APŽVALGINIS STRAIPSNIS

Effects of β-glucans on the immune system

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Key words: *β-glucan; anticarcinogenic activity; monoclonal antibodies; complement receptor.*

Summary. *β-Glucans are naturally occurring polysaccharides. These glucose polymers are constituents of the cell wall of certain pathogenic bacteria and fungi. The healing and immunostimulating properties of mushrooms have been known for thousands of years in the Eastern countries. These mushrooms contain biologically active polysaccharides that mostly belong to group of β-glucans. These substances increase host immune defense by activating complement system, enhancing macrophages and natural killer cell function. The induction of cellular responses by mushroom and other β-glucans is likely to involve their specific interaction with several cell surface receptors, as complement receptor 3 (CR3; CD11b/CD18), lactosylceramide, selected scavenger receptors, and dectin-1 (βGR). β-Glucans also show anticarcinogenic activity. They can prevent oncogenesis due to the protective effect against potent genotoxic carcinogens. As immunostimulating agent, which acts through the activation of macrophages and NK cell cytotoxicity, β-glucan can inhibit tumor growth in promotion stage too. Antiangiogenesis can be one of the pathways through which β-glucans can reduce tumor proliferation, prevent tumor metastasis. β-Glucan as adjuvant to cancer chemotherapy and radiotherapy demonstrated the positive role in the restoration of hematopiesis following by bone marrow injury. Immunotherapy using monoclonal antibodies is a novel strategy of cancer treatment. These antibodies activate complement system and opsonize tumor cells with iC3b fragment. In contrast to microorganisms, tumor cells, as well as other host cells, lack β-glucan as a surface component and cannot trigger complement receptor 3-dependent cellular cytotoxicity and initiate tumor-killing activity. This mechanism could be induced in the presence of β-glucans.*

β-Glucan has been known to scientists as a plant constituent for decades. For over twenty years, it has been studied for the favorable biological effects on mammals. It has been common knowledge in the scientific community that β-glucan is the most known powerful immune stimulant and a very powerful antagonist to both benign and malignant tumors; it lowers cholesterol and triglyceride level, normalizes blood sugar level, heals and rejuvenates the skin and has various other benefits.

β-Glucan sources and structure

β-Glucans are naturally occurring polysaccharides. These glucose polymers are produced by a variety of plants, such as oat, barley, and seaweed. β-Glucans are the constituents of the cell wall of certain pathogenic bacteria (*Pneumocystis carinii, Cryptococcus neoformans, Aspergillus fumigatus, Histoplasma capsulatum, Candida albicans*) and fungi (*Saccharomyces cerevisiae*).

The main components of the fungal cell wall are polysaccharides and glycoproteins.

For example, yeast (*Saccharomyces cerevisiae)* cell wall consists of three layers: an inner layer of insoluble β-glucan (30–35%), middle layer – of soluble β-glucan (20–22%), external layer – of glycoprotein (30%) (1). β-Glucan has been purified from brewer's and backer's yeast (2), from oats and barley bran (3).

The healing and immunostimulating properties of mushrooms have been known for thousands of years. The extracts of these mushrooms were widely used for treatment purpose in East countries. The number of mushrooms on Earth is estimated to be 140 000;

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aproximately 10% (approximately 14 000 named species) are known. These mushrooms contain biologically active polysaccharides in fruit bodies and cultured mycelium. These polysaccharides are of different chemical composition, with most belonging to the group of β-glucans and they have attracted the most attention (4). β-Glucan has been isolated from some mushrooms as shiitake (*Lentinus edodes*), maitake (*Grifola frondosa*) (5), schizophylan (*Schizophillum commune*), and SSG (*Sclerotinia sclerotiorum)* (6). β-Glucan extracts from *Lentinus edodes* and *Schizophillum commune* are used in traditional medicine for cancer treatment in Japan since 1980.

