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REVIEW

Do cereal mixed-linked β-glucans possess immune-modulating activities?

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β-glucans are known for their immune-modulating properties. However, the heterogeneity of these glucose polymers makes a distinction between the different sources and structures necessary—a fact that has been little allowed for in the literature. We have focused on β-glucans from cereals as they are already used as functional food ingredients due to their established cholesterol lowering effect. Cereal β-glucans have shown in vitro activity on cytokine secretion, phagocytic activity and cytotoxicity of isolated immune cells, and activation of the complement system. Animal studies suggest a possible protective effect against an intestinal parasite, against bacterial infection, and a synergistic effect in antibody-dependent cellular cytotoxicity. Animal studies have shown activity of orally applied cereal β-glucans indicating uptake or interaction with cells of the gastrointestinal tract. However, uptake is still debated, interaction with intestinal epithelial cells has been suggested but not clarified, and mechanisms of action remain largely unknown. So far, cereal β-glucans have not shown immune modulation in the few conducted human studies and further studies are needed to clarify their effect.

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1 Introduction

β-glucans are natural cell wall polysaccharides found in yeast, mushrooms, some bacteria, seaweeds and cereals. In recent years β-glucans have received much attention due to their immune-modulating properties. β-glucans show potential in cancer therapy, and mushroom β-glucans such as Lentinan and Schizophyllan from shiitake mushroom (*Lentinus edodes*) and *Schizophyllum commune*, respectively, are currently approved in Japan for clinical use in human cancer treatment [1]. In the context of the emergence of antibiotic-resistant bacteria strains, β-glucans are receiving much attention as a potential strategy to combat infectious diseases by stimulating innate immunity [2]. Since toxicity studies of dietary β-glucans have shown that these polysaccharides are well tolerated [3–5], β-glucans have become increasingly interesting as ingredients for functional foods [6]. Even though recent research has shed some light on the mechanisms of action and structure-functional relationship of β-glucans, there are still major unexplored areas. β-glucans from different sources (fungi, bacteria, seaweed and cereals) share some common structures as they are all composed of β-linked glucose monomers. However, linkage type, branching and molecular weight (Mw) vary considerably among these groups, making it necessary to investigate their respective immune-modulating properties separately. A number of expert reviews on the immune-modulating properties of β-glucans are available [2, 7], but all of them focus on fungal β-glucans without any proper distinction between the different β-glucan groups. In addition, possible mechanisms of uptake and immune-modulating activity of β-glucans following oral administration have received little attention. Cereal β-glucans are a natural part of our everyday diet, and the European Food Safety Authority (EFSA) recently approved a health claim for blood cholesterol reduction by oat β-glucan [8]. It is therefore worthwhile to investigate the immune-modulating properties of cereal β-glucans in a separate review, discussing the effect on the immune

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Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; **CR3**, complement receptor 3; **C3b**, complement component 3b; **DCs**, dendritic cells; **GALT**, gut-associated lymphoid tissue; **GI**, gastrointestinal; **i.g.**, intragastric; **i.p.**, intraperitoneal; **i.v.**, intravenous; **M-cells**, membranous/micro-fold cells; **MLN**, mesenteric lymph nodes; **Mw**, molecular weight; **PBMC**, peripheral blood mononuclear cells; **s.c.**, subcutaneous

system, anti-cancer and anti-infective activities, possible uptake mechanisms, mechanisms of action as well as structure-functional relationships.

2 Cereal β-glucan structure and sources

Barley and oat are the cereals with the highest β-glucan contents, 2.5–11.3% and 2.2–7.8%, respectively, followed by rye (1.2–2.9%), wheat (0.4–1.4%), sorghum (0.1–1%) and rice (0.04%) [9]. Cereal β-glucans are linear macromolecules composed of β (1→3) and β (1→4) linked glucose monomers. Cereal β-glucans are therefore often referred to as mixed-linked β-glucans. The (1→4) linked monomers appear as β (1→3) linked cellotriosyl and cellotetrasyl units that constitute together about 90% of the molecule [10]. Smaller amounts of cellulosic oligosaccharides with degree of polymerisation of 5–15 are also present and may constitute up to 10% in the macromolecule [10, 11]. The ratio of β (1→4) to β (1→3) linkages of β-glucans varies and has been reported to be in the range of 2.3–2.7 for different hulled and hull-less barley varieties [12]. β-glucans from mushrooms and yeast, on the other hand, have quite different structure compared to cereal β-glucans and are composed of a β-(1→3)-linked glucan backbone with β-(1→6)-linked side chains of varying length and distribution [13].

