucomannan, mannogalactoglucan, proteoglucans and riboglucans [3, 8, 10, 15-17, 19, 25, 40]. The polysaccharides' phyto complex is thought to be responsible for its immune-stimulant and anti-tumorigenic properties. Kawagishi [7] was the first to isolate active anticancer compounds purified from the sodium hydroxide extract of the fruit body of AbM. The author detected polysaccharides with apparent antitumor activity [7]. Current clinical studies are yielding conformatory results [26, 41], which may need further validation.

It has been scientifically proven that some forms of beta-glucans can play an important role in human health [42, 43]. They are able to help the immune system because they represent a common "danger signature" of health-threatening mushrooms and fungi [44-48]. Since harmless edible mushrooms such as AbM contain high amounts of these beta-glucan danger signals, this can be exploited by us to enhance the alertness of our immune system for general prevention of disease and as an additive treatment to help combat existing disease.

Mechanism of action

A healthy and strong immune system is crucial for a healthy body. One of the most important properties of AbM is the ability to boost immune responses. Immunomodulation might be caused by a group of extremely efficient immunostimulants such as β -glucans (β -1-3-D-glucans and β -1-6-D-glucan), which are active constituents of AbM. Glucans activate a number of cells involved in immune reactions [49, 50]. AbM also contains other smaller uncharacterized molecules that are important for AbM's health effects (Hetland G, unpublished findings). A beneficial effect has been suggested of beta-glucan on generation of new blood cells in the bone marrow of cancer patients who received such add-on (adjuvant) treatment during chemotherapy [43]. However, in vitro experiments with bone marrow stem cells indicate that AbM does not promote such blood stem cell multiplication (Tangen and Hetland, unpublished results). However, AbM extracts rather enhance innate immunity by targeting and activating the innate immune cells such as macrophages, monocytes, dendritic cells, granulocytes and NK cells. In short, this implies that AbM is an activator of these phagocytic cells, including the non-phagocytic NK cells [24, 26, 48, 49]. In addition, AbM extract stimulates cytokine production, which is needed for activities of these immune cells. The AbM extract stimulates maturation of dendritic cells that present antigen/self-antigen complexes that activate T-cells [24, 25, 51].

The reason for this forceful and swift reaction of innate immunity when in contact with an edible and harmless mushroom such as AbM, is its shearing of pathogen-associated molecular patterns (PAMP) with other highly poisonous and health-threatening fungi and mushrooms. PAMP, such as beta-glucans form the main cell wall skeleton in mushrooms and fungi and are recognized immediately by so-called pattern-recognition receptors, such as Toll-like receptor 2 (TLR2), dectin-1 and complement receptor 3 (CR3) [48, 52-54]. When these receptors are engaged, signals are transmitted into the immune cells, stimulating them to produce and release biologically active mediators such as cytokines [24] that are signal substances needed in the communication with other immune cells, and nitric oxide and hydrogen peroxide [55] that directly kills invading microbes. Since AbM does not have a direct bacteria-killing effect, its

action on bacterial sepsis is via modulation of cells and a cascade defense like the complement system, an important part of the innate immune system [56].

Health Benefits of Agaricus Blazei Murill

Cancer

Cancer is a disease which is characterized by uncontrolled cell growth (a division beyond normal limits), and is the leading cause of death in Western countries. Cancer may affect people at all ages and genders. The risk for most types of cancer increases with age, and is primarily impacted by environmental factors with a 90-95% of cases influenced by lifestyle and environmental factors and 5-10% due to genetics [57].

