

RESEARCH ARTICLE

Arabinoxylan activates Dectin-1 and modulates particulate β -glucan-induced Dectin-1 activation

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Scope: Arabinoxylan is one of the most commonly consumed dietary fiber. Immunomodulation by arabinoxylan is documented but the mechanisms by which these immune-effects are accomplished are unknown.

Methods and results: By applying reporter cell lines for Toll-like receptors (TLRs) and Dectin-1, we demonstrated that arabinoxylan interacts with Dectin-1 receptors and not with TLRs. Arabinoxylan activates Dectin-1 to a similar magnitude as soluble β -glucans. Soluble β -glucans are known to inhibit the particulate β -glucan-induced activation of Dectin-1. As arabinoxylan is also soluble, the inhibiting capacity of arabinoxylan on particulate β -glucan-activated Dectin-1 cell lines was studied. It was found that this inhibition was similar to that of soluble β -glucan and was caused predominantly by inhibition of the Dectin-1A transcript variant. The Dectin-1 inhibitory function of arabinoxylan was further confirmed in human dendritic cells that demonstrated reduced production of IL-10 and TNF- α . The production of the antifungal cytokines IL-4 and IL-23 were increased in dendritic cells stimulated with arabinoxylan and particulate β -glucan. In contrast to soluble β -glucan, arabinoxylan did not enhance production of IL-10, TNF- α , and IL-23.

Conclusion: Arabinoxylan activates Dectin-1 and supports antifungal immune responses in human dendritic cells. The mode of action of arabinoxylan is similar but not identical to that of soluble β -glucans.

Keywords:

β -Glucan / Arabinoxylan / Dectin-1 / Dietary fiber and immunomodulation

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1 Introduction

The reduced intake of dietary carbohydrate fibers in Western society has been implicated in the still growing list of Western immune diseases such as allergy and inflammatory bowel diseases [1]. The mechanisms behind these preventive effects of dietary fibers are still not completely understood. Understanding the mechanisms by which food components act has become an important criterion for health claim [2] and might help in preventing or stopping the growth of Western immune diseases. Until a few decades ago, the main beneficial effect of dietary fiber was considered to be associated

with its fecal bulking effect [3]. During recent years, however, it has become recognized that the beneficial effects may also be associated with fiber-induced immunomodulatory effects in the intestine. Often, these effects are attributed to fermentation of the dietary fibers by commensal microbiota in the gut, resulting in selective enrichment of “good microbiota” and production of immune regulatory SCFAs [4]. However, recently a novel mechanism for immune modulation was suggested. This is through direct interaction of the dietary fibers with so-called pattern recognition receptors (PRRs) on immune cells and intestinal epithelial cells [5]. These PRRs are the sensors of the immune system and regulate immune responses [6]. The carbohydrate fibers inulin-type fructan [7] and β -glucan [8] were shown to have PRR-dependent immunomodulatory effects.

The PRRs involved in recognition of dietary fibers are Toll-like receptors (TLRs) and C-type lectin receptors (CLR) [7,8]. These PRRs are responsible for immune defense against

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Abbreviations: PRR, pattern recognition receptor; SEAP, soluble embryonic alkaline phosphatase; TLR, Toll-like receptor

pathogens and for induction of tolerance to beneficial bacteria such as the commensals in the intestine [9]. TLRs perform this function by recognizing bacterial and viral antigens such as lipids, proteins, and nucleic acids [10]. TLRs can also recognize the dietary fiber inulin-type fructan that modulates TLR2 to enhance the intestinal barrier by which entry of pathogens into the host can be prevented [11]. The C-type lectin receptors, on the other hand, exert their function by binding almost exclusively carbohydrates [12]. The C-type lectin domain family 7 member A (CLEC7A) receptor also known as the Dectin-1 receptor is known to bind the carbohydrate fiber β -glucan [13]. It specifically recognizes β (1-3) and β (1-6) linkages [14] and some also report binding of β (1-4) linkages in β -glucan molecules [15]. The Dectin-1 receptor is unique in its ability to distinguish particulate and soluble β -glucan from pathogenic sources. This ability allows the receptor to regulate and localize the inflammatory response for Dectin-1 bound to particulate β -glucan from adjacent pathogenic fungus and not to the soluble β -glucan from a distant fungal-infected cell [16]. When particulate β -glucan binds to the Dectin-1 receptors, groups of Dectin-1 receptors will be clustered resulting in expulsion of the regulatory receptors such as CD45 and CD148. Soluble β -glucan cannot cluster Dectin-1 receptors and thus induce less strong activation. Soluble β -glucan can even inhibit the particulate β -glucan-induced Dectin-1 activation, by interfering with the binding and clustering of the Dectin-1 receptor [16].

Arabinoxylan is a common dietary fiber that also has some immunomodulatory capacities [17, 18]. The dietary arabinoxylan mainly originates from cereal grains such as wheat, corn, sorghum, and rice bran [19]. It makes up almost 50% of the nonstarch carbohydrate fiber content of wheat bran [20] and is therefore one of the most common dietary fibers in food and feed. Structurally, arabinoxylan consists of β (1-4)-linked xylose residues with arabinose moieties substituted to O-2 and/or O-3 of the xylose units. The substitution level and position can differ depending on the source [21]. Arabinoxylan from rice bran was shown to prime natural killer cell activation and has antineoplastic effects in mice [22]. Wheat bran arabinoxylan was shown to induce the production of immunoregulatory IL-10 by dendritic cells cocultured with media supernatant from arabinoxylan-treated intestinal epithelial cells. This latter study suggests that direct interaction between arabinoxylan and PRRs on immune cells might be involved as modulation of dendritic-cell responses occurred in the absence of microbiota or SCFA [5].