β-glucans extracted from different cereal species have similar structure and provide almost identical nuclear magnetic resonance (NMR) spectra [9, 11]. While the overall structure of β-glucans from different cereal grains is similar, there are differences in the ratio of cellotriosyl to cellotetrasyl units. The molar ratio of tri- to tetrasaccharide (DP3/DP4) is 3.0–4.5 for wheat, 1.8–3.5 for barley and 1.5–2.3 for oat [9]. Furthermore, the Mw of native cell wall polysaccharides varies among the different cereal species. In general, the highest values are reported for oat β-glucan, closely followed by barley, whereas rye and wheat seem to contain lower Mw β-glucans. The values reported for the Mw of cereal β-glucans in the literature vary considerably as not only the extraction conditions but also the method (e.g. standards, solvent and detection) used for determination influences the obtained result. Beer et al. [14] reported peak Mw of 1.3×10^6–1.5×10^6 for barley and 2.1×10^6 to 2.5×10^6 for oat β-glucan using high-performance size exclusion chromatography with calcofluor post column fluorescence detection and β-glucan standards.

The Mw and fine structure of cereal β-glucans influence their solubility and conformation in solution. Pure cereal β-glucans are largely soluble in water even though higher temperatures may be required for complete solubilisation. In solution, cereal β-glucans behave mostly like random coil polysaccharides [15]. However, cereal β-glucans have been reported to form aggregates in aqueous solution [16]. Two different mechanisms for aggregate formation have been proposed. The longer cellulose like sequences in the β-glucan macromolecules may exhibit interchain aggregation via strong hydrogen bonds [11], while the β-(1→3) linkages prevent the formation of an ordered structure. On the other hand, it has been suggested that consecutive cellotriosyl units form helical segments and constitute areas of ordered conformation and aggregate formation to the β-glucan [11, 17]. Aggregate formation is connected to solubility, and recent studies using asymmetrical flow flied-flow fractionation have revealed that boiling of cereal β-glucan samples may disrupt aggregates and thereby increase solubility [18]. The same technique applied on a commercial barley β-glucan sample of defined weight average Mw revealed that the sample solubilised in water consisted of low molar mass species with elongated or rod-like conformation, intermediate size species with random coil conformation and high molar mass species consisting of aggregates [19]. Cereal β-glucan interactions with cell surface receptors on immune and non-immune cells likely depend on structure, molar mass, conformation in solution and solubility. These are parameters that are highly connected to each other and vary even within the same purified cereal β-glucan sample thus making the establishment of structure-functional relationships particularly difficult.

In general, β-glucans can be purified from cereal grains by aqueous extraction in combination with enzyme treatments for removal of proteins, co-extracted arabinoxylans and starch [9, 12]. The extraction of β-glucans from different cereal grains in laboratory scale as well as the large-scale production of β-glucan has been reviewed elsewhere [9]. Mw and purity may be of importance for immune-modulating properties of cereal β-glucan preparations, both of which are influenced by extraction conditions and the subsequent isolation procedure. Enzyme treatment for purification of β-glucan preparations may lead to a decrease in β-glucan Mw as reported by Samuelsen et al. for β-glucan fractions extracted from a Norwegian barley variety [20]. Bhatty reported a β-glucan content of 76 and 50% for β-glucan preparations produced from hull-less barley in laboratory scale and pilot plant, respectively [21]. The main non-β-glucan components found in cereal β-glucan preparations are starch, arabinoxylan and proteins, but antioxidants and polyphenols have also been identified [22]. Finally, the presence of trace amounts of lipopolysaccharide (LPS) may cause false positive results in certain immunological test systems, and therefore has to be taken into consideration.

3 in vitro immune-modulating activities of cereal β-glucan

Cereal β-glucan preparations have shown activity in various in vitro test systems of immune modulation.

In a test system favouring complement activation via the alternative pathway, aggregated barley β-glucan significantly inhibited haemolysis indicating complement activation, whereas high Mw soluble β-glucan showed no activity [23]. In a similar test system favouring complement activation via the classical pathway, we recently demonstrated that high Mw soluble β-glucan fractions extracted from four

different barley varieties inhibited haemolysis. The most active samples had the highest (1→4)/(1→3) ratio, but there was no significant correlation between the parameters or between estimated Mw and activity [20]. The complement system is part of the innate immune system and plays an important role in initiating inflammation, recruitment and activation of phagocytes and opsonisation of pathogens.