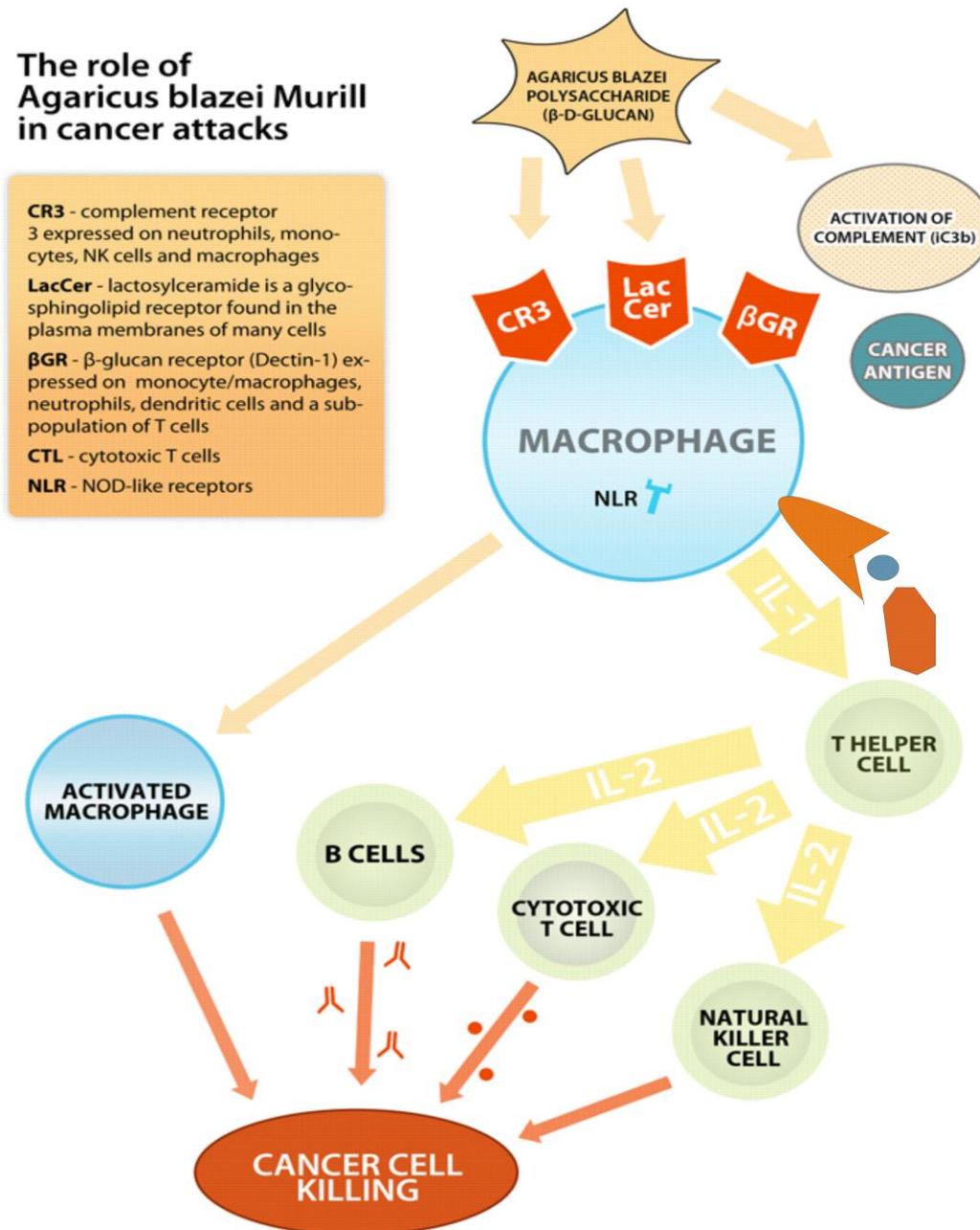
It has been shown that a significant number of cancer patients have been taking complementary medical therapies such as medicinal mushrooms, while receiving their conventional anti-cancer treatments. Mushroom extracts have been proposed to have anti-cancer properties and may act through two separate mechanisms: i) direct cytotoxic effect and ii) indirectly through immunomodulatory action [50].

The orally administered β -glucans were taken up and processed by macrophages via surface receptors such as Dectin-1 with or without TLR-2/6, and complement receptor (CR)-3, eliciting an immune response. One of the actions is the phagocytosis of antibody-tagged tumor cells [1]. Another direct consequence of AbM treatment in cancer patients, and possibly brought about by the mushroom's beta-glucans, is activation of the patients' NK cells. Studies found that a group of active ingredients such as beta-glucans, proteoglycans and ergosterol were responsible for the induced tumor regression in mice [3, 8, 10, 15-17, 25, 40]. Additionally, mice which were supplemented daily with beta-glucan isolated from AbM, exhibited a decreased level of spontaneous metastasis [58]. In another set of experiments, fat soluble ergosterol from AbM proved to be an antiangiogenic substance, which hampers blood vessel formation and as a result, reduces tumor growth and metastasis in sarcoma- and lung carcinoma- bearing mice [59]. Another action of AbM in this context, is the reduction in blood levels of IL-8, an inflammatory and vessel-forming cytokine.

