Endo-glucanase digestion of oat β-Glucan enhances Dectin-1 activation in human dendritic cells

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ABSTRACT

Oat β-Glucans were studied for their immunological impact before and after enzymatic digestion in order to enhance the efficacy of oat β-Glucans for application in functional foods. Oat β-Glucan is reported to have minimal impact compared to its fungal counterpart *in vitro*. Digestion with endo-glucanase enhanced its efficacy towards stimulating MCP-1, RANTES, IL-8, and IL-4 production in human dendritic cells as compared to the nondigested β-Glucan. This effect resulted from an enhanced activation of the Dectin-1 receptor. Our data suggest that the immune-stimulation was dependent on the β-(1-3) linkages and the reduced particle size of digested β-Glucans. Thus, we show that enzymatic pre-digestion of dietary fibres such as oat β-Glucan enhances its impact on specific immune receptors. We also demonstrate that particle size and/or molecular weight of oat β-Glucans and exposure of specific binding sites for the receptors might be important tools for designing efficacious functional feed and food additives.

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1. Introduction

Evidence is accumulating that dietary fibre intake reduces the chance on typical Western diseases (Sonnenburg & Sonnenburg, 2014) . This includes diseases with an immune component such as inflammatory bowel disease (Oliveira et al., 2013). The exact mechanisms behind this are not fully understood, but factors that were suggested to play a role are changes in gut microbiota composition and short chain fatty acid (SCFA) profiles in the intestine. These changes have been reported to attenuate immune responses as shown in several mice studies (Arpaia et al., 2013; Hansen et al., 2013; Smith et al., 2013) and clinical trials (Kiely, Ajayi, Wheeler, & Malone, 2001; Lecerf et al., 2012; Meijer, de Vos, & Priebe, 2010). More recently, it has been shown that many dietary fibres activate the so-called pattern recognition receptors (PRRs) on gut immune cells (Bermudez-Brito et al., 2015) and modulate immune responses as well as gut barrier function (Vogt et al., 2014). Insight in methods to enhance or regulate the impact of immune activating dietary fibres on PRRs can become a helpful tool in designing novel functional food and feed additives.

β-Glucan with β-(1-3) linkages from fungi is one of the first discovered dietary fibre with immune-activating properties via direct binding to PRRs (Brown et al., 2002). The β-Glucan molecule binds the PRR C-type lectin domain family 7 member A (CLEC7A) receptor also known as Dectin-1 receptor (Brown et al., 2002). β-Glucans can be found in many food and feed ingredients like yeast cell walls and oat (Knudsen, 2014). Reportedly, the immune activating capacity of oat depends on its β-(1-3, 1-4) Glucan content (Estrada et al., 1997) and oat glucans are commonly applied in feed and food products (Bartlomiej, Justyna, & Ewa, 2012). Despite these beneficial immune effects, the molecular mechanisms by which the effects of oat β-Glucan are accomplished are not completely understood. While purified oat β-Glucan has been shown to have only limited immune activating capacity *in vitro* compared to for example fungal β-Glucans (Noss, Doekes, Thorne, Heederik, & Wouters, 2013), substantial beneficial effects have been reported for oat β-Glucan *in vivo* (Murphy, Davis, Carmichael, Mayer, & Ghaffar, 2009; Volman et al., 2010). This might be explained by chemical modification of oat β-Glucans by digestion in the intestine.

The difference in β-(1-3), β-(1-6), or β-(1-4) linkages in β-Glucan molecules are considered a major reason for differences in immune activating capacity of different β-Glucans (Adams et al., 2008). β-Glucans are polymers of D-glucose linked by β-(1-3), β-(1-6), and/or β-(1-4) linkages. The lectin binding domain in Dectin-1 receptor is known to be specific for glucans with β-(1-3) and β-(1-6) linkages wherein presence of β-(1-4) along with β-(1-3) and β-(1-6) in the glucan molecule can positively influence the interaction with the Dectin-1 receptor (Brown & Gordon, 2001). The type of linkages in β-Glucan molecules as well as solubility is source dependent (Brown & Gordon, 2003). Oat β-Glucan is mainly composed of β-(1-3) and β-(1-4) linkages (Estrada et al., 1997). In addition to the linkages in the β-Glucan molecule, Dectin-1 interaction is described to be dependent on the particularity of the molecule (Goodridge et al., 2011). Particulate β-(1-3) glucans from fungal source were shown to be stronger stimulators of the Dectin-1 receptor than soluble β-Glucans. Particulate β-Glucans cluster Dectin-1 receptors on the membrane. This clustering leads to expulsion of neighbouring negative regulators such as CD45 and CD148 of Dectin-1 induced immune activation (Goodridge et al., 2011) and to a strong activation in immune cells such as dendritic cells that subsequently produce pro-inflammatory cytokines (Yokota, Takashima, Bergstresser, & Ariizumi, 2001). Soluble β-(1-3) Glucan is not able to cluster Dectin-1 receptors and fails to reduce the negative regulatory pathways resulting in lower activation patterns (Goodridge et al., 2011).

In the present study, we hypothesized that enzymatic degradation of oat β-Glucans into oligomers leads to changes in particle size and will impact the oat β-Glucan induced immune responses in human dendritic cells by changing the binding kinetics to the Dectin-1 receptor. The interaction of the polymer and enzymatic digested molecules were studied with two splice variants of human Dectin-1, i.e. Dectin-1A and Dectin-1B. Dectin-1A has a stalk region between the extracellular domain and the transmembrane domain which is absent in Dectin-1B with possible effects for immune activation (Willment, Gordon, & Brown, 2001; Yokota et al., 2001). Additionally, the relationship between activation patterns of oat β-Glucan with particle size was studied.

2. Material and methods

2.1. β-Glucans and enzymatic modification

Commercial oat β-(1-3, 1-4) Glucan (medium viscosity) was purchased from Megazyme (Wicklow, Ireland). Endo-glucanase from *Aspergillus niger* was provided by DSM Food Specialities (Delft, the Netherlands). β-Glucan (10 mg/mL) was suspended in 10 mM sodium acetate buffer (pH 5.0). Enzyme was added at 0.5 μL/mg β-Glucan. The mixture was incubated at 37 °C for 12 hours in a head-over-trail rotator. Enzyme was inactivated by boiling the mixture for 10 min. Solutions (1.0 mL) were filtered through a 0.22 μm filter membrane for chemical characterization. Other solutions were lyophilized for further analysis.

