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REVIEW ARTICLE

β -Glucans, History, and the Present: Immunomodulatory Aspects and Mechanisms of Action

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The present paper represents a comprehensive up-to-date review of β -glucans, their chemical and biological properties, and their role in immunological reactions. β -D-Glucans belong to a group of physiologically active compounds called biological response modifiers and represent highly conserved structural components of cell walls in yeast, fungi, or seaweed. Despite almost 150 years of research, the exact mechanisms of their action remain unclear. The present review starts with the history of glucans. Next, attention is focused on sources and structure, comparing the effects of physicochemical properties, and sources on biological effects. As glucans belong to natural products useful in preventing various diseases, they have been highly sought after throughout human history. Based on extensive recent research, this paper explains the various mechanisms of effects and the ways glucans mediate their effects on defense reactions against infections. Despite the fact that predominately pharmacological effects of glucans are positive, their unfavorable and potentially toxic side effects were not overlooked. In addition, attention was focused on the future research, possible alternatives such as synthetic oligosaccharides, and on clinical applications.

Keywords defense, glucan, history, immunity

1. INTRODUCTION

Generally, β -glucan is a chemical name of a polymer of β -glucose. There are more of such polymers, differing in glycosidic bond position. Cellulose, (1 \rightarrow 4)- β -D-glucan, is an example. The following data refer to homopolymers of glucose having a linear molecule with (1 \rightarrow 3)- β -D-glycosidic linkages or a branched one, with side chains bound by (1 \rightarrow 6)- β -D-glycosidic linkages. Although chemically heterogeneous, these polysaccharides are usually termed by common name " β -glucans." It is necessary to take into account that these compounds, which otherwise caused

similar or nearly identical immune reactions in macroorganisms, can differ in origin as well as in their primary, secondary, or tertiary structures or their solubility in water or alkalies. Due to these factors, numerous discrepancies can be found in the literature. Some sources of β -glucans are listed in Table 1.

β -Glucans show notable physiological effects; this is their most important quality and the reason why so much attention has been devoted to them. They belong to a group of physiologically active compounds, collectively termed biological response modifiers (BRM). According to their effects, BRM can be classified into two groups – cytokines, responsible for communication between immune system cells and regulation of the system, and immuno-modulators. The immunomodulators are able to positively (i.e., immunopotential) or negatively (i.e., immunosuppression) manipulate the immune system. Different known immunomodulators can be classified into three groups (Werner, 1987): 1) Intact microbes (e.g., *Bacille Calmette-Guérin*) and components of microbial cells (e.g., endotoxin of Gram-negative microorganisms (LPS), muramyl dipeptide (MDP), fungal polysaccharides (zymosan, β -glucans), polynucleotides, bestatine); 2) Natural components of a normal immune system (e.g., thymic hormones, lymphokines, monokines); and, 3) Synthetic compounds (e.g., levamisole, isoprinosine, diethyldithiocarbamate).

Thus far, among many known and tested immunomodulators of the first group, polysaccharides isolated from different microorganisms and plants are the most important. A large number of such polysaccharides, which act only as immunopotentiators, is known (Whistler et al., 1976). As mentioned earlier, β -glucans belong to this group.

2. HISTORY

At the beginning of the 18th Century, it was already known that certain infectious diseases showed a therapeutic effect on malignant processes. Purposeful use of such therapy dates approximately from the middle of the 19th Century, when Bush (Bush, 1850) performed experiments in curing sarcoma

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TABLE 1
Some β -glucans with immunomodulatory effects

Name	Source	Character of polymer
Curdlan	<i>Alcaligenes faecalis</i>	linear
laminaran	<i>Laminaria sp.</i>	linear
pachymaran	<i>Poria cocos</i>	linear
lentinan	<i>Lentinus edodes</i>	branched
Pleuran (HA-glucan)	<i>Pleurotus ostreatus</i>	branched
schizophyllan	<i>Schizophyllum commune</i>	branched
sclerotinan (SSG)	<i>Sclerotinia sclerotiorum</i>	branched
scleroglucan	<i>Sclerotium glaucanicum</i> , <i>S. rolfsii</i>	branched
grifolan	<i>Grifola frondosa</i>	branched
T-4-N, T-5-N	<i>Dictyophora indusiata</i>	branched
yeast glucan	<i>Saccharomyces cerevisiae</i>	branched

by infecting patients with erysipelas, i.e., with β -hemolytic streptococci of the Group A. These therapeutical procedures were repeated toward the end of the 19th Century by Coley (Coley, 1894), who applied less dangerous extracts of microbial cultures, in this case from cultures of *Bacillus prodigiosus* (now *Serratia marcescens*). This extract was at these times known as "Coley's toxin," and was lately determined as interleukin IL-12 (Tsung and Norton, 2006).