β-Glucans derived from different sources have some differences in their structure. Glucans are a heterogeneous group of glucose polymers, consisting of a backbone of β(1,3)-linked β-D-glucopyranosyl units with β(1,6)-linked side chains of varying distribution and length. Oat and barley β-glucans are primarily linear with large regions of β(1,4) linkages separating shorter stretches of β(1,3) structures. Mushrooms β-glucans have short β(1,6)-linked branches coming off of the β(1,3) backbone. Yeast β-glucans have β(1,6) branches that are further elaborated with additional β(1,3) regions. These structural differences can have large implications for the activity of the β-glucan. For example, differences in the length of the polysaccharide chain, extent of branching, and the length of those branches can result in the difference between material extractable by hot water, as mushroom β-glucans, and insoluble cell wall component, as yeast β-glucan, and in different molecular weight. In general, *in vitro* studies have suggested that large molecular weight or particular β-glucans (such as zymosan) can directly activate leukocytes, stimulating their phagocytic, cytotoxic, and antimicrobial activities, including the production of reactive oxygen and nitrogen intermediates. Intermediate or low molecular weight β-glucans (such as glucan phosphate) possess biological activity *in vivo*, but their cellular effects are less clear. Very short β-glucans (<5000–10 000 molecular weight; such as laminarin) are generally considered inactive (7–9). Yeast β-glucan, because it is easily purified, and mushrooms β-glucans, because there are a lot of experiments performed in Japan, China, and Korea, are mostly investigated.

β-Glucan immunostimulating activity

Patients who suffer from systemic fungal infections including those caused by *Candida*, *Aspergillus*, and *Cryptococcus* species have been described to possess high levels of circulating β-glucans in their plasma. It is possible that they may have modulating effects on the immune system by activating of macrophages, phagocytosis of the pathogen, release of proinflammatory cytokines (10). And there was established, β-glucan as a key molecular pattern recognized by neutrophils (or polymorphonuclear leukocytes (PMNs)) in response to *Candida albicans,* because antibody specific for β-glucan, a major component of yeast cell walls, blocks this response (11). This mechanism, to recognize and respond to their conserved structural components, particularly β-glucans, has evolved in mammals as defense against fungal pathogen.

Macrophages play a critical role in all phases of host defense that are both innate and adaptive immune responses in case of an infection. When pathogen crosses an epithelial barrier, it is affected by phagocytosis of macrophages and digested by lysosomal enzymes released from them. Lysosomal enzymes and phagocytic activity determine the macrophage function. The secretion of cytokines (IL-1, IL-6, IL-8, IL-12, TNF-α) and inflammatory mediators (nitric oxide, NO, and hydrogen peroxide, H_2O_2) are other effects of these cells. Therefore, activation of macrophage functions by β-glucans increases host immune defense. However, polysaccharides stimulate a dose-dependent increase in NO and TNF-α, but not in reactive oxygen intermediate production in peritoneum macrophages (12). It is suggested that the ability of polysaccharides upon the up-regulation of these surface molecules involved in antigen-presenting processes may, by inference, activate T-cell-mediated immunity against malignant cells *in vivo*. Taken together, these results suggest that β-glucan acts as an effective immunomodulator and enhances the anti-tumoral activity of peritoneum macrophages.

In vitro studies have demonstrated enhanced microbial killing by monocytes and neutrophils in healthy volunteers after β-glucan administration. Besides activation of macrophages, T cells, natural killer (NK) cells, β-glucan activates complement by alternative activation pathway. Pathogens that activate complement are first coated with the C3b fragment of C3, which is rapidly proteolysed into the iC3b fragment by serum factor I. These iC3b fragments serve to promote a high-avidity attachment of the iC3b-opsonized pathogens to the iC3b receptors (CR3, CD11b/CD18) of phagocytic cells and NK cells, stimulating phagocytosis and/or cytotoxic degranulation (13).

β-Glucan receptors

The induction of cellular responses by mushroom and other β-glucans is likely to involve their specific

interaction with one or more cell surface receptors. This has been the focus of intense research in recent years. Unfortunately, one quite commonly finds the results obtained with one or two β-glucans extrapolated to β-glucans in general, which are then referred to "β-glucan" (14). It should by now be evident that such substance does not exist. Even β-glucans of similar structure, molecular weight, and solution conformation exhibit vastly differing biological activities *in vitro* and *in vivo*, and these differences are yet more pronounced when structurally less similar β-glucans are included in the discussion. To complicate matters even more, much of what is currently known about the molecular interaction of β-glucans and various cell types comes from studies conducted with zymosan. Zymosan is a particle obtained from yeast (*Saccharomyces cerevisiae*) consisting of a variety of different substances, including mannans, glucans, glucosamine, and glycoproteins and is, therefore, not ideally suited for the investigation of β-glucan-specific activities. If, however, a specific effect resulting from the binding of zymosan to a cell can be inhibited by a variety of β-glucans, it can be concluded that these β-glucans bind to the same receptor(s) as the β-glucan part of the zymosan particle.