The antitumor effects of AbM extracts have been attributed to the induction of apoptosis (programmed cell death) of cancer cells and the activation of NK cells [9, 10]. It has been reported that increased NK cell activity correlates with increased infiltration of cells in tumor sites. Moreover, the analysis of leukocytes from hepatitis C virus (HCV) patients supplemented with AbM extracts, revealed an increased expression of genes, which are crucial in antitumor defense [11]. One early clinical trial focused on patients with acute leukemia and demonstrated that extracts from AbM inhibited leukemia cells [60]. However, there are no published follow-up studies on the use of AbM against leukemia.

At the moment, AbM is used as adjuvant treatment alongside traditional treatments for cancer. AbM could prove valuable in both its antitumor effects via immune stimulation and in easing side effects and thus improving the quality of life for cancer patients as seen in one clinical study [26]. The effect of AbM on prostate cancer has been unequivocal [9, 61]. AbM, in a mixture with other medicinal mushrooms, has also been reported to act against breast cancer cells in vitro [62]. However, more clinical trials are needed to confirm this mechanism of action.

In addition to AbM's induction of apoptosis in some cancer cell types, its immunomodulatory role against cancer, is shown in Fig. 2.



Research in progress

Currently, there are some ongoing animal and clinical studies, which are focused on the role of AbM extracts in treating cancer.

Colon cancer is a cancer of the large intestine (colon), the lower part of the digestive system. Rectal cancer is a cancer of the last several inches of the colon. When occurring together they are often referred to as colorectal cancers. Most cases of colon cancer begin as small, noncancerous

“lumps” of cells called polyps. Over time, some of these polyps may become colon cancers.

A scientific study was performed in Oslo, Norway in the last year, with an AbM-based extract, AndoSan™, in a mouse model, which had the same genetic defect as most common predisposing conditions of human colon cancer; this data is now being examined. If it comes out positive, a clinical study in cancer colon patients will follow to determine whether AbM can have a preventive effect on the development of colon cancer in individuals prone to this disease.

Multiple myeloma (from Greek myelo-, bone marrow) is uncontrolled clonal proliferation in the bone marrow of plasma cells that are derived from B cells. Plasma cells are normally responsible for the production of specific antibodies which help fight infections. In multiple myeloma, the multiplying clone of abnormal plasma cells produces large amounts of monoclonal antibodies, which can be detected in blood and urine. Health problems caused by multiple myeloma can affect bones through osteolytic lesions, the immune system, kidneys, and red blood cell count. The number of new cases of multiple myeloma each year in Western Europe is approximately 4 per 100,000 inhabitants [63]. Myeloma is an incurable disease although treatment may help control symptoms and complications and prolong life. The condition is usually progressive and fatal. Symptoms include anemia, renal damage, and increased susceptibility to bacterial infections. Treatment options include local irradiation of myelomas in bone, high-dose chemotherapy followed by stem cell transplantation, and use of new drugs (e.g. lenolidamide).

A clinical study has been done on multiple myeloma in Norway. The trial enrolled 33 patients, who were treated for 7 weeks with the AbM extract (AndoSan™) or with placebo only [<http://clinicaltrials.gov/ct2/show/NCT00970021>]. The supplementation was tested in a double blinded fashion as adjuvant treatment to high-dose chemotherapy followed by transplantation of preharvested autologous hematopoietic stem cells that had been mobilized from bone marrow to peripheral blood. The data will be examined at end of 2012, after the code is broken and depending on who got the AbM extract and who got placebo. A similar multi-center study may ensue, pending a positive outcome of the first trial.