However, Coley does not have priority in this regard. At the time, it was already known that certain components of microorganisms caused in macroorganisms, especially mammalian ones, ferocious reactions comparable to pathophysiological conditions during infection by intact microbes. It is likely that the first investigated substance with these properties was LPS of Gram-negative bacteria (e.g., *Escherichia*, *Salmonella*, *Shigella*, *Pseudomonas*, *Neisseria*, and *Haemophilus spp.*); a paper describing the endotoxin was published in 1865 (Billroth, 1865). In both humans and experimental animals, LPS induces increased phagocytosis with a potential protective effect for host. However, its toxic effects (such as fever, diarrhea, hypotensive shock, intravascular coagulation, multiple organ dysfunctions) completely predominate. A toxic principle of LPS is its lipid moiety, while the saccharidic moiety, with prevailing glucose, galactose and mannose content (Nowotny, 1969), is non-toxic and bears immunomodulating activity as well. It is apparent that even polysaccharides themselves can act as immunomodulators, while their toxicity is negligible.

Proper history of polysaccharides as immunomodulators goes back to the 1940s when Shear and coworkers (Shear et al., 1943) described a substance, again from *Serratia marcescens* cultures, that caused necrosis of tumors. Subsequently, this substance (so-called Shear's polysaccharide) was identified as a mixture of three polysaccharides with the main chain consisting of D-glucose and D-mannose units connected by (1 \rightarrow 3) glycosidic linkages (Srivastava and Adams, 1994).

In time, other polysaccharide immunomodulators were sought and among them were β -glucans. Their investigation began in the 1960s and 1970s. Two lines can be traced in β -glucan history, each based on different starting points but gradually converging. The first took place chiefly in the United States and Europe, the second in Asia, primarily in Japan. Research on β -glucans in the Euro-American milieu was based on knowledge of immunomodulatory effects of zymosan, a mixture of polysaccharides isolated from the cell walls of *Saccharomyces cerevisiae*. Zymosan was for the first time prepared and investigated by Pillemer and Ecker (1941) in the 1940s and since that time, it has been used in many physiological and immunological studies. Zymosan is potent stimulator especially of alveolar macrophages and, among others, it induces the release of a series of cytokines, mainly interleukin (IL-8), from human neutrophils.

Although zymosan was able to stimulate non-specific immune response, at the onset it was not clear what component of this rather crude composition is responsible for that activity. When zymosan was examined in detail, β -glucan was identified as the component of primary effect. It was subsequently isolated and its immunological effects were investigated. This research was pioneered by Nicholas R. DiLuzio (i.e., see DiLuzio and Riggi, 1970, Williams et al., 1980) at Tulane University in New Orleans.

The arrival of β -glucan in Japan was different. In Asian medicine, consumption of different medicinal mushrooms (e.g., shiitake, maitake, reishi, etc.) has a long tradition. In detailed studies of the biological effects of these mushrooms, especially the anticancer actions, β -glucans were again found to be a main cause of non-specific immunomodulation. The source of this investigation is associated with Goro Chihara from Teikyo University in Kawasaki, who isolated β -glucan, named by him lentinan, from mushroom shiitake (*Lentinus edodes*, now *Lentinula edodes*) (Chihara et al., 1969).

All sufficiently-purified polysaccharidic immunomodulators distinguish themselves by very low toxicity (e.g., lentinan has an LD_{50(mouse)} > 1600 mg/kg [Chihara et al., 1982]). Time incorporation of β -glucans in the history of immunomodulation is shown in Table 2.

The history of the investigation of the chemical composition of β -glucans is rather long and not straightforward. Composition of the cell walls of different microbial producers of β -glucan, in particular yeasts, was previously investigated in the 19th Century. Van Wisselingh (1898) published an opinion that in the fungal cell wall, either chitin or cellulose could prevail.

β -Glucan from the cell wall of *Saccharomyces cerevisiae* can serve as an example of how difficult an exact determination of chemical structure was in the first half of the last century. In the 1950s and 1960s, β -glucan isolated from the cell walls of *S. cerevisiae* was subjected to investigation by the technique of sugar analysis, i.e., partial hydrolysis, methylation analysis, periodate oxidation, Smith degradation (periodate oxidation, reduction by NaBH₄, and subsequent partial hydrolysis), etc. However, the