β-Glucan samples were tested using a LPS specific ELISA (ELISA kit from Clone-cloud corp., Houston, TX, USA). The LPS concentration was lower than the detection levels of 4 ng/mL. None of the β-Glucan samples applied in this study were responsive at this concentration.

2.2. Molecular weight distribution measurement by HPSEC

To monitor the molecular weight change of β-Glucan after enzyme treatment, high performance size exclusion liquid chromatography (HPSEC) was performed on an Ultimate 3000 HPLC system (Thermo Scientific, Waltham, MA, USA) using a PWX-guard column (6 mm i.d. × 40 mm, Tosoh Bioscience, Tokyo, Japan) and three TSK-gel columns connected in series (4000, 3000, and 2500 SuperSW, 6 mm i.d. × 150 mm per column, Tosoh Bioscience, Tokyo, Japan). A sample of 10 μL (2.5 mg/mL in 10 mM sodium acetate buffer, pH 5.0) was injected and eluted with 0.2 M sodium nitrate at a flow rate of 0.6 mL/min at 55 °C. The HPLC system was controlled by Chromeleon version 7. Detection was achieved with a refractive index (RI) detector Shodex R101 (Showa Denko, Japan). The molecular mass distribution

of polysaccharides was estimated by applying pullulan standards (Sigma-Aldrich, St Louis, MO, USA).

2.3. Characterization of monosaccharide and oligosaccharides by HPAEC

The enzymatically released monosaccharides and oligosaccharides were analysed by high performance anion exchange chromatography (HPAEC) using an ICS 5000 system (Thermo Scientific) equipped with a CarboPac PA-1 column (2 mm i.d. × 250 mm) in combination with a CarboPac guard column (2 mm i.d. × 50 mm) and pulsed amperometric detection (PAD). A gradient elution was performed by varying the proportion of solvent A (1 M NaOAc in 0.1 M NaOH) to solvent B (0.1 M NaOH) at a flow rate of 0.3 mL/min. The solvent gradient was as follows: 0–36 min from 0 to 40% A; 36–40 min washing step with 100% A; 40–55 min equilibration step with 100% B.

2.4. Particle size measurement

The particle size of β-Glucan with and without enzymatic digestion was determined using laser light diffraction (Mastersizer 2000, Malvern Instruments Ltd, Malvern, UK) equipped with a Hydro SM sample dispersion unit as described before (Bermudez-Brito, Rosch, Schols, Faas, & de Vos, 2015).

2.5. Human Dectin-1 reporter cell lines

HEK-Blue™ Null1 cells (InvivoGen, Toulouse, France) were stably transfected with pUNO1-hDECTIN1a or pUNO1-hDECTIN1b plasmids (InvivoGen). The expression plasmids were linearized with NotI Fast digest enzyme (Thermo scientific). HEK-Blue™ Null1 (InvivoGen) cells were seeded at 500,000 cells/mL in 12-well culture plates (Corning costar, New York, NY, USA) and incubated overnight in DMEM culture media (Lonza, Basel, Switzerland) with 10% de-complemented foetal calf serum (60 °C for 1 hour), 50 U/mL Penicillin (Sigma-Aldrich), 50 µg/mL Streptomycin (Sigma-Aldrich) and 100 µg/mL Normocin (InvivoGen). The next day, transfection was performed using Lipofectamine LTX® (Life technologies, Carlsbad, CA, USA). Purified, 1 µg linear plasmid was diluted in low serum media Opti-MEM® (Life technologies) and mixed with 3.5 µL of Lipofectamine LTX® (Life technologies). The transfection mix was incubated at room temperature for 30 min and subsequently added to the cells for 24 hours incubation; transfected cells were selected using 12 µg/mL blasticidin (InvivoGen) and 100 µg/mL zeocin (InvivoGen). Single cell clones were selected by serial dilution in a 96-well plate.

2.6. Human Dectin-1 reporter cell line assay

HEK-Null1-Dectin-1A and HEK-Null1-Dectin-1B cell lines were cultured and maintained in DMEM culture media (Lonza) with 10% de-complemented foetal calf serum (60 °C for 1 hour), 50 U/mL Penicillin (Sigma-Aldrich), 50 µg/mL Streptomycin (Sigma-Aldrich), 100 µg/mL Normocin (InvivoGen) supplemented with 12 µg/mL Blasticidin (InvivoGen) and 100 µg/mL Zeocin (InvivoGen). The parental cell line, HEK-Blue™ Null1 (InvivoGen) cells express soluble embryonic alkaline phosphatase (SEAP) under control of a NF-κB AP-1 responsive promoter.

Upon stimulation with Dectin-1 agonists, HEK-Null1-Dectin-1A and HEK-Null1-Dectin-1B are activated, leading to transport of NF-κB transcription factor to the nucleus resulting in expression and secretion of SEAP, which can be quantified using Quantiblue (InvivoGen). Cells were stimulated with 1 mg/mL oat β-Glucan for 24 hours at 37 °C. The inactivated enzyme was added as control at a similar concentration as used to digest β-Glucan to exclude potential artefacts due to the enzyme. Supernatant of activated reporter cells were mixed with QUANTI-Blue in a ratio of 1:10 and quantified at 650 nm using a Versa Max ELISA plate reader (Molecular devices, Sunnyvale, CA, USA). The NF-κB activation in unstimulated cells was subtracted from the activation levels in stimulated cells. The assay was performed in 96-well plates (Corning costar) using 10 repeats.

2.7. Human dendritic cell stimulation with β-Glucan

Human dendritic cells (MatTek, Ashland, MA, USA) were seeded in a 96-well culture plate at 1,000,000 cells/mL in 100 µL DC-MM (MatTek) (Bermudez-Brito et al., 2015). After 24 hours at 37 °C, each well was treated with β-Glucan at 100 µg/mL dissolved in DC-MM (MatTek). Untreated dendritic cells were used as negative control. To determine the specificity of activation, Dectin-1 was first blocked with Dectin-1 blocking antibody (InvivoGen, Toulouse, France) at 10 µg/mL for 1 hour at 37 °C and then treated with different β-Glucan samples at 100 µg/mL, as mentioned above. After 24 hours, supernatant was used to quantify different cytokines/chemokines. The experiment was performed six times.