In order to study the binding of arabinoxylan to PRRs, we studied the activation of TLR and Dectin-1 reporter cell lines after exposure to wheat arabinoxylan. As the human Dectin-1 receptor is known to be expressed in two different splice variants known as Dectin-1A (full length) and Dectin-1B (stalkless variant) [23, 24], both Dectin-1A and Dectin-1B reporter cell lines were developed for studies with arabinoxylan. The immune stimulatory effect of arabinoxylan was compared with that of particulate and soluble β -glucan. The immunomodulatory

effects of these fibers were confirmed with use of human dendritic cells and measurement of cytokine production induced by arabinoxylan.

2 Materials and methods

2.1 Arabinoxylan and β -glucan samples

Low viscosity wheat arabinoxylan (Megazyme, Wicklow, Ireland), zymosan depleted (InvivoGen, Toulouse, France), and laminarin (InvivoGen) were used in this article. The applied wheat arabinoxylan is highly water-soluble having a low viscosity [5]. Zymosan depleted is the particulate β (1-3)-glucan from *Saccharomyces cerevisiae* with mean molecular weight 240 kDa and a particle size of 3 μ m [16, 25]. Laminarin is the soluble linear β (1-3)-glucan from *Laminaria digitata* having β (1-6)-glucan (interchain) linkages [26] and has a molecular weight of 6 kDa as mentioned in supplier information. Zymosan depleted is zymosan treated with hot alkali to delete its TLR2 activation potential (InvivoGen). Throughout this article, zymosan depleted will be referred to as zymosan. These samples were tested for endotoxin levels using an LPS-specific ELISA (ELISA kit from Clone-Cloud Corp., Houston, TX, USA). The LPS concentrations were lower than the detection level of 4 ng/mL and none of the reporter cell lines applied in this study were responsive at this concentration.

2.2 Human Dectin-1 reporter cell lines

Dectin-1A and Dectin-1B are the two major transcript variants of the human Dectin-1 gene [27]. Dectin-1 reporter cell lines were developed using HEK-Blue™ Null1 cells (InvivoGen). These cells were stably transfected with pUNO1-hDectin1a or pUNO1-hDectin1b plasmids (InvivoGen) to have the Dectin-1-responsive reporter cell. The expression plasmids were linearized with FastDigest NotI enzyme (Thermo Scientific, Waltham, MA, USA). HEK-Blue™ Null1 (InvivoGen) cells were seeded at 500 000 cells/mL in 12-well culture plates (Corning Costar, NY, USA) and incubated overnight in DMEM culture media (Lonza, Basel, Switzerland) with 10% decomplemented fetal calf serum (60°C for 1 h), 50 U/mL penicillin (Sigma, St. Louis, MO, USA), 50 μ g/mL streptomycin (Sigma), and 100 μ g/mL Normocin (InvivoGen). The following day, transfection was performed by using Lipofectamine LTX® (Life Technologies, Carlsbad, CA, USA). Purified, 1 μ g linear plasmid was diluted in low serum media Opti-MEM® (Life Technologies) and mixed with 3.5 μ L of Lipofectamine LTX® (Life Technologies). The transfection mix was incubated at room temperature for 30 min and then added to the cells in the culture plate. Cells were incubated with transfection medium mix for 24 h and transfected cells were selected using selection antibiotics 12 μ g/mL Blasticidin (InvivoGen) and 100 μ g/mL Zeocin (InvivoGen).

Table 1. Reporter cell lines with their selection antibiotics and agonists used in the assays.

Name of the cell line	Selection antibiotics	Agonist (InvivoGen)	Concentration of agonist
HEK-Blue™ hTLR2	HEK-Blue™ selection	HKLM (Heat-killed <i>Listeria monocytogenes</i>)	10 ⁸ cells/mL
HEK-Blue™ hTLR4	HEK-Blue™ selection	LPS	10 ng/mL
HEK-Blue™ hTLR5	Blasticidin (30 µg/mL) Zeocin (100 µg/mL)	Flagellin	10 ng/mL
HEK-Null1 Dectin-1A	Blasticidin (12 µg/mL) Zeocin (100 µg/mL)	Particulate β-glucan zymosan Soluble β-glucan laminarin Arabinoxylan	100 µg/mL 1000 µg/mL 1000 µg/mL
HEK-Null1 Dectin-1B	Blasticidin (12 µg/mL) Zeocin (100 µg/mL)	Particulate β-glucan zymosan Soluble β-glucan laminarin Arabinoxylan	100 µg/mL 1000 µg/mL 1000 µg/mL

Single-cell clones were selected by serial dilution in a 96-well plate for each newly developed Dectin-1 cell line. The cell lines were cultured and maintained in the above-mentioned culture medium and selection antibiotics. The parental HEK-Blue™ Null1 cells express the soluble embryonic alkaline phosphatase (SEAP) gene construct with NF-κB-responsive promoter. Thus, these lines can be used as reporter cell lines for Dectin-1A and Dectin-1B receptor activity.