Several investigators have reported increased cytokine secretion after cereal β-glucan stimulation primarily of macrophages or monocytes. Incubation of peritoneal macrophages isolated from mice and murine macrophage cell line P338D1 with oat β-glucan was found to significantly increase the secretion of interleukin (IL)-1, an effect that was not changed by the removal of endotoxin traces with immobilised polymyxin B. A moderate increase of TNF-α secretion was also observed in the same experiment [24]. Rye β-glucan fractions of specific Mw ranges have been tested for their effect on TNF-α secretion by human monocytes. All fractions contained low levels of LPS; however, they increased TNF-α secretion to a higher extent than could be accounted for by the LPS contamination. The fraction with weight average Mw 18.9 kDa showed the highest activity [25]. In addition, cereal β-glucans have shown an effect on cytokine secretion by isolated murine spleen cells as they increased IL-2, INF-γ and IL-4 secretion [24]. Moreover, oat β-glucan has been reported to increase phagocytic activity of isolated murine peritoneal macrophages towards fluorescently labelled *Micrococcus lysodeikticus* bacteria [26].

In another study, barley β-glucan did not stimulate peripheral blood mononuclear cell (PBMC) proliferation, maturation of dendritic cells (DCs) or the secretion of cytokines IL-10 or IL-12 by DCs. However, barley β-glucan stimulated DCs and significantly increased the INF-γ production by T-cells in allogeneic DC-–T-cell co-cultures, but showed no effect on T-cell proliferation or the expression of T-cell activation markers [27].

Several fungal β-glucan binding receptors have been identified; dectin-1, complement receptor 3 (CR3), Toll-like receptor (TLR) 2 and 4, lactoslyceramide (LacCer) and scavenger receptors CD5, CD36 and SCARF1. The β-glucan receptors and their immune activation pathways have been reviewed elsewhere [2, 28, 29]. At least two of these receptors, dectin-1 and CR3, also bind cereal β-glucans, but both with lower affinity than yeast β-glucan [30–32]. Dectin-1 is a natural killer (NK)-cell-receptor-like C-type lectin that was first discovered on DCs, but is also highly expressed on macrophages and found on human neutrophilic and eosinophilic granulocytes, monocytes, B-lymphocytes and T-cells [29]. In vitro studies on the binding of barley β-glucan to soluble dectin-1 are contradictory [33, 34]; however, Tada et al. [35] demonstrated in a recent study that barley β-glucan can activate the transcription factor NF-κB via a dectin-1-mediated signalling pathway. A derivate of the human embryonic kidney cell line 293 was co-transfected with dectin-1, Syk, CARD9 and Bcl10. Incubation of the cells with barley β-glucan resulted in NF-κB activation and IL-8 secretion, whereas cells transfected with Syk, CARD9 and Bcl10 but not dectin-1 showed no response. The authors also showed that barley β-glucan increased IL-6 secretion by isolated murine peritoneal macrophages and these macrophages contained significantly higher amounts of phosphorylated Syk; a finding that suggests that dectin-1 is involved in mediating the immune-modulating effect of cereal β-glucan on macrophages/monocytes. Furthermore, barley β-glucan has been demonstrated to increase NK cell cytotoxicity towards different target cells [36], and NK cell and neutrophil mediated haemolysis of complement component 3b (C3b) opsonised erythrocytes [37] by binding to the lectin site of CR3. CR3 (also known as $\alpha_M\beta_2$-integrin, Mac-1 and CD11b/CD18) is expressed on NK-cells (which do not express dectin-1), neutrophils and other leukocytes. β-glucan binding to the lectin site of CR3 induces a primed state of the receptor and triggers killing of C3b (iC3b) opsonised particles such as tumour cells that lack CR3 binding membrane polysaccharides [37]. The lectin site of CR3 is not specific for β-glucans and may also bind polysaccharides containing mannose, N-acetyl-glucosamine as well as glucose [32]. This mechanism is involved in the enhancement of antibody-dependent cell-mediated cytotoxicity (ADCC) by cereal β-glucans and is further discussed below.

It should also be mentioned that fungal β-glucan binding receptors have been detected on human vascular endothelial cells [38] and rat alveolar epithelial cells [39], suggesting that cereal β-glucan binding receptors may also occur on non-immune cells.

4 Activity against cancer

Orally administered oat β-glucan (0.6 mg/mL in drinking water) was found to decrease metastatic spread of lung tumour metastases in mice [40]. Peritoneal macrophage antitumour cytotoxicity increased after treatment, and it was assumed that the protective effect of oat β-glucan was due to macrophage activation. Orally administered barley β-glucan has been shown to enhance the activity of antitumour monoclonal antibodies (mAbs) in mice [41–44]. Barley β-glucan has been found effective in suppressing tumour growth when given in combination with intravenous (i.v.) antitumour mAb treatment. Neither β-glucan nor Abs were effective as single treatments, but β-glucan was equally effective when administered intraperitoneally (i.p.) or orally. Daily administration was required since doses of β-glucan given only once or twice a week were ineffective [41]. The effect correlates with Mw; high Mw barley β-glucan (404 kDa) possessed higher tumour growth suppressive effect than lower Mw fractions [42]. The synergistic effect between β-glucan, leucocytes and antitumour antibodies known as ADCC was also registered when Rituximab, which is an immunoglobulin (Ig)G1 antibody used in human cancer therapy, was administered i.v. to mice and given in combination with oral barley β-glucan [44]. Studies on the mechanism behind the activity have revealed that the monoclonal antitumour antibodies activate

