Available online at www.sciencedirect.com
ScienceDirect
Physiology & Behavior 94 (2008) 276–284
PHYSIOLOGY & BEHAVIOR
www.elsevier.com/locate/phb

Review

Dietary modulation of immune function by β-glucans

Julia J. Volman, Julian D. Ramakers, Jogchum Plat *

Department of Human Biology, Nutrition and Toxicology Institute Maastricht (NUTRIM), Maastricht University, PO Box 616, NL-6200 MD Maastricht, The Netherlands

Received 1 August 2007; received in revised form 20 November 2007; accepted 20 November 2007

Abstract

The immune response can be modulated by nutrients like β-glucans, which are glucose polymers that are major structural components of the cell wall of yeast, fungi, and bacteria, but also of cereals like oat and barley. There is a lot of structural variation in the β-glucans from these different sources, which may influence their physiological functions. In this review the current status concerning possibilities to modulate immune function by β-glucans is discussed. *In vitro* as well as *in vivo* studies in animals and humans show that especially β-glucans derived from fungi and yeast have immune modulating properties. Most frequently evaluated are effects on leukocyte activity, which has been suggested to contribute to the increased resistance against infections observed after β-glucan interventions. Although most studies supply the β-glucans parenteral (e.g. intravenous or subcutaneous), also enteral administrated (dietary) β-glucan influence the immune response. Although more human studies are needed, it is tempting to suggest that dietary β-glucans may be a useful tool to prime the host immune system and increase resistance against invading pathogens.

Keywords: Dietary; β-glucans; Immune function; Resistance to infections

Contents

1. Introduction

There is a constant threat of invasive pathogens attacking the human body. Our immune system composed of two mutually interactive systems, i.e. the innate and adaptive immune system,

* Corresponding author. Tel.: +31 433881309; fax: +31 433670976.
E-mail address: j.plat@hb.unimaas.nl (J. Plat).

0031-9384/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2007.11.045

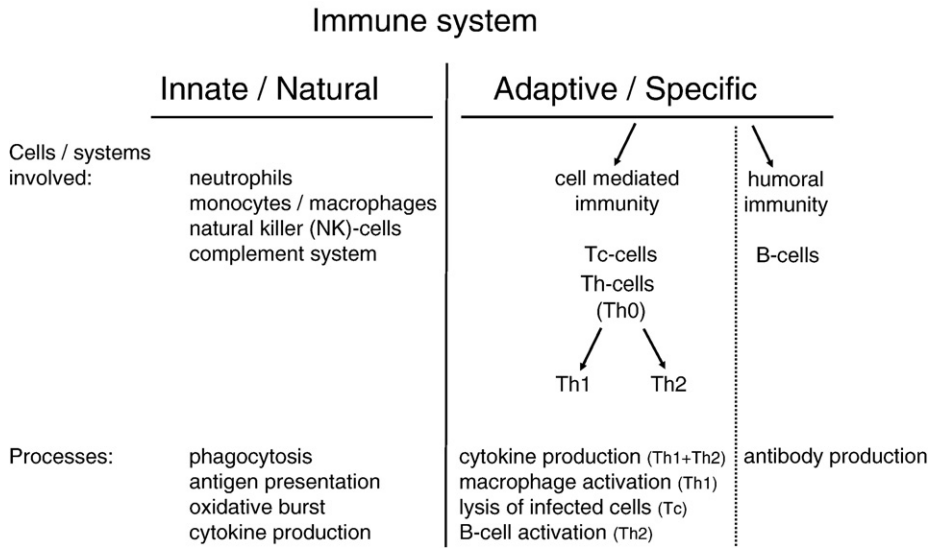
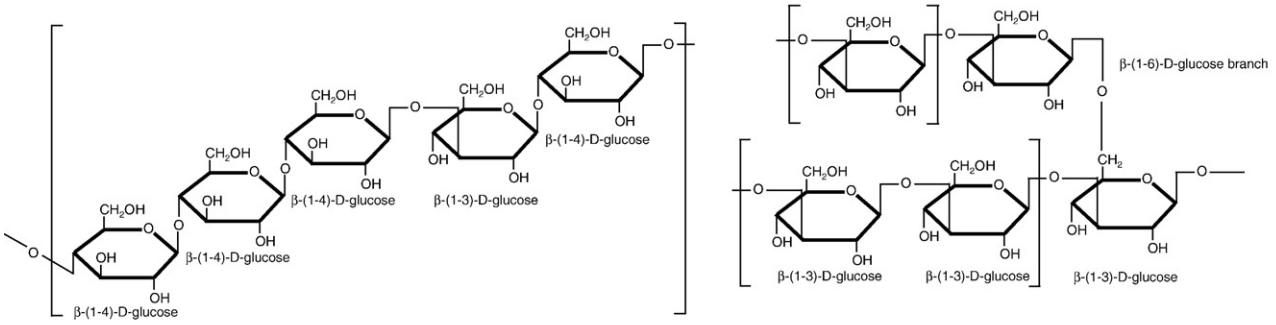


Fig. 1. Simplified schematic overview of the immune system. Abbreviations: Tc-cells, cytotoxic T-lymphocytes; Th-cells, helper T-lymphocytes.

protects the body against these invasions. Evidence accumulates that the composition of the diet influences the functioning of our immune system. Therefore, changing dietary compositions as a tool to improve the immune function, currently receives a lot of attention. β-Glucans, as present in various foods such as cereals and mushrooms, are currently under investigation for this purpose. The objective of this review is to give an overview of the immune modulating properties of (dietary) β-glucans. Therefore we start with a brief overview of the immune system and β-glucans. Next, the results of immune modulation by *in vitro*, parenteral and enteral administrated β-glucans are described. After that the mechanism by which dietary β-glucans may work will be discussed followed by giving examples of potential applications.



β-Glucan type	Structure	Description
Bacterial		Linear β1,3 glucan (i.e. Curdlan)
Fungal		Short β1,6 branched, β1,3 glucan (i.e. Schizophyllan)
Yeast		Long β1,6 branched, β1,3-glucan (i.e. WGP β-glucan, Betafectin™)
Cereal		Linear β1,3/β1,4-glucan (i.e oat, barley, rye)

Fig. 2. Structure of cereal and yeast β-glucan and scheme of various β-glucan sources.