Glucans are thought to mediate their effects via interaction with membrane receptors on macrophages, neutrophils, and NK cells.

β-Glucan receptors were firstly identified on the surface of monocytes by Czop and Austen in 1985 as opsonin-independent receptors for particulate activators of the alternative complement activation pathway (15). Till now, four β-glucan receptors have been identified as candidates mediating these activities. It is namely complement receptor 3 (CR3; CD11b/CD18), lactosylceramide, selected scavenger receptors, and dectin-1 (βGR).

CR3 (complement receptor 3) is a heterodimeric transmembrane glycoprotein, belonging to the β_2-integrin family, consisting of CD11b noncovalently associated with CD18. Distinct functional domains have been identified in the extracellular portion of the CD11b subunit of CR3: the I- or A-domain is essential for binding and phagocytosis of iC3b-coated particles, and the lectin domain located C-terminal to the I-domain is responsible for the nonopsonic binding properties of CR3 (16). The leukocyte $\alpha_M\beta_2$ integrin known also as Mac-1, complement receptor type 3 (CR3), and CD11b/CD18 functions both as an adhesion molecule facilitating diapedesis and as a C3R enabling phagocytosis or degranulation in response to factor I-cleaved C3b fragment of C3 (iC3b)-opsonized microorganisms. The same lectin domain within CD11b regulates both the cytotoxic and adhesion functions of Mac-1/CR3.

CR3 is highly expressed on neutrophils, monocytes, and NK cells and less present on macrophages.

Lactosylceramide (LacCer; CDw17) is a glycosphingolipid found in the plasma membranes of many cells and was identified as a β-glucan receptor from biochemical analyses of the interactions between β-glucan and isolated human leukocyte membrane components (17). It has been suggested that the interaction of β-glucan with this receptor can induce macrophage inflammatory protein (MIP)-2 and the activation of NFκB and can enhance the neutrophil oxidative burst and antimicrobial functions, but the mechanisms behind these activities are unknown.

Adaptive immunity uses somatically generated receptors that recognize antigenic patterns to which the host has been previously exposed. In contrast, innate immunity relies on genetically predetermined pattern recognition receptors (PRRs) that recognize carbohydrates, lipids, and proteins that are unique to microorganisms and are not produced by the host. These macromolecular structures, usually found in the cell wall, are referred to as pathogen-associated molecular patterns (PAMPs). Glucans may be fungal recognition molecules (PAMPs) for the innate-immune system of the host (18).

Dectin-1 (or β-glucan receptor, βGR) was described by Brown and Gordon by using a blocking monoclonal antibody against CR3 and anti-Dectin1 antibody (19). βGR consists of a single C-type, lectin-like, carbohydrate recognition domain, a short stalk, and a cytoplasmic tail possessing an immunoreceptor tyrosine-based activation motif. It recognizes carbohydrates containing β-1,3 and/or β-1,6 glucan linkages. βGR is expressed on cells of the monocyte/macrophages lineage, neutrophils. Dendritic cells and a subpopulation of T cells also express the βGR, but at lower levels (20).

Phagocytosis of non-opsonized microorganisms by macrophages initiates innate immune responses for host defense against infection. Cytosolic phospholipase A_2 is activated during phagocytosis, releasing arachidonic acid for production of substances, which initiate acute inflammation. Dectin-1 receptor was identified as pattern recognition receptors that stimulate arachidonic acid release and cyclooxygenase 2 (COX2) expression in macrophages by pathogenic yeast and yeast cell walls. Pure particulate (1, 3)-β-D-glucan stimulated arachidonic acid release and COX2 expression were augmented in a Toll-like recep-

tor 2 (TLR2)-dependent manner by macrophage-activating lipopeptide-2 (21). There were first results established concerning a significant role for dectin-1, in cooperation with TLR2, to activate a macrophage's proinflammatory response to a mycobacterial infection (22).

