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The Effects of β-glucans on Cancer Metastasis

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Abstract: Beta-glucans (β-glucans), naturally occurring polysaccharides, are present as constituents of the cell wall of cereal grains, mushrooms, algae, or microbes including bacteria, fungi, and yeast. Since Pillemer *et al.* first prepared and investigated zymosan in the 1940s and others followed with the investigation of β-glucans in the 1960s and 1970s, researchers have well established the significant role of β-glucans on the immune system relative to cancer treatment, infection immunity, and restoration of damaged bone marrow. However, information on their biological role in anti-metastatic activity remains limited. As an immunomodulating agent, β-glucan acts through the activation of innate immune cells such as macrophages, dendritic cells, granulocytes, and natural killer cells. This activation triggers the responses of adaptive immune cells such as $CD4^+$ or $CD8^+$ T cells and B cells, resulting in the inhibition of tumor growth and metastasis. Reports have shown that β-glucans exert multiple effects on cancer cells and cancer prevention. However the mechanisms of their actions appear complex due to differences in source, chemical structure, insufficiently defined preparation, and molecular weight, hence the inconsistent and often contradictory results obtained. This review is focused on the potential of β-glucans as anti-metastatic agents and the known mechanisms underlying their biological effects.

Keywords: Anti-metastatic, β-glucan, immunomodulatory, zymosan.

INTRODUCTION

Scientists have considered beta-glucans (β-glucans) as an important constituent for decades. Current knowledge about the molecular interaction of β-glucans with various cell types comes from the first investigative studies conducted with zymosan in 1941. Zymosan, a mixture of polysaccharides obtained from yeast, consists of a variety of substances including mannans, glucans, glucosamine, and glycoproteins; therefore is not ideal for the investigation of β-glucan-specific activities [1].

Humans have recognized and consumed β-glucans, consisting of half the mass of fungal cell wall, as potent immunological stimulators for thousands of years, especially in China and Japan [2-4]. Caregivers have administered β-glucans orally for treatment of human diseases historically with no reports of adverse effects. In addition, the inexpensive β-glucans appear to act by modulating the immune system.

As β-glucans are not synthesized in humans, the immune system recognizes these compounds as non-self molecules, inducing both innate and adaptive immune responses [5]. The anti-tumor activity of β-glucans was first demonstrated around 50 years ago, and since the 1980s several studies on animals have reported on the remarkable effects of β-glucans on a range of tumors [6, 7]. Despite the limited studies on the effect and action mechanism in the role of β-glucans, this review discusses the available literature on the potential anti-metastatic effect of β-glucans and the probable mechanisms underlying their biological effects.

MURINE MODELS

Several investigations have reported on the anti-metastatic effect of β-glucans by using the murine metastasis model. Notably, Inobata *et al.* demonstrated that schizophyllan decreased the frequency of metastasis and prolonged the life span of mice imbedded with Lewis lung cancer cells. In this model, schizophyllan increased the infiltration of macrophages and T cells in the local tumor and lung nodulation [8]. Earlier, we reported the inhibitory effects of β-glucans (IS2) purified from mutated *Saccharomyces cerevisiae* (*S. cerevisiae*) on lung metastasis of colon 26-M3.1 carcinoma; IS2 induced splenocyte proliferation, tumoricidal activity, and cytokines production in peritoneal macrophages and induced natural killer (NK) cell cytotoxicity but did not directly affect cytotoxicity on colon 26-M3.1 cells *in vitro*. We further confirmed toll-like receptor (TLR)4 and MyD88 as crucial molecules and signal mediators for these β-glucans [9-10]. Zhong *et al.* applied human lung cancer cells as a xenograft model in mice and achieved long-term survival when they treated mice with a combination of monoclonal antibodies and β-glucans through the complement deposition and neutrophils infiltration [11].

ANTI-METASTATIC EFFECT OF BETA-GLUCANS FROM MUSHROOMS

Several research groups carried out studies on the anti-metastatic effect of β-glucans from mushrooms. Kodama *et al.* isolated grifolan (GRN, Fig. **1A**), a β-1,6 glucan with β-1,3 branched chains, from *Grifola frondosa* (Maitake mushroom), and reported that GRN containing Maitake D-fraction (PDF) hindered metastatic progress and lessened the expression of tumor markers examined in tumor patients [12, 13]. The acid-insoluble PDF is alkali-soluble and hot water-extractable. This fraction also has been reported to have anti-tumor and anti-metastatic activity by oral and systemic administration in cancer patients as well as tumor-bearing mice. Activation of immune cells such as macrophages, dendritic cells (DCs), NK cells, and T cells participated in this improvement [12-15]. Reports have also shown GRN to inhibit tumor growth and improve significant symptoms when administered in breast and lung cancer patients. Furthermore, in treatment along with chemo-therapy, GRN enhanced immune-competent cell activities 1.2–1.4 times, compared with chemotherapy alone, by enhancing the immune system through activation of macrophages, T cells, and NK cells [12-16].

Chihara *et al.* purified lentinan (Fig. **1B**), a glucan extracted from *Lentinus edodes*, in 1970 [17]. Intraperitoneal (i.p.) administration of lentinan induced cytolytic activity of macrophages against metastatic tumor cells such as Lewis lung carcinoma in an *in vivo* animal models and two human melanomas, and its prophylactic treatment in murine proved effective in tumor

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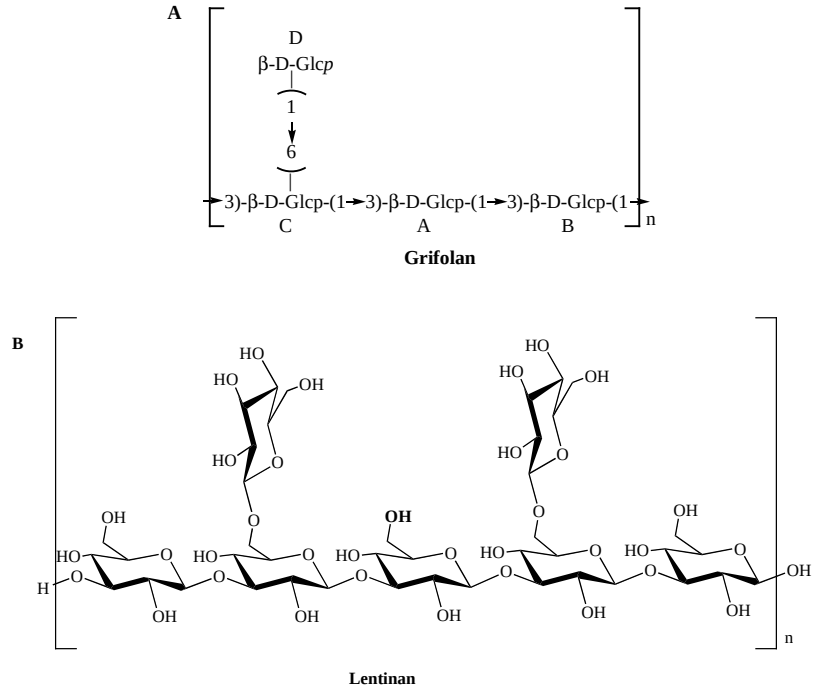

Fig. (1). Chemical structure of Grifolan (**A**) and Lentinan (**B**).

metastasis [17, 18]. In addition, a cancer patient diagnosed as having gastric cancer with multiple hepatic metastases showed a 99% reduction in hepatic metastases when lentinan was co-administered with anti-cancer drugs for 4 months post surgery [19].

Water-soluble β-glucans, with a β-1, 3 glucan branched by 1,4 or 1, 6 branched chains, purified from *Ganoderma lucidum* (*G. lucidum*) have shown antitumor activity in mice [20]. These soluble β-glucans enhance the function of monocytes, macrophages, DCs, neutrophils, and NK cells [21]. Jang *et al*. reported that the ethanol fraction of *G. lucidum* has an inhibitory effect on matrix metalloproteinase (MMP)-2 and MMP-9 activities in human gastric carcinoma cells suggesting that administration of soluble β-glucans can inhibit tumor-induced angiogenesis and metastasis [22].

In mice, oral supplementation of β-glucans extracted from *Agaricus blazel (A. blazel)* reduced spontaneous pulmonary metastasis of 3LL cells and peritoneal disseminated metastasis of HRA cells and inhibited the growth of these metastatic tumors in lung or peritoneal cavity [23]. Oshiman *et al*. further supported the results that oral administration of β-1, 6-D-polyglucose of *A. blazel* showed a significant regression of inoculated tumors in mice [24]. Niu *et al*. ascertained that anti-tumor activity in tumor-bearing mice by linear β-1, 3 glucans isolated from *A. blazel* resulted from the activation of NK cells and production of cytokines [25].

Dong *et al.* isolated HEP3, a β-D-glucan slightly soluble in water, from the alkaline extract of the fruiting bodies of *Hericium erinaceus (H. erinaceus)* in 2006 [26]. Lee *et al.* reported that the β-glucan from this mushroom activated macrophage function by increasing the production of nitric oxide and expression of interleukin (IL)-1β and tumor necrosis factor (TNF)-α [27]. In addition, Kim *et al.* reported that systemic injection of the water and water/ethanol extracts containing β-glucan significantly reduced tumor weights of CT-26 colon cancer cells in mice by activation of macrophages and inhibition of angiogenesis, suggesting that *H. erinaceus* derived β-glucans inhibit tumor metastasis [28].