Unfortunately, a general cure for cancer is still yet to be found, although 2 out of 3 cancer patients can today be treated successfully. Currently, even though there are therapies that induce tumor regression, many people turn to alternative treatment methods. However, it is essential not to discontinue standard methods of treatment until thorough scientific validation of alternative or complementary therapies exist.

Gastro-inflammation

The digestive system plays an important role in the human body by absorbing food nutrients into the blood stream. Uncontrolled intestinal inflammation may lead to different forms of intestinal disorders called Inflammatory Bowel Disease (IBD). The etiology of these inflammatory diseases is unknown, but they are assumed to be autoimmune disorders.

IBD refers to two chronic diseases: Ulcerative Colitis (UC) and Crohn's disease (CD). Although the diseases have some common features, there are important differences. UC is an inflammatory disease of the large intestine or colon. CD most commonly affects the last part of the small intestine and parts of the large intestine. The present treatment of IBD contains anti-inflammatory drugs to decrease the inflammation, and immunosuppressive agents to inhibit the

immune system from destroying the body's own cells and whole tissues. However, alternative treatments are becoming more popular each year because of the lack of effective treatment. Today, costly novel treatment with antibody to the inflammatory cytokine, tumor necrosis factor (TNF), is used against CD, but this therapy is hampered with development of therapy-resistance and huge potential side effects.

Recently, AbM has been tested in a clinical trial in Norway on patients with IBD. Clinical data revealed that patients with UC and CD exhibited a down-regulated level of pro-inflammatory cytokines such as TNF in the serum as an indication for the local effect in the colon wall itself. This is an indication of the potential effectiveness of AbM to inhibit the production of pro-inflammatory cytokines, and reduce calprotectin (a marker for IBD) in UC and CD patients. Twelve patients diagnosed with UC and 12 patients with CD, volunteered to participate in the study of oral intake of a normal dose of AndoSan™; 20 ml thrice daily for 12 days. The collected data demonstrated a reduction in several cytokines (especially pro-inflammatory and chemotactic cytokines) in the serum of UC and CD patients after 12 days of intake of a Basidiomycetes mushroom extract (Andosan™) mainly based on AbM. In patients with UC, there was also a concomitant reduction in levels of fecal calprotectin [41]. Similar results showing such a decline in levels of inflammation-driving cytokines, have been demonstrated in healthy volunteers consuming AndoSan™ in a similar experimental set-up [64]. Collectively, the findings support the notion of a general anti-inflammatory and stabilizing effect of the AbM extract on cytokine release in individuals with good health or IBD. Moreover, the consumption of this AbM-based medicinal mushroom extract by the IBD patients resulted in no side effects. Rather, the patients spontaneously reported less bowel problems and joint pain (common in CD) [41]. Presently, a follow-up placebo-controlled and blinded clinical trial is being conducted in 100 IBD patients at Oslo University Hospital.

There is no known cure neither for UC nor for CD, which is why alternative therapies are popular among this group of people. However, it is important to remember that any alternative treatment should complement, not replace, conventional care.

Hepatitis

The liver is one of the most important organs in our body. Considering all of the liver's special functions, and its anatomical location and drainage of blood from the vessels in the bowels, it is the port of entry (hence the Latin name "Vena porta" for the vein from intestines to liver), of ingredients and impurities in food and drink. Thus, the liver is frequently exposed to a large load of intestinal antigens from pathogens (viruses, bacteria, parasites), toxins, tumor cells, and harmless dietary antigens [65]. Statistics and epidemiological data say that except for alcohol and drug overdose, the main reason for liver damage is viral infection due to hepatitis, especially B (HBV) and C (HCV) forms. The liver is a blood-rich organ containing large numbers of phagocytic cells and NK cells, which can be activated by β -glucans, in addition to Kupffer cells – relatives of macrophages, and also endothelial cells aligning the sinusoids that can be stimulated by AbM.

Ongoing clinical studies

Preliminary data shows clinical effects of the AbM condensed liquid in human volunteers with an elevated activity of γ -glutamic-pyruvate transaminase (γ -GTP), a marker for liver damage. A total of 20 patients (half of which were men) with chronic C-type hepatitis received the extract orally, twice a day, for 8 weeks. Reduced serum γ -GTP levels were established in 80% of the patients without any toxicological findings or other side effects. It was concluded from these results that the AbM extract could be useful for patients with C-type hepatitis [66].

