Glucans as Biological Response Modifiers

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Abstract: β-D-glucans belong to a group of natural, physiologically active compounds, generally called biological response modifiers. Glucans represent highly conserved structural components of cell walls in yeast, fungi, or seaweed. Despite long history of research, the exact mechanisms of glucan action remain unsolved. The present review starts with the history of glucans. Next, the detailed information about the possible glucan sources is followed by a description of the mechanisms of action. Physiological functions of glucan suggest the possible use of glucans not only as non-specific immunomodulator, but also as its possible future use as a drug.

Key Words: Glucan, saccharides, immune system, macrophages, CR3, yeast.

INTRODUCTION

Natural products, useful in treating and/or preventing various diseases, have been sought through the history of man. Most of these natural products are plagued with a common problem, *i.e.*, the fact that they often represent a complex mixture of individual ingredients, each of which can contribute to their biological activity. In a certain extent, it is also problem of β-glucans.The polysaccharides, usually termed "β-glucans", and referred in this paper, are non-cellulosic polymers of β-glucose, with glycosidic bonds in position β(1→3) and with certain portion of β(1→6) bound glucose molecules. They are isolated mostly from different fungi, but they are present also in other sources, such as cereals, bacteria or seaweeds. These compounds isolated from different sources that otherwise caused similar or nearly identical immune reactions in macroorganisms, can differ in their primary, secondary or tertiary structures or their solubility in water or alkalies.

The most important quality of β-glucans and the reason why so much attention has been devoted to them are physiological effects that they show. They are typical biological response modifiers (BRMs) with pronounced immunomodulating activity. Generally, imunomodulators can act both positively (immunostimulators) or negatively (immunosupressants) [1]. A large number of polysaccharides, that act only as immunostimulants, is known [2], but the most effective (and also most studied) are β-glucans. More than 6,000 papers describing the biological activities of glucans exist. Thus far, strong immunostimulating effects of β-glucans have been demonstrated in all tested animal species including earthworms [3,4], shrimp [5], fish [6], mice, rats [7], rabbits, guinea pigs [8], sheep, pigs [9], cattle [10] and, last but not least, humans.

HISTORY

It is likely that the first investigated substance with immunomodulating properties was so called endotoxin - lipopolysaccharide (LPS) of Gram-negative microbes. A paper describing the endotoxin was published in 1865 [11]. LPS induced intensified phagocytosis with a potential protective effect for a host; however, its toxic effects dominated completely. It was found that a saccharidic moiety of LPS, with prevailing glucose, galactose and mannose content [12], is non-toxic but bears immunomodulating activity. It was apparent that even polysaccharides themselves could act as immunomodulators, while their toxicity was negligible.

The history of polysaccharides as immunomodulators goes back to the middle of the last century, when Shear and coworkers [13] described a substance—called Shear's polysaccharide—which caused necrosis of tumors.

Subsequently, other polysaccharidic immunomodulators were researched; among them were β-glucans. Investigation of β-glucans began in the 60s and 70s of the last century. Two lines can be traced in β-glucans history, based on different starting points, but gradually converging. The first one took place chiefly in the U.S.A., the second one in Asia, specifically Japan. Research on β-glucans in the euro-american milieu was based on knowledge of immunomodulatory effects of zymosan [14], a mixture of polysaccharides isolated from the cell walls of *Saccharomyces cerevisiae*. When zymosan was closely examined, β-glucan was identified as a primary effective component: it was subsequently isolated and its immunological effects were investigated (*e.g.*, see [15,16]).

In Japan they came to β-glucan differently. In Asian medicine, consuming different medicinal mushrooms (shiitake, maitake, reishi *etc.*) has a long tradition. In detailed studies of biological effects of these mushrooms, especially their anticancer action, β-glucans were again found to be a main cause of non-specific immunomodulation. This initial investigation was conducted by Goro Chihara at Teikyo University in Kawasaki, who isolated β-glucan, which he referred to lentinan, from mushroom shiitake (*Lentinus edodes*, now *Lentinula edodes* [17]).

