

## CLINICAL APPLICATIONS

### $\beta$ -glucans as Immunomodulating Agents

$\beta$ -glucans are potent immunomodulators with effects on both innate and adaptive immunity. The ability of the innate immune system to quickly recognize and respond to an invading pathogen is essential for controlling infection. Dectin-1, which is a type II transmembrane protein receptor that binds  $\beta$ -1,3 and  $\beta$ -1,6 glucans, can initiate and regulate the innate immune response.

It recognizes  $\beta$ -glucans found in the bacterial or fungal cell wall, with the advantage that  $\beta$ -glucans are absent in human cells. It then triggers effective immune responses including phagocytosis and proinflammatory factor production, leading to the elimination of infectious agents. Dectin-1 is expressed on cells responsible for the innate immune response and has been found in macrophages, neutrophils, and dendritic cells. The Dectin-1 cytoplasmic tail contains an immunoreceptor tyrosine-based activation motif (ITAM) that signals through the tyrosine kinase in collaboration with Toll-like receptors 2 and 6 (TLR-2/6). The entire signalling pathway downstream to dectin-1 activation has not yet been fully mapped out, but several signalling molecules have been reported to be involved. They are NF- $\kappa$ B (through Syk-mediate pathway), signalling adaptor protein CARD9, and the nuclear factor of activated T cells (NFAT).

This will eventually lead to the release of cytokines including interleukin (IL)-12, IL-6, tumour necrosis factor (TNF)- $\alpha$ , and IL-10. Some of these cytokines may play an important role in the cancer therapy. On the other hand, the dendritic cell-specific, ICAM-3-grabbing, non-integrin homolog, SIGN-related 1 (SIGNR1), is another major mannose receptor on macrophages that cooperates with Dectin-1 in the non-opsonic recognition of  $\beta$ -glucans for phagocytosis. Furthermore, it has been found that blocking of TLR-4 can inhibit the production of IL-12 p40 and IL-10, induced by purified Ganoderma glucans (PS-G), suggesting a vital role of TLR-4 signalling in glucan-induced dendritic cell maturation. Such an effect is also operated via the augmentation of the I $\kappa$ B kinase, NF- $\kappa$ B activity, and MAPK phosphorylation.

## Immune Activation Induced by $\beta$ -glucans

$\beta$ -glucans can act on a variety of membrane receptors found on the immune cells. They may act singly or in combination with other ligands. Various signalling pathways are activated and their respective simplified downstream signalling molecules are shown. The reactor cells include monocytes, macrophages, dendritic cells, natural killer cells and neutrophils. The immunomodulatory functions induced by  $\beta$ -glucans involve both innate and adaptive immune responses.  $\beta$ -glucans also enhance opsonic and non-opsonic phagocytosis and trigger a cascade of cytokines release, such as tumour necrosis factor (TNF- $\alpha$ ) and various types of interleukins (ILs).

Other possible receptors and signalling pathways induced by  $\beta$ -glucans are less definite at the moment. For example, lentinan, a form of mushroom derived  $\beta$ -glucans, has been found to bind to a scavenger receptor found on the surface of myeloid cells, and triggers phosphatidylinositol-3 kinase (PI3K), Akt kinase, and p38 mitogen-activated protein kinase (MAPK) signalling pathways. However, no specific  $\beta$ -glucans scavenger receptor has been identified so far.[\[37\]](#) *Candida albicans* derived  $\beta$ -glucans, but not other forms of pathogenic fungal  $\beta$ -glucans, can bind to the LacCer receptor and activate the PI-3K pathway in controlling the neutrophil migration. but such an activation pathway may involve other molecules found in the *Candida* derived  $\beta$ -glucans.

Furthermore, two other receptors known as scavenger and lactosylceramide also bind  $\beta$ -glucans and can elicit a range of responses.  $\beta$ -glucans can enhance endotoxin clearance via the scavenger receptors, by decreasing TNF production, leading to improved survival in rats subjected to *Escherichia coli* sepsis.  $\beta$ -glucans binding to a lactosylceramide receptor can enhance myeloid progenitor proliferation and neutrophil oxidative burst response, leading to an increase in leukocyte anti-microbial activity. It is also associated with the activation of NF- $\kappa$ B in human neutrophils.