β-Glucan increase resistance to infectious challenge

β-Glucan itself can elicit broad anti-infective effects. *Staphylococcus aureus, Escherichia coli, Candida albicans, Pneumocystis carinii, Listeria monocytogenes, Leishmania donovani, Influenza virus* are the microorganisms, against which a protective effect of β-glucan has been established.

The potential antiviral effect of *Saccharomyces cerevisiae* β-glucan was investigated on the pneumonia induced by swine influenza virus (SIV). The microscopic lung lesions induced by SIV infection were significantly more severe than those induced by infection in animals pre-administered β-glucan. Significantly more SIV nucleic acid was detected in the lungs of pigs experimentally infected with SIV only at 5, 7, and 10 days post-inoculation (dpi) compared with lungs from pigs pre-administered beta-glucan and infected with SIV. The concentrations of interferon-gamma (IFN-gamma) and nitric oxide (NO) in bronchoalveolar lavage fluid from pigs pre-administered β-glucan and infected with SIV were significantly higher than for any other group at 7 and 10 dpi for IFN-gamma, and at 5, 7, and 10 dpi for NO (23).

Other study was the hypothesis that systemic β-glucan treatment would result in enhanced migration of neutrophils into a site of inflammation and improve antimicrobial function was tested in a model of acute inflammation in rats. Animals treated with β-glucan showed a 66±6% and 186±42% increase in wound cell number recovered 6 and 18 h postwounding, respectively. Increased migration did not correlate with increased chemo attractant content of wound fluid, alterations in neutrophil-induced loss of endothelial barrier function, or changes in neutrophils adhesion to endothelial cells. Studies also showed a priming effect for chemotaxis and respiratory burst in circulating neutrophils isolated from β-glucan-treated animals (24).

There are some reports that acidic polysaccharide isolated from *Phellinus linteus* (PL) alleviated the septic shock induced by high dose lipopolysaccharide (LPS) injection in mice (25). To examine the origin of this effect, cytokine production in serum and the expression of MHC II in B cells and macrophages in areas of inflammation was investigated. Pretreatment with PL 24 h before LPS administration resulted in a significant inhibition of up to 68% of circulating tumor necrosis factor (TNF)-α, a moderate reduction of 45% of IL-12 and 23% of IL-1β, but no significant reduction in IL-6. The decrease of IL-1β, IL-12 and TNF-α in sera and the down-modulation of MHC II during septic shock may contribute to the long survival of mice by PL. Administration of PL *in vivo* decreases IL-2, IFN-γ and TNF-α production in splenocytes and enhances spontaneous cell apoptosis in macrophages and lymphocytes stimulated with LPS *in vitro*. But β-glucans induce secretion of TNF-α in dose-dependent manner. High concentration (≥500 μg/mL) causes suppression of the TNF-α release.

As previously described, β-glucan administration enhances microbial killing by monocytes and neutrophils. It may play a role in decreasing the infectious complication rate in patients undergoing major surgery.

The safety and efficacy of β-glucan in surgical patients at high risk for postoperative infection were determined in a double-blind, placebo-controlled randomized phase I/II trial (26). Trial was performed in 34 high-risk patients undergoing major abdominal or thoracic surgery. Patients who received β-glucan had significantly fewer infectious complications (3.4 infections per infected patient *vs.* 1.4 infections per infected patient), decreased intravenous antibiotic requirement (10.3 days *vs.* 0.4 days), and shorter intensive care unit length of stay (3.3 days *vs.* 0.1 days).

A dose-response trend with regard to infection incidence among patients who received β-glucan at a dose of 0.1 mg/kg–2.0 mg/kg was observed in a phase II multicenter, double-blind, randomized, placebo-controlled study (27). Perioperative administration of β-glucan also reduces serious postoperative infections or death by 39% after high-risk noncolorectal gastrointestinal operations (28).

Kournikakis et al. (29) have evaluated the anthrax-protective effect of β-glucan in an experimental animal model. β-Glucan was administered by subcutaneous injection to Balb/c mice 2 days prior to anthrax challenge, or daily oral gavage for 7 days prior to challenge, or in drinking water for 10 days postchallenge with a lethal dose of *Bacillus anthracis* spores.

