BETA-GLUCAN CONTRIBUTION TO GLUTRASOL™ FORMULATIONS

I. OVERVIEW

Individuals with a compromised immune system are more susceptible to arthritis; reduced wound healing capacity; reduced bone marrow proliferation with resulting lowered white cell counts and anemia; increased incidence of cancers; and increased incidence of viral, fungal, and bacterial infection. The immune system is impaired by numerous environmental factors and can become overwhelmed.

Glutrasol formulations address compromised immunity and disease symptoms with a food.

Transfer factor is considered the single-most important component of Glutrasol. Beta-glucans (polysaccharides) are the second-most important component. This paper focuses on the known effects of beta-glucans. "Healing" mushrooms are CortControl's source of beta-glucans, and their healing properties have been understood for centuries.

In veterinary field studies, a synergistic effect of combining transfer factor with glucans was discovered. Transfer factor alone or beta-glucan alone significantly increases NK cells. A combination of transfer factor and beta-glucans increases killer T cells by a factor of 4.73 [1].

NK cells target cancer cells. Predictably, multiple publications address the effect of beta-glucans on cancer. However, the same benefits accrue to viral, bacterial, fungal, and parasitic diseases. Joyce Czop (Harvard Medical School) states [2], " Animals pretreated with purified glucan particles are subsequently more resistant to bacterial, viral, fungal, and protozoan challenge, reject antigenically incompatible grafts more rapidly and produce higher titers of serum antibodies to specific antigens." This is consistent with CortControl veterinary studies showing 3-4 times the vaccination response, relative to a vaccines without Glutrasol [3].

An important function of beta-glucans is immuno-modulation. A century and a half of research [4] has shown that beta glucans act as immuno-modulating agents, meaning they trigger a cascade of events that help regulate the immune system, making it more efficient.

Beta glucans effectively bind and activate specific innate immune cells including T-cells, NK (natural killer) cells, and macrophages. The ability of beta-glucans to modify the attack of immune cells on invasive agents supports an efficient and stronger immune response, while causing minimal damage to the rest of the body. "Minimal damage" includes avoidance of over-stimulation, which leads to autoimmune diseases [5].

Immuno-suppression is observed in people with stress-related diseases, such as coronary disease. Under stress, the number of macrophages available are reduced and unable to participate in the immune cascade. This causes even deeper immuno-suppression. Beta-1,3/1,6-glucan has been shown to nutritionally potentiate and activate macrophage cells which may assist in countering these effects.

Beta-glucans provide value in 6 areas:

(1) boost the immune response,
(2) activate and modify macrophage response,
(3) signal via cytokines,
(4) provide response specificity,
(5) prevent over-stimulation, and
(6) opsonize viral, bacterial, fungal, and parasitic invaders.

II. HOW BETA-GLUCANS FUNCTION

A. Boost in the Immune Response

A healthy immune response is triggered when infectious and foreign agents such as bacteria, fungi, and viruses invade the human body. The immune system responds with key inflammatory markers designed to attack the site of infection.

Beta-glucans alone ~~can~~ add a significant boost to the immune system.

B. Biochemistry behind the Boost

In order to function optimally, macrophages must pass through a state of activation which involves certain form and structure changes. Those changes are effected when a human (or mammal) ingests mushrooms with a potent level of beta-glucans.

The boost in response due to beta-glucans begins with macrophage interaction. Macrophages ("big eaters") ingest the beta-glucan particles and become activated in the process. An activated macrophage sends signals (cytokines) to produce more macrophages in the bone marrow.

CR1, CR3, TLR-2/6 and Dectin-1 receptors on the macrophage bind to the beta-glucans. Of these, Dectin-1 has received increasing study and is cited as a major beta-glucan receptor in macrophages, neutrophils, and dendritic cells [6]. Dectin-1 is a type II transmembrane protein receptor that binds β-1,3 and β-1,6 glucans. (SIGNR1) is a major mannose receptor on macrophages that cooperates with Dectin-1.

Dectin-1 is expressed on the surface of all macrophage populations tested as well as on monocytes, dendritic cells, and neutrophils, demonstrating that Dectin-1 is not restricted to cells of the dendritic cell lineage.

Beta-glucans are not highly soluble, and particles that pass through the intestines are relatively large. Enterocytes facilitate the transportation of β(1,3)-glucans and similar compounds across the intestinal wall into the lymph, where they begin to interact with macrophages to activate immune function [7]. Large beta-glucans are broken down in the macrophage and made available to other immune cells.

