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Review

Glucans and Cancer: Historical Perspective

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Received November 15, 2015; Accepted December 16, 2015

This paper represents a review of beta-glucans, their history, chemical and biochemical characteristics, and their role in immune reactions and cancer treatment. Glucans represent a group of biologically active natural compounds and recently are gaining significant attention not only as a food supplement but also as a drug. With more than 50 clinical trials evaluating their effect on cancer suppression, it is not the question if but when.

Key words: Cancer, glucan, history, immunity, structure

INTRODUCTION

Even people in prehistoric time realized that some plants of mushrooms contain medicinal compounds. Inside the mummy of "ice man" Otzi, which is at least 5000 years old, we found a bag with dry mushrooms. From the same period is a written Indian document talking about medicinal effects of mushrooms. Three thousand years ago, Egyptians believed that mushrooms were sacred food being able to prolong life. The Chinese Book of Songs (Shijing) from 1100 BC describes treatment with the mushroom *Poria cocos*. This one is quite common throughout the Far East, in China is known as fuling, in Korea as bok-ryung, and in Japan as bukuryo. Japanese legend indicates that monkeys without cancer or any other disease fed on the mushroom *Lentinula edodes*. This led to Japanese interest in possible chemical components responsible for possible anticancer effects. The effects of mushrooms on cancer were also known to African shamans or Native Americans.^{1,2} Decades of research revealed that the responsible molecules were beta-glucans, which can be also found in cells from bacteria, yeast, and plants. In addition, glucans can also be produced extracellularly, most often by streptococci.^{3,4} Simultaneously, yeast-derived glucans were subject of studies originating in Europe and the USA.

A NEED FOR IMMUNOMODULATION

We are surrounded by potentially pathogenic bacteria and viruses. It is speculated that the number of microorganisms on the earth reaches approximately 10^{30} with a total weight of approximately 8 billion tons, which is significantly more than the weight of all multicellular animals and plants combined. This is the main reason the immune system evolved in such a way that its optimal development requires constant contact with antigenic stimuli from an early age.⁵ However,

our current lifestyle is known for its obsession with cleanness and purity. If the individual is from the earliest age exposed to hygiene going overboard, its immune system will not develop properly, and that person will be sensitive to the constant threat of pathogenic microorganisms.

It has been repeatedly shown that early contamination with a wide spectrum of microorganisms decreases the risk of development allergic reactions and asthma and also decreases the risk of some autoimmune diseases such as Crohn's disease or ulcerative colitis.⁶ It is well-established that natural antigenic stimulation can be replaced by vaccination. However, there are other means of gaining resilience against infectious diseases. Polyvalent extracts of pathogenic microbes were used for stimulation of the immune system already at the end of 18th century.⁷

Immunomodulation is generally defined as a change in the immune system caused by compounds which either activate or suppress immunity. Immunomodulators belong into the groups of biological response modifiers and are routinely used for pushing the level of immune reactions to a desired level, including both stimulation and suppression.⁸⁻¹¹ Generally, immunomodulators represent a diverse array of synthetic, natural, and recombinant molecules, some of which are already approved for the use in patients. Numerous immunomodulating approaches such as the use of monoclonal antibodies or influencing of either negative or

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How to cite this article: Sima P, Vannucci L, Vetvicka V. Glucans and Cancer: Historical Perspective. *Cancer Transl Med* 2015;1 (6):209-14.

positive immunoregulating elements emerged recently. However, successful cancer therapy also requests stimulation of various aspects of immune system.¹² With over 10,000 published studies, glucan has the best position among other immunomodulators.