The important quality of polysaccharidic immunomodulators - β-glucans - is evidenced by the fact that all sufficiently purified ones distinguish themselves by very low

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toxicity (*e.g.*, mouse lentinan has LD_{50} > 1600 mg/kg; [18]). Conversely, the considerable heterogeneity of all natural β-glucans continues to be the cause of a series of mutually contradicting conclusions. An attempt was then made to solve this problem using semisynthetic and synthetic probes, suitable for accurate immunological research [19].

SOURCES OF β-GLUCAN

There are various natural sources of β-glucans. In addition to the often used fungal cell walls, β-glucan is also isolated from seaweed (laminaran from *Laminaria sp.*, [20]), bacteria (curdlan from *Alcaligenes faecalis*) [21], oat and barley. The composition of the cereal β-glucan is somewhat different (it contains in addition β(1→4) bound glucose).

The fungal cell wall comprises an appreciable part of cell mass. In yeasts it represents between 15 and 25 % of the total cell mass. Research on the cell wall of different fungal species did not lead to a straightforward model of its structure, and concepts of its organization underwent certain development. According to Stratford [22], the yeast cell wall resembles reinforced concrete. An armature, representing about 35% of wall mass and formed by fibrils of alkali insoluble β-glucan, is dipped into mannoproteins, bound to the armature through amorphous β-glucan and chitin. Similar model of the fungal cell wall was published by Selitrennikoff [23].

Until recently, biologically efficient β-glucans were supposed to have similar structure—the main chain of β(1→3) bound D-glucopyranose moieties to which some D-glucopyranoses are randomly connected by β(1→6) linkages (Fig. **1**). The degree of branching (DB) of some β-glucans is presented in Table **1**. However, the detailed structure of β-glucans from dissimilar sources differs as well as their biological activity [19,24-27]. In native β-glucans, their fibrils are composed from organized parts in which the main chain is coiled to triple helix. The triple helix, formed by three H-bonds in C-2 position and stabilized by side chains, is most likely present only in high-molecular β-glucans with molecular weight over 90 kDa [28,29]. On the contrary, in isolates of β-glucans this triple-helical structure can by destroyed during an isolation process, for the H-bonds of triple helices are interrupted by increased temperature, high pH or certain solvents [30].

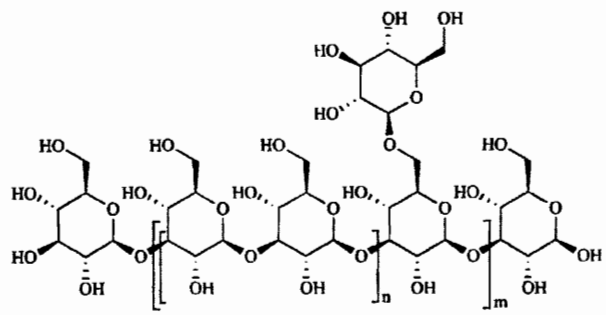

Fig. (1). General structure of glucan.

The number of individual glucans is almost as great as the number of sources used for isolation. Despite extensive research, it is still impossible to say that only one selected β-glucan is the optimal immunomodulator. Different physico-chemical parameters, such as solubility, structure, molecular weight, branching and polymer charge also play a role in determining of an extent in which the polysaccharide modulates immune reactions. In addition, all these parameters depend not only on source of glucan, but also on an isolation procedure. A degree of branching of some glucans is presented in Table **1**.

Table 1. Degree of Branching (DB) of Different β-Glucans

β-glucan	source	DB
chrysolaminaran	*Chaetoceros mülleri*	0.005 - 0.009
pachymaran	*Poria cocos*	0.015 - 0.02
yeast glucan	*Saccharomyces cerevisiae*	0.03 - 0.2
lentinan	*Lentinula edodes*	0.23 - 0.33
pleuran	*Pleurotus ostreatus*	0.25
pleuran	*Pleurotus tuber-regium*	0.3
scleroglucan	*Sclerotium glucanicum*	0.3
schizophyllan	*Schizophyllum commune*	0.33
grifolan	*Grifola frondosa*	0.31 - 0.36
SSG	*Sclerotinia sclerotiorum*	0.5

DB represents a molar ratio of branched and non-branched β-glucoses in a β-glucan molecule.