complement, and iC3b opsonises tumour cells and binds to complement receptor CR3 on leucocytes [43]. Activation of CR3 also requires binding of β-glucan to the lectin site of the receptor resulting in cytotoxic activity such as phagocytosis and the production of O_2 radicals and cytokines [36, 37, 43, 45]. Mice deficient in C3 or CR3 were shown to be unresponsive to combined mAb and β-glucan treatment [43]. In another study, barley β-glucan (400 μg/day) given orally was found to inhibit tumour growth by itself and enhanced the efficacy of photodynamic therapy in mice bearing Lewis lung carcinoma [46]. The mechanisms behind the effects were not revealed, but the authors suggest that complement-dependent cytotoxicity is involved in both cases. Photodynamic therapy is known to activate complement via the alternative pathway [47], and the authors assumed that the necessary antibodies for the effect of β-glucan alone were produced after subcutaneous (s.c.) inoculation of the tumour cells. Barley β-glucan does not seem to affect colon cancer cells directly, as shown by in vitro testing of HT-29 and Caco-2 human epithelial colon cancer cell lines [20].

5 Activity against infections

β-glucans from oat have shown prophylactic activity against parasitic [26, 48, 49], bacterial [24, 26] and viral [50–53] infections in mice after oral or parenteral administration. Pretreatment with oat β-glucan (3 mg administered every other day intragastrically (i.g.)) to mice immunosuppressed with dexamethasone [49] or immunocompetent mice [48] 10 days before challenge with the protozoa *Eimeria vermiformis* decreased oocyst shedding, minimalised clinical signs and decreased mortality and weight loss of infected animals. Immunosuppressed mice obtained increased levels of non-specific serum Ig, and specific serum IgG against sporozoites and merozoites, higher numbers of INF-γ secreting cells in the spleen and an increased number of IL-4 secreting cells in the mesenteric lymph nodes (MLNs). Immunocompetent mice showed increased levels of intestinal antimerozoite IgA and a decreased number of IL-4 secreting MLN cells. Similar results were found after s.c. administration of 500 μg β-glucan every other day 10 days before challenge with *Eimeria vermiformis*. In normal mice, s.c. administration was found to increase unspecific Ig and specific IgG levels in serum and number of INF-γ secreting MLN and spleen cells, parameters that were not seen after i.g. administration into normal mice. In a follow-up experiment by the same research group, immunocompetent mice infected with *Eimeria vermiformis* were treated 12 h before infection with a single dose of oat β-glucan i.p. [26]. Oat β-glucan treatment induced significant increases in the total lymphocyte numbers isolated from MLN, spleen and Peyer`s patches (PPs) for both infected and non-infected animals when compared to their respective untreated controls. *Eimeria vermiformis* infection decreased the percentage of CD4+ T-cells and increased the percentage of CD8+ T-cells in spleen, MLN and PP; an effect that was counteracted by β-glucan treatment.

In a model of bacterial infection, pretreatment of mice with oat β-glucan i.p. (500 μg) three days before challenge with *Staphylococcus aureus* significantly increased survival [24, 26]. I.p. injection of oat β-glucan resulted in the infiltration of the peritoneal cavity with leucocytes, primarily macrophages [24].

Several studies have investigated the effect of oat β-glucan treatment in the drinking water, alone or in the combination with exercise (moderate or fatiguing), on herpes simplex virus type 1 infection in mice models of upper respiratory tract infection [50–53]. Exercise stress increased morbidity, mortality and decreased macrophage antiviral resistance, all of which was counteracted by the administration of β-glucan [51, 52]. Depletion of lung macrophages nullified the effect of β-glucan [52]. Moderate exercise and oat β-glucan treatment showed no synergistic effects in the combat of herpes simplex virus type 1 infection [50]. However, oat β-glucan treatment alone was found to increase peritoneal macrophage resistance to virus infection and to slightly but not significantly decrease mortality, while NK cell cytotoxicity remained unaffected by the β-glucan treatment [50].

Other studies have reported the effect of cereal β-glucan on immune parameters without the use of infectious challenges. Mice receiving oat β-glucan in their drinking water exhibited increased number and respiratory burst activity of peritoneal cavity neutrophils compared to control mice [54]. S.c. injection of oat β-glucan to immunosuppressed beef steers led to a faster return of lymphocyte numbers to the control level and increased antigen-specific antibody production and antigen-triggered lymphocyte proliferation after immunization [55]. β-glucan injection to healthy beef steers showed no effect on tested immune parameters.