$\beta$ -glucans act on a diversity of immune-related receptors particularly in Dectin-1 and CR3, and can trigger a wide spectrum of immune responses. The targeted immune cells of  $\beta$ -glucans include macrophages, neutrophils, monocytes, NK cells, and dendritic cells. The immunomodulatory functions induced by  $\beta$ -glucans involve both innate

and adaptive immune responses.  $\beta$ -glucans also enhance opsonic and non-opsonic phagocytosis. Whether  $\beta$ -glucans polarize the T cells subset toward a particular direction remains to be explored.

## Diabetes

Both oat and fungal  $\beta$ -glucans reduce blood glucose concentrations after oral administration, as seen in animal experiments and clinical trials. In diabetic rats, orally ingested fruiting bodies and the acidic polysaccharides of both *Tremella mesenterica* and *T. aurantia* reduce blood glucose concentrations.  $\beta$ -glucans prepared by hot water extraction of *Agaricus blazei* basidiocarps show antihyperglycemic, antihypertriglyceridaemic, antihypercholesterolemic, and antiarteriosclerotic activity in diabetic rats. Oat  $\beta$ -glucans have been used in several clinical trials to reduce glucose. Studies show that oat  $\beta$ -glucan lowers postprandial glycemia. It has also been shown that oat bran flour is more effective than oat bran crisp, explained by the thrice higher  $\beta$ -glucan content in oat bran flour.

## Hypertension

$\beta$ -glucan has been seen to reduce hypertension. In genetically modeled rats with spontaneous hypertension (SHR), a diet containing 5% Shiitake (*Lentinus edodes*) or maitake (*Grifola frondosa*) causes a decrease in the mean systemic blood pressure. Moreover, consumption of whole maitake basidiocarps and the water-soluble extract also led to a decrease in blood pressure in Zucker fatty rats, in a diabetes rat model.

## Hypertriglyceridemia

Diabetes-associated dyslipidemia is a major risk factor for CVD. The dyslipidemia is caused either by insulin resistance or adipocytokines. In diabetes, the adipose cells are insulin-resistant, thus, the insulin-mediated uptake of free fatty acids in skeletal muscles is impaired. Increased circulating free fatty acids flux to the liver, resulting in increased triglyceride synthesis and the assembly of very low-density lipoprotein (VLDL). Thus, the characteristic of dyslipidemia in patients with diabetes is hypertriglyceridemia. Hyperglycemia and low insulin may also contribute to VLDL production (Hobbs 2006). In diabetes, adiponectin is reduced, which increases the muscle-free fatty acid uptake and reduces the plasma-free fatty acid level. This mechanism is independent of insulin-

resistance. In addition, high-density lipoprotein (HDL) may also decrease.  $\beta$ -glucan has been shown to decrease LDL cholesterol and increase HDL, to possibly alleviate dyslipidemia and reduce CVD.

Oats was first found to have a cholesterol-lowering effect and the active component was identified as  $\beta$ -glucans. Oats reduced both serum total cholesterol and LDL cholesterol compared to the control. In 20 hypercholesterolemic male patients, oat bran was seen to be better than wheat bran in lowering cholesterol. Barley was also seen to have a similar effect (Davy *et al.*).

## Cancer

$\beta$ -glucans, like lentinan (derived from the Shiitake mushroom) and Polysaccharide-K, have been used as an immunoadjuvant therapy for cancer since 1980, primarily in Japan. There is a large collection of research, which demonstrates that  $\beta$ -glucans possess anti-tumor and anti-cancer activities. In a mouse model study, beta 1,3 glucan in conjunction with interferon gamma inhibited tumors and liver metastasis. In some studies,  $\beta$ -1,3 glucans enhanced the effects of chemotherapy. In a cancer experiment, using a mouse model, administration of cyclophosphamide in conjunction with beta-1,3 glucans derived from yeast resulted in reduced mortality. In human patients with advanced gastric or colorectal cancer, the administration of beta-1,3 glucans derived from shiitake mushrooms in conjunction with chemotherapy resulted in prolonged survival time.