An open-label pilot study conducted over a 1 year period, tested whether the AbM extract can improve liver function in patients with hepatitis B. Four patients under 12 months of clinical observation with alanine aminotransferase (ALT) over 100 IU/L, and not taking drugs for hepatitis were given an AbM extract (1500 mg daily for 12 months). The ALT level was taken as a major outcome measurement. At the end of the study, the levels of both aspartate aminotransferase (AST) and ALT decreased. This data indicated the potential benefit of the AbM extract in normalizing liver function of patients with hepatitis B [12].

In other studies, a possible *in vivo* effect of oral AbM (AndoSanTM) was examined in patients with Interferon (IFN)- α -resistant chronic hepatitis C virus infection. It was observed that the viral load was slightly, but not statistically significantly, decreased after 1 week of treatment. The treatment also up-regulated the gene for IFN- α -receptor, implying that AbM may improve the effect of IFN- α treatment in patients with chronic hepatitis [11]. However, one report on three patients with advanced cancers contradicted these studies because it suggested that AbM taken as supplement, induced liver damage [61].

Until today, there has been no good method to compensate for liver failure, especially when secondary to chronic hepatitis type infections. However, AbM could be a promising remedy in hepatitis treatment especially for patients with the IFN- resistant type of the disease.

Allergies and Asthma

Allergies are abnormal immune system reactions to substances that are typically harmless to most people. In this case, the immune system mistakenly identifies a harmless substance such as tree pollen, as a dangerous invader, and produces antibodies (specific IgE) as well as many active “immune activators” against the allergen (Betv1). In consequence, chemicals released by the immune system lead to allergy symptoms, such as a hay fever, itchy eyes or skin reactions. Allergies and asthma often occur together, especially in children where 50% of asthma is induced by allergies in the airway. The diseases are caused by misdirected inflammatory immune responses which AbM may help rectify because it has been shown to have positive influence upon T helper (Th) cell subsets [14, 15].

There are two distinct subsets of Th cells that are different in terms of their cytokine production and biological function, and their responses are balanced against each other (Fig. 1). These subsets are Th1 and Th2. Anti-tumor and anti-infection immunity are both due to Th1 responses, which also do promote autoimmune diseases when excessive. Anti-rejection and anti-parasite immunity is due to Th2 responses, which may also induce IgE-mediated allergies. Th1 and Th2 response is mutually inhibitory, so if Th1 cells are stimulated, Th2 cells are suppressed [67, 68]. This means that Th1 cell activation with AbM will lead to the inhibition of an antibody-mediated immune response, and in consequences allergy symptoms (Fig. 1).

Based on a study with mice, it might be concluded that an AbM extract, may prevent the development of IgE-mediated allergies when given before allergen immunization [14, 69]. The extract seems to have a therapeutic effect when given together with or as late as 3 weeks after the allergen immunization in the mouse model (Fig. 3) [13]. Although one should be careful when extrapolating animal data to a human setting, three weeks for a mouse equals several months for a human, suggesting that an established allergy in patients can be reverted. The latter study was done with AndoSan™.

In summary, the AbM extract may both prevent allergy and allergy-induced asthma development and be used as a therapeutic substance against allergy and asthma.