2. Immune system

The immune system protects the human body against invasion of pathogens (i.e. bacteria, viruses and parasites), which is the result of two mutually interactive systems: the innate and adaptive immune system (Fig. 1). Epithelial barriers like the skin and the linings of the gastrointestinal tract, lungs and urinary tract are the first line of defence of the innate immune system. The innate immune response depends largely on the recognition of conserved microbial structures (pathogen associated molecular patterns, PAMPs) by so-called pattern recognition receptors (PRR). The toll-like receptors (TLRs) and dectin-1 are two examples of PRRs. Examples of PAMPs are cell wall components, such as lipopolysaccharide (LPS) from gram-negative bacteria, and β-glucan from fungi and yeast. Leukocytes that are involved in this early innate response are granulocytes (a.o. neutrophils), monocytes/macrophages and natural killer (NK) cells. These cells play an important role in phagocytosis of pathogens, free radical production (oxidative burst), cytokine production, and antigen presentation to lymphocytes. The innate immune response is rapid and functional, but non-specific. In contrast, the adaptive immune response is somewhat slower, but highly specific. This response depends on 1) the production of antibodies (immunoglobulines (Igs)) by B-lymphocytes directed against specific antigens present on pathogens (called humoral immune response) and on 2) the attack of infected body cells by cytotoxic and helper T-lymphocytes (called cell-mediated immune response). In addition, due to the presence of memory cells, the adaptive immune response is characterized by enhanced and fast responses after repetitive contacts with the same antigen. Ultimately, an immune response is the result of these two interacting systems. After leukocytes of the innate immune system are activated, they produce cytokines and present antigens to T- and B-lymphocytes, which in turn activates the adaptive immune system [1,2].

Cytokines are signalling proteins secreted by a wide variety of cell types and used for inter-cell communication. The types of cytokines produced determine whether a naïve helper T-lymphocyte (Th0) develops into a type 1 helper T-lymphocyte (Th1) or a type 2 helper T-lymphocyte (Th2). For example, interleukin (IL)-12, produced by e.g. activated macrophages, stimulates Th1 cell development. This leads to the production of typical Th1 cytokines like IL-1β, interferon (IFN)γ, IL-2, and tumor necrosis factor (TNF)α, which play an important role in cell-mediated immunity. In contrast, IL-4, results in the development of Th2 cells, producing IL-5, IL-6, IL-10, and IL-13, which are involved in the humoral immune response. Recently, also regulatory T-lymphocytes (also defined as Th3 cells) have been described. Their role is most likely to influence the Th1/Th2 balance by inhibiting Th1 activity by producing anti-inflammatory cytokines like transforming growth factor (TGF)β and IL-10 [3].

The most important regulator in the immune response is the transcription factor nuclear factor κB (NF-κB). As already mentioned, immune cells can be activated directly by binding of PAMPs from pathogens to PRRs or indirectly by binding of cytokines produced by other cells to their respective receptors. After ligand receptor binding, a cascade of intracellular signalling events occurs, ultimately leading to activation of NF-κB. In resting cells, NF-κB is present in the cytoplasm, bound to inhibitor κB (IκB), a complex that inactivates NF-κB. After cellular activation, IκB kinase (IKK) is activated, which in turn phosphorylates IκB. This phosphorylation is followed by ubiquination and degradation of IκB. Next, IκB-free NF-κB migrates from the cytoplasm into the nucleus, binds to response elements and induces gene transcription. NF-κB activation induces transcription of genes

Table 1
Various, commonly used, β-glucans from different sources and their structures [a, b]

Name	Source	Source type	Structure
Glomerellan	*Glomerella cingulata*	Fungus	1,3 1,6 branched
GRN (grifolan)	*Grifola frondosa* (Maitake mushroom)	Fungus/ mushroom	1,3 1,6 branched
LNT (Lentinan)	*Lentinus (lentinula) edode* (shiitake mushroom)	Fungus/ mushroom	1,3 1,6 branched
Pneumocytis carinii	*Pneumocytis carinii*	Fungus/ protozoan	1,3 1,6 branched
P-SG (polysaccharide from *Ganoderma lucidum*)	*G. lucidum*	Fungus/ mushroom	1,3 1,6 branched
SPG (Sonifilan/ schizophyllan)	*Schizophyllum commune*	Fungus	1,3, 1,6 branched
SR (Scleroglucan)	*Sclerotium rolfsii* or *S. glucanicum*	Fungus	1,3, 1,6 single branched
SSG (*Sclerotinia sclerotiorum* glucan)	*S. sclerotiorum* (ascomycotma)	Fungus	1,3 1,6 highly branched
CSBG (*Candida spp.* β-glucan)	*Candida albicans*	Yeast/ fungus	1,3 1,6 branched
Glucan phosphate (GluP)	*Saccharomyces cerevisiae*	Synthetic modified	1,3
PGG (betafectin)	*Saccharomyces cerevisiae*	(Genetically engineered) yeast	1,3 1,6 highly branched
Saccharomyces cerevisiae	*Saccharomyces cerevisiae*	Yeast	1,3 and small numbers of 1,6 branches and 1,6 linked
WGP-glucan (whole glucan particle)	*Saccharomyces cerevisiae* (baker's yeast)	Yeast	1,3 1,6
Zymocel	*Saccharomyces cerevisiae*	Yeast	Crude β-glucan extract
Zymozan	*Saccharomyces cerevisiae*	Yeast	Crude extract with β-glucan and mannan; non-uniform branches and backbone units
Barley, oat, wheat, rye, rice		Cereal (Gramineae (grasses)	1,3 1,4 mixed linkage, unbranched
Curdlan	*Alcaligenes faecalis*	(gram negative) bacteria	1,3 unbranched
LAM (Laminarin/ Laminaran)	*Laminaria* species *(e.g. digitata)*	algae e.g. brown seaweeds	1,3 unbranched (with some branching of 1,6)

[a] Unbranched β-glucans are also regarded as linear.
[b] Yeasts and mushrooms are specific types of fungi.

encoding for inflammatory proteins like cytokines and chemokines and induces free radical production [4].