β-Glucan anticarcinogenic activity

Carcinogenesis can be separated to different stages. The initiation phase involves exposure to a mutagen and often requires its subsequent metabolic transformation into a biologically active form. This exposure,

even if resulting in permanent damage to DNA, is often insufficient by itself to cause cancer. At least in chemically induced tumors in experimental animals, a tumor promoter is often required to stimulate cell division and result in the formation of small, benign tumors. A similar promotion phase is thought to exist in naturally occurring cancers, but the actual events are still only poorly understood. Progression to malignancy occurs when the tight controls that normally govern cell cycle progression break down, resulting in the uncontrolled proliferation of cancerous cells. It also involves the ability of these cells to invade surrounding tissue and to eventually metastasize.

The anticarcinogenic activity of two medicinal mushrooms, such *Ganoderma lucidum* and *Tricholoma lobayense,* extracts was tested against cell transformation induced by a defined *ras* oncogene (30). Investigation was performed using R6/*ras* assay system. *Ras* proteins play a pivotal role in regulating cell growth and the development of human cancer. This study is the first to demonstrate that the polysaccharide-enriched mushroom extracts can inhibit cell transformation induced by a defined oncogene through a novel non-cytocidal route. In this study, the *ras*-transformed cells in focus formation were effectively inhibited during the early stage of transformation, and were equally inhibited when the stably transformed cells were mixed with normal cells. It means that the inhibitory effect of mushroom extracts against *ras*-transformed cells requires the presence of normal cells. These data suggest that the inhibitory effect of mushroom extracts against *ras*-transformed cells requires the presence of normal cells. The demonstration of the inhibitory effect of mushroom extracts on *ras*-induced transformation in this study may have broad implications for cancer prevention.

The protective influence against Dielthylnitrosamine (200 mg/kg i.p.) genotoxicity, cytotoxicity, and carcinogenicity of aqueous extracts of *Agaricus blazei* mushroom was tested in rats (31–33). Dielthylnitrosamine (DEN) is a potent genotoxic carcinogen that has been used as initiating agent in some two-stage (initiation-promotion) alternative protocols for hepatocarcinogenesis (Dragan et al., 1991). It has been reported that after its metabolic biotransformation, DEN produces the promutagenic adducts O^6-ethyldeoxyguanosine and O^4- and O^6-ethyldeoxythymidine that may initiate liver carcinogenesis (Dragan et al., 1994; Verna et al., 1996). The findings were controversial. In one experiment (33) there was not protective effect on post-initiation stage of hepato-carcinogenesis. In others (31, 32) that previous treatment with *A. blazei* exerts a hepatoprotective effect on both liver toxicity and hepatocarcinogenesis process induced by a moderately toxic dose of DEN. However, effect depends on both the dose of the chemopreventive agent and of the carcinogen used. The highest concentration of *A. blazei* extract (11.5 mg/mL) demonstrated procarcinogenic properties by reducing the elimination of damaged cells (there was less apoptosis/necrosis of liver cells after DEN injection in rats receiving the mushroom extract) leading to the formation of an increased number of preneoplastic lesions. It means that mechanism of action is not clear yet.

Natural killer (NK) cells are directly cytotoxic for tumor cells and play a primary role in regulating immune responses. As immunostimulating agent which acts through the activation of macrophages and NK cells cytotoxicity, β-glucan can inhibit tumor growth in promotion stage too. There were monitored levels of NK cell cytotoxic activity MM-46 carcinoma-bearing C3H/HeN (34) and C3H/HeJ (35) mice treated with D-Fraction extracted from maitake mushrooms (*Grifola frondosa*). Results showed that D-Fraction markedly suppressed tumor growth, corresponding with increases in TNF-α and IFN-γ released from spleen cells and significantly increases TNF-α expressed in NK cells. Furthermore, D-Fraction increased macrophage-derived interleukin (IL)-12, which serves to activate NK cells.

Angiogenesis is crucial to tumor growth and metastasis, and interruption of this process is a prime avenue for therapeutic intervention of tumor proliferation. The Sarcoma S180 tumor-bearing mouse model was used to investigate the polysaccharopeptide, PSP, isolated from the edible mushroom *Coriolus versicolor* (36). Quantitative analysis of microcorrosion casting of the tumor tissue showed more angiogenic features such as dense sinusoids and hot spots, in control (untreated) than in PSP-treated animals. Immunostaining of tumor tissues with antibody against the endothelial cell marker (Factor VIII) demonstrated a positive correlation in that both the vascular density and tumor weight were lower in mice treated with PSP. The total amount of new vessels production was reduced, the basic tumor type-specific vascular architecture was retained. However, the expression of vascular endothelial cell growth factor (VEGF) in these tumors was suppressed. So, anti-angiogenesis can be one of the pathways through which β-glucans mediate anticarcinogenic activity.