MECHANISMS OF ACTION

The best known effects of β-glucans consists of the augmentation of phagocytosis of professional phagocytes – granulocytes, monocytes, macrophages and dendritic cells. In this regard, macrophages [18,31,32], considered to be the basic effector cells in host defense against bacteria, viruses, parasites and tumor cells, play the most important role.

Macrophages are constituents of the non-specific (innate, non-adaptive), evolutionary older, immune system, which beyond phagocytes is comprised of a complicated family of serum proteins called a complement and a number of other soluble recognizing and effector molecules. This innate immunity is based on non-clonal receptors (pattern recognition receptors, PRRs), that recognize certain molecules on the surface of invading microorganisms and are collectively termed as pathogen-associated molecular patterns (PAMPs). Regardless of their name, PAMPs are not unique for pathogens only, but are fundamental for the survival and pathogenicity of a given microorganism. The PAMPs differ from host molecules, are not subjected to variability, and are evolutionary highly conserved. Different biopolymers, including β-glucans, belong to the PAMPs.

The first step of β-glucan-macrophage interaction is binding to specific receptors (PRRs) present on a surface of the macrophage cell. For β-glucan recognition, the macrophages keep several receptors at their disposal: TLR-2 (toll-like receptor 2), dectin-1, CR3 (complement receptor 3), lactosylceramide and probably others. However, the interaction between β-glucan and CR3 receptor is established more effectively than at the other receptors.

Toll-like receptors (TLRs) were not discovered until quite recently, although they possibly represent the most important receptor molecules of the non-adaptive component of the immune system. They are typical PRRs, which when bound together with PAMPs facilitate activation of the adaptive immune system in vertebrates. The name of these receptors is derived from sequential homology with a protein coded by the Toll gene. This gene occurs in *Drosophilla* flies where it plays a role in embryogenesis and, in mature flies, helps in defense against fungal infection [33,34]. TLRs are transmembrane proteins with extracellular repetitive sequences rich in leucine. Thus far, approximately eleven TLRs are known. β-Glucan (and also zymosan, intact yeast cells, LPS) is initially bound to TLR-2 [35].

Dectin-1 is a lectin located on the macrophage surface and has special application in the detection and phagocytosis of fungal pathogens. In certain cases, it cooperates with the TLR-2. It is also a transmembrane protein with many particular functions, *e.g.*, binding of a fungal PAMP, uptake and killing of invading cells, and induction of the production of cytokines and chemokines. It consists of four components: an extracellular carbohydrate-recognition domain (CRD), a stalk, a transmembrane region, and an intracellular cytoplasmic tail with an immunoreceptor tyrosine-based activation motif. Dectin-1 consists of 244 aminoacids (six of them are cysteins), all of which are highly conserved. Binding of β-glucans, and also zymosan or intact fungal cells, is mediated by the CRD [36-39].

Lactosylceramid is a glycoprotein containing a hydrophobic ceramide lipid and hydrophilic sacharidic moieties. It recognizes both microbial cells and fugal β-glucans [40]. Its role is not completely elucidated and will require further study.

Scavenger receptors contain a heterogeneous group of proteins with two transmembrane domains, two intacellular domains and one extracellular domain [41,42]. These receptors recognize a range of foreign cells, lipoproteins and polyanions [43]. They can bind lentinan, but no specific scavenger receptor for β-glucan itself have yet been identified [41].

The complement receptor 3 (CR3), known also as Mac-1, $\alpha_M\beta_2$-integrin, or CD11b/CD18, is one of the most promiscuous pattern-recognition receptors. In addition to complement components, it recognizes a large number of other ligands, among them β-glucan. The CR3 is a type of membrane glycoprotein consisting of two non-covalently linked α and β subunits known as CD11b or α_M and CD18 or β_2. This receptor has two major functions. As the Mac-1 molecule, it mediates the diapedesis of phagocytes and natural killer (NK) cells into sites of inflammation by generating a high-affinity binding site for the intercellular adhesion molecule-1 expressed by the stimulated endothelium. As CR3, it triggers phagocytosis and degranulation in response to microorganisms or immune complexes opsonized with the proteolyticaly inactive product of the complement cleavage fragment iC3b [44]. The functions of CR3/Mac-1 require bidirectional changes in conformation that result in "inside-out" and/or "outside-in" signaling that exposes a high-affinity binding site for protein ligands (*e.g.*, intercellular adhesion molecule ICAM-1, iC3b, fibrinogen; [45]).