3. β-Glucan

β-Glucans are carbohydrates consisting of linked glucose molecules, which are major structural components of the cell walls of yeast, fungi and some bacteria. Also cereals, such as barley and oat contain β-glucans as part of their endosperm cell walls. Depending on the source, there are clear differences in macromolecular structure between β-glucans (Fig. 2). The cell wall β-glucans of yeast and fungi consists of 1,3 β-linked glycopyranosyl residues with small numbers of 1,6 β-linked branches. In contrast, the oat and barley cell walls contain unbranched β-glucans with 1,3 and 1,4 β-linked glycopyranosyl residues, whereas β-glucans from bacterial origin are unbranched 1,3 β-linked glycopyranosyl residues [5–7]. Furthermore, besides differences in type of linkage and branching, β-glucans can vary in solubility, molecular mass, tertiary structure, degree of branching, polymer charge and solution conformation (triple or single helix or random coil). All these characteristics may influence their immune modulating effects. For example, Brown and Gordon [6] have recently suggested that high molecular weight (MW) and/or particulate β-glucans from fungi directly activate leukocytes, while low MW β-glucans from fungi only modulate the response of cells when they are stimulated with e.g. cytokines. With respect to the characteristics of the β-glucans, it should be noted that the isolation method may influence these characteristics. Consequently, differences can be expected between various β-glucans differentially isolated from the same source. Table 1 lists various, commonly used, β-glucans from different sources and their structures.

4. Immune modulating effects of *in vitro* and parenteral administrated β-glucans

In vitro and animal studies have shown that 1,3 β-glucans from yeast and fungi are able to enhance the responsiveness or function of immune cells, whereas only a limited number of human studies have been carried out. In the next paragraphs the results of these studies will be discussed in more detail.

4.1. In vitro studies

β-Glucans can enhance the functional activity of macrophages and activate antimicrobial activity of mononuclear cells and neutrophils *in vitro* [9–11]. This enhanced immune response is accomplished by an increased pro-inflammatory cytokine production [12–14], oxidative burst, and chemokine production [9]. Olson *et al.* [12] demonstrated that fungal *S. cerevisiae* β-glucan increased *in vitro* TNFα production by alveolar macrophages isolated from rats. Also the β-glucan rich yeast particle zymosan – that has often been used to study β-glucan effects – stimulated macrophages to secrete TNFα [7]. In addition, human whole blood incubated with soluble yeast β-glucan showed an enhanced production of TNFα, IL-6, IL-8 and monocyte tissue factor (TF). Furthermore, when LPS was added together with this β-glucan TNFα, IL-8, IL-10 and TF concentrations were strongly increased, whereas no further increase in IL-6 concentration was observed. In addition, pre-incubation with β-glucan increased the LPS-induced productions of these parameters [15]. Besides β-glucans from yeast and fungi, also β-glucans from oat show these immune modulating effects *in vitro*. Estrada *et al.* [5] have demonstrated an increased IL-1α production by murine macrophages in the presence of oat β-glucan *in vitro*, while spleen cells showed an enhanced IL-2, IFNγ and IL-4 secretion.

Poly-[1-6]-D-glucopyranosyl-[1-3]-D-glucopyranose (PGG)-glucan from yeast increased the activity of leukocytes against various pathogens. Indeed, PGG-glucan increased migration of neutrophils *in vitro* toward C5a, whereas chemotaxis toward IL-8 was suppressed [16]. Furthermore, pre-incubation of whole blood with PGG-glucan increased the oxidative burst of *in vitro* activated isolated leukocytes. However, PGG-glucan induced immune stimulating responses without effects on inflammatory cytokine production [17]. In addition to macrophages and neutrophils, also the function of dendritic cells can be influenced by β-glucans, since it has been reported that the fungus β-glucan P-SG induced a Th1 type of cytokine response by human dendritic cells *in vitro* [18]. Moreover, besides leukocytes, also epithelial cells can respond to β-glucans, as alveolar epithelial cells isolated from rats secrete the chemokine macrophage inflammatory protein (MIP)-2 after *in vitro* culturing with *Pneumocytis carinii* β-glucan [19]. In contrast to the increased immune response as outlined above, Nakagawa *et al.* [20] reported a dampened LPS-induced IL-6, IL-2 and IFNγ production of cultured human peripheral blood mononuclear cells (PBMCs) when incubated with *Candida spp.* β-glucan (CSBG). However, when monocytes were depleted from the cultured PBMCs the decreased production of IL-2 and IFNγ disappeared. Therefore the authors suggest that the monocytes were responsible for the suppressed production of the Th1 type cytokines IL-2 and IFNγ. When cultured PBMCs were incubated with CSBG without LPS stimulation, IL-6 production was only slightly increased [20]. Altogether, it can be concluded from these *in vitro* studies that β-glucans from various sources overall enhance the immune response of leukocytes and epithelial cells, mainly by modulating the cytokine production.

4.2. Animal studies

Besides *in vitro* studies, numerous animal studies have been conducted to evaluate the immune modulating effects of β-glucans. Most studies have been carried out with isolated leukocytes from animals that were treated with β-glucan supplied via various routes. Subsequently these isolated cells were challenged with LPS or pathogens (*ex vivo*). In general, these *ex vivo* stimulated leukocytes from the treated animals showed an increased pro-inflammatory cytokine production [14,21], oxidative burst [22] and chemotaxis [23]. There are indications that the β-glucan induced response is in fact a Th1 specific response, since splenocytes from mice treated with bacterial 1,4 β-glucan, a polysaccharide from *Ganoderma lucidum* (PS-G), and *Sclerotinia sclerotiorum* glucan (SSG) show an increased ovalbumin-induced IFNγ production [18,24,25], whereas IL-4 [24,25] and IL-5 [18] were decreased. Suzuki *et al.* [25] also reported that isolated

splenocytes from SSG-administered mice showed an increase IgG2a production and a decreased IgG1 production. Since IgG2a responses are induced by IFNγ and suppressed by IL-4, whereas IgG1 production is inhibited by IFNγ and stimulated by IL-4, these antibodies responses are respectively regarded as Th1 and Th2. Overall, these studies indeed suggest that β-glucans induce Th1 specific immune responses. In agreement with *in vitro* studies, PGG-glucans showed no stimulating effects on *ex vivo* cytokine production, since LPS-stimulated lymphocytes and monocytes isolated from PGG-glucan treated mice produced less pro-inflammatory cytokines [26]. Furthermore, the oxidative burst activity of neutrophils was increased in rats, without effects on TNFα and IL-1β levels [27].