Double-blind placebo-controlled randomized study was conducted to evaluate the effects of 28-day administration of polysaccharide peptides (PSP), isolated

from the fungus *Coriolus versicolor,* on patients, who had completed conventional treatment for advanced non-small cell lung cancer (NSCLC) (37). Thirty-four patients, with no significant difference in their baseline demographic, clinical or tumor characteristics, or previous treatment regimes were recruited into each of the PSP and control arms. After 28-day treatment, there was a significant improvement in blood leukocyte and neutrophil counts, serum IgG and IgM, and percent of body fat among the PSP, but not the control, patients. Although the evaluable PSP patients did not improve in NSCLC-related symptoms, there were significantly less PSP patients withdrawn due to disease progression, than their control counterparts (5.9 and 23.5%, respectively). There was no reported adverse reaction attributable to the trial medications. PSP treatment appears to be associated with slower deterioration in patients with advanced NSCLC.

In non-random case series, maitake mushroom (*Grifola frondosa*) MD-fraction was investigated to determine its effectiveness for 36 (22- to 57-year-old) cancer patients in stages II-IV. MD-fraction containing β-1,6 glucan with β-1,3 branched chains. Cancer regression or significant symptom improvement was observed in 58.3% of liver cancer patients, 68.8% of breast cancer patients, and 62.5% of lung cancer patients. The trial found a less than 10–20% improvement for leukemia, stomach cancer, and brain cancer patients. Furthermore, when maitake was taken in addition to chemotherapy, immune-competent cell activities were enhanced 1.2–1.4 times, compared with chemotherapy alone. Animal studies have supported the use of maitake MD-fraction for cancer (38).

All these data suggest that polysaccharides, β-glucans, could influence on initiation phase of carcinogenesis even mechanism of action is not clear. By activating NK cells function, interfering with tumor angiogenesis, β-glucans can inhibit promotion and progression of the tumors.

β-Glucan as adjuvant to cancer chemo- and radiotherapy

The major side effect of most chemotherapeutic drugs is neutropenia. The administration of these anticancer drugs impairs blood forming function. These functions are important to maintain defense system of the patient. As a result, chemotherapy may accelerate risk of infections that decrease the quality of life for cancer patients.

The effect of β-glucan (SCG), purified from edible mushroom *Sparassis crispa,* on cyclophosphamide (CY)-induced leukopenia was tested (39). 200 mg/kg of CY was administered i.p. to ICR mice. Immediately after this SCG was administered (125, 250, 500, 1000, 2000 μg/mouse) i.p. to CY-treated mice. The number of WBCs was reduced significantly within 3 day after CY treatment. The peak of the WBC count appeared on day 7, only in SCG 2000 μg-dose group and CY group – on day 9. The effect of SCG was dose-dependent, but high concentration of SCG showed lower efficacy. IL-6 concentration in spleen cells was also increased. IL-6 is involved in B cells differentiation, T cells activation, induction of acute phase proteins and reduction of G0-residence time of the hematopoietic cells. Therefore, by increasing IL-6 concentration SCG enhances hematopoietic response.

Radiotherapy often results in hematopoietic and immune depletion. Consequently, patients often experience anemia, lymphocytopenia, thrombocytopenia, and granulocytopenia. This leads to high risk of development of serious and lethal infections and increasing the mortality and morbidity of these patients.

A water-soluble glucan from *Lentinus lepideus* was orally administered every day for 24 days to irradiated with 6 Gy mice (40). The levels of IL-1β, IL-6, and GM-CSF were significantly increased in glucan-treated mice. In contrast, the level of TNF-α, whose level had been increased after irradiation, was decreased over time. These results suggested that glucan could increase serum levels of radioprotective cytokines, while decreasing the level of radioinduced TNF-α, which is increased as a consequence of tissue injury and anemia due to radiation. Also the number of CFCs (colony forming cells) was already close to the level seen in nonirradiated mice at Day 8, and continued to show such levels during the 24-day period. All these data suggested that glucan is able to modulate the dysregulation of cytokine production in radiation damage.