Schizophyllan (SPG, Fig. **2A**), a highly purified β-1, 3-D-glucan from the culture broth of *Schizophyllum commune (S. commune)* Fries, decreased the frequency of lung metastasis and prolonged the life span of mice in a spontaneous model using Lewis lung cancer cells [29]. Further, Kano *et al.* reported on the effectiveness of combined administration of SPG with recombinant IL-2 in increasing the survival rate of mice intraperitoneally inoculated with EL-4 lymphoma [30]. In addition, Arika *et al.* reported that intramuscular injection of SPG in combination with local radiation inhibited tumor growth as well as tumor metastasis

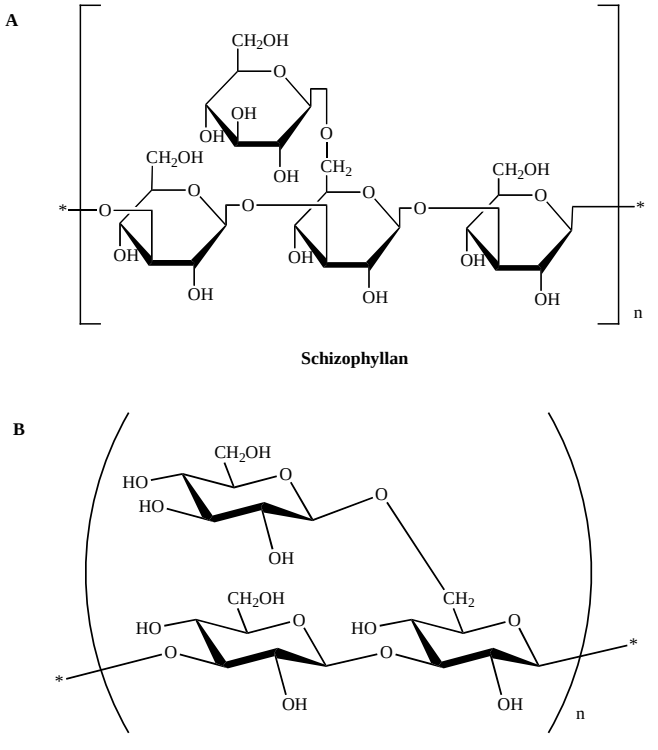

Fig. (2). Chemical structure of Schizophyllan (**A**) and SSG (**B**).

in C3H/He mouse models inoculated with squamous-cell carcinoma NR-S1. They further reported these effects occurred through activation of immune cells and production of cytokines such as TNF-α and interferon (IFN)-γ [31-33]. These reports suggest the availability of β-glucans isolated from mushrooms for co-administration with other anti-cancer agents to treat metastasis.

Jones *et al.* purified a β-1, 3 branched 1, 6 glucan (SSG, Fig. **2B**) from *Sclerotinia sclerotiorum* (*S. sclerotiorum*) [34]. Administration of SSG (oral and i.p.) inhibited experimental pulmonary metastasis of Lewis lung carcinoma through activation of macrophages to enhance phagocytic and lysosomal enzyme activity, active oxygen secretion, and cytokine production [35, 36]. Others purified β-glucans from various mushrooms such as *Auricularia auricula-judae* and *Cordyceps sinensis.* An investigation of the administration of these glucans exhibited anti-tumor activity in mouse models but studies have yet to examine this anti-tumor metastasis activity thoroughly [37, 38]. Table **1** summarizes solubility of β-glucans from different sources of mushrooms and yeasts.

Besides β-glucans, mushrooms contain potential effective anticancer compounds such as polysaccharides, polysaccharide-bound protein and steroids. Several researchers reported the anti-cancer effects of these compounds having cytotoxicity and anti-oxidant, anti-angiogenic, and immuno-modulating activity. Thus these compounds may induce synergistic anti-cancer effects when administered with anti-cancer drugs as well as radiotherapy [39, 40].

ANTI-METASTATIC EFFECT OF BETA-GLUCANS FROM OTHER SOURCES (YEASTS)

Limited investigation of the β-glucans isolated from *S. cerevisiae* has been reported for its anti-metastatic activity, even though there is little evidence whether these glucans inhibit cancer growth by immunomodulation or blocking metastasis [9]. The β-glucans from different sources have specific characteristics in structure and conformation, thus biologic activity depends on their specific characteristics [41, 42]. Whole yeast β-glucan particles can activate DCs and stimulate Th1 dominant immune response, leading to anti-tumor activity. Research shows that administration of yeasts-derived particulate β-glucans activates innate and adaptive immune response and down-regulate immunosuppressive cells to delay tumor progression [41].

In contrast, soluble, yeast-derived 1, 3- β-glucan (PGG; betafectin) shows little evidence in stimulating immune system and inhibiting tumor cell growth and metastasis [43, 44]. We reported that soluble β-glucans (IS-2) purified from mutated *S. cerevisiae* inhibited lung and liver tumor metastasis in murine models [9]. Yeast-derived particulate β-glucans have shown to bind dectin-1 receptor and activate DCs and macrophages to secrete pro-inflammatory cytokines such as TNF-α, IL-12, IL-6 [41, 45].

Zymosan (Fig. **3A**), a cell wall extract of *S. cerevisiae*, contains nonuniform branches and backbone units of β-glucans as well as mannans, mannoproteins, and chitin. Many researchers have carried out the effect of β-glucans with zymosan and have verified their anti-oxidant and immune functions, leading to an anti-tumor activity of S-180 tumor-bearing mice [46, 47].

COMBINED EFFECT OF BETA-GLUCANS ON ANTI-METASTATIC ACTIVITY WITH OTHER THERAPIES

Trials for combination therapy on tumor growth and metastasis carried out with the murine model or human metastatic cancer patients used β-glucans with other treatments such as cytokines, radiation, and antibodies. Among these trials, combinations with antibodies proved the most prominent and other authors mentioned in this review. Further combination trials with chemotherapy or cytokines include schizophyllan with local irradiation using murine model in 1992. This combinatorial treatment showed remarkable inhibition of pulmonary metastasis from the early stage of tumor growth [48].

A combination of β-glucan and IFN-γ in an experimental liver metastasis model almost entirely inhibited the growth of liver metastasis [49]. A combination trial of other cytokine IL-2 with β-glucans against a metastatic murine model showed a synergic anti-metastatic effect and prolonged survival of cancer cell-inoculated mice. $CD4^+$ and $CD8^+$ cells as well as NK cells and macrophages were reported to be involved in these synergistic effects [30, 50, 51].

Mushiake *et al.* explored the combined treatment of lentinan with anticancer drug fluoropyridine and found that this combined treatment could augment the survival period of colon-26-bearing mice. This combined treatment found infiltration into tumor sites and augmentation of functional DCs *in vivo* and *in vitro*. Splenic DCs harvested from combined treated mice showed more potent T

Table 1. Common β-glucans and their sources and characteristics.

Origin	Source	Structure	Solubility in Water	References
Mushroom glucans	*Grifola frondosa* : Grifolan (GRN)	1,3 1,6 branched	soluble	[12]
	Lentinus edodes : Lentinan (LNT)	1,3 1,6 branched	soluble	[18, 19]
	Ganoderma lucidum : P-SG	1,3 1,6 branched	soluble	[20, 22]
	Agaricus blazel	1,3 1,6 branched	soluble	[23, 24]
	Auricularia auricula-judae	1,3 backbone	soluble	[39]
	Cordyceps sinensis	1,3 backbone	insoluble	[127, 128]
	Hericium erinaceus : HEP3	1,3 backbone	slightly soluble	[28]
	Schizophyllum commune Fries. Schizophyllan: sizofiran (SPG)	1,3 1,6 branched	soluble	[29, 30]
	Sclerotinia sclerotiorum : SSG	1,3 1,6 highly branched	soluble	[34, 35]
Yeast glucans	*Saccharomyces cerevisiae* : WGPs	whole yeast b-glucan particles	insoluble	[41]
	Saccharomyces cerevisiae : betafectin (PGG)	1,3 1,6 highly branched	insoluble	[43, 44]
	Mutated *Saccharomyces cerevisiae ;* IS-2		soluble	[9]
	Saccharomyces cerevisiae : Zymosan	cell wall extract containing b-glucan	insoluble	[46, 47]

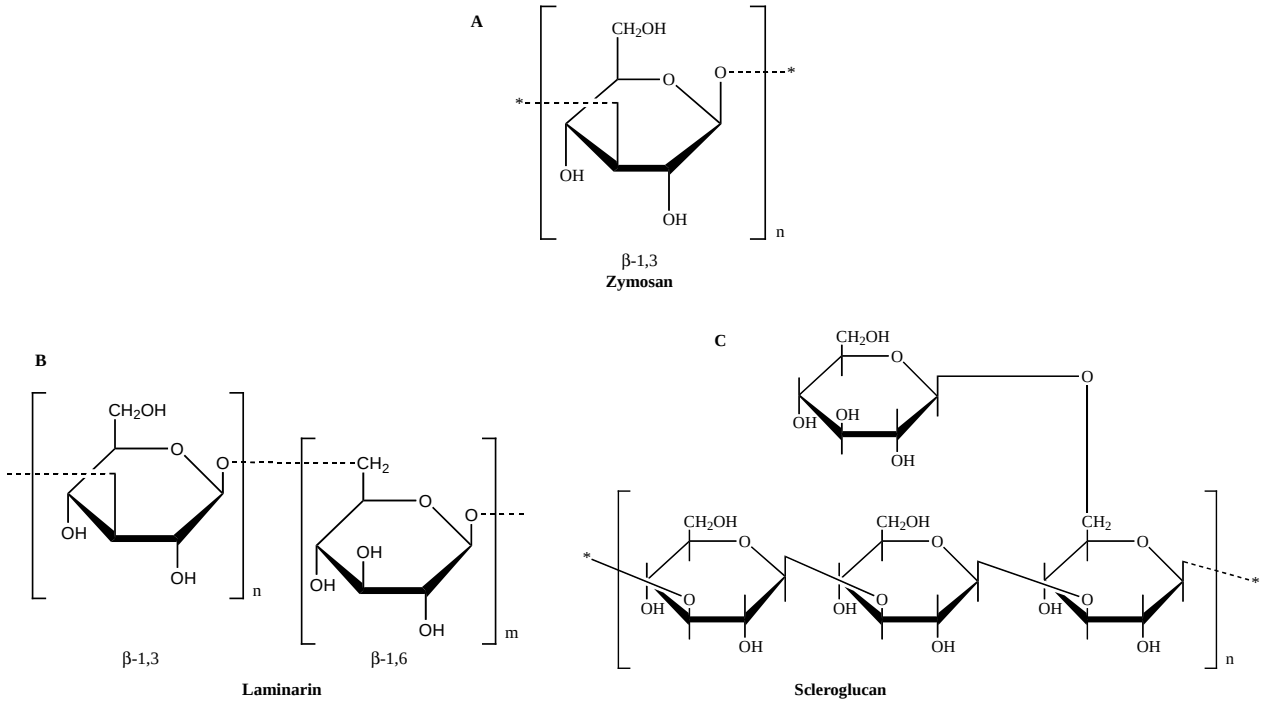

Fig. (3). Chemical structure of Zymosan (**A**), Laminarin (**B**) and Scleroglucan (**C**).

cell proliferation activity than that of DCs from mice treated with fluoropyridine alone. Furthermore, the activity of cytotoxic T lymphocytes (CTLs) in splenocytes of this combined treatment was more specific and potent than that of CTLs from mice treated with fluoropyridine alone. Moreover, the study found that athymic mice do not share the same prolonged survival effect. The above findings indicated that DC activation can induce T cell proliferation and CTL activation [52]. Harnack *et al.* and our group reported the potential availability of β-glucan in a combination therapy with anti-cancer drugs to minimize their side effects [53, 54].