TABLE 2
History of immunomodulators

1865	T. Bilroth: endotoxin (LPS)
1894	W. B. Coley: erysipel toxins, <i>B. prodigiosus</i>
1936	J. Freund: mykobak J. Freund: mycobacterial adjuvants (BCG)
1941	L. Pillemer: zymosan
1943	M. J. Shear: polysaccharides from <i>S. marcescens</i>
1963	R. Prevot, B. Halpern, G. Biozzi: <i>Corynebacterium parvum</i>
1964–1971	A. White, A. Goldstein, J.F. Bach <i>et al.</i> : thyme hormones
1967	H. Okamoto: picibanil (OK-432)
1968	N. DiLuzio: yeast glucan
1969	G. Chihara: lentinan
1967	W. Braun: double-stranded polynucleotides
1970	N. Najjar: tuftsin
1971	G. Renoux: levamisol
1975	E. Lederer, L. Chedid : MDP

results obtained were rather discrepant (Bell and Northcote, 1950; Manners and Patterson, 1966). Only after finding that in the yeast cell wall several types of β -glucans exist (Bacon and Farmer, 1968), detailed fractionation of cell wall components and their characterization was made (Manners *et al.*, 1973). It is believed now that the main component of β -glucan from the yeast cell wall is a slightly branched, high-molecular (1 \rightarrow 3)- β -D-glucan (DP about 1500, molecular weight ca. 240 kDa), with about 3% of β (1 \rightarrow 6) branching.

Considerable heterogeneity of all natural β -glucans, not only from saccharomycetes but also from other sources, obviously was and continues to be the cause of a series of mutually contradicting conclusions. Recently, an attempt was made to solve this problem using semisynthetic and synthetic probes, suitable for accurate immunological research (Jamois *et al.*, 2005).

3. SOURCES AND STRUCTURE OF β -GLUCAN

There are various natural sources of β -glucans; however, they are most frequently prepared from fungal cell walls. In addition to the fungal cell walls, β -glucan is also isolated from seaweed (laminaran from *Laminaria sp.*, linear β (1 \rightarrow 3)-D-glucan [Black *et al.*, 1951], commercially sold as Phycarine [Vetvicka and Yvin, 2005]). It is also produced by bacteria (curdlan from *Alcaligenes faecalis*) (Harada *et al.*, 1968) and included in cereals. The composition of the cereal β -glucan is somewhat different; it contains, in addition, β (1 \rightarrow 4) bound glucose.

Taxonomic conception of kingdom Fungi went through a certain progression; many microorganisms earlier classified as fungi are now categorized into other kingdoms. In the following text, the name fungi will be written in quotation marks to distinguish between taxonomic and practical definition. Examples of composition of cell wall polysaccharides are presented in Table 3. The table makes evident that the most important producers of β -glucans are ascomycetes (where yeasts and certain filamentous moulds pertain) and basidiomycetes. To basidiomycetes belong edible or non-edible mushrooms, either found in nature or artificially cultivated.

The cell wall composes an appreciable part of fungal cell mass; in yeasts it represents between 15% and 25% of total cell

TABLE 3
Composition of cell wall polysaccharides of some “fungi”

Kingdom	Class	Examples		Prevailing wall polysaccharides at vegetative cells
		Order	Genus and Species	
<i>Protista</i>	<i>Acrasiomycetes</i>	<i>Dictyosteliales</i>	<i>Dictyostelium discoideum</i>	cellulose - glycogen
<i>Chromista</i>	<i>Oomycetes</i>	<i>Perenosporales</i>	<i>Plasmopara viticola</i>	cellulose - β -glucan
			<i>Phytophthora infestans</i>	
<i>Fungi</i>	<i>Hyphochytridiomycetes</i>	<i>Hyphochytriales</i>	<i>Rhizidiomyces parasiticus</i>	cellulose - chitin
	<i>Zygomycetes</i>	<i>Mucorales</i>	<i>Mucor mucedo</i>	cellulose - chitin
	<i>Chytridiomycetes</i>	<i>Chytridiales</i>	<i>Blastocladiella emersonii</i>	chitin - β -glucan
	<i>Ascomycetes</i>	<i>Eurotiales</i>	<i>Aspergillus niger</i>	
	<i>Homobasidiomycetes</i>	<i>Agaricales</i>	<i>Agaricus bisporus</i>	
			<i>Lentinula edodes</i>	
			<i>Pleurotus ostreatus</i>	
			<i>Grifola frondosa</i>	
			<i>Schizophyllum commune</i>	
	<i>Hemiascomycetes</i>	<i>Saccharomycetales</i>	<i>Saccharomyces cerevisiae</i>	mannan - β -glucan
<i>Hypomycetes</i>	<i>Sporobolomycetaceae</i>	<i>Sporobolomyces roseus</i>	mannan - chitin	

mass. The cell wall is not an inert structure but a vital organelle that performs a range of functions – mechanical protection, osmotic stabilization, compounds binding, mutual cellular adhesion, etc. The cell wall also serves as enzyme support and a selectively permeable barrier.