In other experiment was demonstrated, that soluble yeast β-glucan could enhance the proliferation of hematopoietic cells, promote leukocyte recovery following sublethal irradiation, and increase the survival of lethally irradiated animals following allogeneic hematopoietic cells transplantation in a CR3-dependent manner. Taken together, these observations suggest a novel role for complement, CR3, and β-glucan in the restoration of hematopiesis following by bone marrow injury (41).

Monoclonal antibodies and β-glucan

Immunotherapy using monoclonal antibodies (mAbs) is a novel cancer treatment strategy. It holds a great promise because of ability to target cancer cells specifically and minimize damage to normal tissues.

It is an important advantage over the chemotherapy and radiotherapy. Monoclonal antibodies are antibodies produced by using hybridoma technology. They react with specific antigens on the surface of certain types of cancer cells. Some of mAbs are already used in clinical oncology for treatment of malignant diseases.

Monoclonal antibodies typically use a combination of mechanisms in directing cytotoxic effect to a tumor cells. Most interact with components of the immune system through antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). Many of them alter signal transduction within the tumor cell or act to eliminate a critical cell-surface antigen. Monoclonal antibodies can also be used to target payloads, as radioisotopes, drugs or toxins, to directly kill tumor cells or to activate prodrugs specifically within the tumor (antibody-directed enzyme prodrug therapy, ADEPT) (43).

In particular, humanased or chimerased mouse mAb containing the human IgG1 Fc region trigger both ADCC and complement activation (42). ADCC occurs when antibodies bind to antigens on tumor cells and the antibody Fc domains engage Fc receptors (FcR) on the surface of immune effector cells (43). ADCC can be enhanced by CR3-dependent mechanism (44). According this mechanism, CR3 binds to iC3b, thus enhancing FCR-mediated effector cell binding to opsonized cell.

Complement-dependent cytotoxicity (CDC) is another cell-killing method that can be directed by antibodies. As with ADCC, the different subclasses of antibodies have varying abilities to elicit CDC responses. IgM is the most effective isotype for complement activation, but it is not widely used in clinical oncology because IgM does not readily extravasate from vascular structures. IgG1 and IgG3 are both very effective at directing CDC via the classical complement-activation pathway. The complement cascade ends in the formation of a membrane attack complex (MAC), with creates pores in the cell membrane that facilitate free passage of water and solutes into and out of the cell (42).

There is the third mechanism of cytotoxicity – CR3-dependent cellular cytotoxicity (CR3-DCC) (45). But CR3 priming for cytotoxic function requires ligation of both, the I-domain and lectin-like domain of CR3 (44). This mechanism is normally reserved for yeast and fungi and some microorganisms, which have β-glucan as an exposed component of their cell wall. Yeast cell wall β-glucan binds to a C-terminal lectin domain of CD11b, and also iCR3b binds to N-terminal I-domain binding site of CD11b. After this dual ligation efficient cytotoxic degranulation and phagocytosis are primed. In contrast to microorganisms, tumor cells, as well as other host cells, lack β-glucan as a surface component and cannot trigger CR3-DCC and initiate tumor-killing activity (45). This mechanism could be induced in the presence of β-glucans.

However mAb therapy is not very effective, because in some patients tumors don't express a high level of tumor antigens or tumor cells are protected from complement-mediated injury by membrane regulators of the complement system, such as CD55 and CD59 (45), that are overexpressed on tumors (51). Therefore, there is a need for agents that might increase the effectiveness of anticancer mAbs.

The effect of mAb against ganglioside GD2 (experimental neuroblastoma model) (48), G250 (renal carcinoma model) (49) and CD20 (Rituximab) ($CD20^+$ lymphoma model) (50) together with β-glucan was investigated. Synergistic effect of mAk and β-glucan for tumor regression was demonstrated in all these experiments.