Cheung-VKN *et al.* labeled β-glucans with fluorescein to track their oral uptake and processing *in vivo*. The orally administered β-glucans were taken up by macrophages via the Dectin-1 receptor and were subsequently transported to the spleen, lymph nodes, and bone marrow. Within the bone marrow, macrophages degraded the large β-1,3-glucans into smaller soluble β-1,3-glucan fragments. These fragments were subsequently taken up via the complement receptor 3 (CR3) of marginated granulocytes. These granulocytes with CR3-bound β-glucan-fluorescein were shown to kill inactivated complement 3b (iC3b)-opsonized tumor cells after they were recruited to a site of complement activation [8].

Beta-glucan action is mediated via the activated complement receptor 3 (CR3, also known as CD11b/CD18), which is found on natural killer (NK) cells, neutrophils, and lymphocytes. This pathway is responsible for opsonic recognition of β-glucans leading to phagocytosis and reactor cell lysis. Beta-glucans bind to the lectin domain of CR3 and prime it for binding to inactivated complement 3b (iC3b) on the surface of reactor cells.

Two other receptors known as scavenger [9] and lactosylceramide [10,11] also bind β-glucans and can elicit a range of responses. β-glucans can enhance endotoxin clearance via scavenger receptors by decreasing TNF production.

β-glucans binding to lactosylceramide receptor can enhance myeloid progenitor proliferation and neutrophil oxidative burst response, leading to an increase in leukocyte anti-microbial activity. Beta-glucan binding is also associated with the activation of NF-κB in human neutrophils.

C. Modulation and Genetic Expression

Complement receptor type 1(CR1) also known as C3b/C4b receptor or CD35 (cluster of differentiation 35) is a protein that in humans is encoded by the CR1 gene [12,13].

Das [14] notes, "This gene is a member of the regulators of complement activation (RCA) family and is located in the 'cluster RCA' region of chromosome 1. The gene encodes a monomeric single-pass type I membrane glycoprotein found on erythrocytes, leukocytes, glomerular podocytes, hyalocytes, and splenic follicular dendritic cells. The protein mediates cellular binding to particles and immune complexes that have activated complement. Decreases in expression of this protein and/or mutations in its gene have been associated with gallbladder carcinomas, mesanggiocapillary glomerulonephritis, systemic lupus erythematosus, and sarcoidosis. Mutations in this gene have also been associated with a reduction in Plasmodium falciparum rosetting, conferring protection against severe malaria."

In primates, CR1 serves as the main system for processing and clearance of complement opsonized immune complexes. It has been shown that CR1 can act as a negative regulator of the complement cascade, mediate immune adherence and phagocytosis, and inhibit both the classic and alternative pathways. The number of CR1 molecules decreases with aging of erythrocytes in normal individuals and is also decreased in pathological conditions such as systemic lupus erythematosus (SLE), HIV infection, some haemolytic anaemias, and other conditions featuring immune complexes.

Certain alleles of this gene have been statistically associated with an increased risk of developing late-onset Alzheimer's disease [15].

D. Signaling with Cytokines

Cytokines act as protein internal regulators of the immune system, and activated macrophages release cytokines. Cytokines functionally resemble hormones, but they do not arise from a specific gland.

Activated lymphocytes communicate with other macrophages and lymphocytes via cytokines. Both innate or adaptive pathways are involved.

For example, orally administered yeast-glucan was reported to decrease the levels of IL-4 and IL-5 cytokines responsible for the clinical manifestation of allergic rhinitis, while increasing the levels of IL-12 [16]. Cytokine release results in a coordinated attack.

A signaling cascade arises when immune receptors interact with β-1,3 and β-1,6 glucans. Several signaling molecules have been reported to be involved. They are NF-κB (through Syk-mediate pathway), signaling adaptor protein CARD9 and nuclear factor of activated T cells (NFAT) [17]. This will eventually lead to the release of cytokines including interleukin (IL)-12, IL-6, tumor necrosis factor (TNF)-α, and IL-10. Some of these cytokines may play an important role in the cancer therapy.

E. Preventing Over-stimulation

Beta-glucans balance the immune response and prevent over-stimulation.

More isn't always better.