CLINICAL TRIALS OF BETA-GLUCANS ON HUMAN METASTASIS

Although several reports have discussed on the effect of β-glucans on clinical cancer treatment and have shown possible treatment benefits, the reports and data dealing with the effects on metastasis remain preliminary and controversial [55].

Okuyama *et al*. conducted the first clinical trial with β-glucans against metastasis in 1985. This group used lentinan as β-glucan for a subsidiary chemotherapy measure in patients with post-operation of gastric cancer with liver metastasis and showed an increased survival rate compared to results of treatment using only 5-fluorouracil (5-FU) plus mitomycin [56]. Recent studies revealed lentinan was effective in patients with advanced pancreatic, colorectal, and gastric cancers [57-59].

The effect of lentinan on lymphocyte subset in peripheral blood, lymph nodes, and tumor tissues in gastric cancer patients with or without lymph node metastasis was also investigated. The study revealed no significant changes in the lymphocyte subsets, such as $CD3^+$, $CD4^+$, $CD8^+$, CD16 ($Leu11^+$, NK cell subsets), and CD14 (LeuM3, monocytes) of peripheral blood with lentinan treatment. However, increasing $CD4^+$ cell ratios in lymph nodes and a prominent increase in the number of $CD4^+$, CD16 ($Leu11^+$), and CD14 (LeuM3) cells appeared in tumor-infiltrating lymphocytes without metastasis. In addition, lentinan was reported to enhance T cell functions in cancer patients [60, 61]. These data indicate the possible role of T cells by β-glucan treatment in advanced cancer patients.

Studies have looked at the effect of oral administration of β-glucans on peripheral blood monocytes and their expression of activation markers in advanced breast cancer patients. Oral administration of β-glucans for 2 weeks affected the proliferation and activation of peripheral blood monocytes, increased the expression of CD95 and $CD45RA^+$, and activated monocytes. This short-term investigation (only 2 weeks), showed that oral β-glucan administration seems to stimulate proliferation and activation of peripheral blood monocytes in advanced breast cancer patients [62].

In another clinical studies, β-glucans proved effective in metastatic cancer and improved the survival rate of patients treated with a combination chemotherapy or hormonochemotherapy [12, 63, 64]. Shimizu *et al.* reported that CD4-, CD3-stained cells, and IL-2R-stained cells tend to increase in the metastatic pelvic lymph nodes in patients with advanced cervical cancer receiving preparative schizophyllan [65].

Despite the observed effect of β-glucans on cancer metastasis, the data proved contradictory and scarce due to the limitation of cases and investigation in the clinical study. Furthermore, the proposed mechanisms from these authors were based on *in vitro* and animal experiments. Therefore, future efforts should go toward studies with the generalized structure of β-glucan and well-designed protocols to verify the clinical efficacy of β-glucan alone or in combination.

MECHANISMS OF ANTI-METASTATIC ACTION BY BETA-GLUCANS

β-glucans at Innate and Adaptive Immune System

Most β-glucans are considered as non-digestible carbohydrates and are fermented to various degrees by the intestinal microbial flora [66-68]. Further, β-glucans did not show any action on apoptotic pathways and no direct effect on telomerase [69]. Therefore, it has been speculated that β-glucan actions in immune cells or immunomodulatory properties may be partly attributed to their functional effects [2, 70]. β-glucans stimulate innate immune cells such as macrophages, DCs to produce cytokines, and local

immunomodulators, and these in turn activate adaptive immune cells such as T cells and B cells [18, 71-75]. β-glucans enhanced IFN-γ production from tumor-infiltrating T cells, CTL responses and DC infiltration within tumors, leading to reduced tumor burden [76, 77]. As a consequence, both innate and adaptive responses can be modulated by β-glucans in their anti-metastatic actions.

Many investigations have undertaken on the immune and antitumor activity of lentinan, a β-D-glucan derivate isolated from *L. edodes*, and reported that this compound not only acts as T cell oriented adjuvant through innate immune cell activation but having augmented activities of NK cells, lymphokine activated killers (LAKs), and CTLs [78, 79]. Lentinan also accelerates the infiltration of granulocytes into target tissues and activates secretion of active oxygen and production of cytokines as well as cytotoxicity in peritoneal macrophages against metastatic tumors. Investigators have reported on the role of the complement system together with macrophage activation by lentinan [80]. Schizophyllan structurally related to lentinan activates macrophages *in vitro* and *in vivo*, leading to augmentation of T cell activation, but do not directly activate T cells [81, 82]. In addition, glucan SSG from *S. sclerotiorum* induces the development of Th1 cells *via* the IL-12 pathway and stimulates Th1 cells to produce IFN-γ, which activates macrophages [83]. In animal studies, the orally administered specific backbone 1-3 linear and β-glycosidic chain of β-glucans enter the proximal small intestines as undigested form. Where in some are captured by the macrophages *via* the dectin-1 receptor, and transport them to the spleen, lymph nodes, bone marrow and endothelial reticular system. The large β-1, 3-glucans in the macrophages are degraded into smaller soluble β-1, 3-glucan fragments, which are released from macrophages and taken up by other immune cells through complement receptors 3 (CR3) expressed on neutrophils and NK cells, leading to the killing of opsonized tumor cells after they were recruited to complement activation sites coated with monoclonal antibodies [69, 84].

We can also consider administration of soluble β-glucans because they can be delivered directly to the CR3 on circulating granulocytes [84]. Soluble β-glucans such as laminarin (Fig. **3B**) and scleroglucan (Fig. **3C**) can be directly bound and internalized by intestinal epithelial cells and gut-associated lymphoid tissue (GALT) cells [85]. This internalization does not take place through dectin-1 alone, rather, dectin-1 and TLR2 are accountable for uptake of soluble β-glucans by GALT cells. The use of thymectomized mice or anti-lymphocyte serum has elucidated the role of T cells and lymphocytes in these immune responses. This group further suggested the function of lentinan to restore humoral immune responses [86]. Li *et al.* examined the trafficking of orally administered particulate β-glucans and their process *in vivo*. β-glucans trafficked into spleen and lymph nodes and activated DC that captured dying tumor cells, leading to the expansion and activation of antigen-specific $CD4^+$ and $CD8^+$ T cells [76].

Qi *et al.* compared functional characteristics between particulate and soluble β-glucans and suggested rational design of immuno-therapeutic protocols. They reported that yeast-derived β-glucans activate DCs and macrophages *via* dectin-1. By using dectin-1 knock out mice, they found particulate β-glucans but not soluble β-glucans activate DCs that promote Th1 and CTL priming and differentiation. However, soluble β-glucans were found to bind DCs and macrophages *via* dectin-1 independently and did not activate DCs. Although soluble β-glucans had no therapeutic effect, they augmented antibody-cotreatment efficacy *via* complement activation pathways [41].

Mishiake *et al*. suggested chemoimmunotherapy combined with β-glucans *in vivo*. Anticancer drugs combined with lentinan prolonged the survival period through augmentation of DC infiltration into the cancer site, T cell proliferation and CTL activity [52].

Signal Events Involved in Anti-metastatic Action of β-glucans

Generally, polysaccharides are considered as classic T-cell independent antigens that do not elicit cell-mediated immune responses. Likewise, glucans are thought to mediate their effects *via* interaction with membrane receptors on innate immune cells such as macrophages, neutrophils, and NK cells [1]. To date, research has identified six categories of β-glucan receptors supposed to mediate these activities. These include, dectin-1; TLR-2 and TLR4; CR3 (CD11b/CD18); lactosyl ceramide (LacCer); glycosphingolipid; and selected scavenger receptors [1, 70, 87-91] (Table **2**, Fig. **4**).

Among these receptors, dectin-1 commonly expresses on macrophages, neutrophil lineages, DCs, and some T cells but not on NK cells [92-95]. Evidence suggests that dectin-1 is most important in the activation of innate immune responses in macrophages as mediating activation of phagocytosis and production of cytokines [96-98]. Dectin-1 and SIGN-related 1(SIGNR1) are known to be the major receptor for the β-glucan-dependent non-opsonic recognition of zymosan on macrophages, and TNF-α production was induced by these receptor molecules *in vitro* [72, 95, 99, 100]. *In vivo* evidence by using dectin-$1^{-/-}$ mice also revealed dectin-1 as the major receptor for zymosan and involved in leukocyte responses to β-glucan [98].