The recognition of iC3b on microorganisms by the CR3 of phagocytes and NK cells triggers phagocytosis and/or cytotoxic degranulation responses that are important in host defense. This mode of action represents a form of innate pattern recognition that allows discrimination between microorganisms and host cells. A key finding was that β-glucan could bind to the lectin domain of CR3 and prime the receptor for cytotoxic degranulation in response to tumors that bore iC3b. Many human tumors generate an immune response that results in the deposition of antibody and iC3b on membrane surfaces. This iC3b serves as a specific target for cells bearing CR3 that has been primed with soluble β-glucan. When tumors lack such iC3b, research with mouse tumor models has demonstrated that antibodies to tumor antigens can be administered in combination with soluble β-glucan to restore tumor-bound iC3b and assure tumor-specific targeting [46].

In 1987 it was shown that CR3-dependent phagocytosis or degranulation in response to iC3b-opsonized yeast required ligation of two distinct sites in CR3 - one for iC3b and a second site for β-glucans [47]. Later research mapped these two sites to CD11b. All protein ligands bind to overlapping sites contained within the I-domain of CD11b. The C-terminal region was found to contain the lectin-like site for β-glucans. First, using flow cytometry with FITC-labeled β-glucan and CHO cells expressing recombinant chimeras between CD11b and CD11c, the lectin site was mapped to a region of CD11b located C-terminal to the I-domain [48]. Next, using baculovirus, rCD11b fragments from which the I-domain had been deleted were shown to have the same affinity for ^{125}I-β-glucan when expressed on insect cells without CD18 as did the neutrophil CR3 heterodimer [49].

Recent data gathered by several laboratories suggest that the lectin domain plays an essential role in adhesion, as well as in cytotoxicity, by promoting membrane complexes between CR3 and specific cell surface receptors that have no transmembrane domain or apparent mechanisms for transmembrane signaling. Such lectin-dependent complexes allow CR3 to function as a transmembrane signaling adapter for GPI-anchored receptors. When CR3 forms a membrane complex with CD87 [50], the CR3 portion of the complex is induced to express its high affinity binding site for ICAM-1. Neutrophils from mice deficient in CD87 were shown to be unable to migrate into sites of inflammation and *in vitro* experiments showed that activation of CD87-deficient neutrophils could not stimulate adhesion to ICAM-1 [50].

The C-terminal location of the lectin site was confirmed in a study of rCR3 binding to *C. albicans* that suggested that ligation of polysaccharides to the lectin site caused an increased affinity of protein-binding sites in the I-domain. Our studies implicated a site located C-terminal to both the I-domain and the divalent-cation binding repeats that became covered when antibodies were attached to the distal I-domain [50]. Further studies with rCD11b expressed on insect cells showed that the binding of labeled glucan could be blocked by monoclonal antibodies (mAbs) to the I-domain, as well as by mAbs to C-terminal epitopes [50]. In our studies, we had shown that staining by all available mAbs specific for C-terminal domain epitopes could be blocked to varying degrees by a small soluble polysaccharide that bound with high

affinity to the lectin site of CR3. When the data from these two reports were combined for analysis, it became apparent that the membrane proximal epitope defined by mAb CBRM1/23 was blocked more efficiently than other C-terminal epitopes by polysaccharide attachment to CR3 and is the prime candidate region for the lectin domain. Recently, we have also shown that this epitope is blocked selectively when CR3 forms lectin-dependent complexes with CD87 [50].