Decreased immunity results when the immune system is over-stimulated with real and perceived threats. With time, this leads to a weakened immune system, and the individual becomes more susceptible to development of cancer and infections. Individuals with over-stimulated immune systems have an increased risk of chronic inflammatory conditions such as autoimmune disorders and asthma. Complement receptors [18] are related to glucan modulation -

- Membrane cofactor protein is a widely distributed C3b/C4b binding regulatory glycoprotein of the complement system;
- Decay-accelerating factor (DAF: CD55: Cromer antigen) protects host cells from complement-mediated damage by regulating the activation of C3 convertases on host cell surfaces;

- Complement receptor 2 is the C3d receptor.
- Factor H, another immuno-regulatory protein, also maps to chromosome 1 at band 32.

Scientists refer to beta-glucans as “biological response modifiers.” A modified response supports immuno-modulation by preventing hyper inflammation and ~~an~~ autoimmune response.

F. Specificity

Immune specificity to fight viruses, fungi, bacteria, and parasites arises from the following facts:

1. viruses, fungi, and bacteria contain beta-glucans,
2. healthy humans do not manufacture beta-glucans,
3. a macrophage that has been trained to detect beta-glucans will eliminate viruses, fungi and bacteria without attacking human cells,
4. in a summary statement, "self" is distinguished from "invader." The immune response is specific and doesn't harm the host.

G. Opsonization

Beta-glucan is an opsonin. An opsonin is any molecule that enhances phagocytosis by marking an antigen for an immune attack or marking dead cells for recycling.

Opsonization (also, opsonisation) is the molecular mechanism whereby molecules, microbes, or apoptotic cells are chemically modified so as to have stronger interactions ~~ (to be more "delicious" to)~~ with cell surface receptors on phagocytes and NK cells. In a personified view, they become more "delicious" to immune cells. With the antigen coated in opsonins, binding to immune cells is greatly enhanced.

A coating of beta-glucans on an invader makes the invader more susceptible to leukocyte removal. Binding reactions are greatly enhanced. Opsonin translates as "to prepare for eating." Opsonization further mediates phagocytosis via signal cascades from cell surface receptors [19].

Opsonins aid the immune system in a number of ways. In a healthy individual, they mark dead and dying self cells for clearance by macrophages and neutrophils, activate complement proteins, and target problems for destruction through the action of natural killer (NK) cells.

Cell membranes have negative charges (zeta potential), which makes it difficult for two cells to come close together. When opsonins bind to their targets, they boost the kinetics of phagocytosis by favoring interaction between the opsonin and cell surface receptors on immune cells. This overrides the negative repulsive charges from cell membranes. This principal holds true for clearance of pathogens and dead or dying "self" cells.

A mechanism of β-glucan action is mediated via the activated complement receptor 3 (CR3, also known as CD11b/CD18), which is found on natural killer (NK) cells, neutrophils, and lymphocytes. This pathway is responsible for opsonic recognition of β-glucans, leading to phagocytosis and reactor cell lysis. β-glucans bind to the lectin domain of CR3 and prime it for binding to inactivated complement 3b (iC3b) on the surface of reactor cells.

III. CONFORMATIONAL CONSIDERATIONS

Solubilized beta-glucans present multiple conformations. Conformations, in turn, affect stereochemistry, reaction possibilities, and reaction rates.

Conformations are basically rotations around single bonds. In aqueous solution, β-glucans undergo conformational change into triple helix, single helix or random coils. Conformation possibilities are determined by branching. The immune functions of β-glucans are apparently dependent on their conformational complexity [20].

It has been suggested that a higher degree of structural complexity is associated with more potent immuno-modulatory and anti-cancer effects. All β-glucans are glucose polymers linked together by a 1→ 3 linear β-glycosidic chain core and they differ from each other by their length and branching structures [21]. The branches derived from the glycosidic chain core are highly variable and the 2 main groups of branching are 1→4 or 1→6 glycosidic chains.

IV. SOURCES OF BETA-GLUCANS

CortControl uses hybridized mushrooms for the Glutrasol formulations.

Hybridization embodies complexity and functionality, combining the best features of multiple species. Healing and immuno-stimulating properties are documented by animal studies.

V. BETA-GLUCANS AND CANCER

Cancer is perhaps the largest studied area of beta-glucan treatment. Beta-Glucans clearly show anticarcinogenic activity without direct cytotoxic effects. Beta-glucans have been used as an immuno-adjuvant therapy for cancer since 1980, mostly in Japan.

This is supported by CortControl's cancer success with animals.

NK cells are crucial to inhibiting cancer growth and are mostly responsible for detecting and controlling tumors. NK cells seek and destroy tumor growth.