Research on the cell wall of different “fungal” species does not lead to a straightforward model of its structure, and concepts of its organization underwent certain development. According to Stratford (Stratford, 1994), the yeast cell wall resembles reinforced concrete. An armature, representing about 35% of wall mass and formed by fibrils of alkali insoluble $\beta(1\rightarrow3)$ -glucan, is dipped into mannoproteins (about 25–35% of wall mass) and bound to the armature through amorphous β -glucan and chitin. The cell wall of other “fungi” is constructed in a similar way. Essentially, the same model of the fungal cell wall was published by Selitrennikoff (2001).

Recently, Grün (2001) published another model of the cell wall of ascomycetes and basidiomycetes. According to this model, the cell wall of most “fungi” contains five main components: $(1\rightarrow3)$ - β -D-glucan, $(1\rightarrow6)$ - β -D-glucan, $(1\rightarrow3)$ - α -D-glucan, chitin, and glycoproteins. On the other hand, $(1\rightarrow3)$ - α -D-Glucan is not present in yeasts (e.g., in *S. cerevisiae* and *Candida albicans*). However, in many other species of the *Ascomycetes* and *Basidiomycetes*, it forms 9–46% of cell wall mass. Chitin at saccharomycetes is found only in bud scars.

Though different models of the fungal cell wall differ somewhat, they agree that β -glucan is not located on the surface of the wall but is more or less immersed in the wall material. With regard to both immunological research and pharmaceutical utilization of β -glucans, an important conclusion can be reached. In macroorganisms, β -glucans act first of all as markers of fungal invasion so that activity of β -glucan preparations will increase with the degree of denudation of glucan fibrils. A general definition of glucans of any origin is given in Table 4.

Until recently, biologically efficient β -glucans were supposed to have similar structure – the main chain of $\beta(1\rightarrow3)$ bound D-glucopyranose moieties to which some D-glucopyranoses are randomly connected by $\beta(1\rightarrow6)$ linkages (Figure 1). The degree of branching (DB) of some β -glucans is presented in Table 5. However, the detailed structure of β -glucans from dissimilar sources differs as well as their biologi-

cal activity (Rees and Scott, 1971; Wagner et al., 1988; Adachi et al., 1989; Jamas et al., 1991; Kraus and Franz, 1992). In native β -glucans, their fibrils are composed from organized parts in which the main chain is coiled to triple helix. These regions are combined with single or double filaments of $\beta(1\rightarrow3)$ -D-glucopyranoses (Saito et al., 1987, Ohno et al., 1988). The triple helix, formed by three H-bonds in C-2 position and stabilized by side chains, is probably present only in high-molecular β -glucans with molecular weight over 90 kDa (Chuah et al., 1983; Ohno et al., 1988). The H-bonds of triple helices can be interrupted by increased temperature, high pH, or certain solvents.

Diverse data on the comparison of structure, molecular size, and biological effect can be found in the literature. For example, anti-tumor activity of schizophyllan is supposedly conditioned by triple helix presence and a molecular weight higher than 100 kDa (Kojima et al., 1986). However, the triple helix structure most likely should not be a solely effective form of β -glucan because alkaline treatment, used in most of isolation procedures, destroys this structure (Young and Jacobs, 1998; Saito et al., 1991; Miura et al., 1995). In addition, the most recent opinions do not confirm established ideas of the necessity of high molecular mass and branching of biologically active β -glucans. Thirty years ago, Kabat (1976) found that for antigen polysaccharidic determinants, the size of the binding site on an antibody corresponds to six or seven monosaccharide units. Size of the binding site for β -glucan – in this case on a receptor of an immunocompetent cell, e.g., the macrophage – seems also to correspond to this number of glucose residues.

4. GLUCAN AND IMMUNOLOGICAL REACTIONS

Natural products useful in preventing or treating disease have been highly sought after throughout human history. A major problem in characterizing many natural products is that they represent a complex mixture of ingredients, each one of which may contribute to their bioactivity. β -Glucans from fungi, yeast and seaweed are well-known biologic response modifiers that function as immunostimulants against infectious diseases and cancer (Borchers et al., 1999; Brown and Gordon, 2003). Unlike most other natural products, purified β -glucans retain their

TABLE 4
General division of β -glucans of any origin

Type	Types
Gel-forming β -glucans (“soluble”)	High-molecular branched β -glucans (e.g., grifolan, schizophyllan, scleroglucan) Linear β -glucans (e.g., laminaran from brown sea algae), Chemically modified particular β -glucans (e.g., carboxymethylated, sulfonated or phosphorylated β -glucans).
Particular (“insoluble”) β -glucans	Yeast β -glucan

β -Glucans from the first group are usually soluble in alkalies. More recently a premise was raised that insolubility of β -glucan in alkalies is driven only by the extent of binding to chitin (Brown et al., 1993).