2.3 Human PRR reporter assay

The HEK-Blue™ reporter cell line for human TLR2, TLR4, TLR5 (InvivoGen), HEK-Null1 Dectin-1A, and HEK-Null1 Dectin-1B cells were cultured and maintained in DMEM culture media (Lonza) with 10% decompemented fetal calf serum (60°C for 1 h), 50 U/mL penicillin (Sigma), 50 µg/mL streptomycin (Sigma), and 100 µg/mL Normocin (InvivoGen). In addition, the culture medium was supplemented with selection antibiotics to maintain stable expression of the PRRs genes (see Table 1 for type of media). These reporter cell lines express the SEAP gene. This SEAP gene construct is expressed through NF-κB and the AP-1 transcription factor-responsive promoter. Thus, when these reporter cell lines are activated with their respective agonists, NF-κB is transferred to the nucleus and the SEAP gene is expressed. The SEAP from the supernatant can be measured using QUANTI-Blue reagent (InvivoGen). The agonists (positive control) used in these assay are mentioned in Table 1. The assay was performed in above-mentioned culture medium devoid of selection antibiotics. The cell viability was assessed microscopically after the assay was performed. We never observed changes in viability.

2.4 Stimulation of reporter cells with arabinoxylan

The Dectin-1 and TLR reporter cell lines were seeded at 500 000 cells/mL concentration in 100 µL/well volume in a 96-well culture plate overnight. The following day, reporter

cell lines were treated with their respective agonists (dissolved in culture medium at the concentrations mentioned in Table 1) or with 2000 µg/mL (TLR reporter cell lines) or with 1000 µg/mL arabinoxylan (Dectin-1 reporter cell lines). To study the inhibition capacity of arabinoxylan and soluble β-glucan laminarin for Dectin-1 receptors, arabinoxylan and soluble β-glucan laminarin at 1000 µg/mL were preincubated with Dectin-1 reporter cells for 1 h. This was followed by incubation with particulate β-glucan zymosan at 100 µg/mL concentration. Thus, the Dectin-1 reporter cells were treated with a combination of particulate β-glucan zymosan with either soluble β-glucan laminarin or arabinoxylan. After 24 h of incubation, the media supernatant from different treatment groups was analyzed at 1:10 dilution with QUANTI-Blue solution. The color change was read at 650 nm using ELISA plate reader VERSA MAX (Molecular Devices, CA, USA). The assay was performed with five repeats.

2.5 Human dendritic cell stimulation with β-glucan

Human dendritic cells (MatTek, MA, USA) were seeded in a 96-well culture plate at 1 000 000 cells/mL in 100 µL/well volume of Dendritic Cell Maintenance Medium (DC-MM) (MatTek) culture media [5]. After 24 h of incubation at 37°C, each well was treated with arabinoxylan or soluble β-glucan laminarin at 500 µg/mL concentration, dissolved in DC-MM (MatTek) culture media. To study the inhibition potential of arabinoxylan, arabinoxylan and laminarin (control) were pretreated at 500 µg/mL concentration for 1 h followed by treatment with 100 µg/mL particulate β-glucan zymosan in the same wells. As positive control for inhibition, Dectin-1 receptor was blocked by pretreatment with Dectin-1 blocking antibody (InvivoGen) at 10 µg/mL concentration for 1 h followed by treatment with 100 µg/mL particulate β-glucan zymosan in the same wells. To test the activation potential, arabinoxylan, soluble β-glucan laminarin, and particulate β-glucan zymosan were treated sep-

arately at the above-mentioned concentrations. Untreated dendritic cells served as negative control for the experiment. After 24 h incubation at 37°C, media supernatant from dendritic cells was used to quantify cytokines/ chemokines using multiplex magnetic bead-based assay (Affymetrix, CA, USA). The experiment was performed with five repeats.

2.6 Cytokine detection

The multiplex kit was used to measure concentration of IL-10, TNF- α , IL-4, and IL-23. The antigen standards provided with the multiplex kit (Affymetrix) were dissolved and diluted four-fold to have seven serially diluted standards. DC-MM (Mat-Tek) culture media was used as blank. The magnet beads were dispersed in a clear base, black 96-well plate and washed with a hand-held magnetic plate holder with 150 μ L of the wash buffer provided in the kit. The standards (duplicates) and samples (50 μ L) were applied to the magnetic beads, mixed on a plate shaker, and incubated overnight at 4°C on a stable flat surface. Followed by overnight incubation, magnetic beads were washed three times as mentioned earlier and incubated with 25 μ L/well of detection antibody mix for 30 min on a plate shaker at room temperature. The plate was washed three times and incubated with 50 μ L/well streptavidin-PE for 30 min at room temperature on a plate shaker. In the last step, the 96-well plate was washed three times and beads were dispersed in 120 μ L of reading buffer per well and read in a Luminex[®] 100™ instrument with STarStation software.

2.7 Statistical analysis

Parametric distribution of data points was confirmed using the Kolmogorov–Smirnov test. Statistical differences were analyzed using unpaired *t*-tests and with two-way Analysis of Variance (ANOVA) in grouped analysis. Data are expressed as mean \pm SD. *p*-Values < 0.05 were considered to be statistically significant. The data were analyzed using GraphPad Prism 5 program (La Jolla, CA, USA).