A human intervention study with trained cyclists receiving 5.6 g oat β-glucan per day over 2 weeks followed by 3 days of extensive exercise bouts revealed no difference in plasma cytokine levels, leukocyte counts, leukocyte activity or reported upper respiratory tract infections between the placebo and β-glucan group [56]. Other human clinical trials with hypercholesterolemic subjects have shown that daily consumption of 4.8 or 6 g oat β-glucan for 4 or 6 weeks, respectively, did not change cytokine production (IL-6, IL-8 and TNF-α) by LPS-stimulated PBMC or the expression of 84 genes involved in atherosclerosis [57], neither was C-reactive protein level affected [57, 58]. It might be important to note that cereal β-glucan doses per kg body weight are similar in the human and animal studies we report here.

6 β-glucan in the gastrointestinal (GI) tract

β-glucans are indigestible by mammalian enzymes and assumed to reach the large intestine intact. Studies with ileostomy patients consuming cereal β-glucans and pigs fed

β-glucans revealed a slight decrease of β-glucan Mw for high Mw β-glucans in the upper GI tract, while low Mw β-glucans remained largely unchanged [59–62]. β-glucan recovery after passage through the upper GI tract in a human ileostomy model was over 80% [62]. In vitro fermentation of oat and barley β-glucan has been reported to increase short chain fatty acid production towards a propionate-rich profile (51:32:17; acetate:propionate:butyrate), but did not selectively stimulate the growth of probiotic *Bifidobacteria* or *Lactobacilli* [63]. Instead barley β-glucan is fermented by *Bacteroides* spp. and *Clostridium beijerinckii* [64]. A clinical study where 0.75 g barley β-glucan was consumed daily for 30 days did not show significant increase in probiotic bacteria compared with placebo [65].

Questions remain unanswered regarding the possible uptake of β-glucans from the gut lumen and into the systemic circulation during their passage through the upper GI tract. Fluorescein labelled barley β-glucans have been detected in spleen, lymph nodes and bone marrow following oral administration in mice [43], indicating uptake from the intestine. Labelling was conducted with fluorescein dichlorotriazine, which covalently reacts with hydroxyl groups of β-glucan and may also cross-link the glucan chains. Obviously, this labelling may change the properties of the polymer, alter the affinity to receptors or influence uptake mechanisms. The isolated fluorescent cells were confirmed to be macrophages. The uptake from the gut did not involve the CR3 receptor as labelled β-glucan had also been detected in CR3-deficient mice. Instead authors hypothesised that dectin-1 might be involved in the uptake of β-glucan by GI macrophages [43]. The uptake of fungal and seaweed-derived β-glucans from the gut has been demonstrated in different animal models using fluorescent labelling of solely the reducing end of the β-glucan polymers [66] or a commercial assay kit for detection of fungal infection, which is based on the reaction of limulus amoebocyte lysate (LAL) factor G with β-glucan and does not require β-glucan derivatization [67]. The uptake mechanism is still unclear, but fungal β-glucans are assumed to be engulfed by the pinocytic function of microfold or membranous (M) cells of the PP [68, 69]. M-cells are specialised epithelial cells for the uptake and transport of macromolecules and antigens in follicle-associated epithelium (e.g. over the PP) throughout the intestine [70, 71]. Rice et al. [66] found that fungal and seaweed β-glucans were internalised by a subset of intestinal epithelial cells that might be M-cells by a dectin-1-independent mechanism. In addition, β-glucans were detected in gut-associated lymphoid tissue (GALT) cells from murine PP nodules after oral administration. The supernatants from PP cells isolated from mice treated with the fungal β-glucan Scleroglucan have been shown to activate alveolar macrophages [72]. Additionally, treatment with Scleroglucan increased PP cells proliferative response to T- and B-cell mitogens concanavalin A and LPS [68], indicating a role of PP cells in mediating the immune-modulating activity of β-glucans. Due to the structural similarity of fungal and cereal β-glucan, it is not unlikely that they share a common uptake mechanism, but this remains to be shown. The nature of a possible interaction of β-glucans with M-cells also remains to be revealed; if dectin-1 is not involved, there may be other β-glucan-specific receptors present. The surface of M-cells has binding sites for secretory IgA (sIgA) [73] and immune complexes of IgA and antigens such as certain immune-modulating pectic polysaccharides have been suggested to incorporate to PP through this route and thereby modulate the immune system [74]. However, immune complexes of sIgA and cereal β-glucans have, to our knowledge, not yet been identified. Alternatively, β-glucans may be taken up via normal intestinal enterocytes. Absorptive enterocytes are not specialised for the uptake and transport of antigens and macromolecules, but they are highly abundant in the GI-tract and have been demonstrated to transcytose soluble protein antigens [75] and nanoparticles [76].