GLUCANS

Some biological response modifiers can have undesirable effects on some parts of the immune system and subsequently have negative effects on some diseases. Glucans and beta-glucans, in particular, hold a different position – they are highly active but have no side effects. In addition, they are one of the few natural immunomodulators with well-defined chemical structure and well-established mechanisms of action.^{13,14}

Various configurations of glucan exist in nature. From the chemical point of view, glucans are polysaccharides, i.e., polymers of beta-glucose with the main chain of (1–3) bound D-glucopyranose moieties to which some D-glucopyranoses are randomly connected by (1–6) linkages [Figure 1]. It is important to note that beta-glucans also exist. As an example, important beta-glucans are dextran ([1–6] glucan); starch ([1–4] and [1–6] glucan); and glycogen ([1–4] and [1–6] glucan). Compared to beta-glucan, our knowledge of possible biological effects of beta-glucans is limited. Stimulation of immunity was found in beta-glucans from *Agaricus bisporus*, *Tinospora cordifolia*, and *Ramalia celastri*.¹⁵ Interesting comparison of effects of beta-glucans was written.¹⁶

HISTORY OF GLUCANS

The real investigation of glucans began in the 1960s and 1970s. Two lines can be traced in the history of glucan, based on different starting points but gradually converging. The foremost origins were in the USA, Europe, and Japan, respectively. Research on glucans in the Euro-American milieu was based on knowledge of the immunomodulatory effects of zymosan, a mixture of polysaccharides isolated from the cell walls of the well-known and widely-used baker's yeast *Saccharomyces cerevisiae*. Although zymosan was able to stimulate a nonspecific immune response, initially it was not clear which component of this rather crude composition was responsible for the activity. When zymosan was examined in detail, glucan was identified as the component of primary effect. It was subsequently isolated, and its immunological effects were evaluated. This research was pioneered by Di Luzio and Riggi.¹⁷ In a number of papers, he demonstrated that glucan administration caused significant phagocytic stimulation of the

reticuloendothelial system, enhanced host defense mechanisms (such as anti-infectious immunity), and resistance to experimental tumors. Subsequent studies focused on possible receptors. After observation of the first receptors,¹⁸ CR3 (CD11b/CD18) was found¹⁹ followed by Dectin-1.²⁰

Separately, intensive research of immunomodulating activities of beta-glucan was also conducted in Japan. Japanese scientists arrived at beta-glucan via a different route. In Asian medicine, consuming different medicinal mushrooms (shiitake, maitake, reishi, etc.,) has been a long tradition as folk medicine. In earlier Japanese studies, mice with tumors that received beta-glucans, including lentinan, experienced a rapid decrease in the number of tumor cells as well as a notable increase in neutrophils in solid tumors. In detailed studies of the biological effects of these mushrooms, in particular, their anticancer action, beta-glucans were again found to be the main cause of nonspecific immunomodulation.

This initial investigation was conducted by Goro Chihara, who isolated beta-glucan from the shiitake mushroom, which he referred to as “lentinan” (*Lentinula edodes*).²¹ This glucan, with some subsequent modification, was later approved as a drug and has been successfully used for almost 30 years. The most commonly used glucans are summarized in Table 1.

STRUCTURE

Individual glucans differ in numerous characteristics, including their structure, molecular size, branching, and solubility. All these individual aspects can influence the biological effects of glucans; the problem is that we do not fully know how. The considerable heterogeneity of all natural beta-glucans not only from *Saccharomycetes* but also from other sources was obvious and continued to be the cause of a series of mutually contradicting conclusions. Diverse data on the comparison of structure, size, and effects can be found in the scientific literature.²² Some studies suggested that the triple helix presence and high molecular weight support anticancer effects of schizophyllan,²³ but the fact that most isolation processes (such as increased temperature, high pH, or some solvents) destroy the triple helix configuration²⁴ do not support this theory. Similarly, numerous publications published recently suggested that small polysaccharides or even glucan-based oligosaccharides are more active than their high-molecular weight counterparts.^{25–27}

Research on the cell wall of different fungal species has not led to a straightforward model of its structure and the concepts of its organization have undergone certain development. According to Stratford,²⁸ the yeast cell wall resembles reinforced concrete. An