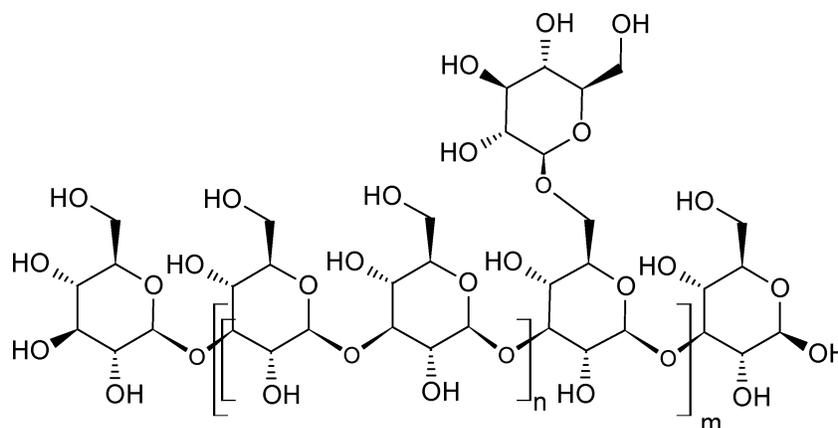


FIG. 1. General structure of glucan.

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 (Takeshita et al., 1991; Kimura et al., 1994; Matsuoka et al., 1997; Yan et al., 1999). Another activity demonstrated with β -glucan in the mid-1980s was its ability to stimulate hematopoiesis in an analogous manner as granulocyte monocyte-colony stimulating factor (Patchen et al., 1983). Both particulate and soluble β -glucans, all of which were administered intravenously, caused significantly enhanced recovery of blood cell counts after gamma radiation (Patchen et al., 1985). Others showed that β -glucan could reverse the myelosuppression produced with chemotherapeutic drugs (Wagnerova et al., 1993).

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 *Leishmania major* (Al Tuwaiji et al., 1987) and *L. donovani* (Cook and Holbrook, 1984), *C. albicans* (Bacon and Farmer, 1968), *Toxoplasma gondii* (Bousquet et al., 1988), *Streptococcus suis* (Dritz

et al., 1995), *Plasmodium berghei* (Kumar and Ahmad, 1985), *Staphylococcus aureus* (Liang et al., 1998), *Escherichia coli* (Rasmussen and Seljelid, 1990), *Mesocestoides corti* (White et al., 1988), *Trypanosoma cruzi* (Williams et al., 1989), *Eimeria vermiformis* (Yun et al., 1998), and anthrax infection (Vetvicka et al., 2002). Most published studies described effects of injected β -glucans (either intraperitoneal [IP], intravenous [IV], or subcutaneous [SC]). It is however necessary, for possible clinical practice, to evaluate the possibility of oral delivery. These experiments are summarized in Table 6.

Yeast β -glucan is able to absorb mycotoxins (such as zearalenon, aflatoxin B1, deoxynivalenol, ochratoxin A, and patulin), probably through hydrogen bonds and van der Waals forces; this β -glucan effect is important particularly for livestock.

The influence of certain cereals (barley, oats) and edible mushrooms (e.g., *Grifola frondosa*, *L. edodes* and *Flammulina velutipes* [Fukushima et al., 2001], *Pleurotus ostreatus* [Bobek et al., 1996], or *Agaricus bisporus* [Fukushima et al., 2000]) on decreasing levels of serum cholesterol and liver low-density lipoproteins, leading to lowering of arteriosclerosis and heart disease hazards, is also mediated by β -glucan. It is known that cereals, mushrooms and yeast facilitate bowel motility and can be used in amelioration of intestinal problems, particularly constipation (Battilana et al., 2001; Dongowski et al., 2002). Non-digestible β -glucans, forming a remarkable portion of these materials, are also able to modulate mucosal immunity of the intestinal tract (Tsukada et al., 2003). In the central nervous system, β -glucans activate microglial cells (Muller et al., 1994). These cells act as scavengers of the brain cell debris and play a positive role in Alzheimer's disease, AIDS, and multiple sclerosis (Haga et al., 1989).

Possible effects of β -glucans on macroorganisms are thus very diverse and impinge upon not only the immune system, but probably in most of the described activities, are in some form more or less connected with that system.