In addition to a possible uptake of β-glucans from the intestine, β-glucans may also interact with different types of cells during their passage through the GI-tract and thereby exert immune modulation. Oat β-glucan containing fecal water has been shown to increase IL-8 secretion by different intestinal epithelial cell lines under pro-inflammatory conditions [61]. The interaction was dectin-1-independent as the enterocyte cell lines were later shown to lack functional dectin-1 [77]. We have previously shown that commercial low Mw (40 kDa) barley β-glucan increased IL-8 secretion from intestinal epithelial cell line HT-29 [78], while high Mw β-glucan extracted from Norwegian barley showed no effect on cytokine secretion from Caco-2 or HT-29 [20]. However, it has been shown that differentiated Caco-2 cells respond to the presence of apical stimuli like non-pathogenic bacteria only in the presence of leukocytes [79]. Mice given oral oat β-glucan showed increased activation of the transcription factor NF-κB in both intestinal epithelial cells and leukocytes [80], which of the two cell types was activated first remains unknown. Apart from intestinal epithelial cells, there are two other cell types that may get in direct contact with luminal β-glucan; DCs, which may extend through the intestinal epithelium, and intraepithelial lymphocytes. Intraepithelial lymphocytes consist primarily of CD8 positive effector T-cells [81] with smaller numbers of CD4 T-cells. Tsukada et al. [82] showed an increased number of intraepithelial lymphocytes, especially γδT-cells, after oral administration of yeast β-glucan to mice, while lymphocyte numbers in the liver remained unchanged. The authors also report the production of INF-γ in the intestine after β-glucan administration. Whether the observed effect was due to direct interaction of β-glucan with the intraepithelial lymphocytes remains unclear.

7 Structure-functional relationship

The use of different β-glucan preparations in various immunological test systems (Table 1) complicates attempts to draw conclusions on structure-functional relationships of the observed effects. β-glucan Mw and fine structure, such as

Table 1. Effects of different cereal β-glucan preparations in immunological tests

Main effect	β-glucan source	Purity (%)	Mw (kDa)	Solubility	LPS content	Reference
in vitro						
Complement activation						
Activation of alternative complement pathway	Commercial barley β-glucan, Sigma	n.s.	–	Aggregates	Not relevant for test	[23]
Activation of classical complement pathway	Extracted from Norwegian barley varieties	90; 96.5	1090; 886	Soluble	Not relevant for test	[20]
Cytokine secretion						
Increased cytokine secretion by murine macrophages and spleen cells	Commercial oat β-glucan, Ceapro Inc.	n.s.	1100	Soluble	Below 10 pg/mg (LAL test); Polymyxin treatment resulted in no changes	[24]
TNF-α secretion by human monocytes 18.9 kDa fraction most active	Extracted from Swedish rye flour milling fraction	n.s.	13.9–79.8	Soluble	8 ng/mg (LAL test)	[25]
NF-κB activation in dectin-1 transfected cells, IL-6 secretion by murine macrophages	Purified by freeze thawing from commercial barley β-glucan sample E70-S, ADEKA Co.	91.6	n.s.	Soluble	n.s.	[35]
Beta-glucan primed DCs induce INF-γ secretion by T-cells	Provided by another research group	n.s.	High	Soluble	n.s.	[27]
40 kDa sample increased IL-8 secretion by intestinal epithelial cell line HT-29	Commercial cereal β-glucan molecular weight standards, Megazyme	n.s.	40; 123; 245; 359	Soluble	No changes by the presence of Polymyxin	[78]
Fecal water containing the β-glucan increased secretion of pro-inflammatory cytokines by intestinal epithelial cell lines	Extracted from Swedish oat	n.s.	60	Soluble	n.s.	[61]
Activity against cancer						
Increased NK cell antibody-dependent cell-mediated cytotoxicity	Commercial barley β-glucan, Sigma	n.s.	n.s.	Suspension, supernatant used	n.s.	[36]