Despite extensive research, the mechanisms involved in the *in vivo* priming of cells by β-glucans and the signaling events still remain to be elucidated. It seems that glucan exhibits its biological properties through a cascade of events, including the specific binding, receptor interaction (in case of CR3 dual ligation), and signaling. Recently, the involvement of the Syk-phoshatidylinoitol 3-kinase pathway has been suggested [51]. Our preliminary results suggested that the signal formed by interaction of membrane receptor with glucan is transmitted to the cell nucleus where it affects expression of genes involved in regulation of cell proliferation, apoptosis and invasion. Changes in expression of these genes may result in increased proliferation, invasion and altered apoptosis in affected cells.

Signaling pathways involved have not been identified yet. However, some β-glucans have been found to induce salicylic acid signaling pathway in plants [52]. Recently, we have shown that glucan-treatment of ZR-75-1 cells induced expression of genes involved in signal transduction, cell cycle regulation, apoptosis, tumor invasion and metastasis [53]. Whether these changes at the gene level are also seen at protein level is yet to be determined. Therefore, we hypothesize that glucan exerts its biological functions through binding to its receptor, which then mediates a downstream signal by activating certain signaling pathways. In fact, our preliminary studies have shown a significant downregulation of phosphorylated extracellular signal-regulated kinase (phospho-ERK) levels in cell extracts of NCI-H23 cells treated with glucan, when compared to control cells. Importantly, reduced phospho-ERK levels coincided with decreased proliferation.

Our observation of significant upregulation of CDC42 and NF-κB2 expression level indeed suggests novel molecular mechanism that might be responsible for the anti-cancer activity of glucan. CDC42 has recently been shown to contribute to enhanced mitogenic signaling [54] and silencing CDC42 inhibited MDA-MB-231 and BT20 cell proliferation and migration due to reduced activity of ERK signaling in MDA-MB-231 cells [55]. Another study has suggested that CDC42 can activate NF-κB by a distinct pathway. Although further detailed analyses would be necessary in this context, our results suggest that activity of glucan is directly or indirectly related to CDC42 and NF-κB2 expression.

Generally, binding of β-glucan to a receptor activates macrophages and other professional phagocytes. The activation consists of several interconnected processes including increased chemokinesis, chemotaxis, and degranulation leading to increased expression of adhesive molecules on the macrophage surface and adhesion to the endothelium. In addition, β-glucan binding also triggers intracellular processes, characterized by the respiratory burst-formation of reactive oxygen and nitrogen species and free radicals. In addition, increasing of level and activity of hydrolytic and metabolic enzymes and Ca^{2+} influx through receptor-operated channels has been described (for review see [56]).

Activation of macrophages and other professional phagocytes represents a part of more complicated processes, when mediator molecules secreted by them (such as interleukin-1 (IL-1), interleukin-9 (IL-9), or tumor necrosis factor (TNF-α)) initiate inflammation reactions. Inflammation is an essential protective process preserving the integrity of organisms against physical, chemical and infective attacks. However, the inflammatory response to several attacks can lead to damage of normal tissues. Physiological inflammation runs to the extent and to the rate corresponding with an inducing noxa, so to the β-glucan present. If the noxious impact persists, a pathological inflammation can take place, manifested by excessive tissue damage subsequently ending in immune disorders and development of immunopathological (*e.g.*, autoimmune) processes. In the most serious cases, generalization of inflammatory processes can be considered, with shock development and subsequent fatal multiple organ dysfunction syndrome.

Results of one research group suggested that β-glucan-induced inflammatory processes could competitively interact with simultaneously administered nonsteroidal antiinflammatory drugs. The lethal toxicity due to septic shock, elicited by a sequential administration of β-glucan and a nonsteroidal anti-inflammatory drug indomethacin, was described in mice [57-59]. Antibiotic treatment protects mice against septic shock evoked by this drug combination [60]. However, these results were never independently confirmed.