Investigators have shown TLR-2 as involved in the response to zymosan [73, 101]. They further investigated the role of TLR-2 associated with dectin-1 for recognition of β-glucan. Dectin-1 synergized with TLR-2 induced production of IL-2, IL-10, IL-12 and TNF-α. The authors additionally elucidated the role of Syk kinase in the production of IL-2 and IL-10 from DCs [77, 102-105]. Interestingly, Syk plays a major role in the induction of IL-2 and IL-10 in DC, and this induction requires ERK MAP kinase but remains unaffected by MyD88 deficiency, indicating the important role of dectin-1 in DC from β-glucan stimulation. Furthermore,

Table 2. β-glucan Receptors

Name	Main Cellular Expression	Known or Proposed Functions	Major References
Dectin-1	Macrophages, neutrophils, DCs, B cells, T cells	Phagocytosis, cytokine production (TNF-a, IL-1 IL-2, IL-10, IL-12), MIP-1α, MIP-1b, CXCL-1	[115]
TLR-2 and 4	Macrophages, DCs, B cells, T cells, endothelial cells	Independent or association with dectin-1,	[95]
CR3(CD11b/CD18)	Neutrophils, monocytes, NK cells, lymphocytes	Adhesion, migration, phagocytosis, cytotoxicity	[129]
LacCer	Neutrophils, endothelial cells	Required for CR3 mediated phagocytosis, MIP-2, TNF-a	[130]
Glycosphingolipid	DCs, airway epithelial cells	IL-8 production, co-localization with TLR or dectin-1, DC activation,	[115]
Selected scavenger receptors	Macrophages, neutrophils	phagocytosis	[110]

MIP-2: macrophage inflammatory protein-2, TLR: Toll-like receptor, LacCer: lactosylceramide, DC: dendritic cell, CXCL: C-X-C motif ligand

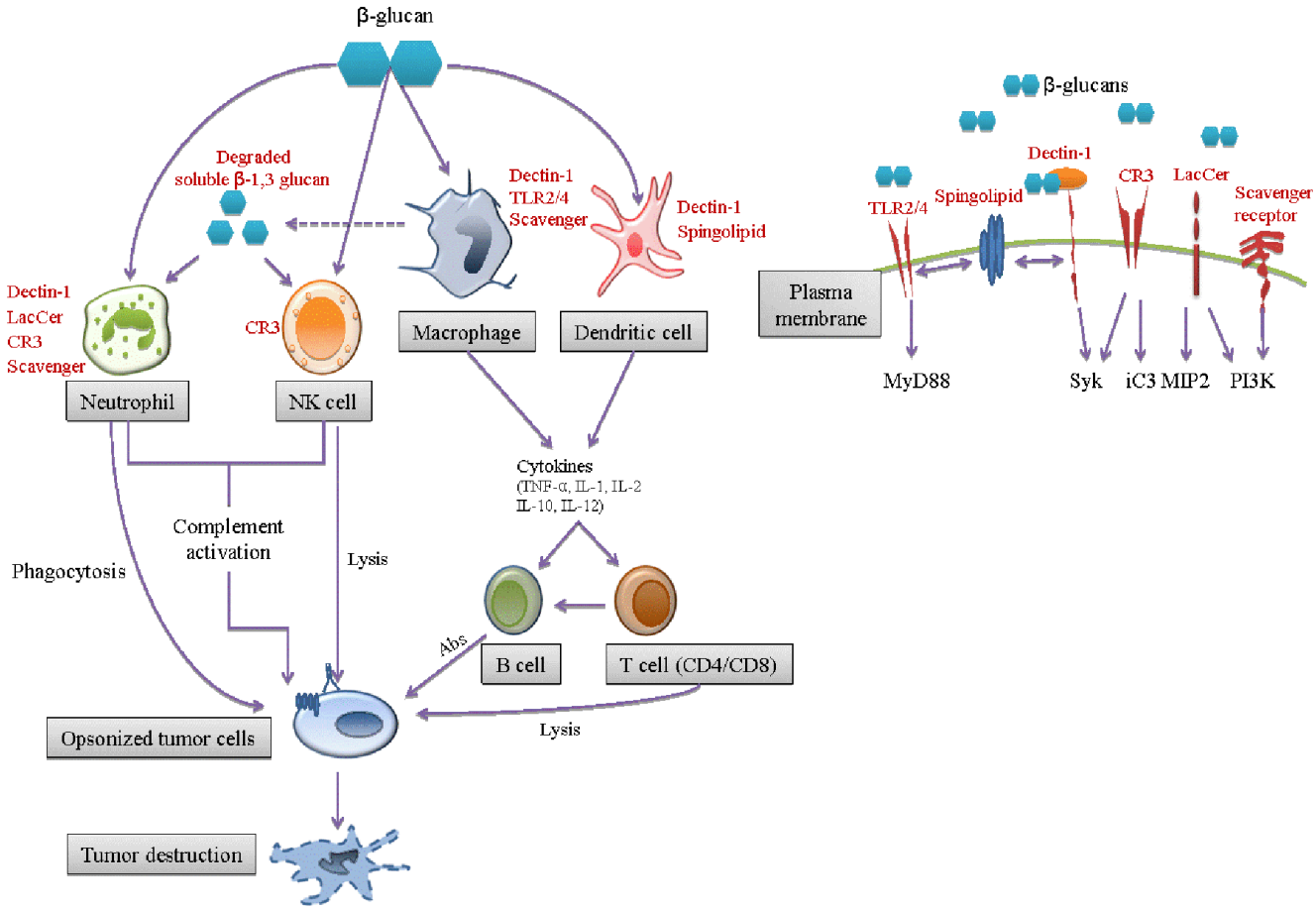

Fig. (4). Schematic diagram of possible pathways of β-glucan action.

Brown *et al*. suggested that zymosan can induce the co-localization of dectin-1 with TLR-2 to make a signal complex [39]. But pro-inflammatory cytokine production or phagocytosis by macrophages can occur by dectin-1 only without TLR [94, 106, 107].

The action mechanism of β-glucan on cancer includes the C3 fragment of complement and antibodies [108]. The β_2 integrin, CR3 expresses mainly on neutrophils, monocytes, NK cells, and lymphocytes, but not macrophages [69, 92]. Zymosan increased NK cell-mediated killing of tumor cells *via* activation of CR3 [6, 109]. Among these, CD11b, the subunit of CR3 regulates both cytotoxic and adhesion functions of Mac-1/CR3 [1]. The possible role of scavenger receptors, if any, in the biological effects mediated by β-glucans remains unknown, and has no evidence that they play major roles in anti-cancer activity [4, 110].

LacCer located in neutrophil and endothelial cells, was supposed to have the role for signal transduction platforms for CD11b/CD18-dependent neutrophil phagocytosis, but the role played by β-glucans in metastasis remains unclear [111].

Sphingolipid as component of lipid rafts on plasma membrane was supposed to mediate signal complex together with dectin-1 or TLR2/4, leading to activation of cells through these receptors [112-115]. Considering the results of the above-mentioned literature, we can assume dectin-1 as the main receptor for β-glucan signaling, but research has not yet determined its role in anti-metastasis *in vivo*.

Investigations concerning the interrelation between the chemical structure of β-glucan and its antitumor effects suggested the major significance of the functional importance of water solubility and location of substitute groups of β-glucan on antitumor activity. Furthermore, the biological effects of β-glucans differ in molecular weight (MW) and chemical modifications of degree of branching, polymer length and tertiary structure. In general, large MW or particulate β-glucans can more effectively activate phagocytic activity, and stimulate proinflammatory mediator production [116, 117]. However, different groups reported contradictory data [118-120]. Likewise another group considered soluble β-glucans as stronger immunostimulators than insoluble ones [121-124]. Intermediate or low-MW β-glucans possess biological activity *in vivo*, but their *in vitro* activities were less clear [125]. Smaller molecular size β-glucan binds to CR3 in NK cells, and that of large molecular size binds dectin-1 and TLRs in macrophages and DCs [75]. In addition, O'Brien elucidated the role of CR3 for signal mediators from soluble β-glucan and showed the conformational change of CR3 and functional proteomics profile after the binding of β-glucan on CR3 [126]. Cramer *et al*. examined the role of signal mediators from fungal β-glucan particles to secrete proinflammatory cytokines by resident macrophages and reported that dectin-1 and CR3 were not crucial for cytokine secretion but partially dependent on MyD88 pathway. This indicates that the receptor and signal pathway for β-glucan differ on source of β-glucan and cell origin [45]. Further, β-glucan can make a single or triple helical conformation in solution by hydrogen bonds between polymer chains. At least one study regarded those with a triple helical configuration as more powerful immunomodulators [118]. However, their individual anti-tumor activities with different branching frequencies have not been studied. Although physico-chemical studies of β-glucan with different structures and affinity study with different sources were

examined, information on the structural variations of β-glucans on anti-metastasis is limited [120].

CONCLUSIONS

In summary, β-glucans prime the innate immune cells such as blood neutrophils, macrophages, DCs, and NK cells, specifically recognizing complement-antibody complexes and killing tumor cells. Binding of β-glucan to specific receptors (especially dectin-1, TLR2, and CR3) activates macrophages, leading to increased chemokinesis, chemotaxis, and migration of macrophages. However, no evidence exists on whether these migration activities set the tumor cells in motion and into other tissues resulting in metastasis. While researchers have proved the role of β-glucans on anti-metastatic activity and explored the intrinsic mechanisms at cellular and molecular levels, we have insufficient data on whether β-glucans inhibit the metastatic progress or protect the cell growth by direct killing or immunomodulating activity. Traditionally, the beneficial effects of β-glucans *in vitro* and *in vivo* experiments including human clinical trials did not involve single pure β-glucan, resulting in conflicting reports from most of the available information on relationships between structure and functions. Controlled experiments and generalized treatments with β-glucans require well-defined structure and characterized standards since we have limited reports of clinical trials using purified β-glucans for cancer patients. Most of the available evidence comes from *in vitro* or animal studies and clinical studies have just now begun to appear. Additionally, combination treatments may also prove helpful after detailed preclinical examinations. It is to be noted that the effect and action mechanism of β-glucan *in vivo* can differ markedly according to the structures, MWs, and solution conformations. Further, investigators have yet to clearly understand the metabolic fate of ingested β-glucans. Therefore, future studies must follow careful protocols in clinical application of β-glucans. Finally, this review offers some hope for future research results to emerge on the role of β-glucans as potent anti-metastaic agents.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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REFERENCES

[1] Akramiene, D.; Kondrotas, A.; Didziapetriene, J.; Kevelaitis, E. Effects of beta-glucans on the immune system. *Medicina (Kaunas)*, **2007**, *43*(8), 597-606.