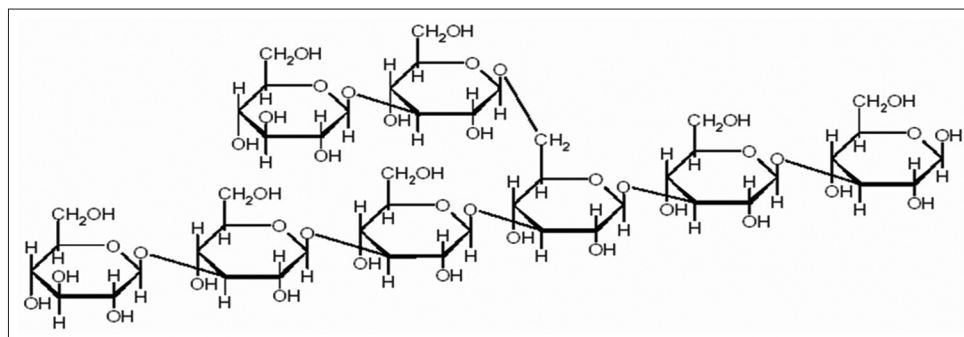


Figure 1. Basic structure of beta-glucan

Table 1: The most common glucans

Name	Origin
AM-ASN	<i>Amanita muscaria</i>
Beta-glucan I (AAG)	<i>Auricularia auricula-judae</i>
Flammulin	<i>Flammulina velutipes</i>
Ganopol	<i>Ganoderma lucidum</i>
Grifolan	<i>Grifola frondosa</i>
Chrysolaminarin	<i>Chaetoceros mülleri</i>
Krestin	<i>Trametes versicolor</i>
Curdlan	<i>Alcaligenes faecalis</i> , <i>Alcaligenes</i> , <i>Agrobacterium</i> , <i>Rhizobium</i>
Yeast glucan	<i>Saccharomyces cerevisiae</i>
Laminarin	<i>Laminaria</i> sp.
Lentinan	<i>Lentinus edodes</i>
Pleuran	<i>Pleurotus ostreatus</i>
Schizophyllan	<i>Schizophyllum commune</i>
Sklerotinan	<i>Sclerotinia sclerotiorum</i>
Skleroglucan	<i>Sclerotinum glucanicum</i>
Tylopilan	<i>Tylopilus felleus</i>
T-4-N, T-5-N	<i>Dictyophora (Phallus) indusiata</i>
Zymosan	<i>Saccharomyces cerevisiae</i>

armature, representing about 35% of the wall mass and formed by fibrils of alkali insoluble $\beta(1 \rightarrow 3)$ -glucan, is dipped into mannoproteins (about 25–35% of the wall mass) and bound to the armature through amorphous β -glucan and chitin. An excellent review of the chemistry of yeast and fungal cell wall can be found.²²

GLUCANS AND IMMUNE SYSTEM

Glucans represent evolutionary highly conserved structure often labeled as Pathogen Associated Molecular Patterns (PAMPs). Multicellular organisms developed the ability to recognize these molecules as nonself and upon recognition, react via defense mechanisms. It means that the ability to recognize beta-glucans as potential pathogens is phylogenetically coded in all creatures, from invertebrates to humans.²⁹ The immunostimulating effects of glucans were described in earthworms, fish, chicken, mouse, rats, rabbits, guinea pigs, dogs, pigs, sheep, and horses.^{30,31} In addition, some studies demonstrated the activation of plant defense systems.³²

In vertebrates, PAMPs including glucans are specifically recognized via receptors generally called Pattern Recognition Receptors expressed on membranes of effector cells of natural immunity, i.e., macrophages, monocytes, dendritic cells, leukocytes, and natural killer (NK) cells. To successfully exploit the biological effects of these carbohydrates and to improve host defense against fungal pathogens, it is important to continue investigating the receptors involved in beta-glucan recognition. The most important receptors are Dectin-1 and CR3 (CD11b/CD18) receptors. Additional receptors include Toll-2, lactosylceramide, and scavenger receptor family. Recent studies employing both cell lines and different animal models suggest that these individual receptors are able to bind glucan and collaborate with each other.