There is an evidence that β-glucan plays a considerable role in increased production of nitric oxide, one of the most effective reactive nitrogen species, by inducible nitric oxide synthase (iNOS) in macrophages [61,62] from L-arginine. Various types of nitric oxide synthase (NOS) are known (besides iNOS also a neuronal and epithelial ones). Function of formed nitric oxide is double-edged. It induces a cytotoxic effect upon tumor cells [63] and shows distinct impact on many pathogens [64]. On the other hand, it can damage tissues and DNA [65] and its high concentrations can cause septic shock. The activator's sustained action induces expression of iNOS and increased formation of nitric oxide (NO) results in vasodilatation of veins. The vasodilatation brings about an intense drop of venous resistance and blood pressure [66]. Other side effects imputed to β-glucan-induced nitric oxide production are bioaerosol-induced respiratory symptoms seen in both occupational and residential environments [67]. Generally, the dual role of NO as a protective or toxic molecule is due to several factors such as: the isoform of NOS involved, concentration of NO and the type of cells in which it is synthesized, the availability of L-arginine, formation of cGMP by soluble guanylate cyclase and the overall extra and intracellular environment in which NO is produced [68].

Our knowledge of possible negative effects of various β-glucans is limited. In addition to the possible negative effects of inflammation reactions, as well as nitric oxide, several other adverse reactions were reported.

Particulate β-glucan applied parenterally was reported to cause granuloma formation, microembolization, local inflammation and pain [69,70]. Inhalation of intact cells or cellular detritus of fungi or yeasts—ingredients of home dust [71] or different agricultural and industrial dusts [72]—induces the so called syndrome of toxic organic dust (STOD) which is characterized by lung reactions that include pneumonia, cough and chronic bronchitis [73], rhinitis, headache and irritation of eyes and throat. β-Glucans were reported as important causal factors of these complaints [74,75] which, through activation of macrophages, monocytes and leukocytes, causes increased secretion of inflammatory components (*e.g.*, TNF-α and interleukin-8 (IL-8)). β-Glucans have also been suggested as important agents in the inflammatory reactions seen in the so called Sick Building Syndrome [76].

PHYSIOLOGICAL EFFECTS OF β-GLUCANS

β-Glucans are well-known biologic response modifiers that function as immunostimulants against infectious diseases and cancer [77,78]. Unlike most other natural products, purified β-glucans retain their bioactivity, which permits the characterization of how β-glucans work on a cellular and molecular level.

β-Glucan has been used as an immunoadjuvant therapy for cancer since 1980, primarily in Japan [79-81]. Another activity demonstrated with β-glucan in the mid 1980's was its ability to stimulate hematopoiesis in an analogous manner as granulocyte monocyte-colony stimulating factor [82]. Both particulate and soluble β-glucans, all of which were administered intravenously, caused significantly enhanced recovery of blood cell counts after gamma irradiation [83, 84]. Later studies demonstrated that glucan is similarly effective when hematopoiesis is compromised by various types of chemotherapy [85].

In addition to the effect in treatment of cancer, β-glucans have been demonstrated to protect against infection with both bacteria and protozoa in several experimental models and were shown to enhance antibiotic efficacy in infections with antibiotic-resistant bacteria. The protective effect of β-glucans was shown in experimental infection with *Escherichia coli* [86], *Streptococcus suis* [87], *Staphylococcus aureus* [88], *Candida albicans* [89], aspergillosis [90], *Leishmania major* [91], *Toxoplasma gondii* [92], *Plasmodium berghei* [93], *Mesocestoides corti* [94], *Trypanosoma cruzi* [95], and *Eimeria vermiformis* [96]. Regarding the threat of bioterrorism, it is particularly interesting that glucan has been found to prophylactically protect against anthrax infection [97].

Most published studies described effects of injected β-glucans (either *i.p.*, *i.v.* or *s.c.*). Only recently the effects of glucan delivered *p.o.* were evaluated. (see Table **2**).

The influence of certain barley and mushrooms glucans on decreasing levels of serum cholesterol and liver low-density lipoproteins, leading to lowering of arterosclerosis and heart disease hazards, was also described [98]. It is also