[2] Seviour, R.J.; Stasinopoulos, S.J.; Auer, D.P.D.; Gibbs, P.A. Production of pullulan and other Exopolysaccharides by Filamentous Fungi. *Crit. Rev. Biotechnol.*, **1992**, *12*, 279-298.

[3] Klis, F.M.; de Groot, P.; Hellingwerf, K. Molecular organization of the cell wall of Candida albicans. *Med. Mycol.*, **2001**, *39*, 1-8.

[4] McIntosh, M.; Stone, B.A.; Stanisich, V.A. Curdlan and other bacterial (1/3)-b-D-glucans. *Appl. Microbiol. Biotechnol.*, **2005**, *68*, 163-173.

[5] Brown, G.D.; Gordon, S. Immune recognition of fungal β-glucans. *Cell. Microbiol.*, **2005**, *7*, 471-479.

[6] Vetvicka, V.; Yvin, J.C. Effects of marine b-1,3 glucan on immune reactions. *Int. Immunopharmacol.*, **2004**, *4*, 721–730.

[7] Nakao, I.; Uchino, H.; Orita, K.; Kaido, I.; Kimura, T.; Goto, Y.; Kondo, T.; Takino, T.; Taguchi, T.; Nakajima, T. Clinical evaluation of schizophyllan (SPG) in advanced gastric cancer--a randomized comparative study by an envelope method. *Gan. To. Kagaku. Ryoho.*, **1983**, *10*, 1146-1159.

[8] Inomata, T.; Goodman, G.B.; Fryer, C.J.; Chaplin, D.J.; Palcic, B.; Lam, G.K.; Nishioka, A.; Ogawa, Y. Immune reaction induced by X-rays and pions and its stimulation by schizophyllan (SPG). *Br. J. Cancer*, **1996**, *27*, S122-S125.

[9] Yoon, T.J.; Kim, T.J.; Lee, H.; Shin, K.S.; Yun, Y.P.; Moon, W.K.; Kim, D.W.; Lee, K.H. Anti-tumor metastatic activity of beta-glucan purified from mutated Saccharomyces cerevisiae. *Int. Immunopharmacol.*, **2008,** *8*, 36-42.

[10] Li, H.; Kim, W.J.; Jiang, J.; Lee, S.H.; Youn, H.S,; Moon, E.Y.; Kim, T.J.; Ye, S.K.; Ryu, J.H.; Kang, T.B.; Koppula, S.; Park, P.J.; Choi, D.K.; Lee, K.H. MyD88-dependent Toll-like receptor signaling is required for murine macrophages response to IS2. *Int. Immunopharmacol.*, **2011**, *11*(10), 1578-1583.

[11] Zhong, W.; Hansen, R.; Li, B.; Cai, Y.; Salvador, C.; Moore, G.D.; Yan, J. Effect of yeast-derived beta-glucan in conjunction with bevacizumab for the treatment of human lung adenocarcinoma in subcutaneous and orthotopic xenograft models. *J. Immunother.*, **2009**, *32*(7), 703-712.

[12] Kodama, N.; Komuta, K.; Nanba, H. Can maitake MD-fraction aid cancer patients? *Altern. Med. Rev.*, **2002**, *7*, 236-239.

[13] Kodama, N.; Murata, Y.; Asakawa, A.; Inui, A.; Hayashi, M.; Sakai, N.; Nanba, H. Maitake D-Fraction enhances antitumor effects and reduces immunosuppression by mitomycin-C in tumor-bearing mice. *Nutrition.*, **2005**, *21*, 624-629.

[14] Kodama, N.; Asakawa, A.; Inui, A.; Masuda, Y. Nanba H. Enhancement of cytotoxicity of NK cells by D-Fraction, a polysaccharide from Grifola frondosa. *Oncol. Rep.*, **2005**, *13*, 497-502

[15] Mayell, M. Maitake extracts and their therapeutic potential. *Altern. Med. Rev.*, **2001,** *6*, 48-60.

[16] Kodama, N.; Komuta, K.; Nanba, H. Effect of Maitake (Grifola frondosa) D-Fraction on the activation of NK cells in cancer patients. *J. Med. Food.*, **2003**, *6*, 371-377.

[17] Chihara, G.; Hamuro, J.; Maeda, Y.; Arai, Y.; Fukuoka, F. Fractionation and purification of the polysaccharides with marked antitumor activity, especially lentinan, from Lentinus edodes (Berk.) Sing. (an edible mushroom). *Cancer Res.*, **1970**, *30*, 2776-2781.

[18] Ladányi, A.; Tímár, J.; Lapis, K. Effect of lentinan on macrophage cytotoxicity against metastatic tumor cells. *Cancer Immunol. Immunother.*, **1993**, *36*, 123-126.

[19] Takita, M.; Onda, M.; Tokunaga, A.; Shirakawa, T.; Ikeda, K.; Hiramoto, Y.; Teramoto, T.; Oguri, T.; Fujita, I.; Okuda, T.; Mizutani, T.; Kiyama, T.; Yoshiyuki, T.; Matsukura, N. Successful treatment of hepatic metastasis of gastric cancer with 5'-DFUR and Lentinan. *Gan To Kagaku Ryoho.*, **1998**, *25*, 129-133.

[20] Zhang, L.; Zhang, M.; Zhou, Q.; Chen, J.; Zeng, F. Solution properties of antitumor sulfated derivative of alpha-(1-->3)-D-glucan from Ganoderma lucidum. *Biosci. Biotechnol. Biochem.*, **2000**, *64*, 2172-2178.

[21] Sze, D.M.; Chan, G.C. Supplements for immune enhancement in hematologic malignancies. *Hematology Am. Soc. Hematol. Educ. Program.*, **2009**, 313-319.

[22] Jang, K.J.; Son, I.S.; Shin, D.Y.; Yoon, H.M.; Choi, Y.H. Anti-invasive activity of ethanol extracts of Ganoderma lucidum through tightening of tight junctions and inhibition of matrix metalloproteinase activities in human gastric carcinoma cells. *J. Acupunct. Meridian. Stud.*, **2011**, *4*, 225-235.

[23] Kobayashi, H.; Yoshida, R.; Kanada, Y.; Fukuda, Y.; Yagyu, T.; Inagaki, K.; Kondo, T.; Kurita, N.; Suzuki, M.; Kanayama, N.; Terao, T. Suppressing effects of daily oral supplementation of beta-glucan extracted from *Agaricus blazei* Murill on spontaneous and peritoneal disseminated metastasis in mouse model. *J. Cancer Res. Clin. Oncol.*, **2005**, *131*, 527-538.

[24] Oshiman, K.; Fujimiya, Y.; Ebina, T.; Suzuki, I.; Noji, M. Orally administered beta-1,6-D-polyglucose extracted from *Agaricus blazei* results in tumor regression in tumor-bearing mice. *Planta. Med.*, **2002**, *68*, 610-614.

[25] Niu, Y.C.; Liu, J.C.; Zhao, X.M.; Su, F.Q.; Cui, H.X. Immuno-stimulatory activities of a low molecular weight antitumoral polysaccharide isolated from *Agaricus blazei* Murill (LMPAB) in Sarcoma 180 ascitic tumor-bearing mice. *Pharmazie.*, **2009**, *64*(7), 472-476.

[26] Dong, Q.; Jia, L.M.; Fang, J.N. A beta-D-glucan isolated from the fruiting bodies of *Hericium erinaceus* and its aqueous conformation. *Carbohydr. Res.*, **2006**, *341*, 791-795

[27] Lee, J.S.; Min, K.M.; Cho, J.Y.; Hong, E.K. Study of macrophage activation and structural characteristics of purified polysaccharides from the fruiting body of *Hericium erinaceus*. *J. Microbiol. Biotechnol*., **2009**, *19*, 951-959.

[28] Kim, S.P.; Kang, M.Y.; Kim, J.H.; Nam, S.H.; Friedman, M. Composition and mechanism of antitumor effects of *Hericium erinaceus* mushroom extracts in tumor-bearing mice. *J. Agric. Food Chem*., **2011**, *59*, 9861-9869.

[29] Inomata, T.; Goodman, G.B.; Fryer, C.J.; Chaplin, D.J.; Palcic, B.; Lam, G.K.; Nishioka, A.; Ogawa, Y. Immune reaction induced by X-rays and pions and its stimulation by schizophyllan (SPG). *Br. J. Cancer Suppl*., **1996**, *27*, S122-125.

[30] Kano, Y.; Kakuta, H.; Hashimoto, J. Inhibitory effect of metastasis by combined administration with interleukin-2 and sizofiran, a single glucan--immunohistochemical study. *Biotherapy*., **1996**, *9*, 263-269.

[31] Arika, T.; Amemiya, K.; Nomoto, K. Combination therapy of radiation and Sizofiran (SPG) on the tumor growth and metastasis on squamous-cell carcinoma NR-S1 in syngeneic C3H/He mice. *Biotherapy*., **1992**, *4*, 165-170.

[32] Sakurai, T.; Ohno, N.; Yadomae, T. Effects of fungal beta-glucan and interferon-gamma on the secretory functions of murine alveolar macrophages. *J. Leukoc. Biol*., **1996**, *60*, 118-124.

[33] Minari, J.; Mochizuki, S.; Matsuzaki, T.; Adachi, Y.; Ohno, N.; Sakurai, K. Enhanced cytokine secretion from primary macrophages due to Dectin-1 mediated uptake of CpG DNA/β-1,3-glucan complex. *Bioconjug. Chem*., **2011**, *22*, 9-15.

[34] Jones, D.; Gordon, A.H.; Bacon, J.S. Co-operative action by endo- and exo-beta-(1 leads to 3)-glucanases from parasitic fungi in the degradation of cell-wall glucans of Sclerotinia sclerotiorum (Lib.) de Bary. *Biochem. J*., **1974**, *140*, 47-55.