2.8. Cytokine detection

The production of MCP-1, RANTES, IL-8, IL-4, TNF-α, and IL-23 in human dendritic cells were quantified using the multiplex kit (Affymetrix, Santa Clara, CA, USA). The antigen standards provided were dissolved and diluted 4-fold to have seven serially diluted standards. Magnetic beads were dispersed in a 96-well black plate and washed with a hand-held magnetic plate holder with 150 µL wash buffer. The standards (in duplicate) and samples (50 µL) were added to the magnetic beads, mixed on a plate shaker and incubated overnight at 4 °C on a stable flat surface. Next, the magnetic beads were washed three times and incubated with 25 µL/well of antibody detection mix for 30 min on a plate shaker at room temperature. The plate was washed three times and incubated with 50 µL/well streptavidin-PE for 30 min at room temperature on a plate shaker. The 96-well plate was washed three times and beads were dispersed in 120 µL of reading buffer per well and read in a Luminex-100 instrument with StarStation software (Luminex, Austin, TX, USA).

2.9. Statistical analysis

The data were analysed using Graphpad Prism 5 program (La Jolla, CA, USA) and represented with standard deviation. The data were tested to have non-parametric distribution. The statistical differences were analysed using Mann–Whitney U-test. p value < 0.05 was considered statistically significant (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

## 3. Results

3.1. Characterization of β-Glucan and its digests

Molecular mass distribution of untreated and enzymatic digested β-(1-3, 1-4) Glucan was analysed with HPSEC (Fig. 1). Incubation with endo-glucanase decreased soluble high molecular mass β-Glucan (370 kDa) to low molecular mass oligosaccharides (<3.0 kDa). Oligosaccharides formed upon degradation of oat β-Glucan were further characterized using HPAEC (Fig. 2). Cello-oligosaccharides were used as standards to identify the oligomers. In addition to monomeric glucose a range of (1-4)-β-D-gluco-oligosaccharides with different degrees of polymerisation (DP; DP2-7) were formed after digestion with endo-glucanase. The other unidentified peaks presumably represent various (1-3),(1-4)-β-D-gluco-oligosaccharides and (1-4)-β-D-gluco-oligosaccharides with a DP > 10.

3.2. Digested oat β-Glucan is a more potent stimulators of dendritic cells than the β-Glucan polymer

To study the immune modulating capacity of nondigested and digested oat β-Glucan, human dendritic cells were incubated
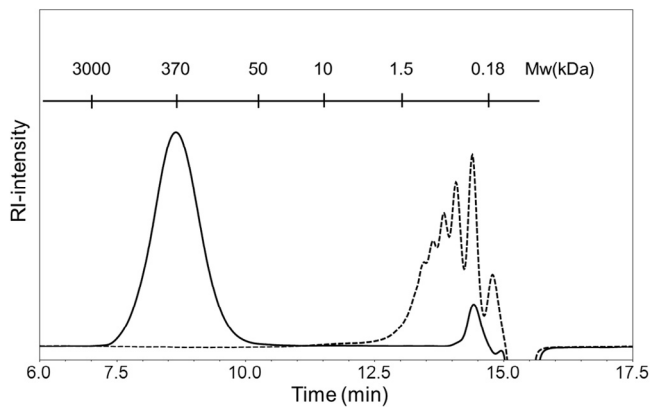
Fig. 1 – HPSEC elution patterns of oat β-Glucan before (solid line) and after (dotted line) endo-glucanase digestion. Molecular weight (Mw) calibration as indicated is based on pullulans with distinct Mw.
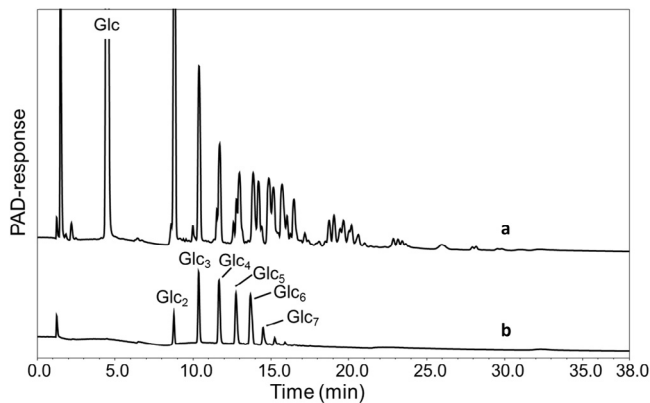
Fig. 2 – HPAEC elution patterns of oat β-Glucan digested with endo-glucanase (a) and of standard cellodextrins (b).

with soluble nondigested or endo-glucanase digested oat β-Glucan and studied for cytokine/chemokine production. Dendritic cells were stimulated with oat β-Glucan in the presence or absence of Dectin-1 blocking antibody, to study the Dectin-1 dependency of the cytokine production. After 24 hours, supernatants were analysed for the presence of innate cytokines MCP-1, RANTES, IL-8, the Th2 promoting cytokines IL-4, the Th1 stimulating cytokines TNF-α, and the Th17 supporting cytokines IL-23.

When compared to nondigested oat β-Glucan, the digested oat β-Glucan had a stimulating effect on several chemokines and cytokines. The digested oat β-Glucan induced a 1.4 fold higher production of MCP-1 ($p < 0.05$) and a 4.6 fold enhancement of RANTES production ($p < 0.01$) as compared to nondigested oat β-Glucan (Fig. 3). Also the production of the chemotactic cytokine IL-8 ($p < 0.01$) and the Th2 promoting cytokine IL-4 ($p < 0.05$) were increased. The production of TNF-α and IL-23 were not statistically significantly different between the digested and the nondigested oat β-Glucan, but we found a consistent trend of increased production with the digested oat β-Glucan. The statistically increased production of MCP-1, RANTES, IL8, and IL-4 by the digested oat β-Glucan was not observed in the presence of a Dectin-1 blocking antibody illustrating that the enhancement of these cytokines is Dectin-1 dependent (Fig. 3). Production of IL-8 and IL-23 after Dectin-1 antibody treatment with digested β-Glucan was less reduced compared to nondigested β-Glucan (p < 0.01).