Besides *ex vivo* effects of β-glucans, also *in vivo* effects of a variety of β-glucans have been reported on the response towards pathogen infections in animals. In general, these studies showed an increased microbial clearance and a reduced mortality of lethally infected animals [28–31]. For example. subcutaneous injection of yeast β-glucan (PGG-glucan) and whole glucan particle (WGP) β-glucan increased survival rate, diminished bacterial load in the lungs and increased the proportion of bacteria-free animals after infection with anthrax in mice [32]. In addition, bacterial counts in blood of *S. aureus* challenged rats treated intramuscular with PGG-glucan were lower than in control rats, and also the number of monocytes and neutrophils were increased [27]. Bedirli *et al.* [31] found an increased survival rate after intramuscular β-glucan (Sigma) treatment of rats in an experimental model of sepsis, however the elevated TNFα, IL-1β and IL-6 concentrations in the blood were blocked by β-glucan. The authors suggest that the protective effects of β-glucan were due to a dampened inflammatory response *in vivo* [31]. Whereas most studies showed an increased *in vitro* and *ex vivo* cytokine production after β-glucans, these cytokine effects *in vivo* might be dampened because of feedback mechanism, that protects the host against excessive inflammatory responses. Besides fungal and yeast β-glucans, intraperitoneal injection of β-glucans from oat also enhanced survival after bacterial and parasitic infection in mice [5,33]. In contrast to the studies mentioned above, Dritz *et al.* [34] found no effect on neutrophil or macrophage function of weanling pigs and instead an increased susceptibility to bacterial infection after a β-glucan (MacroGard-S™) diet.

In general, *ex vivo* responses of leukocytes isolated from β-glucans treated animals are in line with *in vitro* results. When animals were treated with β-glucan this ultimately translated into an enhanced survival against pathogen infections.

4.3. Human studies

Effects of various β-glucans have been examined *in vitro* and in several animal models, while only a few human studies have been carried out. Three clinical studies demonstrated that pre-treatment of high-risk surgical patients with PGG-glucan supplied intravenously (1) decreased the infection incidence and need for antibiotics, (2) shortened intensive care unit length stay, and (3) ultimately improved survival compared to a saline placebo injection [35–37]. In another study, a soluble yeast β-glucan isolated from *S. cerevisiae* – also supplied intravenously – decreased overall mortality and septic morbidity compared to a not further specified placebo injection in trauma patient's [38,39]. Besides effects on clinical parameters, almost no effects on cytokine concentrations and immunoglobulin concentrations in humans have been reported. Only 3 days after trauma serum IL-1 concentrations were elevated in β-glucan treated trauma patients as compared to control treated patients, while TNFα concentrations remained unchanged [39]. Also Lehne *et al.* [40] found no difference in cytokine and immunoglobulin concentrations in the blood of humans after 5 days treatment with three different concentrations of yeast β-glucan (SBG) compared to baseline, although IgA concentrations were increased in saliva, only when using a high dose of β-glucan. To summarize, the protective effects of β-glucans on survival after pathogen infection in humans are in agreement with effects observed in animals. Furthermore, the finding that clinical parameters are improved without a severe elevation in concentrations of circulating cytokines and immunoglobulines suggests an immune regulatory function of β-glucan that goes further than simply immune activation, as they may prime instead of activate leukocytes *in vivo* to combat invading pathogens.

5. Immune modulating effects of enteral administrated β-glucans

Although the previous section may give the impression that only parenteral administration of β-glucans protects against pathogen infections, a number of reports have shown that enteral administration of β-glucans (further mentioned as dietary β-glucan) may also have biological effects. This implies that the addition of β-glucans to the diet may be used to modulate immune function and by that way might improve the resistance against invading pathogens in humans. Besides intraperitoneal or subcutaneous injection of oat β-glucans, also intragastric administration of oat β-glucans in mice enhanced resistance to bacterial and parasitic infections [33,41,42]. Furthermore, Davis *et al.* [43] demonstrated that daily ingestion of oat β-glucan counteracted the decrease in macrophage antiviral resistance induced by exercise stress in mice. Besides oat β-glucans also other β-glucans have been supplied orally and showed immune modulating activities. Rice *et al.* [44] found an increased survival in mice challenged with *S. aureus* or *Candida albicans* after oral administration of glucan phosphate (GluP). Furthermore, Kournikakis *et al.* [32] showed that orally administrated WGP β-glucan increased survival in mice challenged with the anthrax bacteria.

Also after oral administration, effects on immune cell functions have been reported. Phagocytosis, bactericidal killing and oxidative burst of isolated heterophils (neutrophil like cells in chicken) were all increased in immature chickens fed a purified β-glucan of an unknown source, ultimately showing an increased protection against *Salmonella enterica* organ invasion [45]. The oxidative burst (NO) of *ex vivo* LPS-stimulated isolated peritoneal adherent cells from mice was also increased after oral administration of β-glucans from *S. cerevisiae* [22]. Li *et al.* [46] reported an increased LPS-induced IL-6 and TNFα production *in vivo* in the blood from weaned pigs after oral *S. cerevisiae* β-glucan

supplementation. In contrast, another study by the same group with nearly the same design showed a decreased IL-6 and TNFα production in response to LPS, whereas IL-10 concentrations were increased. Moreover, the specific antibody response to ovalbumin in the first week after injection was increased [47]. This study does not specify the type of antibody that is produced, which is important regarding Th1 or Th2 phenotypes. In this respect, Saito *et al.* reported a decreased *in vivo* production of ovalbumin-specific IgG2a antibodies in mice after oral treatment with soluble branched bacterial 1,4 β-glucan, whereas the concentration IgE and IgG1 antibodies was increased [24]. This indicates that β-glucan induces a Th1 response, which is in agreement with the results of *ex vivo* animal studies.