Preclinical studies have shown that a soluble yeast  $\beta$ -glucan product, Imprime PGG, when used in combination with certain monoclonal antibodies or cancer vaccines, offers significant improvements in long-term survival versus monoclonal antibodies alone. This benefit, however, does not result from Betafectin enhancing the specific killing action of the antibody. The anti-tumor activity is caused by a unique killing mechanism that involves neutrophils that are primed with Betafectin and that are not normally involved in the fight against cancer. Recent research by Hong *et al.*, demonstrates that this mechanism of action is effective against a broad range of cancers when used in combination with specific monoclonal antibodies that activate or cause the complement to be bound to the tumor.

Multinational research has successfully demonstrated that the oral form of yeast  $\beta$ -1,3-D glucan has similar protective effects as the injected version, including defense against infectious diseases and cancer. Of late, orally delivered glucan has been found to significantly increase

proliferation and activation of monocytes in the peripheral blood of patients with advanced breast cancer. The technology has wide applicability in cancer therapy. Each form of the cancerous tumor cell has specific antigens on the cell surface, some of which are common to other types of cancer. (e.g., Mucin 1 is present on about 70% of all types of cancer cells). Different immunotherapies target different antigens for binding monoclonal antibodies to tumor cells. This has resulted in the development of hundreds of monoclonal antibodies, many targeting a different specific antigen on cancer cells. In research studies, Betafectin has improved the effectiveness of all complement activating monoclonal antibodies tested, including breast, liver, and lung cancer (company data). The magnitude of success varies based on the specific monoclonal antibody used and the type of cancer.

### **Prevention of Infection**

There have been numerous studies and clinical trials conducted with soluble yeast  $\beta$ -glucans and the whole glucans particulate. These studies have ranged from the impact of  $\beta$ -glucans on post-surgical nosocomial infections to the role of yeast  $\beta$ -glucans in treating anthrax infections. Post-surgical infections are a serious challenge following major surgery, with estimates of 25–27% infection rates post-surgery. Alpha–Beta Technologies conducted a series of human clinical trials in the 1990s, to evaluate the impact of  $\beta$ -glucan therapy on controlling infections in high-risk surgical patients. In the initial trial 34 patients were randomly (double-blind, placebo-controlled) assigned to treatment or placebo groups. The patients who received PGG-glucan had significantly fewer infectious complications than the placebo group (1.4 infections per infected patient for the PGG-glucan group vs. 3.4 infections per infected patient for the placebo group). Additional data from the clinical trial revealed that there was decreased use of intravenous antibiotics and shorter stays in the intensive care unit (ICU) for patients receiving PGG-glucan versus patients receiving placebo.

There have been studies with humans and animal models that further support the efficacy of  $\beta$ -glucan in combating various infectious diseases. One human study demonstrated that the consumption of oral whole glucan particles increased the ability of immune cells to consume a bacterial challenge (phagocytosis). The total number of phagocytic cells and the efficiency of phagocytosis in healthy human study participants increased when a commercial particulate yeast  $\beta$ -glucan was consumed which shows

the potential for yeast  $\beta$ -glucan to increase the reaction rate of the immune system to infectious challenges. The study concluded that oral consumption of whole glucan particles represented the fact that it was a good enhancer of natural immunity.

### **Antimicrobial Effect**

$\beta$ -Glucan from oats has been demonstrated to have antimicrobial effects against *E. coli* and *B. subtilis*. On comparing cationic and native  $\beta$ -glucans, the latter has been seen to inhibit the growth of these bacteria by approximately 35%, while the cationic one has been seen to cause 80% inhibition in both microorganisms, indicating that  $\beta$ -glucan amination promotes antimicrobial effects. In this same study, cationic  $\beta$ -glucan has been seen to be more effective against *E. coli* (Gram-negative) than *B. subtilis* (Gram-positive), which can be explained by the interaction of the polycations with the negatively charged bacterial surface, altering membrane permeability and thereby inhibiting growth.