3.3. Digested oat β-Glucan activate Dectin-1 more than nondigested oat β-Glucan

Next, we investigated whether the difference in immune activation between the nondigested β-Glucan and the digested β-Glucan can be explained by a difference in activation of the Dectin-1 receptor. To this end, we developed HEK293 reporter cell lines with Dectin-1 receptors and an NF-κB responsive SEAP reporter gene. This was done both for Dectin-1A (full length) and Dectin-1B (stalk-less variant), which are the two different human splice variants of the Dectin-1 receptor. As shown in Fig. 4, the enzymatic digested oat β-Glucan had a statistically significant higher impact on both Dectin-1A and Dectin-1B ($p < 0.001$) when compared to the nondigested oat β-Glucans. These differences could not be explained by the presence of enzyme in the digested samples as the control containing inactivated enzyme could not activate any of the Dectin-1 reporter cell lines.

3.4. Difference in activation of Dectin1 by nondigested and digested oat β-Glucan is particle size dependent

As it has been reported that the impact of β-(1-3) Glucan molecules on Dectin1 is dependent on the particular nature of the β-Glucans (Goodridge et al., 2011), we questioned whether this could be an explanation for the differences in Dectin1 activation between nondigested and enzymatic digested oat β-Glucan. The majority (90%) of the particles of nondigested β-Glucan were found to have a wide size range of 51.4–2000 µm, with an average median particle size of 251.1 µm. Enzymatic digestion by endo-glucanase resulted in a shift of the particle size distribution towards smaller particles (Fig. 5). The average
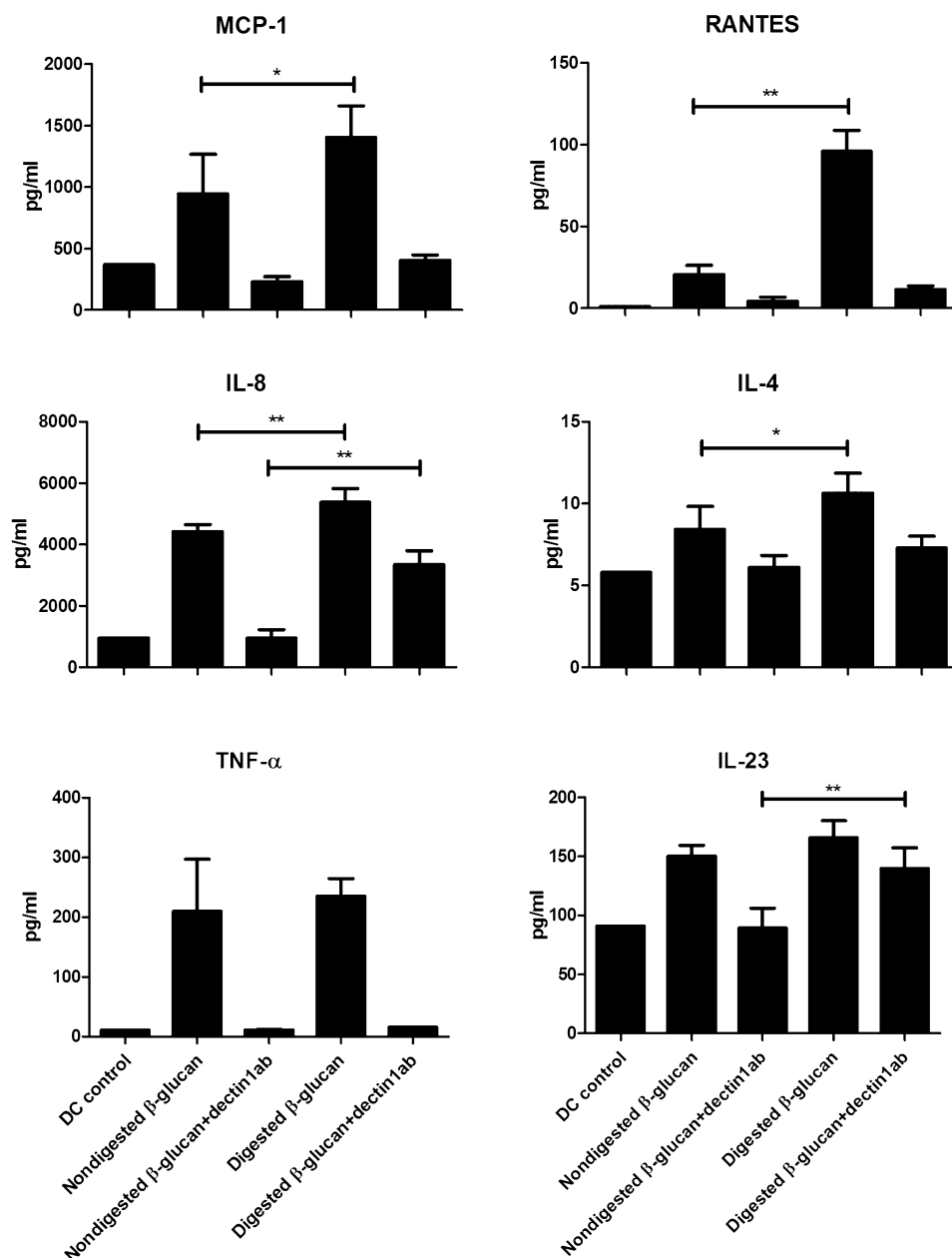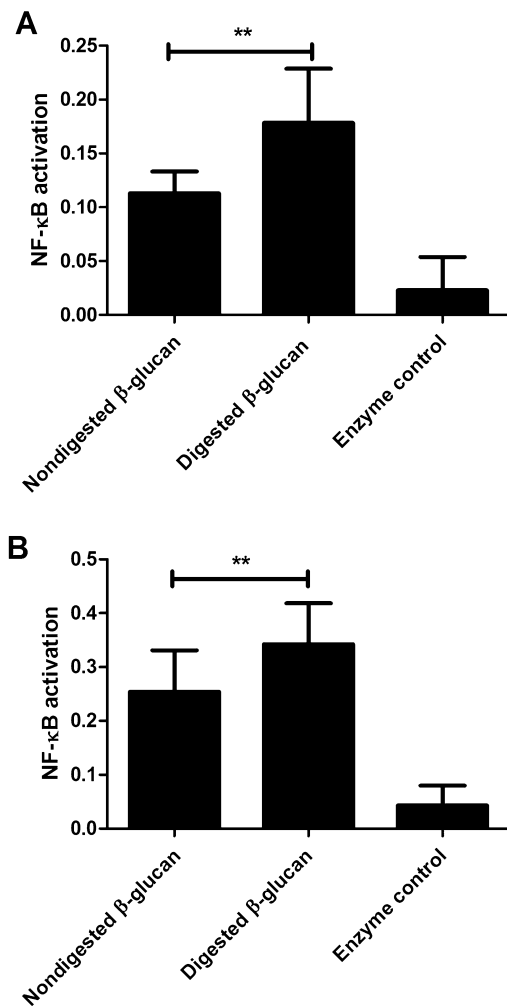
Fig. 3 – Enzymatic digested oat β-Glucan activates dendritic cells stronger than the nondigested samples. Human dendritic cells treated with soluble nondigested or enzyme digested β-Glucan. Co-incubation with Dectin-1 blocking antibody ('+dectin1 ab) and unstimulated dendritic cells is (DC) were used as control. The indicated statistical differences were measured by Mann–Whitney U-test at n = 6 (*p < 0.05; **p < 0.01).