3 Results

3.1 Arabinoxylan activated Dectin-1 but not TLRs

Wheat arabinoxylan was characterized previously and had an arabinose to xylose ratio of 35:67 resulting in on average one arabinosyl substitution on every second xylose. The molecular weight of arabinoxylan was determined using high-performance size exclusion chromatography and was between 10⁵ and 10⁶ Da [5]. To determine if the immunomodulating effects of arabinoxylan can be explained by binding to PRRs, the activation of TLR2, 4, 5, and Dectin-1 by

arabinoxylan was studied. All these PRRs either recognize carbohydrate groups [13] or are implicated in the recognition of dietary fibers [28–30]. Reporter cell lines with either human TLR2, TLR4, TLR5, Dectin-1A, or Dectin-1B receptors were stimulated with arabinoxylan fibers. The activation of TLR receptors was compared to known agonists and unstimulated cells (Table 1). None of the HEK-Blue TLR reporter cells expressing TLR2, TLR4, or TLR5 showed activation by arabinoxylan (Fig. 1A–C). This, however, was different when arabinoxylan was tested on reporter cells expressing Dectin-1A or Dectin-1B. Arabinoxylan was able to activate both the A and B splice variants of the Dectin-1 receptor (*p* < 0.001). Activation was not observed in the parental HEK-Blue™ Null1 cells (Fig. 1D and E).

3.2 Arabinoxylan stimulate Dectin-1 receptors similar to soluble β -glucan

Dectin-1 activation depends on the particle size and solubility of β -glucan. Particulate β -glucan is known to be a stronger activator of Dectin-1 than soluble β -glucan [16]. Thus, we compared the magnitude of the activation of arabinoxylan with the two β -glucan sources, i.e. a particulate and a soluble β -glucan. As shown in Fig. 2A, the activation of Dectin-1A by arabinoxylan was 7.7-fold less than the activation by the particulate β -glucan zymosan (*p* < 0.001). Dectin-1B activation was lower than Dectin-1A in both arabinoxylan and particulate β -glucan, but also here, there was a 3.9-fold less activation by arabinoxylan compared to the particulate zymosan β -glucan (*p* < 0.001; Fig. 2B).

Compared to soluble β -glucan, the pattern of activation was different. Arabinoxylan stimulated both Dectin-1A (Fig. 3A) and Dectin-1B (Fig. 3B) in a similar manner as soluble β -glucan laminarin. Arabinoxylan is also a soluble fiber. Both the soluble β -glucan laminarin and the arabinoxylan impacted Dectin-1A stronger than Dectin-1B (*p* < 0.05).

3.3 Arabinoxylan differentially inhibited Dectin-1 receptor splice variants

As it has been reported that soluble β -glucan can inhibit the action of particulate β -glucan, we studied whether arabinoxylan has this capacity as well. The mechanism of this inhibition of particulate β -glucan-induced Dectin-1 activation by soluble β -glucan is suggested to be from receptor-binding competition [31]. To study the potential inhibiting effects of arabinoxylan, Dectin-1 reporter cell lines were pretreated with arabinoxylan followed by the particulate β -glucan zymosan. No pretreatment and soluble β -glucan laminarin pretreatment served as control. As shown in Fig. 4A, arabinoxylan showed a pronounced inhibition of the particulate β -glucan-induced Dectin-1A receptor with up to 3.9-fold (*p* < 0.001) inhibition. Similar levels of inhibition were