[35] Sakurai, T.; Ohno, N.; Suzuki, I.; Yadomae, T. Effect of soluble fungal (1,3)-beta-D-glucan obtained from *Sclerotinia sclerotiorum* on alveolar macrophage activation. *Immunopharmacol.*, **1995**, *30*, 157-166.

[36] Sakurai, T.; Suzuki, I.; Kinoshita, A.; Oikawa, S.; Masuda, A.; Ohsawa, M.; Yadomae, T. Effect of intraperitoneally administered beta-1,3-glucan, SSG, obtained from *Sclerotinia sclerotiorum* IFO 9395 on the functions of murine alveolar macrophages. *Chem. Pharm. Bull.*, **1991**, *39*, 214-217.

[37] Misaki, A.; Kakuta, M.; Sasaki, T.; Tanaka, M.; Miyaji, H. Studies on interrelation of structure and antitumor effects of polysaccharides: antitumor action of periodate-modified, branched (1 goes to 3)-beta-D-glucan of *Auricularia auricula*-judae, and other polysaccharides containing (1 goes to 3)-glycosidic linkages. *Carbohydr. Res*., **1981**, *92*, 115-129.

[38] Yoshikawa, N.; Kunitomo, M.; Kagota, S.; Shinozuka, K.; Nakamura, K. Inhibitory effect of cordycepin on hematogenic metastasis of B16-F1 mouse melanoma cells accelerated by adenosine-5'-diphosphate. *Anticancer Res*., **2009**, *29*, 3857-3860.

[39] Fisher, M.; Yang, L.X. Anticancer effects and mechanisms of polysaccharide-K (PSK): implications of cancer immunotherapy. *Anticancer Res.*, **2002**, *22*, 1737-1754.

[40] Xu, T.; Beelman, R.B.; Lambert, J.D. The Cancer Preventive Effects of Edible Mushrooms. *Anticancer Agents Med. Chem.*, **2012**.

[41] Qi, C.; Cai, Y.; Gunn, L.; Ding, C.; Li, B.; Kloecker, G.; Qian, K.; Vasilakos, J.; Saijo, S.; Iwakura, Y.; Yannelli, J.R.; Yan, J. Differential pathways regulating innate and adaptive antitumor immune responses by particulate and soluble yeast-derived β-glucans. *Blood*., **2011**, *117*, 6825-6836.

[42] Lee, J.N.; Lee, D.Y.; Ji, I.H.; Kim, G.E.; Kim, H.N.; Sohn, J.; Kim, S.; Kim, C.W. Purification of soluble beta-glucan with immune-enhancing activity from the cell wall of yeast. *Biosci. Biotechnol. Biochem*., **2001**, *65*, 837-841.

[43] Cramer, D.E.; Wagner, S.; Li, B.; Liu, J.; Hansen, R.; Reca, R.; Wu, W.; Surma, E.Z.; Laber, D.A.; Ratajczak, M.Z.; Yan, J. Mobilization of hematopoietic progenitor cells by yeast-derived beta-glucan requires activation of matrix metalloproteinase-9. *Stem Cells.*, **2008**, *26*, 1231-1240.

[44] LeBlanc, B.W.; Albina, J.E.; Reichner, J.S. The effect of PGG-beta-glucan on neutrophil chemotaxis *in vivo*. *J. Leukoc. Biol*., **2006**, *79*, 667-675.

[45] Li, B.; Cramer, D.; Wagner, S.; Hansen, R.; King, C.; Kakar, S.; Ding, C.; Yan, J. Yeast glucan particles activate murine resident macrophages to secrete proinflammatory cytokines *via* MyD88- and Syk kinase-dependent pathways. *Clin. Immunol*., **2007**, *124*, 170-181.

[46] Liu, X.L.; Lin, N.; Zan, D.; Yuan, J.J.; Cai, D.L. Effect of zymosan on antioxidant and immune function of S180 tumor-bearing mice. *Cell Biochem. Biophys*., **2011**, *60*, 225-229.

[47] Mariani, C.L.; Rajon, D.; Bova, F.J.; Streit, W.J. Nonspecific immunotherapy with intratumoral lipopolysaccharide and zymosan A but not GM-CSF leads to an effective anti-tumor response in subcutaneous RG-2 gliomas. *J. Neurooncol*., **2007**, *85*, 231-240.

[48] Arika, T.; Amemiya, K.; Nomoto, K. Combination therapy of radiation and Sizofiran (SPG) on the tumor growth and metastasis on squamous-cell carcinoma NR-S1 in syngeneic C3H/He mice. *Biotherapy*., **1992**, *4*(2), 165-170.

[49] Sveinbjørnsson, B.; Rushfeldt, C.; Seljelid, R.; Smedsrød, B. Inhibition of establishment and growth of mouse liver metastases after treatment with interferon gamma and beta-1,3-D-glucan. *Hepatology*, **1998**, *27*(5), 1241-1248.

[50] Hamuro, J.; Takatsuki, F.; Suga, T.; Kikuchi, T.; Suzuki, M. Synergistic antimetastatic effects of lentinan and interleukin 2 with pre- and post-operative treatments. *Jpn. J. Cancer Res.*, **1994**, *85*(12), 1288-1297.

[51] Mizuno, T. Development of antitumor polysaccharides from mushroom fungi. *Foods Food Ingred. J. Jpn*., **1996**, *167*, 69–85.

[52] Mushiake, H.; Tsunoda, T.; Nukatsuka, M.; Shimao, K.; Fukushima, M.; Tahara, H. Dendritic cells might be one of key factors for eliciting antitumor effect by chemoimmunotherapy *in vivo*. *Cancer Immunol. Immunother*., **2005**, *54*(2), 120-128.

[53] Harnack, U.; Eckert, K.; Pecher, G. Beta-(1-3),(1-6)-D-glucan enhances the effect of low-dose cyclophosphamide treatment on A20 lymphoma in mice. *Anticancer Res*., **2011**, *31*(4), 1169-1172.

[54] Kim, W.J.; Yoon, T.J.; Kim, D.W.; Moon, W.K.; Lee, K.H**.** Immuno-stimulating activity of beta-glucan isolated from the cell wall of mutant saccharomyces cerevisiae, and its anti-tumor application with cisplatin. *Korean J. Food Nutr*., **2010**, *23*(2), 141-146.

[55] Mizuno, M.; Minato, K.; Ito, H.; Kawade, M.; Terai, H.; Tsuchida, H. Anti-tumor polysaccharide from the mycelium of liquid-cultured *Agaricus blazei* mill. *Biochem. Mol. Biol. Int.*, **1999**, *47*, 707-714.

[56] Okuyama, K.; Isono, K.; Juan, I.K.; Onoda, S.; Ochiai, T.; Yamamoto, Y.; Koide, Y.; Satoh, H. Evaluation of treatment for gastric cancer with liver metastasis. *Cancer*, **1985**, *55*(10), 2498-2505.

[57] Shimizu, K.; Watanabe, S.; Watanabe, S.; Matsuda, K.; Suga, T.; Nakazawa, S.; Shiratori, K. Efficacy of oral administered superfine dispersed lentinan for advanced pancreatic cancer. *Hepatogastroenterol*., **2009**, *56*(89), 240-244.

[58] Hazama, S.; Watanabe, S.; Ohashi, M.; Yagi, M.; Suzuki, M.; Matsuda, K.; Yamamoto, T.; Suga, Y.; Suga, T.; Nakazawa, S.; Oka, M. Efficacy of orally administered superfine dispersed lentinan (beta-1,3-glucan) for the treatment of advanced colorectal cancer. *Anticancer Res*., **2009**, *29*(7), 2611-2617.

[59] Kataoka, H.; Shimura, T.; Mizoshita, T.; Kubota, E.; Mori, Y.; Mizushima, T.; Wada, T.; Ogasawara, N.; Tanida, S.; Sasaki, M.; Togawa, S.; Sano, H.; Hirata, Y.; Ikai, M.; Mochizuki, H.; Seno, K.; Itoh, S.; Kawai, T.; Joh, T. Lentinan with S-1 and paclitaxel for gastric cancer chemotherapy improve patient quality of life. *Hepatogastroenterology*, **2009**, *56*(90), 547-550.

[60] Yoshino, S.; Tabata, T.; Hazama, S.; Iizuka, N.; Yamamoto, K.; Hirayama, M.; Tangoku, A.; Oka, M. Immunoregulatory effects of the antitumor polysaccharide lentinan on Th1/Th2 balance in patients with digestive cancers. *Anticancer Res.*, **2000**, *20*(6C), 4707-4711.

[61] Takeshita, K.; Watanuki, S.; Iida, M.; Saito, N.; Maruyama, M.; Sunagawa, M.; Habu, H.; Endo, M. Effect of lentinan on lymphocyte subsets of peripheral blood, lymph nodes, and tumor tissues in patients with gastric cancer. *Surg. Today*., **1993**, *23*(2), 125-129.

[62] Demir, G.; Klein, H.O.; Mandel-Molinas, N.; Tuzuner, N. Beta glucan induces proliferation and activation of monocytes in peripheral blood of patients with advanced breast cancer. *Int. Immunopharmacol*., **2007**, *7*(1), 113-116.

[63] Tari, K.; Satake, I.; Nakagomi, K.; Ozawa, K.; Oowada, F.; Higashi, Y.; Negishi, T.; Yamada, T.; Saito, H.; Yoshida, K. Effect of lentinan for advanced prostate carcinoma. *Hinyokika Kiyo.*, **1994**, *40*(2), 119-123.

[64] Yang, P.; Liang, M.; Zhang, Y.; Shen, B. Clinical application of a combination therapy of lentinan, multi-electrode RFA and TACE in HCC. *Adv. Ther.*, **2008**, *25*(8), 787-794.

[65] Shimizu, Y.; Hasumi, K.; Masubuchi, K. Augmenting effect of sizofiran on the immunofunction of regional lymph nodes in cervical cancer. *Cancer*, **1992**, *69*(5), 1184-1194.