median particle size of digested β-Glucan was decreased to 106.9 μm with a range between 2.4 to 590.1 μm. This suggests that smaller particles of endo-glucanase digested oat β-Glucan might be associated with a more pronounced impact on human dendritic cells.

4. Discussion

Oat is one of the main sources for β-(1-3, 1-4)Glucan in food and feed (Knudsen, 2014). Its efficacy as immunomodulating 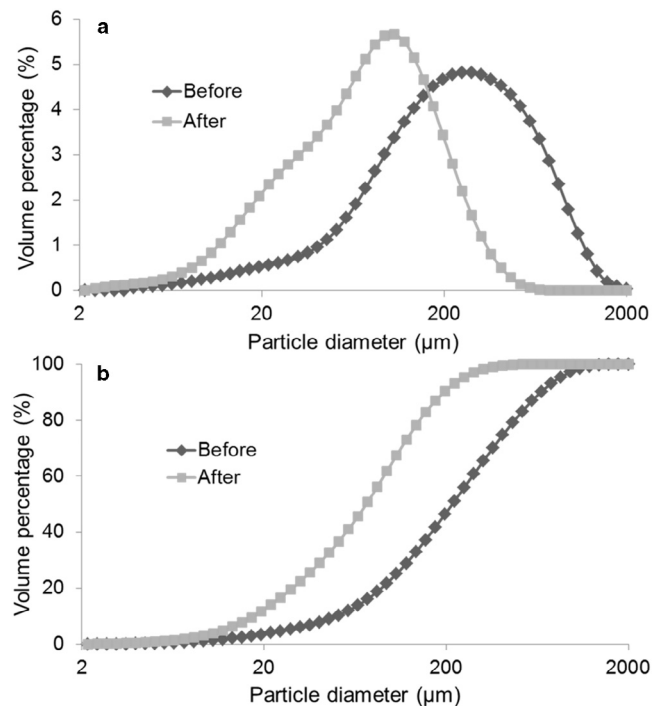dietary fibre was subject of debate because of the reported limited immune activating capacity of oat β-Glucan in vitro (Noss et al., 2013). Our study shows that discrepancies between in vitro and in vivo observations of oat β-Glucan might be caused by the fact that the immunological potency of oat β-Glucan is developed in vivo upon degradation of oat β-Glucan by microbiota-derived enzymes in the gastrointestinal tract. In this study, we applied the enzyme endo-glucanase in our design to follow the changes in efficacy of Dectin-1 activation by oat β-Glucan as model for the fate of oat β-Glucan in vivo. Endo-glucanase is one of the carbohydrate digesting enzymes produced by commensal microbiota degrading β-(1-3, 1-4)

Fig. 4 – Enzymatic digested oat β-Glucan activates Dectin-1 stronger than the nondigested β-Glucan. Dectin-1A (4A) and Dectin1B (4B) activation was represented as NF-κB activation and measured at 650 nm. The statistical differences were calculated using Mann–Whitney U-test at n = 10 (***p < 0.001).

Fig. 5 – Enzymatic digestion reduces median particle size of oat β-Glucan. (a) Particle size distribution curves and (b) cumulative particle size distributions before and after enzyme digestion.

Glucans from the diet (Beckmann, Simon, & Vahjen, 2006; El Kaoutari et al., 2013). As shown, endo-glucanase digestion induced chemical as well as particle size changes and was associated with enhanced activation of Dectin-1. The digestion of oat β-Glucan resulted in a stronger immune response in dendritic cells through stimulation of both splice variants of Dectin-1, i.e. Dectin-1A and Dectin-1B.

Based on our observations, we propose the following mechanisms by which the efficacy of Dectin-1 activation and associated immune activation is enhanced (Fig. 6). Upon digestion by the endo-glucanase, oat β-Glucan undergoes cleavage of the β-(1-4) linkages, as confirmed by presence of cellodextrins and β-(1-3) linkage enriched (1-3, 1-4)-β-D-gluco-oligosaccharides in the digests. This cleavage does bring many changes in the molecular weight and the mean particle size of oat β-Glucans. It is likely that more β-(1-3) linkages were accessible to the Dectin-1 receptor after the endo-glucanase cleavage of oat glucan. These β-(1-3) linkages in β-Glucan are the primary

linkages responsible for Dectin-1 binding and activation (Adachi et al., 2004). The availability of β-(1-3) linkages for Dectin-1 might be further enhanced by the presence of smaller size glucan particles having a higher surface area and therefore facilitate the interaction between Dectin-1 and the enzymatically exposed β-(1-3) linkages of oat β-Glucan. Also, the particle size of digested oat β-Glucan is in the range described to cluster receptors leading to a strong activation in immune cells (Goodridge et al., 2011). This clustering of Dectin-1 receptors has been described for particulate fungal β-Glucans with a size of 3 µm. Endo-glucanase digestion of oat-β-Glucan reduced the particle size from 51.4–2000 µm to 2.4–590.1 µm particles, and thus includes particles sizes reported to cluster Dectin-1 receptors (Goodridge et al., 2011).