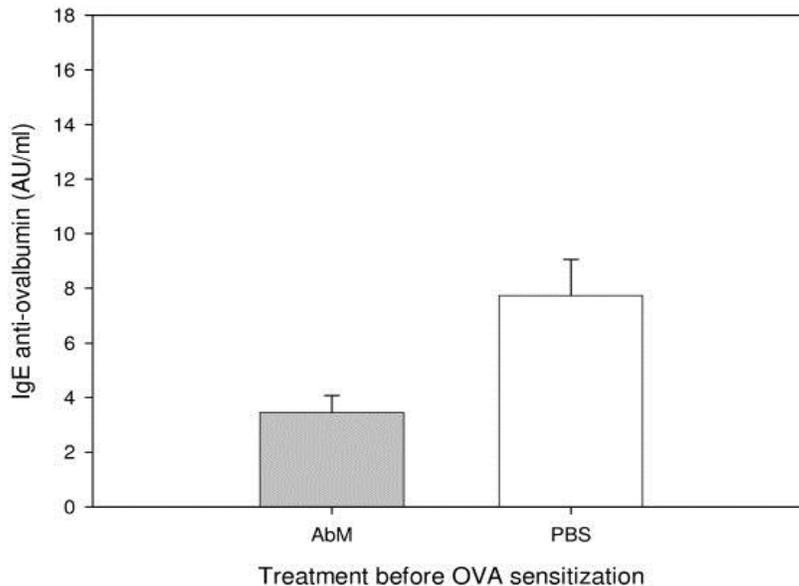


Figure 3. Mice were supplemented either with the AbM-based extract, AndoSan™ or PBS before OVA immunization, and 26 days later serum was collected for the evaluation of specific antibodies. Results: The level of IgE anti-ovalbumin were lower in the AbM than PBS treated groups. Similar results were found if AndoSan™ extract or PBS was given 3 weeks after the allergen immunization (data not shown on this graph).

Diabetes

Diabetes mellitus can be divided into type I that requires insulin treatment to lower blood sugar levels by promoting sugar transport into cells, and type II which is insulin-independent. Type I diabetes is an autoimmune disease with specific destruction of insulin-producing pancreatic beta-cells that culminates in a state of hypoinsulinemia and hyperglycemia. Type II diabetes is considered a life style-induced disease caused by unhealthy eating/drinking habits and physical inactivity, or there is also a hereditary element. The latter type is increasing epidemically worldwide and is especially seen in inner-city youths, and in people experiencing a rapid improvement in living conditions, e.g. people emigrating from poorer conditions with high physical demands in undeveloped countries, to richer, developed countries.

AbM has been shown to reduce blood glucose levels in diabetic rat models [70]. There is also clinical evidence that AbM combined with anti-diabetic drugs can improve insulin resistance

in type II diabetes patients [71]. The authors speculated that an increase in so-called adiponectin concentration could be the mechanism behind the AbM effect. Another group has suggested that the AbM's anti-diabetic effect in diabetic rats is due to AbM's suppression of oxidative stress and proinflammatory cytokine production, which then results in improvement of pancreatic beta-cells mass [72].

Comparison of AbM extracts

There are many different AbM extracts available. Glucans, one of the most active and beneficial components of AbM, are known to augment immune responses against viral and bacterial infections, as well as against cancer [52]. It is important to point out that not all available AbM extracts give the same result because they are prepared by using different mushroom strains and in accordance to different protocols. Latest reports show that there are various compositions of beta-glucans in AbM extracts [73], and the concentration of active ingredients in each component depends on the methods of extraction [70, 74] and on the substrate (rotting woods) they are grown on.

A scientific study compared the efficacy of the leading products available in the market, revealing extracts with the strongest anti-infection properties. In order to evaluate antibacterial efficacy of AbM extracts, five AbM products described as A, B, C, D, E were compared in a mouse model for deadly pneumococcal (Gram-positive) sepsis. The day before the sepsis was induced, the mice received similar volumes of AbM extracts orally: Extract described as A, which was a mixture of 82% AbM, 15% *Hericium erinaceum* and 3% *Grifola frondosa*, all members of the Basidiomycetes mushroom family, appeared to be the only one which had a statistically significant protective effect [52]. Extract A was the most effective in reducing the number of bacteria in the blood of the infected mice and increasing the survival rate of the animals. In another study, using Gram-negative bacteria in mice, the efficacy of AbM extract in decreasing bacteremia and increasing survival rate was confirmed [29]. One may speculate whether this is due to the presence of additional biological components and synergies with the two other Basidiomycetes mushrooms ingredients as well as the protocols for cultivation and processing methods [52].

Extract A currently represents the AndoSan™ product, which appeared to be the only one that gave a significant protection to overcome deadly bacterial sepsis. Therefore, this particular mushroom extract was chosen for studies in other animal models and clinical studies (allergy, HCV infection, IBD, multiple myeloma, colon cancer). Hence, it may be a choice for prevention or treatment of different illnesses as an adjuvant.