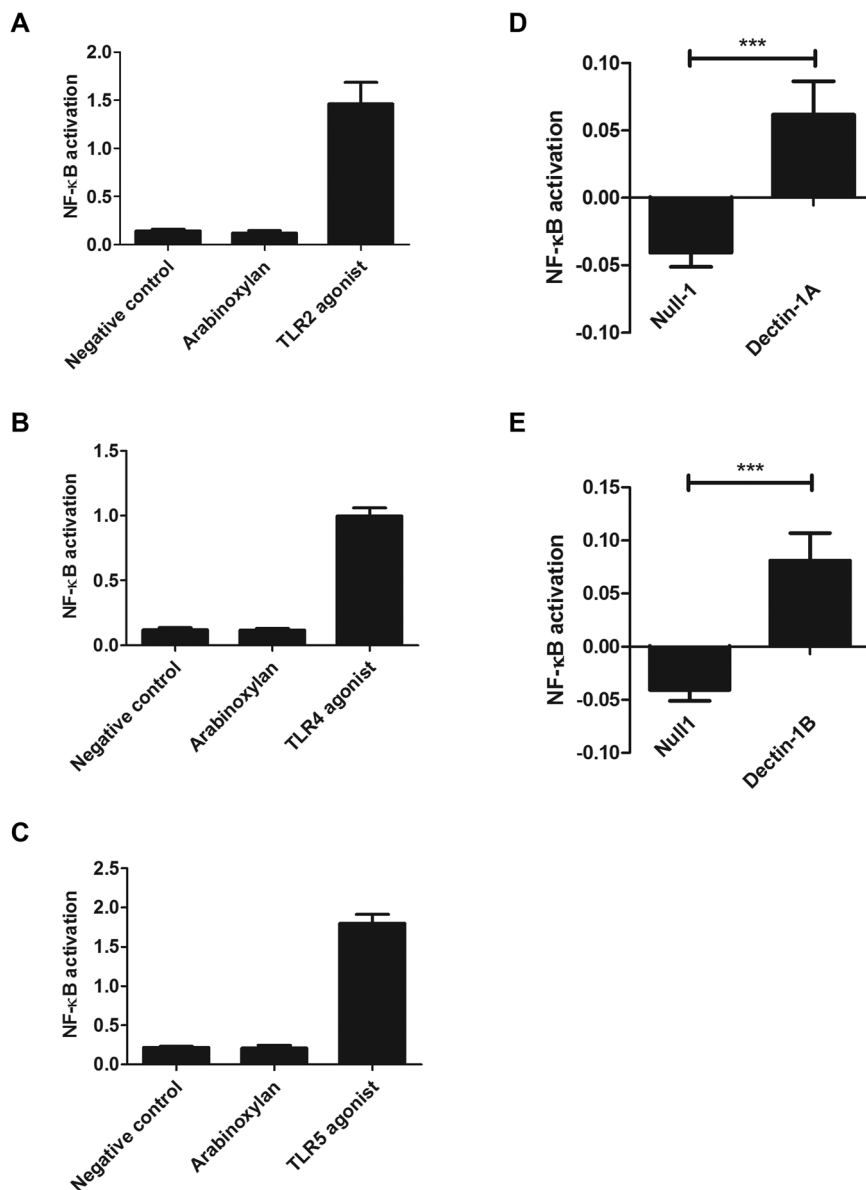


Figure 1. Arabinoxylan stimulates Dectin-1A and -1B but not TLR2, 4, and 5. HEK reporter cells for TLR2 (A), TLR4 (B), TLR5 (C), Dectin-1A (D), and Dectin-1B (E) were stimulated with arabinoxylan at 2000 $\mu\text{g}/\text{mL}$ for TLR reporter cells and 1000 $\mu\text{g}/\text{mL}$ for Dectin-1 reporter cells for 24 h. The activation of the TLR reporter cell lines was compared with unstimulated cells as negative control and TLR-specific agonists (Table 1). For Dectin-1 reporter cells, activation by arabinoxylan was compared to the arabinoxylan-treated parental HEK-Blue™ Null1 cells. The optical density at 650 nm was measured with QUANTI-Blue and expressed as NF- κ B activation. The bar graphs show the data from five replicates with SD. Statistical significance was measured using unpaired *t*-test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

achieved with soluble β -glucan laminarin at 2.7-fold ($p < 0.001$) inhibition.

Arabinoxylan was less potent in inhibition of the Dectin-1B receptor compared to the Dectin-1A receptor stimulated with particulate β -glucan (Fig. 4B). This inhibition was at 1.2-fold ($p < 0.05$). Soluble β -glucan laminarin was more efficient than arabinoxylan on the Dectin-1B receptor than arabinoxylan. The inhibition of the Dectin-1B receptor by soluble β -glucan laminarin was 1.7-fold ($p < 0.001$) and it was statistically significantly higher than inhibition observed with arabinoxylan ($p < 0.05$). These results suggest that arabinoxylan mainly inhibit the Dectin-1A splice variant stimulated with particulate β -glucan while soluble β -glucan inhibit both splice variants.

3.4 Arabinoxylan did not enhance cytokine production in human dendritic cells

To study the impact of arabinoxylan on cytokine release from dendritic cells, dendritic cells were stimulated with arabinoxylan. The tolerogenic cytokine IL-10, the Th2 promoting IL-4, the Th17 stimulating IL-23, and TNF- α were studied. Soluble β -glucan laminarin served as control. As shown in Fig. 5, we did not observe any elevation in production of cytokines by arabinoxylan. The basal levels of cytokine production in dendritic cells under control conditions were not statistically significantly different from that of dendritic cells incubated with arabinoxylan. Soluble β -glucan laminarin, on the other hand was able to enhance production of IL-10

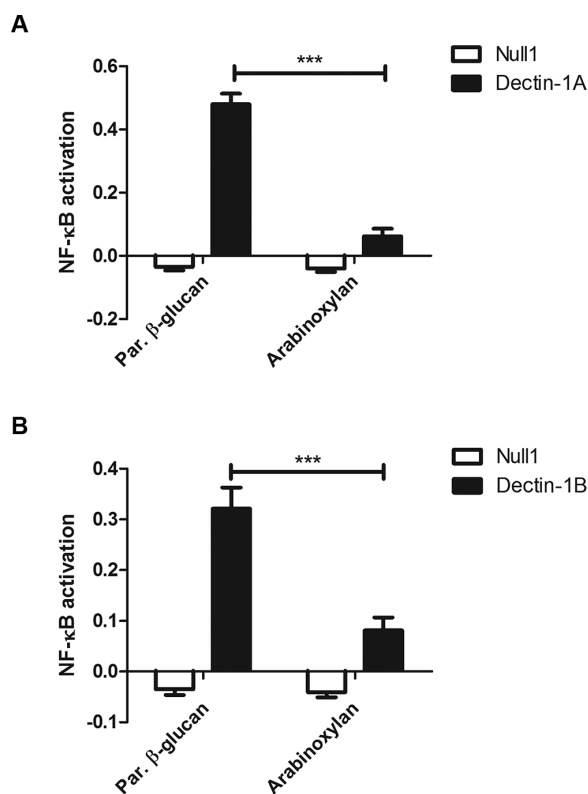


Figure 2. Arabinoxylan is a mild stimulator for Dectin-1A and -1B compared to particulate β -glucan. Dectin-1A (A), Dectin-1B (B) reporter cells, and parental HEK-Blue™ Null1 (A and B) cells were stimulated with particulate (Par.) β -glucan zymosan at 100 μ g/mL and arabinoxylan at 1000 μ g/mL for 24 h. The optical density at 650 nm measured with QUANTI-Blue is represented as NF- κ B activation. The bar graphs show the data from five replicates with SD. Statistical significance was measured using two-way ANOVA test (***) $p < 0.001$.