[66] Knudsen, K.E.; Jensen, B.B.; Hansen, I. Digestion of polysaccharides and other major components in the small and large intestine of pigs fed on diets consisting of oat fractions rich in beta-D-glucan. *Br. J. Nutr.*, **1993**, *70*(2), 537-556.

[67] Ohno, N.; Terui, T.; Chiba, N.; Kurachi, K.; Adachi, Y.; Yadomae, T. Resistance of highly branched (1-->3)-beta-D-glucans to formolysis. *Chem. Pharm. Bull.*, **1995**, *43*(6), 1057-1060.

[68] Wang, H.; Weening, D.; Jonkers, E.; Boer, T.; Stellaard, F.; Small, A.C.; Preston, T.; Vonk, R.J.; Priebe, M.G. A curve fitting approach to estimate the extent of fermentation of indigestible carbohydrates. *Eur. J. Clin. Invest.*, **2008**, *38*(11), 863-868.

[69] Chan, G.C.; Chan, W.K.; Sze, D.M. The effects of beta-glucan on human immune and cancer cells. *J. Hematol. Oncol.*, **2009**, *2*, 25.

[70] Chen, J.; Seviour, R. Medicinal importance of fungal beta-(1-->3), (1-->6)-glucans. *Mycol. Res.*, **2007**, *111*(pt6), 635-652.

[71] Kurashige, S.; Akuzawa, Y.; Endo, F. Effects of Lentinus edodes, Grifola frondosa and Pleurotus ostreatus administration on cancer outbreak, and activities of macrophages and lymphocytes in mice treated with a carcinogen, N-butyl-N-butanolnitrosoamine. *Immunopharmacol. Immunotoxicol.*, **1997**, *19*(2), 175-183.

[72] Brown, G.D.; Taylor, P.R.; Reid, D.M.; Willment, J.A.; Williams, D.L.; Martinez-Pomares, L.; Wong, S.Y.; Gordon, S. Dectin-1 is a major beta-glucan receptor on macrophages. *J. Exp. Med.*, **2002**, *196*(3), 407-412.

[73] Sato, M.; Sano, H.; Iwaki, D.; Kudo, K.; Konishi, M.; Takahashi, H.; Takahashi, T.; Imaizumi, H.; Asai, Y.; Kuroki, Y. Direct binding of Toll-like receptor 2 to zymosan, and zymosan-induced NF-kappa B activation and TNF-alpha secretion are down-regulated by lung collectin surfactant protein A. *J. Immunol.*, **2003**, *171*(1), 417-425.

[74] Young, R.E.; Thompson, R.D.; Larbi, K.Y.; La, M.; Roberts, C.E.; Shapiro, S.D.; Perretti, M.; Nourshargh, S. Neutrophil elastase (NE)-deficient mice demonstrate a nonredundant role for NE in neutrophil migration, generation of proinflammatory mediators, and phagocytosis in response to zymosan particles *in vivo*. *J. Immunol.*, **2004**, *172*(7), 4493-4502.

[75] Kim, H.S.; Hong, J.T.; Kim, Y.; Han, S.B. Stimulatory Effect of β-glucans on Immune Cells. *Immune. Netw.*, **2011**, *11*(4), 191-195.

[76] Li, B.; Cai, Y.; Qi, C.; Hansen, R.; Ding, C.; Mitchell, T.C.; Yan, J. Orally administered particulate beta-glucan modulates tumor-capturing dendritic cells and improves antitumor T-cell responses in cancer. *Clin. Cancer Res.*, **2010**, *16*(21), 5153-5164.

[77] Moradali, M.F.; Mostafavi, H.; Ghods, S.; Hedjaroude, G.A. Immunomodulating and anticancer agents in the realm of macromycetes fungi (macrofungi). *Int. Immunopharmacol.*, **2007**, *7*(6), 701-724.

[78] Chihara, G.; Hamuro, J.; Maeda, Y.Y.; Shiio, T.; Suga, T.; Takasuka, N.; Sasaki, T. Antitumor and metastasis-inhibitory activities of lentinan as an immunomodulator: an overview. *Cancer Detect. Prev. Suppl.*, **1987**, *1*, 423-443.

[79] Nakano, H.; Namatame, K.; Nemoto, H.; Motohashi, H.; Nishiyama, K.; Kumada, K. A multi-institutional prospective study of lentinan in advanced gastric cancer patients with unresectable and recurrent diseases: effect on prolongation of survival and improvement of quality of life. Kanagawa Lentinan Research Group. *Hepatogastroenterol.*, **1999**, *46*(28), 2662-2668.

[80] Aoki, T. Lentinan. In: Fenichel RL, Chirgis MA (eds) Immune modulation agents and their mechanisms. *Immunol. Stud.*, **1984**, *25*, 62-77.

[81] Mizuno, T.; Yeohlui, P.; Kinoshita, T.; Zhuang, C.; Ito, H.; Mayuzumi, Y. Antitumor activity and chemical modification of polysaccharides from Niohshimeji mushroom, Tricholoma giganteum. *Biosci. Biotechnol. Biochem.*, **1996**, *60*, 30-33.

[82] Hobbs, C. Medicinal mushrooms: an exploration of tradition, healing and culture. Botanica Press, Santa Cruz, Calif. **1995**

[83] Ohno, N.; Jinushi, T.; Yadomae, T. Th1-Oriented Immunomodulating Activity of Gel-Forming Fungal (1→3)-Beta-Glucans. *Int. J. Med. Mushroom*, **2002**, *2*, 19-35.

[84] Hong, F.; Yan, J.; Baran, J.T.; Allendorf, D.J.; Hansen, R.D.; Ostroff, G.R.; Xing, P.X.; Cheung, N.K.; Ross, G.D. Mechanism by which orally administered beta-1,3-glucans enhance the tumoricidal activity of antitumor monoclonal antibodies in murine tumor models. *J. Immunol.*, **2004**, *173*(2), 797-806.

[85] Rice, P.J.; Adams, E.L.; Ozment-Skelton, T.; Gonzalez, A.J.; Goldman, M.P.; Lockhart, B.E.; Barker, L.A.; Breuel, K.F.; Deponti, W.K.; Kalbfleisch, J.H.; Ensley, H.E.; Brown, G.D.; Gordon, S.; Williams, D.L. Oral delivery and gastrointestinal absorption of soluble glucans stimulate increased resistance to infectious challenge. *J. Pharmacol. Exp. Ther.*, **2005**, *314*(3), 1079-1086.

[86] Ooi, V.E.C.; Liu, F. A review of pharmacological activities of mushroom polysaccharides. *Int. J. Med. Mushrooms.*, **1999**, *1*, 195-206.

[87] Ross, G.D.; Cain, J.A.; Myones, B.L.; Newman, S.L.; Lachmann, P.J. Specificity of membrane complement receptor type three (CR3) for beta-glucans. *Complement.*, **1987**, *4*(2), 61-74.

[88] Thornton, B.P.; Větvicka, V.; Pitman, M.; Goldman, R.C.; Ross, G.D. Analysis of the sugar specificity and molecular location of the beta-glucan-binding lectin site of complement receptor type 3 (CD11b/CD18). *J. Immunol.*, **1996**, *156*(3), 1235-1246.

[89] Zimmerman, J.W.; Lindermuth, J.; Fish, P.A.; Palace, G.P.; Stevenson, T.T.; DeMong, D.E. A novel carbohydrate-glycosphingolipid interaction between a beta-(1-3)-glucan immunomodulator, PGG-glucan, and lactosylceramide of human leukocytes. *J. Biol. Chem.*, **1998**, *273*(34), 22014-22020.

[90] Rice, P.J.; Kelley, J.L.; Kogan, G.; Ensley, H.E.; Kalbfleisch, J.H.; William Browder, I.; Williams, D.L. Human monocyte scavenger receptors are pattern recognition receptors for (1→3)-β-D-glucans. *J. Leukoc. Biol.*, **2002**, *72*(1), 140-146.

[91] Shao, B.M.; Dai, H.; Xu, W.; Lin, Z.B.; Gao, X.M. Immune receptors for polysaccharides from Ganoderma lucidum. *Biochem. Biophys. Res. Commun.*, **2004**, *323*(1), 133-141.

[92] Ross, G.D. Regulation of the adhesion versus cytotoxic functions of the Mac-1/CR3/α(m)β2-integrin glycoprotein. *Crit. Rev. Immunol.*, **2000**, *20*, 197-222.

[93] Herre, J.; Gordon, S.; Brown, G.D. Dectin-1 and its role in the recognition of β-glucans by macrophages. *Mol. Immunol.*, **2004**, *40*, 869-876.

[94] Underhill, D.M.; Rossnagle, E.; Lowell, C.A.; Simmons, R.M. Dectin-1 activates Syk tyrosine kinase in a dynamic subset of macrophages for reactive oxygen production. *Blood.*, **2005**, *106*, 2543-2550.

[95] Brown, G.D.; Gordon, S. Immune recognition. A new receptor for beta-glucans. *Nature*, **2001**, *413*, 36-37.

[96] Steele, C.; Marrero, L.; Swain, S.; Harmsen, A.G.; Zheng, M.; Brown, G.D.; Gordon, S.; Shellito, J.E.; Kolls, J.K. Alveolar macrophage-mediated killing of Pneumocystis carinii f. sp. muris involves molecular recognition by the dectin-1 β-glucan receptor. *J. Exp. Med.*, **2003**, *198*, 1677-1688.

[97] Willment, J.A.; Marshall, A.S.; Reid, D.M.; Williams, D.L.; Wong, S.Y.C.; Gordon, S.; Brown, G.D. The human β-glucan receptor is widely expressed and functionally equivalent to murine Dectin-1 on primary cells. *Eur. J. Immunol.*, **2005**, *35*, 1539-1547.

[98] Taylor, P.R.; Tsoni, S.V.; Willment, J.A.; Dennehy, K.M.; Rosas, M.; Findon, H.; Haynes, K.; Steele, C.; Botto, M.; Gordon, S.; Brown, G.D. Dectin-1 is required for β-glucan recognition and control of fungal infection. *Nat. Immunol.*, **2007**, *8*, 31-38.