Intragastric administration of SSG showed an enhanced functionality of peritoneal macrophages in mice [48]. In addition, oral administration of SSG influenced the function of cells of the Peyer's patches, which in their turn stimulated the *in vitro* lysosomal enzyme activity of alveolar macrophages. Alveolar macrophages isolated from the SSG treated mice also showed increased phagocytic activity and IL-1 production, whereas hydrogen peroxide production was not affected. Moreover, also the number of alveolar macrophages increased. The authors suggested that after SSG administration via the oral route, the alveolar macrophages were stimulated by activation of cells of the Peyer's patches [49]. Also Tsukada *et al.* [50] reported an increased number of intra-epithelial lymphocytes in the intestine after oral administration of β-glucan in mice.

It can be concluded that oral administration of β-glucan is as effective as parenteral administration to protect against pathogen infections. Moreover, the effect seems independent of the source of β-glucan used, although there have been no careful side-by-side comparisons of β-glucans obtained from different sources. Therefore, enriching our diet with β-glucans might be relevant in terms of enhancing our daily protection against pathogens, although further human studies are needed. In this respect it is important to mention that in animal feeding β-glucan is already applied as a food additive to strengthen the immune response [51]. In addition, the idea of β-glucan as a food additive is supported by Ikewaki *et al.* who recently showed that an already available health food supplement (*Sophy* β-glucan) has immune modulating properties in human PBMCs *ex vivo* [52].

6. Mechanism underlying the effect of dietary β-glucans

After oral administration β-glucans come in contact with the mucosal immune system (Fig. 3). The intestinal epithelial cells together with the immune cells of the Peyer's patches play an important role in regulating immune responses. As indicated in the previous section, oral administration of β-glucans can modulate the mucosal immune response by cells of the Peyer's patches as well as intestinal intraepithelial lymphocytes [48,50]. It has been suggested that the protective effects of orally administered 1,3 β-glucans are mediated through receptor-mediated interactions with Microfold (M)—cells—specialized epithelial cells for the transport of macromolecules-in the Peyer's patches, which lead to increased cytokine production and enhanced resistance to infection [48].

Besides these direct effects on immune function in the intestine it has also been suggested that orally administered barley and yeast WGP β-glucans are taken up by intestinal macrophages and then transported to lymph nodes, spleen and bone marrow. Whether the β-glucans are shuttled to these organs via the lymph or blood compartments has not yet been established. In the bone marrow, it has been described that β-glucans were degraded by macrophages and taken up by bone marrow granulocytes. Since *in vitro* experiments have shown that degraded β-glucan particles were released in the medium [53], this may imply that *in vivo* they could be released into the circulation to induce systemic effects. This assumption suggests that orally administered β-glucans reach the circulation in low amounts. However, the amount of

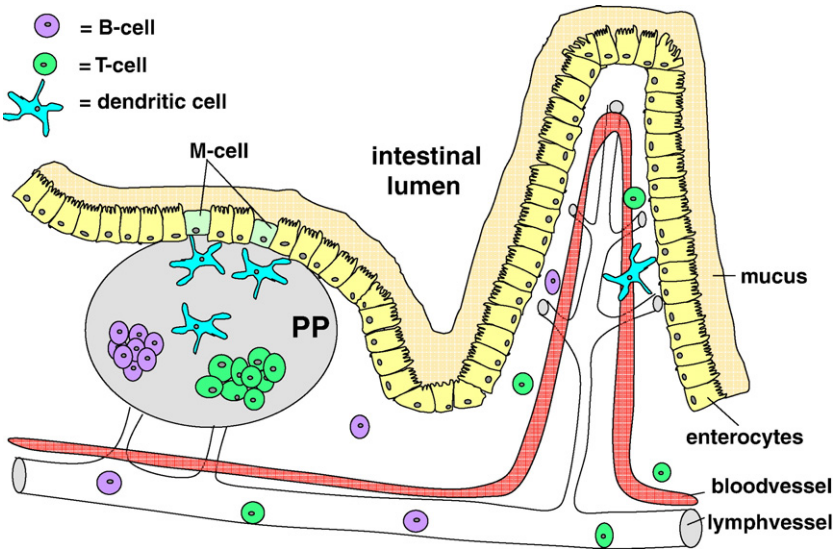


Fig. 3. Simplified schematic view of the intestinal immune system. Two important components of the intestinal immune system are the enterocytes that form a physical barrier of one single cell layer and the gut-associated lymphoid tissue (GALT) system consisting of various immune cells (T-cells, B-cells, dendritic cells and M-cells) in the small intestine clustered in follicles known as Peyer's Patches (PP).

dietary β-glucan that appears in the circulation by this route is lower than after subcutaneous administration [54]. Rice *et al.* [44] suggested that especially β-glucans with a high molecular mass (>2 MDa) may be taken up by M-cells from the intestinal lumen to the underlying lymphoid tissue. *In vivo* effects of β-glucan may depend on their molecular weight, which might be caused by differences in uptake from the intestinal lumen.

Also effects on non-immune cells have been shown [19]. Regarding dietary immune modulation particularly effects on enterocytes are interesting. In this respect we have earlier demonstrated in an *in vitro* study that various enterocyte cell lines showed an enhanced immune response when incubated with fecal water prepared from ileostomic contents obtained from subjects who had consumed an oat β-glucan diet versus a placebo diet [55].

Irrespective if β-glucans are absorbed or not, there seems to be general consensus that the reported immune modulating effects of fungal and yeast β-glucans are dectin-1 dependent although also other receptors might play a role. Alterative receptors involved may be TLR2 and TLR6, complement receptor 3 (CR3), scavenger receptors or lactosylceramide [8]. The dectin-1 receptor (in humans also called the β-glucan receptor (βGR)) is highly expressed on immune cells, such as dendritic cells, neutrophils, eosinophils, macrophages, monocytes, and some T-cells and in humans also on B-cells. Also intestinal cells might express dectin-1 in lower amounts, although this expression is under debate [28,56].