($p < 0.001$), TNF- α ($p < 0.05$), and IL-23 ($p < 0.05$) by the dendritic cells. Thus, the cytokine activation potential of arabinoxylan and soluble β -glucan differ in human dendritic cells.

3.5 Arabinoxylan inhibited IL-10, TNF- α and enhanced IL-4, IL-23 expression in Dectin-1-stimulated dendritic cells

The reporter assay studies suggested an inhibitory action of arabinoxylan for particulate β -glucan stimulated Dectin-1 activation. To address whether this has functional implications, human dendritic cells were treated with arabinoxylan followed by particulate β -glucan zymosan stimulation. Soluble β -glucan laminarin pretreatment served as control. Dectin-1 neutralizing antibody was applied to demonstrate the Dectin-1 dependency of cytokine expression.

Arabinoxylan was able to inhibit the particulate β -glucan-induced elevation of IL-10 ($p < 0.01$) and TNF- α ($p < 0.05$).

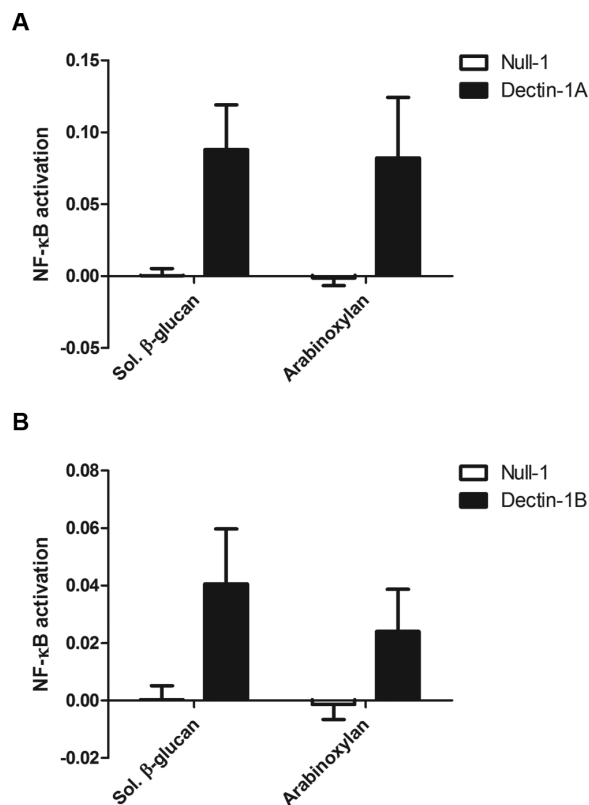


Figure 3. Arabinoxylan activates Dectin-1A and -1B similar to soluble β -glucan. Dectin-1A and -1B reporter cells, and parental HEK-Blue™ Null1 (A and B) cells were stimulated with soluble (Sol.) β -glucan laminarin at 1000 μ g/mL and arabinoxylan at 1000 μ g/mL for 24 h. The optical density at 650 nm measured with QUANTI-Blue is represented as NF- κ B activation. The bar graphs show the data from five replicates with SD. Statistical significance was measured using two-way ANOVA test.

This was Dectin-1-dependent as Dectin-1 blocking antibodies combined with particulate β -glucan also inhibited both IL-10 (4.9-fold; $p < 0.001$) and TNF- α (3.9-fold; $p < 0.001$). Unlike arabinoxylan, soluble β -glucan was not able to statistically significantly inhibit production of IL-10 and TNF- α (Fig. 6).

Interestingly, we did not only observe inhibition of cytokines but also some enhancements after cotreatment of arabinoxylan and soluble β -glucan with particulate β -glucan-induced activation of dendritic cells. In particulate β -glucan stimulated dendritic cells, the expression of IL-4, after pretreatment with arabinoxylan was increased 1.4-fold ($p < 0.05$) and even more increase was observed in soluble β -glucan pretreated cells at 1.8-fold ($p < 0.01$). Production of IL-23 was also increased in particulate β -glucan-stimulated and arabinoxylan or laminarin pretreated cells. IL-23 production was increased by 1.9-fold ($p < 0.001$) and 3.3-fold ($p < 0.01$) by arabinoxylan and laminarin pretreatment, respectively. For both IL-4 and IL-23, this enhancement in cytokine production was Dectin-1-dependent as Dectin-1 blocking antibodies were able to reduce production of IL-4 (2.4-fold; $p < 0.001$) and of IL-23 (4.6-fold; $p < 0.001$; Fig. 6).