Beta-glucans prevent oncogenesis due to the protective effect against potent genotoxic carcinogens. As an immuno-stimulating agent, which acts through the activation of macrophages and NK cell cytotoxicity, beta-glucans can also inhibit tumor growth in promotion stage.

Researchers (at Teikyo University's Biotechnology Research Center in Kawasaki, Japan) showed that beta-glucans have anti-tumor properties, suppressing the formation and development of tumors. The study concluded -

"Results of the clinical application of lentinan have proven prolongation of life span of the patients with advanced and recurrent stomach, colorectal, and breast cancer with only little toxic side effect. It also appears that beta-glucans restore or boost the responsiveness of cytokines, which interact with immune cells and regulate the response to the disease."

In an earlier Japanese study, mice with tumors that received beta-glucans experienced (1) a rapid decrease in the number of tumor cells and (2) a notable increase in neutrophils in solid tumors. Neutrophils are a type of white blood cell that destroys invaders—in this case, cancerous cells—by ingesting them and using chemicals to break them down.

β-glucan exerts anti-tumor effects irrespective of antigens (GD2, GD3, CD20, epidermal growth factor-receptor, and HER-2) or human tumor types (neuroblastoma, melanoma, lymphoma, epidermoid carcinoma, and breast carcinoma) or tumor sites (subcutaneous versus systemic). The effect was correlated with the molecular size of the β-1,3;1,4-glucan [22]. It was found that despite a relatively low initial white cell count, oral β-glucan can stimulate proliferation and activation of peripheral blood monocytes in patients with advanced breast cancer.

VI. BETA GLUCANS AND HEART HEALTH

In a study [23] conducted by the US Department of Agriculture's Beltsville Human Nutrition Research Center in Maryland, beta-glucan was concentrated so it could be easily incorporated into a typical diet. Male and female study participants with mildly high cholesterol were put on a maintenance diet for one week and then were given an oat fiber extract containing either 1% or 10% beta-glucan. After five weeks of receiving the beta-glucan extract, both groups showed a significant reduction of total cholesterol and low density lipoprotein (LDL). Also, total cholesterol levels were significantly lower in the group that received the 10% beta-glucan.

VII. HELPING THE BODY CONQUER INFECTIONS

Beta-glucans help the body do battle with antibiotic-resistant bacteria plus viruses that cause upper respiratory infections. At Brigham and Women's Hospital in Boston, Massachusetts, researchers found that beta-glucans enhance antibiotic efficacy in rats infected with antibiotic-resistant bacteria. Rats with intra-abdominal sepsis due to antibiotic-resistant bacteria—namely, Escherichia coli or Staphylococcus aureus—were given a beta-glucan that enhances the function of macrophages and neutrophils.

Researchers looked at beta-glucan's ability to work in partnership with antibiotics to decrease mortality of the rats. Results of these studies demonstrated that prophylaxis with beta-glucan in

combination with antibiotics provided enhanced protection against lethal challenge with Escherichia coli or Staphylococcus aureus as compared with the use of antibiotics alone [24].

CortControl observed the same result in animal studies with a Glutrasol formulation. Combining Glutrasol with antibiotics worked when antibiotics alone did not.

Belgian scientists orally administered three different beta-glucans in pigs with an ETEC infection that had just been weaned. The study found that pigs fed with the glucans for two weeks after weaning were less susceptible to the infection (evidenced by a lower incidence of diarrhea) when compared to the control group. "This study showed that beta-glucans can protect against an ETEC infection," concluded the researchers.

The Montana Center for Work Physiology and Exercise Metabolism examined beta-glucans' ability to mitigate upper respiratory infections in a single blind, randomized trial in 2008 [25]. Scientists chose firefighters as their subjects since they are regularly bombarded with smoke and fumes. They are more susceptible to respiratory troubles as a result.

Participants who consumed the beta-glucan supplement had fewer (23%) upper respiratory tract infections when compared to the group of firefighters taking a placebo.

VIII. STRESS

Physical and emotional stress [26] and intense physical exercise negatively affect the immune system. Cortisol is believed to be contributory. Under stress, the number of macrophages available are reduced. Beta-1,3/1,6-glucan has been shown to nutritionally both potentiate and activate macrophage cells which can assist in countering these effects.

The Glutrasol formula reduces cortisol in calves. Results were significant at the 95% confidence level. See U.S. Patent 9,463,218.

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