[99] Taylor, P.R.; Brown, G.D.; Herre, J.; Williams, D.L.; Willment, J.A.; Gordon, S. The role of SIGNR1 and the beta-glucan receptor (dectin-1) in the nonopsonic recognition of yeast by specific macrophages. *J. Immunol.*, **2004**, *172*(2), 1157-1162.

[100] Brown, G.D. Dectin-1: A signalling non-TLR pattern-recognition receptor. *Nat. Rev. Immunol.*, **2006**, *6*(1), 33-43.

[101] Underhill, D.M.; Ozinsky, A.; Hajjar, A.M.; Stevens, A.; Wilson, C.B.; Bassetti, M.; Aderem, A. The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. *Nature*, **1999**, *401*(6755), 811-815.

[102] Brown, G.D.; Herre, J.; Williams, D.L.; Willment, J.A.; Marshall, A.S.; Gordon, S. Dectin-1 mediates the biological effects of beta-glucans. *J. Exp. Med.*, **2003**, *197*(9), 1119-1124.

[103] Gantner, B.N.; Simmons, R.M.; Canavera, S.J.; Akira, S.; Underhill, D.M. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *J. Exp. Med.*, **2003**, *197*(9), 1107-1117.

[104] Rogers, N.C.; Slack, E.C.; Edwards, A.D.; Nolte, M.A.; Schulz, O.; Schweighoffer, E.; Williams, D.L.; Gordon, S.; Tybulewicz, V.L.; Brown, G.D.; Reis e Sousa, C. Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. *Immunity*, **2005**, *22*(4), 507-517.

[105] Yadav, M.; Schorey, J.S. The beta-glucan receptor dectin-1 functions together with TLR2 to mediate macrophage activation by mycobacteria. *Blood*, **2006**, *108*(9), 3168-3175.

[106] Yoshitomi, H.; Sakaguchi, N.; Kobayashi, K.; Brown, G.D.; Tagami, T.; Sakihama, T.; Hirota, K.; Tanaka, S.; Nomura, T.; Miki, I.; Gordon, S.; Akira, S.; Nakamura, T.; Sakaguchi, S. A role for fungal {beta}-glucans and their receptor Dectin-1 in the induction of autoimmune arthritis in genetically susceptible mice. *J. Exp. Med.*, **2005**, *201*(6), 949-960.

[107] Herre, J.; Marshall, A.S.; Caron, E.; Edwards, A.D.; Williams, D.L.; Schweighoffer, E.; Tybulewicz, V.; Reis e Sousa, C.; Gordon, S.; Brown, G.D. Dectin-1 uses novel mechanisms for yeast phagocytosis in macrophages. *Blood*, **2004**, *104*(13), 4038-4045.

[108] Vetyicka, V. Glucan-immunostimulant, adjuvant, potential drug. *World J. Clin. Oncol.*, **2011**, *2*(2), 115-119.

[109] Li, B.; Allendorf, D.J.; Hansen, R.; Marroquin, J.; Ding, C.; Cramer, D.E.; Yan, J. Yeast β-glucan amplifies phagocyte killing of iC3b-opsonized tumor cells *via* complement receptor 3-Syk-phosphatidylinositol 3-Kinase pathway. *J. Immunol.*, **2006**, *177*, 1661-1669.

[110] Józefowski, S.; Yang, Z.; Marcinkiewicz, J.; Kobzik, L. Scavenger receptors and β-glucan receptors participate in the recognition of yeasts by murine macrophages. *Inflamm. Res.*, **2012**, *61*(2), 113-126.

[111] Nakayama, H.; Yoshizaki, F.; Prinetti, A.; Sonnino, S.; Mauri, L.; Takamori, K.; Ogawa, H.; Iwabuchi, K. Lyn-coupled LacCer-enriched lipid rafts are required for CD11b/CD18-mediated neutrophil phagocytosis of nonopsonized microorganisms. *J. Leukoc. Biol.*, **2008,** *83*(3), 728-741.

[112] Soong, G.; Reddy, B.; Sokol, S.; Adamo, R.; Prince, A. TLR2 is mobilized into an apical lipid raft receptor complex to signal infection in airway epithelial cells. *J. Clin. Invest.*, **2004**, *113*(10), 1482-1489.

[113] Nakahira, K.; Kim, H.P.; Geng, X.H.; Nakao, A.; Wang, X.; Murase, N.; Drain, P.F.; Wang, X.; Sasidhar, M.; Nabel, E.G.; Takahashi, T.; Lukacs, N.W.; Ryter, S.W.; Morita, K.; Choi, A.M. Carbon monoxide differentially inhibits TLR signaling pathways by regulating ROS-induced trafficking of TLRs to lipid rafts. *J. Exp. Med.*, **2006**, *203*(10), 2377-2389.

[114] Triantafilou, M.; Gamper, F.G.; Haston, R.M.; Mouratis, M.A.; Morath, S.; Hartung, T.; Triantafilou, K. Membrane sorting of toll-like receptor (TLR)-2/6 and TLR2/1 heterodimers at the cell surface determines heterotypic associations with CD36 and intracellular targeting. *J. Biol. Chem.*, **2006**, *281*(41), 31002-31011.

[115] Xu, S.; Huo, J.; Gunawan, M.; Su, I.H.; Lam, K.P. Activated dectin-1 localizes to lipid raft microdomains for signaling and activation of phagocytosis and cytokine production in dendritic cells. *J. Biol. Chem.*, **2009**, *284*(33), 22005-22011.

[116] Czop, J.K. The role of beta-glucan receptors on blood and tissue leukocytes in phagocytosis and metabolic activation. *Pathol. Immunopathol. Res.*, **1986**, *5*(3-5), 286-296.

[117] Williams, D.; Mueller, A.; Erowder, W. Glucan-based macrophage stimulators a review of their anti-infective potential. *Clinical Immunotherapy*, **1996**, *5*, 392-399.

[118] Falch, B.H.; Espevik, T.; Ryan, L.; Stokke, B.T. The cytokine stimulating activity of (1→3)-β-D-glucans is dependent on the triple helix conformation. *Carbohydr. Res.*, **2000**, *329*, 587-596.

[119] Zhang, L.; Li, X.; Xu, X.; Zeng, F. Correlation between antitumor activity, molecular weight, and conformation of lentinan. *Carbohydr. Res.*, **2005**, *340*, 1515-1521.

[120] Mueller, A.; Raptis, J.; Rice, P.J.; Kalbfleisch, J.H.; Stout, R.D.; Ensley, H.E.; Browder, W.; Williams, D.L. The influence of glucan polymer structure and solution conformation on binding to (1-->3)-beta-D-glucan receptors in a human monocyte-like cell line. *Glycobiol.*, **2000**, *10*(4), 339-346.

[121] Wang, Y.; Zhang, L.; Li, Y.; Hou, X.; Zeng, F. Correlation of structure to antitumor activities of five derivatives of a beta-glucan from Poria cocos sclerotium. *Carbohydr. Res.*, **2004**, *339*(15), 2567-2574.

[122] Di Luzio, N.R.; Williams, D.L.; McNamee, R.B.; Edwards, B.F.; Kitahama, A. Comparative tumor-inhibitory and anti-bacterial activity of soluble and particulate glucan. *Int. J. Cancer.*, **1979**, *24*(6), 773-779.

[123] Di Luzio, N.R.; Williams, D.L.; McNamee, R.B.; Malshet, V.G. Comparative evaluation of the tumor inhibitory and antibacterial activity of solubilized and particulate glucan. *Rec. Results Cancer Res.*, **1980**, *75*, 165-172.

[124] Xiao, Z.; Trincado, C.A.; Murtaugh, M.P. Beta-glucan enhancement of T cell IFNgamma response in swine. *Vet. Immunol. Immunopathol.*, **2004**, *102*(3), 315-320.

[125] Brown, G.D.; Gordon, S. Fungal β-Glucans and Mammalian Immunity. *Immunity.*, **2003**, 19, 311-315.

[126] O'Brien, X.M.; Heflin, K.E.; Lavigne, L.M.; Yu, K.; Kim, M.; Salomon, A.R.; Reichner, J.S. Lectin site ligation of CR3 induces conformational changes and signaling. *J.Biol. Chem.*, **2012**, *287*(5), 3337-3348.

[127] Xiao, G.; Miyazato, A.; Abe, Y.; Zhang, T.; Nakamura, K.; Inden, K.; Tanaka, M.; Tanno, D.; Miyasaka, T.; Ishii, K.; Takeda, K.; Akira, S.; Saijo, S.; Iwakura, Y.; Adachi, Y.; Ohno, N.; Yamamoto, N.; Kunishima, H.; Hirakata, Y.; Kaku, M.; Kawakami, K. Activation of myeloid dendritic cells by deoxynucleic acids from Cordyceps sinensis *via* a Toll-like receptor 9-dependent pathway. *Cell Immunol.*, **2010**, *263*, 241-250

[128] Pao, H.Y.; Pan, B.S.; Leu, S.F.; Huang, B.M. Cordycepin stimulated steroidogenesis in MA-10 mouse leydig tumor cells through the protein kinase C Pathway. *J. Agric. Food Chem.*, **2012**, *60*, 4905-4913.

[129] Xia, Y.; Vetvicka, V.; Yan, J.; Hanikýrová, M.; Mayadas, T.; Ross, G.D. The beta-glucan-binding lectin site of mouse CR3 (CD11b/CD18) and its function in generating a primed state of the receptor that mediates cytotoxic activation in response to iC3b-opsonized target cells. *J. Immunol.*, **1999**, *162*(4), 2281-2290.

[130] Wang, J.; Gigliotti, F.; Maggirwar, S.; Johnston, C.; Finkelstein, J.N.; Wright, T.W. Pneumocystis carinii activates the NF-kappaB signaling pathway in alveolar epithelial cells. *Infect. Immun.*, **2005**, *73*(5), 2766-2777.

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