Upon β-glucan binding to dectin-1, a cascade of intracellular signals is initiated which will ultimately lead to NF-κB activation. Indeed several studies reported that various β-glucans activate NF-κB *in vitro* as measured by EMSA in leukocytes [13,17,57,58] and in alveolar epithelial cells [59]. NF-κB activation initiates various processes – also described to be modulated by β-glucans – such as cytokine production, phagocytosis and respiratory burst. Confirmation of the crucial role for dectin-1 in the effects of β-glucans comes from recent studies with dectin-1 deficient mice. Taylor *et al.* [60] showed that these mice are more susceptible to fungal infections with *C. albicans* than wild-type mice, whereas, Saijo *et al.* [61] demonstrated similar findings for protection against *P. carinii*. Interestingly, they found no difference in susceptibility between dectin-1 deficient mice and wild-type mice against *C. albicans*. This discrepancy may be explained by the use of different strains of *C. albicans* and the genetic background of the mice. These studies however do not provide insight which cells are obligatory for the dectin-1 mediated response towards β-glucans, and/or whether absorption of β-glucans into the circulation is an essential step in explaining their immune modulating characteristics. For this a tissue-specific dectin-1 knock-out approach seems warranted. These questions are important in light of dietary β-glucan to distinguish between effects on enterocytes and intestinal immune cells.

7. Conclusion and applications

β-Glucans are not only present as major structural components of the cell walls of yeast, fungi and some bacteria, but are also present in our daily diet as part the endosperm cell wall in cereals, such as barley and oat. Results both from *in vitro* studies as well as *ex vivo* stimulated leukocytes isolated from animals treated with β-glucans suggest that β-glucans enhance the immune response in leukocytes and epithelial cells. Since they influence the immune response of the host, 1,3 β-glucans are often described as biological response modifiers [62]. In the *in vivo* situation there is now substantial evidence that these effects ultimately translate into an enhanced survival after infection with pathogens. In this respect, effects are observed irrespective of the β-glucan source and/or route of administration. Therefore, it might be possible to modulate immune function by increasing the dietary β-glucan intake, for example by developing functional foods. This may have benefits for specific target populations like for example elderly or type II diabetic patients, who are both characterized by a suppressed (Th1) immune response [63–65]. Alternatively, subjects with an overactive Th2 immune response, such as observed in allergic reactions and asthma, may benefit in terms of restoring the balance by the described Th1 stimulation. Both concepts should however be confirmed in future well-controlled human intervention trials.

References

[1] Abbas AK, Lichtman AH. Basic immunology; functions and disorders of the immune system. Philadelphia: Saunders; 2003.
[2] Plat J, Mensink RP. Food components and immune function. Curr Opin Lipidol 2005;16:31–7.
[3] Kidd P. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. Altern Med Rev 2003;8:223–46.
[4] Karin M, Delhase M. The I kappa B kinase (IKK) and NF-kappa B: key elements of proinflammatory signalling. Semin Immunol 2000;12:85–98.
[5] Estrada A, Yun CH, Van Kessel A, Li B, Hauta S, Laarveld B. Immunomodulatory activities of oat beta-glucan in vitro and in vivo. Microbiol Immunol 1997;41:991–8.
[6] Brown GD, Gordon S. Fungal beta-glucans and mammalian immunity. Immunity 2003;19:311–5.
[7] Brown GD, Herre J, Williams DL, Willment JA, Marshall AS, Gordon S. Dectin-1 mediates the biological effects of beta-glucans. J Exp Med 2003;197:1119–24.
[8] Brown GD. Dectin-1: a signalling non-TLR pattern-recognition receptor. Nat Rev, Immunol 2006;6:33–43.
[9] Williams DL. Overview of (1→3)-beta-D-glucan immunobiology. Med Inflamm 1997;6:247–50.
[10] Tzianabos AO. Polysaccharide immunomodulators as therapeutic agents: structural aspects and biologic function. Clin Microbiol Rev 2000;13:523–33.
[11] Zekovic DB, Kwiatkowski S, Vrvic MM, Jakovljevic D, Moran CA. Natural and modified (1→3)-beta-D-glucans in health promotion and disease alleviation. Crit Rev Biotechnol 2005;25:205–30.
[12] Olson EJ, Standing JE, Griego-Harper N, Hoffman OA, Limper AH. Fungal beta-glucan interacts with vitronectin and stimulates tumor necrosis factor alpha release from macrophages. Infect Immun 1996;64:3548–54.
[13] Young SH, Ye J, Frazer DG, Shi X, Castranova V. Molecular mechanism of tumor necrosis factor-alpha production in 1→3-beta-glucan (zymosan)-activated macrophages. J Biol Chem 2001;276:20781–7.
[14] Adachi Y, Okazaki M, Ohno N, Yadomae T. Enhancement of cytokine production by macrophages stimulated with (1→3)-beta-D-glucan, grifolan (GRN), isolated from *Grifola frondosa*. Biol Pharm Bull 1994;17:1554–60.
[15] Engstad CS, Engstad RE, Olsen JO, Osterud B. The effect of soluble beta-1,3-glucan and lipopolysaccharide on cytokine production and coagulation activation in whole blood. Int Immunopharmacol 2002;2:1585–97.
[16] Tsikitis VL, Albina JE, Reichner JS. Beta-glucan affects leukocyte navigation in a complex chemotactic gradient. Surgery 2004;136:384–9.
[17] Wakshull E, Brunke-Reese D, Lindermuth J, Fisette L, Nathans RS, Crowley JJ, et al. PGG-glucan, a soluble beta-(1,3)-glucan, enhances the