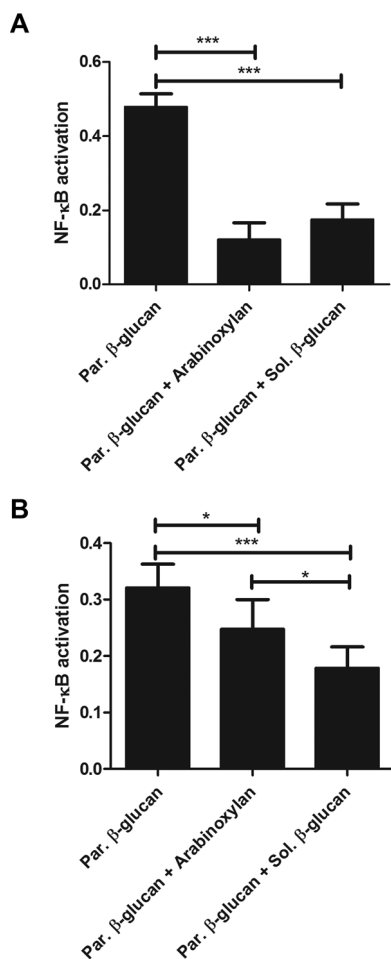


Figure 4. Arabinoxylan inhibits Dectin-1A activation stronger than Dectin-1B. Dectin-1A (A) and Dectin-1B (B) reporter cells were pretreated with soluble (Sol.) β -glucan laminarin at 1000 $\mu\text{g}/\text{mL}$ and arabinoxylan at 1000 $\mu\text{g}/\text{mL}$ for 1 h and followed by stimulation with particulate (Par.) β -glucan zymosan at 100 $\mu\text{g}/\text{mL}$. The inhibition potential of arabinoxylan and soluble (Sol.) β -glucan laminarin was compared with stimulation with particulate (Par.) β -glucan alone. The cells were incubated for 24 h and activation status was measured with QUANTI-Blue. The optical density measurements at 650 nm are represented as NF- κ B activation. The bar graphs show the data from five replicates with SD. Statistical significance was measured using unpaired *t*-test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

4 Discussion

To the best of our knowledge, we demonstrate for the first time that a soluble dietary carbohydrate can activate Dectin-1 in a similar fashion as β -glucan and can also inhibit the actions of particulate β -glucan in a similar fashion as soluble β -glucan. The reasons to investigate these actions of arabinoxylan were twofold. First, to gain insight into possible mechanisms by which arabinoxylan, as one of the major dietary component in food and feed [32, 33], can modulate

the immune response in consumers. And, secondly, to study whether common (degradation) products in food and feed can compete for binding to intestinal receptors such as Dectin-1 and inhibit the actions of other dietary fibers such as shown here for particulate $\beta(1-3)$ -glucan linkages, as present in Western diets mainly from yeast and mushrooms sources. To our best knowledge, dynamics of binding of digestion products to receptors in the intestine has not been studied up to now but is to our opinion instrumental in understanding the efficacy of bioactive food products in the intestine.

Dectin-1 receptors have the capacity to distinguish more pathogenic, particulate β -glucans from fungal cell walls from that of soluble β -glucan. Zymosan, a particulate $\beta(1-3)$ -glucan was used to mimic more proinflammatory responses, while $\beta(1-3/1-6)$ -laminarin was used as model for soluble β -glucan. Although soluble β -glucan laminarin is not common in Western diet, it was used as a model for its known inhibitory action on the particulate β -glucans from yeast [31]. The efficacy of Dectin-1 activation of these two β -glucans was compared to that of arabinoxylan by which it was demonstrated that the soluble arabinoxylan had similar kinetics of activation as the soluble β -glucan. However, the inhibition of Dectin-1 was not totally equal. Soluble β -glucan inhibits both the Dectin-1A and -1B receptors. This was different with arabinoxylan that predominantly inhibited the full-length Dectin-1A subunit and only very weakly the stalkless variant Dectin-1B. The difference in inhibition potential can be explained by differences in molecular weights and different sugar linkages and variations in the structural complexity of the soluble arabinoxylan and β -glucan laminarin. Wheat arabinoxylan is mainly composed of arabinose and xylose with $\beta(1-4)$ -xylan in the backbone whereas soluble β -glucan is composed of *D*-glucose in $\beta(1-3)$ and (1-6) linkages [5]. The carbohydrate-binding domain in the Dectin-1 receptor has, to our best knowledge, not been described to bind $\beta(1-4)$ linkages in xylose or to the arabinose, although $\beta(1-3/1-4)$ -mixed linked glucans from cereals have been mentioned to react with the Dectin-1 receptor [15]. This suggests that the binding site of arabinoxylan and β -glucan on the Dectin-1 receptor might be different. However, the observation that arabinoxylan preferably bind to Dectin-1A suggests that also the *N*-linked glycosylation sites in the stalk region of Dectin-1A receptor might be involved as these sites are absent in the Dectin-1B receptor [23, 24].

The similarity in activation of Dectin-1A receptors by arabinoxylan and soluble β -glucan can be explained by structural similarities between the xylan and β -glucan backbones. This might be due to the $\beta(1-4)$ along with $\beta(1-3)$ linkages that are present in cereal β -glucans from, e.g. barley. These linkages are responsible for interaction with Dectin-1 [15]. Conformationally, the $\beta(1-4)$ linkages in the xylan backbone of arabinoxylan and the $\beta(1-4)$ -stretches in the glucan are similar as both sugars differ only in carbon atoms at position 6 [34]. These structural similarities between arabinoxylan and β -glucan might be responsible for recognition and activation of Dectin-1 receptor by arabinoxylan.