### **Radiation Exposure**

$\beta$ -glucan is a well-known biological response modifier (BRM), isolated from the yeast cell wall polysaccharides and is made up entirely of glucose  $\beta$  (1,3), linked together in linear chains with a variable frequency of  $\beta$  (1,6)-linked side chains. A specific hematopoietic activity was first demonstrated with  $\beta$ -glucan in the mid-1980s, in an analogous manner, as a granulocyte monocyte colony stimulating factor (GM-CSF). The anti-infective activity of  $\beta$ -glucan combined with its hematopoiesis stimulating activity resulted in the enhanced survival of mice receiving a lethal dose of 900–1200 cGy of radiation. *In vitro* studies showed that  $\beta$ -glucan could enhance granulocyte and megakaryocyte colony formation by hematopoietic stem progenitor cells when used in combination with GM-CSF and interleukin-3 (IL-3), respectively.

### **Septic Shock**

$\beta$ -glucan reduces septic shock by the mechanisms of the immune enhancing ability. Early research by Onderdonk *et al.* investigated the ability of yeast  $\beta$ -glucan to reduce septic infections using *in vivo* models. Onderdonk *et al.* found that mice challenged with *E. coli* or *S. aureus* bacteria are protected against septic infections when they are injected with PGG-glucan four to six hours prior to infection. Additional research

further supports that yeast  $\beta$ -glucan reduces septic shock by killing bacteria present in blood. A study by Kernodle *et al.* has demonstrated that preventative dosing of yeast  $\beta$ -glucan, prior to infection with *S. aureus*, prevented sepsis in a guinea pig model. Research on the use of yeast  $\beta$ -glucan immunomodulators as a means of treating and preventing bacterial sepsis is well-documented. Recent reports on glucan and sepsis revealed another possible mechanism – glucan protects against oxidative organ injury.

### **Chemoprotective Effects**

In recent times, several *in vitro* studies have demonstrated that  $\beta$ -glucans of different origin have effective protective activity against different mutagenic agents. The barley  $\beta$ -glucan was found to have a protective effect against damage induced by methyl methanesulfonate (MMS), in the CHO-K1 cell line (deficient in drug metabolism). The effects of the inducers MMS and 2-aminoanthracene (2AA) in the HTC cell line (proficient in drug metabolism), using different treatment protocols (pre-treatment, simultaneous, simultaneous with pre-incubation and post-treatment), indicated that the simultaneous protocol with preincubation provided the greatest reduction in DNA damage, suggesting that the  $\beta$ -glucan may react with mutagenic agents impeding their interaction with the DNA. The protective effect against 2AA and MMS, in lower concentrations, was also seen in CHO-K1, in the presence or absence of a DNA polymerase inhibitor (Ara-C).

### **Surgery**

There have been numerous studies and clinical trials conducted with the soluble yeast  $\beta$ -glucan particle and the whole glucan particle. Immunomodulators that enhance macrophage function have been shown to be beneficial in humans, as well as in animal models. One such study that looked at this correlation examined wound tensile strength and collagen biosynthesis, and positive effects were observed.

### **Wound Healing**

Macrophage activity is known to play a key role in wound healing from surgery or trauma. In both animal and human studies, therapy with  $\beta$ -glucan has provided improvements such as fewer infections, reduced mortality, and stronger tensile strength of scar tissue.

## Allergic Rhinitis

This disease is caused by an IgE-mediated allergic inflammation of the nasal mucosa. Orally administered yeast glucan decreases the levels of IL-4 and IL-5 cytokines responsible for the clinical manifestation of this disease, while it increases the levels of IL-12. Based on these studies, glucan may have a role as an adjunct to standard treatment in patients with allergic diseases.

## Arthritis

Using paramagnetic resonance spectroscopy, yeast-derived glucan is found to cause a decline in oxidative tissue damage during the progress of arthritic diseases, suggesting a role in the treatment of arthritis.

## $\beta$ -D-glucan's Role in Diagnostics

$\beta$ -D-glucan (properly known as (1 $\rightarrow$ 3)  $\beta$ -D-glucan, but also incorrectly called 1, 3- $\beta$ -D-glucan or even just glucan) forms part of the cell wall of certain medically important fungi (medicinal mushrooms), especially the *Aspergillus* and *Agaricus* species. An assay to detect the presence of (1 $\rightarrow$ 3)  $\beta$ -D-glucan in the blood has been produced by Fungitell and is marketed as a means of diagnosing invasive fungal infection in patients. One of the limitations of the assay is the presence of fungal contaminants in amoxicillin-clavulanate, which may result in false-positive results in those patients receiving these antibiotics.

Reference : <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3217690/>