oxidative burst response, microbicidal activity, and activates an NF-kappa B-like factor in human PMN: evidence for a glycosphingolipid beta-(1,3)-glucan receptor. Immunopharmacology 1999;41:89–107.
[18] Lin YL, Lee SS, Hou SM, Chiang BL. Polysaccharide purified from *Ganoderma lucidum* induces gene expression changes in human dendritic cells and promotes T helper 1 immune response in BALB/c mice. Mol Pharmacol 2006;70:637–44.
[19] Hahn PY, Evans SE, Kottom TJ, Standing JE, Pagano RE, Limper AH. *Pneumocystis carinii* cell wall beta-glucan induces release of macrophage inflammatory protein-2 from alveolar epithelial cells via a lactosylceramide-mediated mechanism. J Biol Chem 2003;278:2043–50.
[20] Nakagawa Y, Ohno N, Murai T. Suppression by *Candida albicans* beta-glucan of cytokine release from activated human monocytes and from T cells in the presence of monocytes. J Infect Dis 2003;187:710–3.
[21] Abel G, Czop JK. Stimulation of human monocyte beta-glucan receptors by glucan particles induces production of TNF-alpha and IL-1 beta. Int J Immunopharmacol 1992;14:1363–73.
[22] Mucksova J, Babicek K, Pospisil M. Particulate 1,3-beta-D-glucan, carboxymethylglucan and sulfoethylglucan-influence of their oral or intraperitoneal administration on immunological respondence of mice. Folia Microbiol (Praha) 2001;46:559–63.
[23] Leblanc BW, Albina JE, Reichner JS. The effect of PGG-{beta}-glucan on neutrophil chemotaxis in vivo. J Leukoc Biol 2006.
[24] Saito K, Yajima T, Nishimura H, Aiba K, Ishimitsu R, Matsuguchi T, et al. Soluble branched beta-(1,4)glucans from *Acetobacter* species show strong activities to induce interleukin-12 in vitro and inhibit T-helper 2 cellular response with immunoglobulin E production in vivo. J Biol Chem 2003;278:38571–8.
[25] Suzuki Y, Adachi Y, Ohno N, Yadomae T. Th1/Th2-balancing immunomodulating activity of gel-forming (1→3)-beta-glucans from fungi. Biol Pharm Bull 2001;24:811–9.
[26] Soltys J, Quinn MT. Modulation of endotoxin-and enterotoxin-induced cytokine release by in vivo treatment with beta-(1,6)-branched beta-(1,3)-glucan. Infect Immun 1999;67:244–52.
[27] Liang J, Melican D, Cafro L, Palace G, Fisette L, Armstrong R, et al. Enhanced clearance of a multiple antibiotic resistant *Staphylococcus aureus* in rats treated with PGG-glucan is associated with increased leukocyte counts and increased neutrophil oxidative burst activity. Int J Immunopharmacol 1998;20:595–614.
[28] Saegusa S, Totsuka M, Kaminogawa S, Hosoi T. *Candida albicans* and *Saccharomyces cerevisiae* induce interleukin-8 production from intestinal epithelial-like Caco-2 cells in the presence of butyric acid. FEMS Immunol Med Microbiol 2004;41:227–35.
[29] Hetland G, Ohno N, Aaberge IS, Lovik M. Protective effect of beta-glucan against systemic *Streptococcus pneumoniae* infection in mice. FEMS Immunol Med Microbiol 2000;27:111–6.
[30] Czop JK. The role of beta-glucan receptors on blood and tissue leukocytes in phagocytosis and metabolic activation. Pathol Immunopathol Res 1986;5:286–96.
[31] Bedirli A, Kerem M, Pasaoglu H, Akyurek N, Tezcaner T, Elbeg S, et al. Beta-glucan attenuates inflammatory cytokine release and prevents acute lung injury in an experimental model of sepsis. Shock 2007;27:397–401.
[32] Kournikakis B, Mandeville R, Brousseau P, Ostroff G. Anthrax-protective effects of yeast beta 1,3 glucans. MedGenMed 2003;5:1.
[33] Yun CH, Estrada A, Van Kessel A, Park BC, Laarveld B. Beta-glucan, extracted from oat, enhances disease resistance against bacterial and parasitic infections. FEMS Immunol Med Microbiol 2003;35:67–75.
[34] Dritz SS, Shi J, Kielian TL, Goodband RD, Nelssen JL, Tokach MD, et al. Influence of dietary beta-glucan on growth performance, nonspecific immunity, and resistance to *Streptococcus suis* infection in weanling pigs. J Anim Sci 1995;73:3341–50.
[35] Babineau TJ, Hackford A, Kenler A, Bistrian B, Forse RA, Fairchild PG, et al. A phase II multicenter, double-blind, randomized, placebo-controlled study of three dosages of an immunomodulator (PGG-glucan) in high-risk surgical patients. Arch Surg 1994;129:1204–10.
[36] Babineau TJ, Marcello P, Swails W, Kenler A, Bistrian B, Forse RA. Randomized phase I/II trial of a macrophage-specific immunomodulator (PGG-glucan) in high-risk surgical patients. Ann Surg 1994;220:601–9.
[37] Dellinger EP, Babineau TJ, Bleicher P, Kaiser AB, Seibert GB, Postier RG, et al. Effect of PGG-glucan on the rate of serious postoperative infection or death observed after high-risk gastrointestinal operations. Betafectin Gastrointestinal Study Group. Arch Surg 1999;134:977–83.
[38] de Felippe Junior J, da Rocha e Silva Junior M, Maciel FM, Soares Ade M, Mendes NF. Infection prevention in patients with severe multiple trauma with the immunomodulator beta 1-3 polyglucose (glucan). Surg Gynecol Obstet 1993;177:383–8.
[39] Browder W, Williams D, Pretus H, Olivero G, Enrichens F, Mao P, et al. Beneficial effect of enhanced macrophage function in the trauma patient. Ann Surg 1990;211:605–12 [discussion 612–613].
[40] Lehne G, Haneberg B, Gaustad P, Johansen PW, Preus H, Abrahamsen TG. Oral administration of a new soluble branched beta-1,3-D-glucan is well tolerated and can lead to increased salivary concentrations of immunoglobulin A in healthy volunteers. Clin Exp Immunol 2006;143:65–9.
[41] Yun CH, Estrada A, Van Kessel A, Gajadhar A, Redmond M, Laarveld B. Immunomodulatory effects of oat beta-glucan administered intragastrically or parenterally on mice infected with *Eimeria vermiformis*. Microbiol Immunol 1998;42:457–65.
[42] Yun CH, Estrada A, Van Kessel A, Gajadhar AA, Redmond MJ, Laarveld B. beta-(1→3, 1→4) oat glucan enhances resistance to *Eimeria vermiformis* infection in immunosuppressed mice. Int J Parasitol 1997;27:329–37.
[43] Davis JM, Murphy EA, Brown AS, Carmichael MD, Ghaffar A, Mayer EP. Effects of oat beta-glucan on innate immunity and infection after exercise stress. Med Sci Sports Exerc 2004;36:1321–7.
[44] Rice PJ, Adams EL, Ozment-Skelton T, Gonzales A, Goldman MP, Lockhart BE, et al. Oral delivery and gastrointestinal absorption of soluble glucans stimulate increased resistance to infectious challenge. J Pharmacol Exp Ther 2005;314:1079–86.
[45] Lowry VK, Farnell MB, Ferro PJ, Swaggerty CL, Bahl A, Kogut MH. Purified beta-glucan as an abiotic feed additive up-regulates the innate immune response in immature chickens against *Salmonella enterica* Serovar Enteritidis. Int J Food Microbiol 2005;98:309–18.
[46] Li J, Li DF, Xing JJ, Cheng ZB, Lai CH. Effects of beta-glucan extracted from *Saccharomyces cerevisiae* on growth performance, and immunological and somatotropic responses of pigs challenged with *Escherichia coli* lipopolysaccharide. J Anim Sci 2006;84:2374–81.
[47] Li J, Xing J, Li D, Wang X, Zhao L, Lv S, et al. Effects of beta-glucan extracted from *Saccharomyces cerevisiae* on humoral and cellular immunity in weaned piglets. Arch Anim Nutr 2005;59:303–12.
[48] Suzuki I, Tanaka H, Kinoshita A, Oikawa S, Osawa M, Yadomae T. Effect of orally administered beta-glucan on macrophage function in mice. Int J Immunopharmacol 1990;12:675–84.
[49] Sakurai T, Hashimoto K, Suzuki I, Ohno N, Oikawa S, Masuda A, et al. Enhancement of murine alveolar macrophage functions by orally administered beta-glucan. Int J Immunopharmacol 1992;14:821–30.
[50] Tsukada C, Yokoyama H, Miyaji C, Ishimoto Y, Kawamura H, Abo T. Immunopotentiation of intraepithelial lymphocytes in the intestine by oral administrations of beta-glucan. Cell Immunol 2003;221:1–5.
[51] http://www.myfineequine.com/betaglucan.htm.
[52] Ikewaki N, Fujii N, Onaka T, Ikewaki S, Inoko H. Immunological actions of *Sophy* beta-glucan (beta-1,3-1,6 glucan), currently available commercially as a health food supplement. Microbiol Immunol 2007;51:861–73.
[53] Hong F, Yan J, Baran JT, Allendorf DJ, Hansen RD, Ostroff GR, et al. Mechanism by which orally administered beta-1,3-glucans enhance the tumoricidal activity of antitumor monoclonal antibodies in murine tumor models. J Immunol 2004;173:797–806.
[54] Sandvik A, Wang YY, Morton HC, Aasen AO, Wang JE, Johansen FE. Oral and systemic administration of beta-glucan protects against lipopolysaccharide-induced shock and organ injury in rats. Clin Exp Immunol 2007;148:168–77.
[55] Ramakers JD, Volman JJ, Biorklund M, Onning G, Mensink RP, Plat J. Fecal water from ileostomic patients consuming oat beta-glucan enhances immune responses in enterocytes. Mol Nutr Food Res 2007;51:211–20.
[56] Taylor PR, Brown GD, Reid DM, Willment JA, Martinez-Pomares L, Gordon S, et al. The beta-glucan receptor, dectin-1, is predominantly expressed on the