The immune activating potential of oat β-Glucan was studied in both human dendritic cells and Dectin-1 reporter cell lines. Surprisingly, the effects of digested oat β-Glucan were more pronounced in dendritic cells than in reporter cell lines. Activation of the Dectin-1 receptor in the reporter cells lines is demonstrated only via NF-κB mediated production of SEAP. Although, NF-κB is an important transcription factor, it is not the only downstream pathway activated through Dectin-1 activation in normal immune cells such as dendritic cells. In dendritic cells, other transcription factors like NFAT are also activated downstream and contribute to production of pro-inflammatory cytokines (Goodridge, Simmons, & Underhill, 2007; Gringhuis et al., 2009). Production of IL-8 and IL-23 by digested oat β-Glucan was less reduced by dectin-1 antibody in dendritic cells compared to nondigested β-Glucan. These differences might be due to interaction of digested oat β-Glucan with other
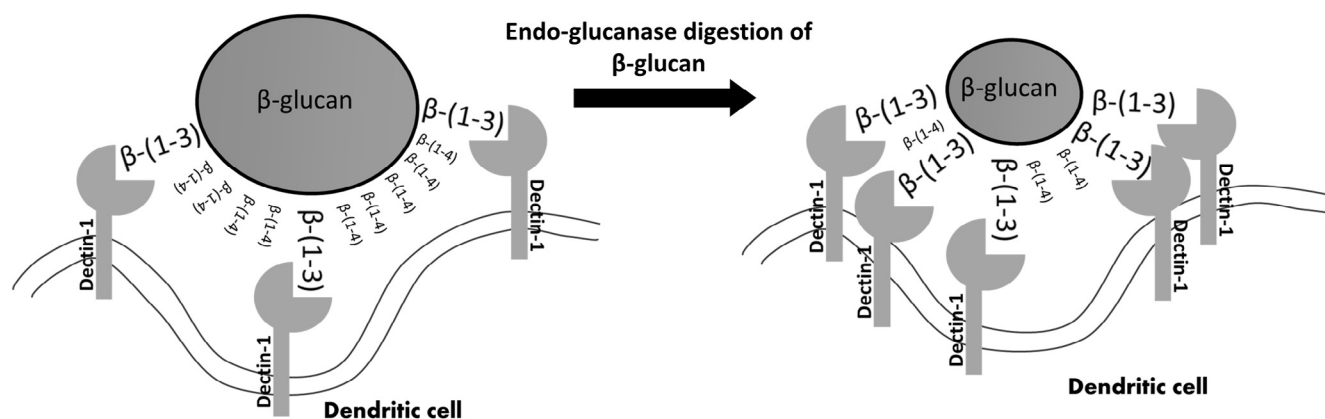
Fig. 6 – Proposed mechanism of increased immune-activity of enzyme digested oat β-Glucan. The endo-glucanase digestion of oat β-Glucan leads to reduced particle sizes of β-Glucan and more exposed β-(1-3) linkages that interact with the Dectin-1 receptor. Smaller particle size provides increased surface area, supporting better stimulation of dendritic cells through clustering and activation of Dectin-1 receptors.

immune receptors like complement receptor 3 on dendritic cells than Dectin-1 receptors (Tsoni & Brown, 2008).

The previously reported differences between *in vitro* and *in vivo* efficacy of oat β-Glucan should not be interpreted as a suggestion that undigested oat β-Glucan has no Dectin-1 stimulatory capacity at all. Our dendritic cell study shows that digested β-Glucan was more immunostimulatory but also nondigested β-Glucan enhanced production of MCP-1, RANTES, IL-8, IL-4, TNF-α, and IL-23 in dendritic cells. This corroborates the findings of others (Murphy et al., 2012) demonstrating enhanced production of IL-6, TNF-α, and IL-1β by peritoneal and lung macrophages from mice, after stimulation with undigested oat β-Glucan. Our finding that degradation to oligomers enhances the efficacy of oat β-Glucan induced immunomodulation is in line with other *in vivo* study. This study demonstrates that specifically low molecular weight oat β-Glucan was more efficacious in preventing LPS induced enteritis in mice than a high molecular weight counterpart (Wilczak et al., 2015).

Our data demonstrate that enzymatic modification of dietary fibres such as oat β-Glucan in the gastro-intestinal tract might enhance the impact of dietary fibres directly on pattern recognition receptors. To our opinion, this is an important finding as it might lead to better design of immune-active oat β-Glucan for both feed and food. We feel that our system in which treatment by fungal enzymes is mimicking *in vitro* digestion might contribute to a better understanding of the efficacy and mechanisms of action of dietary fibres *in vivo* and enhance the predictive value of *in vitro* assays. Also, it reveals to our opinion a novel type of interplay between dietary fibres, microbiota, and immunity. Up to now, the immune effects of dietary fibres are predominantly assumed to occur via stimulation of immune cells by intact dietary fibres and the formation of immune response attenuating SCFA after fermentation/utilisation by the microbiota (Arpaia et al., 2013; Hansen et al., 2013; Smith et al., 2013). Here, we demonstrate that microbiota derived enzymes can transform dietary fibres into more bioactive oligosaccharides or structures that after digestion have enhanced impact on pattern recognition receptors. This may be another novel process in the interplay between dietary fibres, intestinal microbial ecology, and immune responses. A better understanding

of this interplay may lead to design of better and more targeted efficacy of dietary fibres and might be microbiota composition dependent.

Application of enzymatically digested oat β-Glucan in diets might result in improvement of specific immune responses. Normally, oat β-Glucan is fermented in the colon (Knudsen, Jensen, & Hansen, 1993) and has, as a consequence, minimal effects in the small intestine (Mowat & Agace, 2014) where the majority of immune signalling occurs. The immune effects can be introduced in the small intestine by applying enzymatic digested oat β-Glucan in food and feed. It enhances chemokine RANTES, which is an important mediator against viral infections (Crawford, Angelosanto, Nadwodny, Blackburn, & Wherry, 2011; Gudmundsdottir & Risatti, 2009) and bacterial infections (Chen et al., 2015; Kikuchi et al., 2000). The use of digested oat β-Glucan might therefore lead to reduction of the use of antibiotics in the live-stock industry (McEwen & Fedorka-Cray, 2002). Also, our approach of physicochemical analysis in combination with demonstrating biological efficacy enhancement might be helpful tools for food and feed companies to controllably modify structure and particle size of β-Glucans or other dietary fibres for targeted purposes.