Table 1. Continued

Main effect	β-glucan source	Purity (%)	Mw (kDa)	Solubility	LPS content	Reference
in vivo						
Activity against cancer						
Anti-cancer activity in mouse model of Lewis lung carcinoma	Commercial barley β-glucan, Sigma	95	n.s.	Soluble	n.s.	[46]
Increased antibody-dependent cell-mediated cytotoxicity	Barley β-glucan	n.s.	n.s.	Soluble	n.s.	[44]
Increased antibody-dependent cell-mediated cytotoxicity (highest effect for highest Mw)	Commercial barley β-glucan, Sigma and Megazyme	n.s.	45;149; 297; 284; 404	Soluble, high Mw more random coil conformation, low Mw more sphere	Below 0.1 EU/mg (LAL test)	[42]
Increased antibody-dependent cell-mediated cytotoxicity	Barley β-glucan	High	High	Soluble	n.s.	[43]
Activity against infection						
Increased resistance to herpes virus infection; increased respiratory burst activity of neutrophils; decreased spread of lung tumour metastasis	Commercial oat β-glucan, OatVantage, Nurture	50; 68	n.s.	Soluble	n.s.	[40,50,52,54]
Increased resistance of mice to *Eimeria vermiformis* and *Stapyhlococcus aureus* infection, increased immune parameters in immunosupressed beef steer	Commercial oat β-glucan, Ceapro Inc.	68.2	1100	1–3 μm particles	Below 10 pg/mg (LAL test)	[24,26,48,49,55]
NF-κB activation						
NF-κB activation of mice intestinal leukocytes and epithelial cells	Commercial oat β-glucan, medium viscosity oat β-glucan, Megazyme	97	187	Soluble	n.s.	[80]

n.s. = not stated.

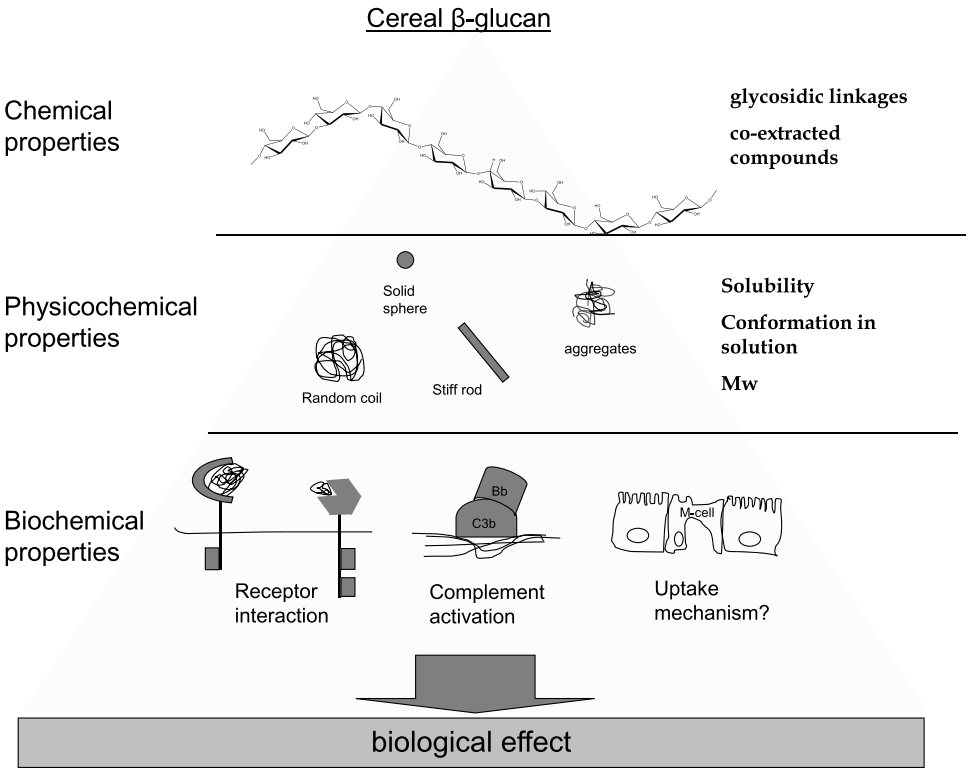

Figure 1. The complexity of cereal β-glucan properties with respect to their biological effect.

1→3 to 1→4 linkage ratio, cellulosic oligosaccharides length, number and distribution, will together with amount and nature of co-extracted compounds in a β-glucan preparation influence solubility, aggregate formation and polymer conformation. In fungal β-glucans, fine structure, Mw, conformation and solubility have been shown to influence biological activity [7, 13, 83], and it is likely that these parameters also affect the activity of cereal β-glucan, as illustrated in Fig. 1. Cereal β-glucans with different Mw distributions have been found active in different studies and test systems (see Table 1). However, systematic investigations using the same methods for Mw determination are mostly lacking making it difficult to draw clear relationships between Mw and activity. Cheung et al. [42] compared the effect of barley β-glucan with different weight average Mw in a mouse model of tumour treatment with mAbs, and found the highest activity for the β-glucan preparation with the highest weight average Mw. Two in vitro studies with monocytes and intestinal epithelial cell lines using rye and barley β-glucan fractions of different Mw revealed higher cytokine secretion in response to the lower Mw fractions compared to higher Mw fractions [25, 78]. It might be that cereal β-glucan activity is determined by uptake in in vivo situations, possibly favouring high Mw fractions, while the outcome of in vitro studies obviously skips the uptake stage and is only related to receptor interaction favouring lower Mw fractions. However, this is speculative and has to be confirmed by future research.