surface of cells of the monocyte/macrophage and neutrophil lineages. J Immunol 2002;169:3876–82.
[57] Battle J, Ha T, Li C, Della Beffa V, Rice P, Kalbfleisch J, et al. Ligand binding to the (1→3)-beta-D-glucan receptor stimulates NFkappaB activation, but not apoptosis in U937 cells. Biochem Biophys Res Commun 1998;249:499–504.
[58] McCann F, Carmona E, Puri V, Pagano RE, Limper AH. Macrophage internalization of fungal beta-glucans is not necessary for initiation of related inflammatory responses. Infect Immun 2005;73:6340–9.
[59] Evans SE, Hahn PY, McCann F, Kottom TJ, Pavlovic ZV, Limper AH. *Pneumocystis* cell wall beta-glucans stimulate alveolar epithelial cell chemokine generation through nuclear factor-kappaB-dependent mechanisms. Am J Respir Cell Mol Biol 2005;32:490–7.
[60] Taylor PR, Tsoni SV, Willment JA, Dennehy KM, Rosas M, Findon H, et al. Dectin-1 is required for beta-glucan recognition and control of fungal infection. Nat Immunol 2007;8:31–8.
[61] Saijo S, Fujikado N, Furuta T, Chung SH, Kotaki H, Seki K, et al. Dectin-1 is required for host defense against *Pneumocystis carinii* but not against *Candida albicans*. Nat Immunol 2007;8:39–46.
[62] Bohn JA, BeMiller JN. (1→3)-beta-D-glucans as biological response modifiers: a review of structure–functional activity relationships. Carbohyd Pol 1995;28:3–14.
[63] Chang FY, Shaio MF. Decreased cell-mediated immunity in patients with non-insulin-dependent diabetes mellitus. Diabetes Res Clin Pract 1995;28:137–46.
[64] Muller LM, Gorter KJ, Hak E, Goudzwaard WL, Schellevis FG, Hoepelman AI, et al. Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. Clin Infect Dis 2005;41:281–8.
[65] Lesourd BM. Nutrition and immunity in the elderly: modification of immune responses with nutritional treatments. Am J Clin Nutr 1997;66:478S–84S.