TABLE 5
Degree of branching (DB) of different β -glucans

β -Glucan	Source	DB
pachymaran	<i>Poria cocos</i>	0.015–0.020
yeast glucan	<i>Saccharomyces cerevisiae</i>	0.03–0.20
lentinan	<i>Lentinula edodes</i>	0.23–0.33
pleuran	<i>Pleurotus ostreatus</i>	0.25
grifolan	<i>Grifola frondosa</i>	0.31–0.36
scleroglucan	<i>Sclerotium glaucanicum</i>	0.30
schizophyllan	<i>Schizophyllum commune</i>	0.33
SSG	<i>Sclerotinia sclerotiorum</i>	0.50

TABLE 6
Oral effects of glucan

Source	Indication	Species	Results	Reference
Yeast	Cancer	Human	Inhibition	Ueno, 2000
	Cancer	Mouse	Inhibition	Hong et al., 2004
Lentinan	Cancer	Mouse	Reduction	Kurashige et al., 1997
Schizophyllan	Antiviral	Mouse	Increased Ab	Hotta et al., 1993
	Cancer	Mouse	No effects	Miura et al., 1995
SSG	Immunity	Mouse	Increase	Sakurai et al., 1992
	Cancer	Mouse	Inhibition	Suzuki et al., 1991
Maitaki	Cholesterol	Rat	Decrease of Lipids	Kubo and Hanba, 1996
	Cancer	Human	Reduction	Nanba, 1995
PSK	Cancer	Mouse	Inhibition	Nio et al., 1988
	Cancer	Human Survival	Increased	Kaibara et al., 1976
<i>Agricus blazei</i>	Cancer	Mouse	Enhanced clearance	Ebina and Fujimiya, 1997
<i>Sparassis crispa</i>	Cancer	Mouse	Inhibition	Ohno et al., 2000
Seaweed	Cancer	Mouse	Inhibition	Vetvicka et al., 2007

5. MECHANISMS OF ACTION

The most pronounced effect of β -glucans consists of augmentation of phagocytosis and proliferative activities of professional phagocytes—granulocytes, monocytes, macrophages, and dendritic cells. In this regard, macrophages (Chihara et al., 1982; Quinn, 1990; Vetvicka et al., 1996), considered the basic effector cells in host defense against bacteria, viruses, multicellular parasites, tumor cells, and erroneous clones of own somatic 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 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.

Macrophages detect PAMPs through a number of different receptors. 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 even other. Generally, the receptors are not too specific and usually detect several different PAMPs.

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 *Drosophila* flies where it plays a role in embryogenesis and, in mature flies, helps in defense against fungal infection (Lemaitre et al., 1996; Bilak et al., 2003). 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, and/or LPS) is initially bound to TLR-2 (Underhill et al., 1999).

Dectin-1 is a lectin located on the macrophage surface and has special involvement 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.

The complement receptor 3 (CR3, also denoted as integrin CD11b/CD18, (M,2 and MAC1) 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 receptor is created from several domains; for saccharide recognition, it is a lectin domain (Ross et al., 1999) (Figure 2). When a fragment iC3b of complement is simultaneously bound, binding of β -glucan triggers phagocytosis and degranulation.

Lactosylceramide (also CDw17 or Gal β 4Glc β 1Cer) is a glycosphingolipid PRR located in the plasmatic membrane. β -Glucan recognition mediated by lactosylceramide causes different cellular responses, including production of cytokines and respiratory burst (Brown, 2006). Examples of macrophage receptors and the corresponding PAMPs are given in Table 7.