8 Summary and future aspects

As outlined above, cereal β-glucans have been found active in various in vitro and in vivo immunological tests (Table 1). In mice models, cereal β-glucans have shown anti-cancer activity in combination with mAb treatment or photodynamic therapy [42–44, 46]; an effect whose mechanism has been partly revealed. The suggested mechanism involves uptake of cereal β-glucan by GI macrophages as fluorescence-marked cereal β-glucan has been detected inside these cells [43]. How cereal β-glucans reach the GI macrophages is, however, not known. An uptake via intestinal M-cells in the PP or other follicle-associated epithelium is a possible explanation, but this remains to be shown experimentally.

Less is known about the mechanisms behind the anti-infective effects cereal β-glucans have demonstrated in animal models. In general, orally applied cereal β-glucans showed stronger activity in immunosuppressed animals versus healthy animals. While prophylactic treatment to mice infected with the parasite *Eimeria vermiformis* seems to stimulate adaptive immunity [26, 48, 49], cereal β-glucan treatment in models of viral and bacterial infections seems to involve the activation of macrophages [26, 52, 54]. Macrophage stimulation by β-glucan has also been demonstrated in vitro leading to increased secretion of the pro-inflammatory cytokines IL-1, IL-6 and TNF-α in addition to increased phagocytosis [24–26, 35]. It has been

suggested that macrophage stimulation by β-glucan is mediated via dectin-1; however, this remains to be explored.

Despite the positive effect of cereal β-glucans in in vitro test systems and animal models, there is still a long way to go to identify possible mechanisms of action and structure-functional relationships, and several challenges will have to be overcome. One of these challenges is the purity of cereal β-glucan preparations. The presence of additional compounds such as LPS, a powerful immunomodulator, or other co-extracted compounds (such as arabinoxylans and polyphenols) may influence the measured biological activity of β-glucan preparations and, depending on the test system, lead to false positive results. LPS contamination in polysaccharide samples can be determined by several methods, which have been summarised by Schepetkin and Quinn [84]. A different approach to check β-glucan specificity in a test system is to compare the effect of intact β-glucan with that of β-glucan totally degraded by specific enzymes. If the activity remains after enzyme degradation, β-glucan is obviously not responsible for the measured effect.

Animal studies have shown activity of orally applied cereal β-glucans [40–44, 46, 50–52, 80], indicating uptake or interaction with cells of the GI-tract. Labelling techniques that introduce little or no change to the β-glucan structure will be important tools to clarify the question of cereal β-glucan uptake from the GI-tract. In vitro models using intestinal epithelial cell lines have been used to investigate possible interactions of cereal β-glucans with cells of the GI-tract. Only low Mw cereal β-glucan increased cytokine secretion [78], however, the presence of leukocytes maybe required for a reaction to apical stimuli by differentiated Caco-2 cells [79]. Co-culture studies with leukocytes aiming at identifying involved β-glucan receptors may help to clarify the question of cereal β-glucan interaction with intestinal epithelial cells.

Apart from their immune-modulating properties, cereal β-glucans have been suggested to reduce serum cholesterol and postprandial blood glucose levels [85–89]. While the reduction in serum cholesterol by cereal β-glucans is considered well documented [8, 90], their long-term effect on blood glucose levels remains inconclusive [90]. These metabolic effects are believed to relate to reduced or delayed nutrient (e.g. glucose) uptake and bile acid reabsorption because of increased viscosity in the small intestine following intake of high Mw and sufficient amounts of cereal β-glucan [91, 92]. A daily intake of at least 3 g β-glucan is recommended for LDL-cholesterol lowering activity in both normocholesterolemic and hypercholesterolemic subjects [8], a dosage which according to the trials is well tolerated. Clinical trials on either athletes or hypercholesterolemic individuals, ingesting up to 6 g oat β-glucan daily did not change immune parameters [56–58]. It might be that additional immune parameters need to be tested for in human trials. It is also possible that higher doses are required for effect, and the high Mw and the solubility of the preparations tested might have been unfavourable. Potentially cereal β-glucans only show immunomodulation in immunocompromised individuals, as they seem to display stronger immune-modulating effects in immunocompromised animals than immunocompetent animals [48, 49, 52, 53, 55]. However, it is also possible that oat β-glucan does not interact with the human immune system after oral administration. Clearly, further human studies are needed to explore these questions.

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