Safety issues

Many researchers have studied AbM, as well as other medicinal mushrooms for close to 50 years. AbM, cultivated on quality-controlled substrate, does not contain toxic substances and is an effective supplement with good biological effects [11, 26, 75]. AbM can safely be used alone or in combination with other treatments for patients, as long as the user is not allergic to mushrooms as such and possible content of harmful substances such as heavy metals, has been controlled for and excluded. Laboratory tests showed that AbM extract may modulate and activate the immune cells in their effort to prevent cancer and assist in regulating a dysfunctional

immune system (Fig. 2). The safety of the AbM-based extract, AndoSan™, was shown both in healthy volunteers [64], and in patients with HCV infection [11] or IBD [41]. Also, no side effects have been reported in multiple myeloma patients taking this extract as adjuvant treatment for 7 weeks. A substantial amount of both in vitro [24, 25, 59, 74, 76-78] and in vivo [4, 10, 11, 26, 41, 52, 66, 79] studies and the presence of similar results, supports the assumed inhibition of tumor growth and immune system stimulation.

There are contradicting reports regarding the effect of AbM on liver function. There is one report stating that three patients with advanced cancer who took an AbM supplement, suffered from severe liver damage [61]. A link between the AbM extract and liver damage was suggested, although several other factors could not be completely ruled out as the causes of liver damage. On the other hand, studies on AbM intake in patients with chronic hepatitis C virus infection did not show any adverse side effects on the liver function [11]. Patients in this study were administered with the AndoSan™ AbM extract, which proved its non-hepatotoxic properties. What is more, the intake of AbM in the form of AndoSan™ was tested in healthy volunteers and patients with IBD. A standardized daily dose of 60 ml of the AbM extract for 12 days, as well as a high daily dose of 360 ml during each of two days was evaluated in the context of safety [64]. Collected data from healthy volunteers [64] and IBD patients [41] revealed no pathological effects on hematological parameters including those for liver, pancreatic and renal function.

Conclusions

AbM medicinal mushroom modulates the immune system by binding to receptors such as TLR2, dectin 1 and CR3 on the innate immune cells; NK cells, monocytes and dendritic cells, which further communicate with T helper cells and bring about an enhanced Th1 response and concomitant reduction of Th2 response. This immunomodulation explains the different beneficial health effects attributed to AbM. In addition, AbM possesses a direct apoptotic property towards different cancer cells, which together with the raised Th1 response and antiinflammation, generates the AbM-induced antitumor effect. The resulting decreased Th2 response, together with the anti-inflammatory effect, explains the observed anti-allergic and anti-asthmatic effects and possibly also anti-diabetic properties of AbM in animal models. Lately, results from several preliminary studies with cancer cells and animal models have prompted a series of clinical trials, the results of which may reveal the beneficial effects of AbM as adjuvant treatment for patients [10-12, 26, 41, 61, 64, 71, 80-82]. This bears promise for exploration of AbM as a therapeutic regimen in the clinical setting.

List of abbreviations: *Agaricus blazei* Murill (AbM), alanine aminotransferase (ALT), aspartate aminotransferase (AST), Complement receptors (CR), Crohn's disease (CD), γ -glutamic-pyruvate transaminase (γ -GTP), Hepatitis C virus (HCV), Inflammatory Bowel Disease (IBD), interferon (IFN), natural killer (NK), pathogen-associated molecular patterns (PAMP), T helper cells (Th), Toll like receptors (TLR), tumor necrosis factor (TNF), Ulcerative Colitis (UC)

Competing interests: Biedron R, Tangen JM and Maresz K have no competing interests. Hetland G is stock holder in Immunopharma, which aims to develop AndoSan™ into adjuvant hospital medicine.

Authors' contributions: All authors have written and/or contributed to the scientific content of this review article.

Acknowledgements and Funding: The International Science and Health Foundation (<http://www.ishf.org>), Cracow, Poland, and Immunopharma, The Norwegian Institute of Public Health and Oslo University Hospital, Norway, have contributed with funding and /or human resources for work reviewed in this article.

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