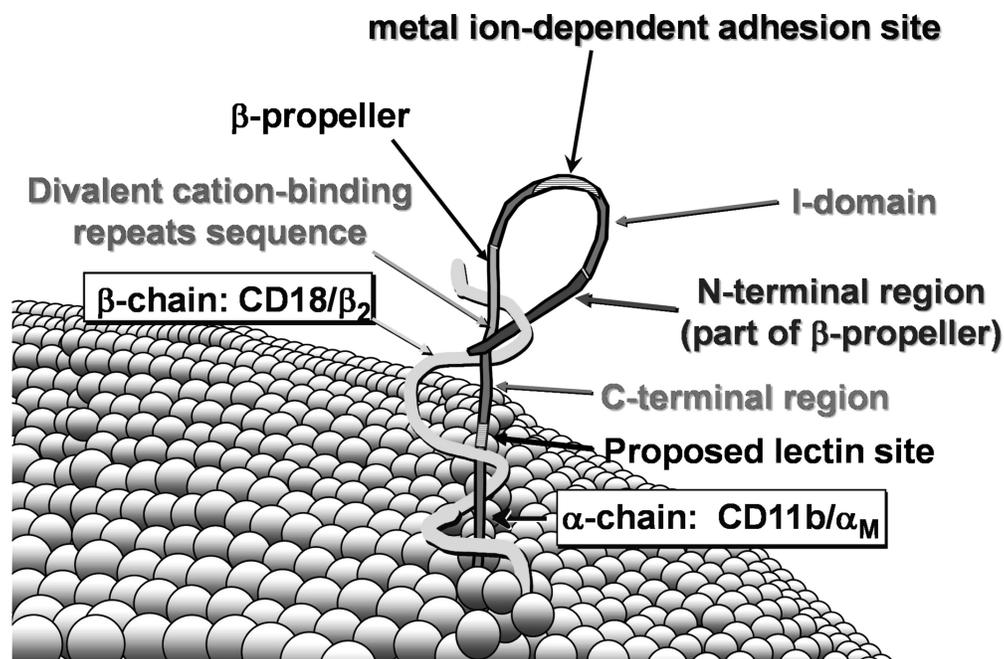


FIG. 2. Schematic representation of CR3 showing its intertwined two-chain structure and the major domains of CD11b (From Ross, G.D. et al., Immunopharmacology, 42, 61, 1999. With permission).

Binding of β -glucan to a receptor activates macrophages. The activation consists of several interconnected processes including increased chemokinesis, chemotaxis, migration of macrophages to particles to be phagocytosed, degranulation leading to increased expression of adhesive molecules on the macrophage surface, adhesion to the endothelium, and migration of macrophages to tissues. In addition, β -glucan binding also triggers intracellular processes, characterized by the respiratory burst after phagocytosis of invading cells (formation of reactive oxygen species and free radicals - hydrogen peroxide, superoxide radical, NO, HOCl [hypochlorous acid], HOI [hypoiodous acid] etc.), increasing of content and activity of hydrolytic and metabolic enzymes, and signaling processes leading to activation of other phagocytes and secretion of cytokines and other substances initiating inflammation reactions (e.g., IL-1, IL-9, tumor necrosis factor- α

[TNF α]). For an excellent review regarding the interaction of glucans with macrophages, see Schepetkin and Quinn (2006).

It is important for the pharmacological effect of β -glucan that activated macrophages do not act only against the activator but also against any present antigen, microorganism or tumor cell. Due to the fact that mammals lack β -glucanases in their enzyme equipment, macrophages represent what is probably the only tool for liquidation of β -glucan in the body. Within the macrophages, phagocytized β -glucan is degraded by an oxidative pathway (Nono et al., 1991).

6. SIDE EFFECTS

While the predominant pharmacological effects of β -glucans may be positive, their unfavorable side effects cannot be

TABLE 7
Examples of macrophage receptors and corresponding PAMPs

Character	Receptor	Primary PAMP
transmembrane proteins	CR3	iC3b opsonized particles, LPS, β -glucan, <i>Candida albicans</i> , <i>Mycobacterium tuberculosis</i> , <i>Cryptococcus neoformans</i>
	dectin-1	<i>Saccharomyces cerevisiae</i> , β -glucan, <i>C. albicans</i> , <i>Pneumocystis carinii</i> , <i>Aspergillus fumigatus</i>
	TLR-2	bacterial lipoproteins, LPS, zymosan, β -glucan
glycosphingolipid	lactosylceramide	β -glucan, <i>C. neoformans</i> , <i>C. albicans</i> , <i>Helicobacter pylori</i>
glycoproteins	scavenger receptors	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Listeria monocytogenes</i> , <i>M. tuberculosis</i> , <i>Neisseria meningitidis</i> , <i>Streptococcus spp.</i> , <i>H. pylori</i> , LPS, bacterial DNA

overlooked. At this time, few adverse effects have been described. It can be presumed, however, that with improved knowledge of the effects of β -glucans, this area will broaden.

Intramuscularly administered β -glucan induces an inflammatory reaction and granuloma formation at the puncture site. This is a painful method of application. The fact that β -glucan can be the cause of the inflammatory reaction itself represents a certain risk. Physiological inflammation occurs at the extent and rate corresponding with an inducing noxa, and also 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 most serious cases, generalization of inflammatory processes can occur with shock development and subsequent fatal multiple organ dysfunction syndrome (MODS) (Tanriverdi et al., 2005).