However, the precise mechanisms still remain unclear. Greater understanding of these interactions, particularly *in vivo*, will be required if the further development of glucans, or the generation of novel therapeutics based on glucans and/or their receptors, is to become a reality.¹¹

Detailed studies revealed CR3 as a most promising target of glucan. To establish the role of CR3 in the glucan-mediated induction of immune reactions, both a panel of cell lines transfected with various parts of the CR3 receptors and Cr3-knock-out mice have been used. Subsequent detailed analysis of the interaction of human cells with glucans has demonstrated that this CR3 receptor is primarily responsible for the binding and biological effects of glucans. CR3 is considered to be the most important receptor mediating clearance of opsonized immune complexes by the phagocytic system.¹⁹

In addition to its function as a receptor for cytotoxicity and phagocytosis, it also serves as an adhesion molecule responsible for leukocyte diapedesis. For these functions, the CR3 molecule goes through a series of inside-out and outside-in signaling steps resulting in exposure of high-affinity binding sites. In 1987, it was shown that neutrophil CR3-dependent phagocytosis and degranulation in response to iC3b-opsonized particles required ligation of two different binding sites in CR3, one for iC3b and one for β -glucan. Using fluorescent-labeled glucan and Chinese hamster ovary cells expressing recombinant chimeras, the binding site was mapped to a region of CR3 located C-terminal to the I-domain. This information revealed the mechanisms of glucan action. After the binding, the CR3 is primed for cytotoxic degranulation in response to the binding of iC3b fragment to a different part of the CR3 molecule. Detailed studies later showed that soluble beta-glucan binding to the lectin site of neutrophil or NK cell CR3 generates a primed state of that receptor capable of mediating cytotoxicity of iC3b-opsonized target cells.³³ These data were further validated by the use of cells from CR3-deficient mice that were resistant to the effects of glucan. Similar to the situation with leukocytes, CR3 that is present on NK cells functions in a like manner. Most of these studies were performed in cancer models; however, a similar mechanism applies to microbial pathogens. Details about how the CR3 receptor worked and how this hypothesis was confirmed by the use of CR3-deficient mice were reviewed.^{34,35}

Upon binding of glucan, receptors transfer the signal resulting in cell activation. In macrophages, glucan binding causes not only increase in phagocytosis but also the production of numerous cytokines such as interleukin-1 (IL-1), IL-2, and IL-6. Phagocytosis is extremely important for elimination of pathogenic microbes and other materials from inside of cells. Increased level of cytokines subsequently stimulates effector cells of both specific and nonspecific immunity, resulting in increased anti-infectious and anticancer response.³⁶ Besides direct effects on immunity, glucans also act as scavengers of free radicals. It is important to note that free radicals are the main risk factors in cancer development.^{37,38}

Direct effects of glucans on cell lines are less clear. On one hand, whereas there are no doubts that glucan treatment results in activation of various receptors³⁹ or direct activation of some pathways,⁴⁰ direct toxic effects are rare. On the other hand, findings of significant effects on expression of several cancer-related genes in human fibroblasts and breast cancer cells suggest that this area deserves more attention.⁴¹

GLUCANS AND ANTICANCER IMMUNITY

Links between cancer and immunity are well-established, despite the fact that the original theory about immunosurveillance is currently not considered valid. Currently, the scientific consensus moved toward the theory of tumor escape.⁴² However, the importance of the healthy and functional immune system in the fight against cancer was never questioned. Based on the multiple biological effects of glucan, it is not surprising that this natural immunomodulator is also involved in the fight against cancer. Despite the fact that most tumors are recognized by the immune system, the antibody response is usually not strong enough to kill a cancer growth. Regardless of their origin, chemical structure or molecular weight, glucans are evaluated in both animal and human cancer models since 1980.⁴³ Since the first direct scientific study 40 years ago, the antitumor activity of glucan has been clearly demonstrated.³⁸ These studies confirmed that glucans have strong activity against a full scale of different cancers including lung, breast, and gastrointestinal cancers.⁴⁴⁻⁴⁸