Table 2. Oral Effects of Glucan

SOURCE	INDICATION	SPECIES	RESULTS	REFERENCE
Yeast	Cancer	Human	Inhibition	[101]
	Cancer	Mouse	Inhibition	[46]
	Lipids	Mouse	Reduction	[102]
	Antiviral	Mouse	Reduction	[103]
Lentinan	Cancer	Mouse	Reduction	[104]
Schizophyllan	Antiviral	Mouse	Increased Ab	[105]
	Cancer	Mouse	No effects	[106]
SSG	Immunity	Mouse	Increase	[107]
	Cancer	Mouse	Inhibition	[108]
Maitaki	Cholesterol	Rat	Decrease of Lipids	[110]
	Cancer	Human	Reduction	[109]
PSK	Cancer	Mouse	Inhibition	[111]
	Cancer	Human	Increased Survival	[112]
Agricus blazei	Cancer	Mouse	Enhanced Clearance	[113]
Sparassis crispa	Cancer	Mouse	Inhibition	[114]
Seaweed	Cancer	Mouse	Inhibition	[85]
Oat	Blood glucose	Human	Reduction	[115]

known that β-glucans, due to their non-digestibility and swelling, can facilitate bowel motility and can be used in amelioration of intestinal problems, particularly obstipation [99]. At the same time, the non-digestible β-glucans, are able to modulate mucosal immunity of the intestinal tract [100].

It is well established that the administration of glucans enhances the efficacy of anticancer immunotheraphy, both in clinical and experimental conditions. However, little is known about the transfer of orally administered glucan through the gastrointestinal tract into different tissues and organs. The studies of the Ross group suggested that orally-adminstered glucan is taken up by gastrointestinal macrophages and subsequently shuttled to the reticuloendothelial system and bone marrow [101]. Rice *et al.* has reported significant differences among various glucans in plasma concentration and binding of glucan by gastrointestinal epithelial and gut-associated lymphatic tissue (GALT) cells [41]. These studies were performed in adult rodents with fully developed digestive and immune systems. In our recent study, we evaluated the absorption of β-glucan during the suckling period when intestinal barrier function and transport function are not fully established. Results from our studies suggest that only a limited amount of β-glucan is absorbed by the gut and transferred into the systemic blood. The majority of β-glucan was detected in the gastrointestinal tract and the liver. Thus, we speculate that the gastrointestinal epithelium, GALT cells, and Kuppfer cells are likely the most affected systems by orally administered β-glucan. Further studies established relation among β-glucan, antibodies and tumor growth [46]. The detailed study of tissue distribution and gastrointestinal transfer is therefore necessary and has high potential to yield data important for possible clinical use of glucans.

FUTURE OF GLUCANS

There are two problems with natural β-glucans that make them undesirable as drugs. First, there is considerable variation in the structure of β-glucans isolated from the same strain of fungi (or any other suitable material). Over the last 10 years, we have obtained more than 90 preparations of β-glucan from colleagues or the pharmaceutical industries in Japan, Brazil, Taiwan, USA and Czech Republic. On several occasions, it was noted that multiple lots of one type of β-glucan had extraordinary affinity for CR3, whereas the next several lots had low or undetectable affinity for CR3. The same biological variability has been found for effects on immune reactions. The problem is that the structure of cell walls varies with growth conditions leading to considerable variation among batches of yeast, mushrooms or seaweed in glucan branching frequency as well as linkage to chitins and mannoproteins. The second problem in the development of a drug from a natural product is that it is difficult to make it proprietary and protect the investment required for development. It is possible, however, that these problems will no longer exist when synthetic oligosaccharides are used.

ABBREVATIONS

BRMs	=	Biological response modifiers
PRRs	=	Pattern recognition receptors
PAMPs	=	Pathogen-associated molecular patterns
TLR-2	=	Toll-like receptor 2
CR-3	=	Complement receptor 3
FITC	=	Fluorescein isothiocyanate
CRD	=	Carbohydrate-recognition domain
ICAM-1	=	Intercellular adhesion molecule
iC3b	=	Proteolyticaly inactive product of the complement cleavage fragment
phospho-ERK	=	Phosphorylated extracellular signal-regulated kinase
IL-1	=	Interleukin-1
IL-8	=	Interleukin-8
IL-9	=	Interleukin-9
TNFα	=	Tumor necrosis factor
iNOS	=	Inducible nitric oxide synthase
NOS	=	Nitric oxide synthase
NO	=	Nitric oxide
GALT	=	gut-associated lymphatic tissue
LPS	=	Lipopolysaccharide
NK	=	Natural killer cells
mAbs	=	Monoclonal antibodies

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