The function of nitric oxide is double-edged. Nitric oxide, the "Molecule of Year, 1992" for which the discoverers of its physiological effects earned the Nobel Prize in Physiology and Medicine in 1998, is produced in macrophages by inducible nitric oxide synthase (iNOS). Synthesis of this enzyme is triggered by binding of β -glucan to a PRR on the macrophage surface. Formed nitric oxide induces a cytotoxic effect upon tumor cells (Hibbs et al., 1984; Stuehr and Nathan, 1989) and shows distinct impact on many pathogens (Grangeur et al., 1988; James and Claven, 1989). On the other hand, it can also damage tissues and DNA (Billar, 1995) and high concentrations can cause septic shock. The sustained action of the activator induces expression of iNOS, and increased formation of NO results in vasodilatation of veins. The latter, in turn, brings about an intense drop of venous resistance and blood pressure (Vincent et al., 2000). As of now, such an effect of β -glucan has not been described, yet it is quite conceivable.

Inhalation of intact cells or cellular detritus of fungi or yeasts - ingredients of home dust (Beijer et al., 2002) or different agricultural and industrial dusts (Rylander et al., 1999) - induces so-called syndrome of toxic organic dust (STOD) which is characterized by lung reactions that include pneumonia, cough, chronic bronchitis (Sato and Sano, 2003), rhinitis, headache, and irritation of the eyes and throat (Alwis et al., 1999). The cause of these complaints is β -glucan which, through activation of macrophages, monocytes, and leukocytes, causes increased secretion of inflammatory components (i.e., TNF α and IL-8).

Due to the fact that β -glucan administration brings about inflammatory processes in the human body, it is possible to hypothesize competitive interaction with anti-inflammatory drugs. Induced lethal toxicity elicited by a combination of β -glucan and a nonsteroidal anti-inflammatory drug (indomethacin), was described in mice (Yoshioka et al., 1998). Results strongly suggest that such a combination induces lethality by compromising the cytokine network that leads to systemic inflammation response syndrome (SIRS) and death.

7. POSSIBLE ALTERNATIVES OF FURTHER RESEARCH

Different natural sources, polymer character, methods of isolation, insolubility or limited solubility of preparations, (and thus unrealistic fractionation), all resulted in the fact that each preparation of β -glucan, especially the particulate one, is necessarily heterogeneous. Even if in some cases the heterogeneity of β -glucan (from the point of view of molecular size, branching, and crystalline or amorphous structure) does not principally change the *in vivo* activities, for trustworthy pharmacological research as well as for regulatory authorities (such as the USFDA) it represents substantial complications. It follows then that most preparations containing β -glucan - with the aim of avoiding complications - are classified as healthy food or nutritional supplements. As a result, the market is saturated with many products containing greater or lesser amounts of β -glucan that are often of questionable quality, with uncertain effects, and recommended by delusory advertisement.

Reliable research techniques allow the problems of heterogeneity and nonexistent standards of various natural β -glucans to be solved. A first possibility is to improve isolation techniques to obtain products with closely defined chemical composition and to use up-to-date physicochemical methods for infallible identification and analyses of these products. Chemically modified β -glucans, which due to their solubility can be easily fractionated, represents a second possibility. Unfortunately, biological activity of some of these products is decreased. Recently, an attempt has been made to solve this problem by construction of semi-synthetic or synthetic probes that are suitable for exact immunological research. A general solution is the binding of short oligomers of glucose containing $\beta(1\rightarrow3)$ and $\beta(1\rightarrow6)$ linkages to a polymer carrier of defined size and structure. A reasonable assumption is that such "synthetic" β -glucans will interact with receptors of immunocompetent cells and elicit analogous reactions as natural β -glucan (Descroix et al., 2006). From the immunopharmacological point of view, such probes would eventually replace the natural β -glucan.

8. CONCLUSION

Among the many thus far known and tested immunomodulators, polysaccharides isolated from various natural sources occupy a prominent position. An important group of these polysaccharides is represented by homopolymers of β -glucose, β -glucans. These contain either linear chains with $(1\rightarrow3)$ - β -D-glycosidic linkages or branched ones containing additional $(1\rightarrow6)$ - β -D-glycosidic linkages.

Due to their very low-to-negligible toxicity, there was a time when β -glucans were considered merely a matter of fashion. This cannot be said about many other immuno-modulators. Effects of β -glucans on a variety of diseases, such as infections, irradiation diseases, and foremost on neoplastic growth, were investigated. A flood of various food additives and "alternative remedies," usually offered by faintly enlightened non-specialists, is a holdover from these pioneer years. After some

time, naturally, wave of enthusiasm declined. β -Glucans were criticized by many regulatory authorities, the main reasons being insufficiently defined preparations and non-specific and/or complex effects.

Fortunately, in the last ten years, research in reputable laboratories has reached a phase where the basic mechanisms of glucan effects are known and the relationship between structure and activity is clearly outlined. It seems now that β -glucans will finally take the position which was ascribed to them fifty years ago.

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