5.   Conclusions

In summary, we show that endo-glucanase digestion of oat β-Glucan increases its immune activating potential in human dendritic cells through increased activation of Dectin-1 receptors. The increased immune activity of digested oat β-Glucan might be attributed to enhanced exposure of β-(1-3) linkages after digestion and by reduced particle size. The endo-glucanase digestion mimics the β-Glucan digestion by microbiota derived enzymes. Thus, enzymatic digestion of oat β-Glucan is a mechanism for *in vivo* enhancement of immune activating effects of oat β-Glucan and also a possible strategy for industry to enhance oat β-Glucan immune stimulating effects in feed and food applications.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgements

REFERENCES

Adachi, Y., Ishii, T., Ikeda, Y., Hoshino, A., Tamura, H., Aketagawa, J., … Ohno, N. (2004). Characterization of beta-glucan recognition site on C-type lectin, dectin 1. Infection and Immunity, 72(7), 4159–4171. doi:10.1128/IAI.72.7.4159-4171.2004.

Adams, E. L., Rice, P. J., Graves, B., Ensley, H. E., Yu, H., Brown, G. D., … Williams, D. L. (2008). Differential high-affinity interaction of dectin-1 with natural or synthetic glucans is dependent upon primary structure and is influenced by polymer chain length and side-chain branching. Journal of Pharmacology and Experimental Therapeutics, 325(1), 115–123. doi:10.1124/jpet.107.133124.

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., de Roos, P., … Rudensky, A. Y. (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature, 504(7480), 451–455. doi:10.1038/nature12726.

Bartlomiej, S., Justyna, R. K., & Ewa, N. (2012). Bioactive compounds in cereal grains – Occurrence, structure, technological significance and nutritional benefits – A review. Food Science Technology International, 18(6), 559–568. doi:10.1177/1082013211433079.

Beckmann, L., Simon, O., & Vahjen, W. (2006). Isolation and identification of mixed linked beta-glucan degrading bacteria in the intestine of broiler chickens and partial characterization of respective 1,3-1,4-beta -glucanase activities. Journal of Basic Microbiology, 46(3), 175–185. doi:10.1002/jobm.200510107.

Bermudez-Brito, M., Rosch, C., Schols, H. A., Faas, M. M., & de Vos, P. (2015). Resistant starches differentially stimulate Toll-like receptors and attenuate proinflammatory cytokines in dendritic cells by modulation of intestinal epithelial cells. Molecular Nutrition and Food Research, doi:10.1002/mnfr.201500148.

Bermudez-Brito, M., Sahasrabudhe, N. M., Rosch, C., Schols, H. A., Faas, M. M., & de Vos, P. (2015). The impact of dietary fibers on dendritic cell responses in vitro is dependent on the differential effects of the fibers on intestinal epithelial cells. Molecular Nutrition and Food Research, 59(4), 698–710. doi:10.1002/mnfr.201400811.

Brown, G. D., & Gordon, S. (2001). Immune recognition. A new receptor for beta-glucans. Nature, 413(6851), 36–37. doi:10.1038/35092620.

Brown, G. D., & Gordon, S. (2003). Fungal beta-glucans and mammalian immunity. Immunity, 19(3), 311–315.

Brown, G. D., Taylor, P. R., Reid, D. M., Willment, J. A., Williams, D. L., Martinez-Pomares, L., … Gordon, S. (2002). Dectin-1 is a major beta-glucan receptor on macrophages. Journal of Experimental Medicine, 196(3), 407–412.

Chen, Y., Zhou, S., Hua, K., Xiao, H., Li, Z., Liu, M., … Jin, H. (2015). Haemophilus parasuis infection activates chemokine RANTES in PK-15 cells. Molecular Immunology, 67(2 Pt B), 661–666. doi:10.1016/j.molimm.2015.06.029.

Crawford, A., Angelosanto, J. M., Nadwodny, K. L., Blackburn, S. D., & Wherry, E. J. (2011). A role for the chemokine RANTES in regulating CD8 T cell responses during chronic viral infection. PLoS Pathogens, 7(7), e1002098. doi:10.1371/journal.ppat.1002098.

El Kaoutari, A., Armougom, F., Leroy, Q., Vialettes, B., Million, M., Raoult, D., & Henrissat, B. (2013). Development and validation of a microarray for the investigation of the CAZymes encoded by the human gut microbiome. PLoS ONE, 8(12), e84033. doi:10.1371/journal.pone.0084033.

Estrada, A., Yun, C. H., Van Kessel, A., Li, B., Hauta, S., & Laarveld, B. (1997). Immunomodulatory activities of oat beta-glucan in vitro and in vivo. Microbiology and Immunology, 41(12), 991–998.

Goodridge, H. S., Reyes, C. N., Becker, C. A., Katsumoto, T. R., Ma, J., Wolf, A. J., … Underhill, D. M. (2011). Activation of the innate immune receptor Dectin-1 upon formation of a 'phagocytic synapse. Nature, 472(7344), 471–475. doi:10.1038/nature10071.

Goodridge, H. S., Simmons, R. M., & Underhill, D. M. (2007). Dectin-1 stimulation by Candida albicans yeast or zymosan triggers NFAT activation in macrophages and dendritic cells. Journal of Immunology, 178(5), 3107–3115.

Gringhuis, S. I., den Dunnen, J., Litjens, M., van der Vlist, M., Wevers, B., Bruijns, S. C., & Geijtenbeek, T. B. (2009). Dectin-1 directs T helper cell differentiation by controlling noncanonical NF-kappaB activation through Raf-1 and Syk. Nature Immunology, 10(2), 203–213. doi:10.1038/ni.1692.

Gudmundsdottir, I., & Risatti, G. R. (2009). Infection of porcine alveolar macrophages with recombinant chimeric porcine reproductive and respiratory syndrome virus: Effects on cellular gene transcription and virus growth. Virus Research, 145(1), 145–150. doi:10.1016/j.virusres.2009.06.009.