Glucans are extremely important, as they are able to cooperate with antibodies. After the tumor cells have been recognized as foreign, specific antibodies are released and subsequently bind to the cancer cells. Following this binding of antibodies, the C3 fragment of complement coats the surface of cancer cells. The glucan-primed cells, such as macrophages and specifically NK cells and neutrophils, then recognize these antibody-C3 coated cells and kill them. Without glucan, the destruction would not take place, and the situation would be compounded very quickly. To fully investigate the mechanisms and potential utility of glucan in immunotherapy, it was necessary to develop a suitable mouse system. First, mouse leukocyte CR3 was shown to function as a receptor for glucans in the same way as human CR3. Next, it was shown that the primed state of macrophages and NK cells remained detectable for up to 24 h after a short interaction with glucan.⁴⁹

We investigated the occurrence of antibodies and complement fragments (most of all C3 fragment) in animal and human models. Our investigation showed that the majority of malignant cells in mammary carcinomas are naturally targeted with C3 for cytotoxicity by NK cells bearing CR3 receptor that has been primed with glucan. Freshly excised human mammary tumors and established breast cancer cell lines were examined and published reports of both circulating antibodies to tumors and tumor opsonization with immunoglobulins and C3 were confirmed.⁵⁰

Numerous recent studies have shown that glucan is extremely active in cooperation with antibodies that naturally occur in the case of cancer.^{51,52} Similar effects can be achieved when we combine antitumor antibodies with glucan.⁵³ Numerous humanized antitumor monoclonal antibodies (Herceptin™, Rituximab™, Avastin™, Zevalin™, Campath-1H™ and Erbitux™) are now being used to treat patients with metastatic breast carcinoma, non-Hodgkin's lymphoma, chronic lymphocytic leukemia, and metastatic colon carcinoma. Avastin™ (bevacizumab) is particularly successful and has been approved to treat a number of cancers. This antibody targets a growth signal called vascular endothelial growth factor that cancer cells send out to attract new blood vessels. Avastin™ intercepts a tumor's vascular endothelial growth factor signals and stops it from reaching its targets. The list of similar monoclonal antibodies is growing each month.

Since the preliminary experiments on animals were so promising, it is no wonder that these experiments are currently

repeated in several clinical trials (among others, by researchers in The Memorial Sloan-Kettering Cancer Center and in the Brown Cancer Center in Louisville). Additional currently running clinical trials are focusing on soluble glucan Imprime PGG™ in combination with monoclonal antibody.

CONCLUSION

The fact that glucans elicit strong and positive immune responses is well-established. Since the 1980s, we know that glucans stimulate hematopoiesis and are useful before and during radio- and chemotherapy and during intoxication with heavy metals.³⁷ In addition, recent years showed that glucans also influence homeostatic processes and can help to neutralize physical, mental, or environmental stress and can help in the treatment of chronic fatigue syndrome.⁵⁴ However, we have to keep in mind that despite these significant effects, glucans, similarly to other drugs, will never become a universal cure.

Beta-glucans are heavily used as food supplements. Their effects are mostly manifested via correction of free radicals, decreasing cholesterol and blood sugar levels, and also as nonspecific fibers supporting sorption in the gut. Glucan's effects on gut-associated lymphoid tissue are systemic, which answers the question if orally-given glucans have any effects. In addition, glucans are added into cosmetic creams due to their anti-inflammatory effects. Glucans, being nontoxic and having no side effects, have a good chance of becoming as effective in the western medicine as they are in Japan.³⁸

Financial support and sponsorship

This work was supported by Institutional Research Concept Grant (No. RVO 61388971).

Conflicts of interest

There are no conflicts of interest.

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