The mechanism by which orally (46) and i.v. (47) administered β-glucans enhance the tumoricidal activity of antitumor mAb was demonstrated in murine tumor models. Oral and i.v. β-glucans function by a similar mechanism. Orally administered β-glucan goes through an intermediate step in which gastrointestinal macrophages process and deliver soluble β-glucan to the CR3 of granulocytes in bone marrow and tissue macrophages. Soluble i.v. administered β-glucan is delivered directly to the bone marrow and tissue macrophages. There was shown that β-glucan-mediated tumor regression requires antitumor Ab that activates complement and deposition of iCR3 on the tumor cells. This was demonstrated by failures of therapy in mice deficient in CR3 ($CD11b^{-/-}$) or C3 ($C3^{-/-}$) (46, 47). The mechanism involved in the *in vivo* priming of CR3 by β-glucan and signaling pathway that activate effector cells was elucidated in the recent years (52). There was shown that *in vivo* intact β-glucan is first taken up by macrophages and cleaved in to a 25-kDa molecular weight active fragment that binds to CR3, and primes the effector cells for target killing through the activation of 3-Syk-Phosphatidylinositol 3-Kinase signaling pathway. Its mean, that this fragment, but not parent β-glucan, can bind and elicit CR3-dependent cellular cytotoxicity *in vitro*. However, the up taking of either soluble or particular β-glucan *in vivo* is CR3 independent.

Conclusions

β-Glucan has been reported to act as immune system activator and cell response modifier. Binding of β-glucans to its specific receptors can elicit a serial cellular response through the modulating of activities of various factors including cytokines, chemokines, transcriptional factors, and growth factors. These effects are beneficial in by chemo- and radiotherapy induced immune suppression and depleted hematopiesis. β-Glucan shows anticarcinogenic activity, prevent oncogenesis and prevent metastasis. In addition, it demonstrates promised results as adjuvant to antitumor mAb by initiating additional tumor-killing mechanism.

Beta gliukanų poveikis imuninei sistemai

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Raktažodžiai: beta gliukanas, prieškancerogeninis poveikis, monokloniniai antikūnai, komplemento receptorius-3.

Santrauka. Beta (β) gliukanai yra natūralūs polisacharidai. Šie gliukozės polimerai yra tam tikrų patogeninių bakterijų ir grybelių ląstelės sienelės sudedamoji dalis. Rytų šalyse žaizdų gijimą skatinančios ir imuninę sistemą stimuliuojančios grybų savybės žinomos jau tūkstančius metų. Šių grybų sudėtyje yra biologiškai aktyvių polisacharidų, kurie priklauso daugiausia β-gliukanų grupei. Šios medžiagos didina imuninę apsaugą, aktyvindamos komplemento sistemą, stiprindamos makrofagų ir natūralių ląstelių-žudikių funkciją. Grybų ir kitų β-gliukanų ląsteliniai efektai sukeliami sąveikaujant jiems su tam tikrais ląstelių paviršiuje esančiais receptoriais, t. y. komplemento receptoriumi (CR3; CD11b/CD18), laktozilceramidu, dektinu-1, kuris vadinamas beta gliukanų receptoriumi (βGR). β-gliukanai turi ir prieškancerogeninį poveikį. Dėl protekcinio veikimo prieš potencialius genotoksinius kancerogenus jie gali užkirsti kelią onkogenezei. Kaip imuninę sistemą stimuliuojantis veiksnys, kuris veikia aktyvinančiai makrofagų ir natūralių ląstelių-žudikių citotoksiškumą, β-gliukanas gali inhibuoti naviko augimą ir promocijos stadijoje. Angiogenezės slopinimas gali būti vienas iš mechanizmų, kuriam veikiant β-gliukanai gali mažinti naviko proliferaciją ir užkirsti kelią metastazavimui. Kaip adjuvantas kartu su navikų chemoterapija ir radioterapija įrodytas teigiamas β-gliukano vaidmuo hematopoezės normalizavimosi procese po kaulų čiulpų pažeidimo. Imunoterapija monokloniniais antikūnais yra nauja navikų gydymo kryptis. Šie antikūnai aktyvuoja komplementą ir opsonizuoja navikines ląsteles iC3b fragmentu. Priešingai nei mikroorganizmai, navikinės ląstelės, kaip ir kitos žmogaus ląstelės, savo sienelės sudėtyje neturi β-gliukanų, todėl negali sukelti nuo komplemento receptoriaus-3 priklausomo ląstelės citotoksiškumo ir sužadinti naviko naikinimo proceso. Šis mechanizmas gali būti indukuojamas esant β-gliukanams.

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