Hansen, C. H., Frokiaer, H., Christensen, A. G., Bergstrom, A., Licht, T. R., Hansen, A. K., & Metzdorff, S. B. (2013). Dietary xylooligosaccharide downregulates IFN-gamma and the low-grade inflammatory cytokine IL-1beta systemically in mice. Journal of Nutrition, 143(4), 533–540. doi:10.3945/jn.112.172361.

Kiely, E. M., Ajayi, N. A., Wheeler, R. A., & Malone, M. (2001). Diversion procto-colitis: Response to treatment with short-chain fatty acids. Journal of Pediatric Surgery, 36(10), 1514–1517. doi:10.1053/jpsu.2001.27034.

Kikuchi, T., Kato, K., Ohara, S., Sekine, H., Arikawa, T., Suzuki, T., … Shimosegawa, T. (2000). The relationship between persistent secretion of RANTES and residual infiltration of eosinophils and memory T lymphocytes after Helicobacter pylori eradication. Journal of Pathology, 192(2), 243–250. doi:10.1002/1096-9896(2000)9999:9999<::AID-PATH688>3.0.CO;2-D.

Knudsen, K. E. (2014). Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. Poultry Science, 93(9), 2380–2393. doi:10.3382/ps.2014-03902.

Knudsen, K. E., Jensen, B. B., & Hansen, I. (1993). Digestion of polysaccharides and other major components in the small and large intestine of pigs fed on diets consisting of oat fractions rich in beta-D-glucan. British Journal of Nutrition, 70(2), 537–556.

Lecerf, J.-M., Dépeint, F., Clerc, E., Dugenet, Y., Niamba, C. N., Rhazi, L., … Pouillart, P. R. (2012). Xylo-oligosaccharide (XOS)

in combination with inulin modulates both the intestinal environment and immune status in healthy subjects, while XOS alone only shows prebiotic properties. British Journal of Nutrition, 108(10), 1847–1858. doi:10.1017/s0007114511007252.

McEwen, S. A., & Fedorka-Cray, P. J. (2002). Antimicrobial use and resistance in animals. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America, 34(Suppl. 3), S93–S106. doi:10.1086/340246.

Meijer, K., de Vos, P., & Priebe, M. G. (2010). Butyrate and other short-chain fatty acids as modulators of immunity: What relevance for health? Current Opinion in Clinical Nutrition and Metabolic Care, 13(6), 715–721. doi:10.1097/MCO.0b013e32833eebe5.

Mowat, A. M., & Agace, W. W. (2014). Regional specialization within the intestinal immune system. Nature Reviews. Immunology, 14(10), 667–685. doi:10.1038/nri3738.

Murphy, E. A., Davis, J. M., Brown, A. S., Carmichael, M. D., Ghaffar, A., & Mayer, E. P. (2012). Effects of oat beta-glucan on the macrophage cytokine response to herpes simplex virus 1 infection in vitro. Journal of Interferon and Cytokine Research, 32(8), 362–367. doi:10.1089/jir.2011.0067.

Murphy, E. A., Davis, J. M., Carmichael, M. D., Mayer, E. P., & Ghaffar, A. (2009). Benefits of oat beta-glucan and sucrose feedings on infection and macrophage antiviral resistance following exercise stress. American Journal of Physiology – Regulatory Integrative and Comparative Physiology, 297(4), R1188–R1194. doi:10.1152/ajpregu.00396.2009.

Noss, I., Doekes, G., Thorne, P. S., Heederik, D. J., & Wouters, I. M. (2013). Comparison of the potency of a variety of beta-glucans to induce cytokine production in human whole blood. Innate Immunology, 19(1), 10–19. doi:10.1177/1753425912447129.

Oliveira, M. C., Menezes-Garcia, Z., Henriques, M. C., Soriani, F. M., Pinho, V., Faria, A. M., … Ferreira, A. V. (2013). Acute and sustained inflammation and metabolic dysfunction induced by high refined carbohydrate-containing diet in mice. Obesity (Silver Spring), 21(9), E396–E406. doi:10.1002/oby.20230.

Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly, Y. M., … Garrett, W. S. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science, 341(6145), 569–573. doi:10.1126/science.1241165.

Sonnenburg, E. D., & Sonnenburg, J. L. (2014). Starving our microbial self: The deleterious consequences of a diet deficient in microbiota-accessible carbohydrates. Cell Metabolism, 20(5), 779–786. doi:10.1016/j.cmet.2014.07.003.

Tsoni, S. V., & Brown, G. D. (2008). beta-Glucans and dectin-1. Annals of the New York Academy of Sciences, 1143, 45–60. doi:10.1196/annals.1443.019.

Vogt, L. M., Meyer, D., Pullens, G., Faas, M. M., Venema, K., Ramasamy, U., … de Vos, P. (2014). Toll-like receptor 2 activation by beta2→1-fructans protects barrier function of T84 human intestinal epithelial cells in a chain length-dependent manner. Journal of Nutrition, 144(7), 1002–1008. doi:10.3945/jn.114.191643.

Volman, J. J., Mensink, R. P., Ramakers, J. D., de Winther, M. P., Carlsen, H., Blomhoff, R., … Plat, J. (2010). Dietary (1→3), (1→4)-beta-D-glucans from oat activate nuclear factor-kappaB in intestinal leukocytes and enterocytes from mice. Nutrition Research (New York, N.Y.), 30(1), 40–48. doi:10.1016/j.nutres.2009.10.023.

Wilczak, J., Blaszczyk, K., Kamola, D., Gajewska, M., Harasym, J. P., Jalosinska, M., … Gromadzka-Ostrowska, J. (2015). The effect of low or high molecular weight oat beta-glucans on the inflammatory and oxidative stress status in the colon of rats with LPS-induced enteritis. Food Function, 6(2), 590–603. doi:10.1039/c4fo00638k.

Willment, J. A., Gordon, S., & Brown, G. D. (2001). Characterization of the human beta-glucan receptor and its alternatively spliced isoforms. Journal of Biological Chemistry, 276(47), 43818–43823. doi:10.1074/jbc.M107715200.

Yokota, K., Takashima, A., Bergstresser, P. R., & Ariizumi, K. (2001). Identification of a human homologue of the dendritic cell-associated C-type lectin-1, dectin-1. Gene, 272(1–2), 51–60.