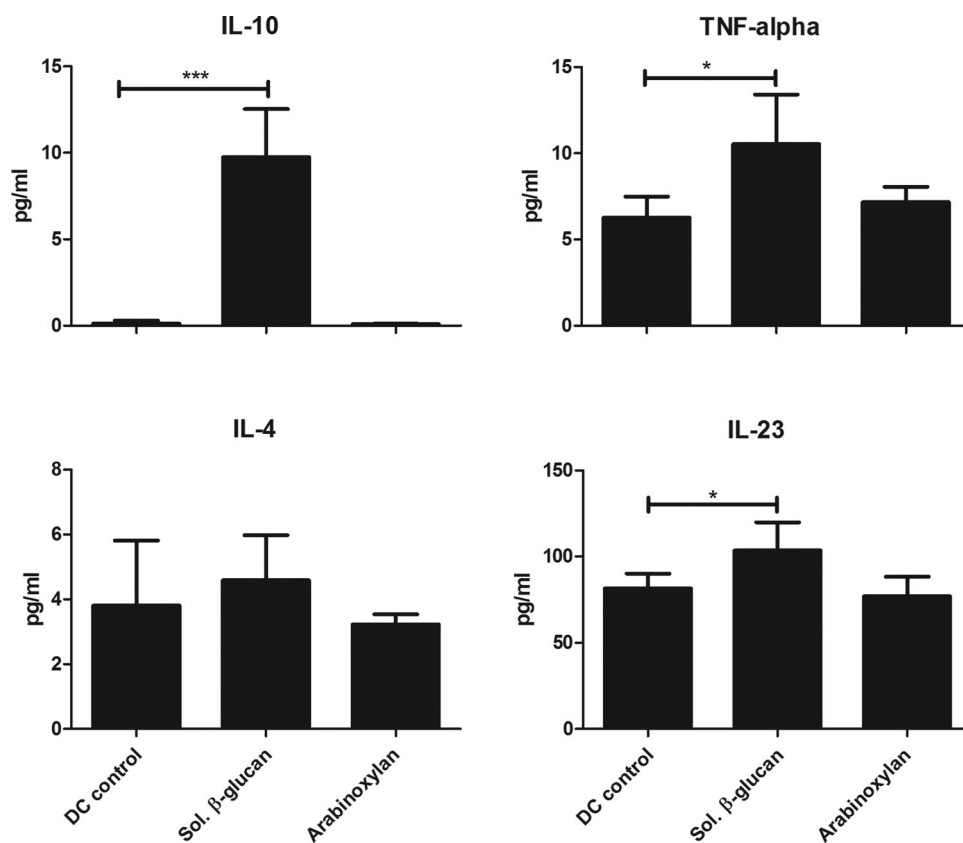


Figure 5. Arabinoxylan does not enhance cytokine expression in human dendritic cells. Human dendritic cells were stimulated with arabinoxylan and soluble (Sol.) β -glucan laminarin (500 μ g/mL) for 24 h. Untreated dendritic cells (DC control) served as control. After incubation, expression of IL-10, TNF- α , IL-4, and IL-23 was measured with multiplex analysis. The statistical differences were measured with unpaired *t*-test with five replicates (**p* < 0.05; ***p* < 0.01; ****p* < 0.001).

The differences between composition of arabinoxylan and soluble β -glucan might also be responsible for the differences in cytokine released by arabinoxylan- and soluble β -glucan-challenged human dendritic cells. The soluble β -glucan stimulated production of IL-10, TNF- α , and IL-23, whereas arabinoxylan did not have such an effect. IL-10 is an immune regulatory cytokine [35], whereas TNF- α and IL-23 are important for immune stimulation to fight fungal infection [36]. Arabinoxylan, thus, acts differently in this respect. Additionally, arabinoxylan inhibited production of both IL-10 and TNF- α , whereas soluble β -glucan failed to do so. Again, this difference in inhibition potential of arabinoxylan and soluble β -glucan might be because of preferential inhibition of Dectin-1A splice variant by arabinoxylan.

The observation that arabinoxylan not only inhibits but also stimulates cytokine release in particulate β -glucan-challenged dendritic cells is interesting and needs some further consideration as it suggests that arabinoxylan just like soluble β -glucan can specifically support antifungal responses. Both arabinoxylan and soluble β -glucan enhanced the production of IL-4 and IL-23 in particulate β -glucan-challenged dendritic cells. IL-4 has been implicated in Th2 stimulation that is considered to be deleterious for fungi [37]. The other, more enhanced cytokine, IL-23, is one of the major cytokines secreted by antigen-presenting cells during fungal infections. Pathogenic fungi induce IL-23 leading to stimula-

tion of IL-17 producing Th17 cells and deletion of the fungi [38].

In conclusion, our data demonstrate that Dectin-1 is not solely activated by β -glucan but also by the dietary fiber arabinoxylan. Arabinoxylan is one of the most commonly consumed dietary fibers and, as shown here, seems to provoke antifungal immune responses. Its cytokine-potentiating effect is not identical to that of soluble β -glucan that might be explained by different binding kinetic to the splice variants of the Dectin-1 receptor. The observation that arabinoxylan competes with particulate β -glucan for binding and activation of Dectin-1 suggests that binding of particulate β -glucan to Dectin-1 in the intestine might be more complex than previously assumed, as arabinoxylan is present in high amounts either as degradation product from feed and food [32] or as ingredient [33, 39]. Due to these types of competitions, bioactive food components, such as in this case β -glucan, might lose efficacy. This type of knowledge is to our opinion essential for designing effective bioactive food and feed.

N.M.S. and P.D.V. conceived and designed the experiments. N.M.S. performed the experiments and analyzed data. N.M.S., H.A.S., M.M.F., and P.D.V. wrote the paper.

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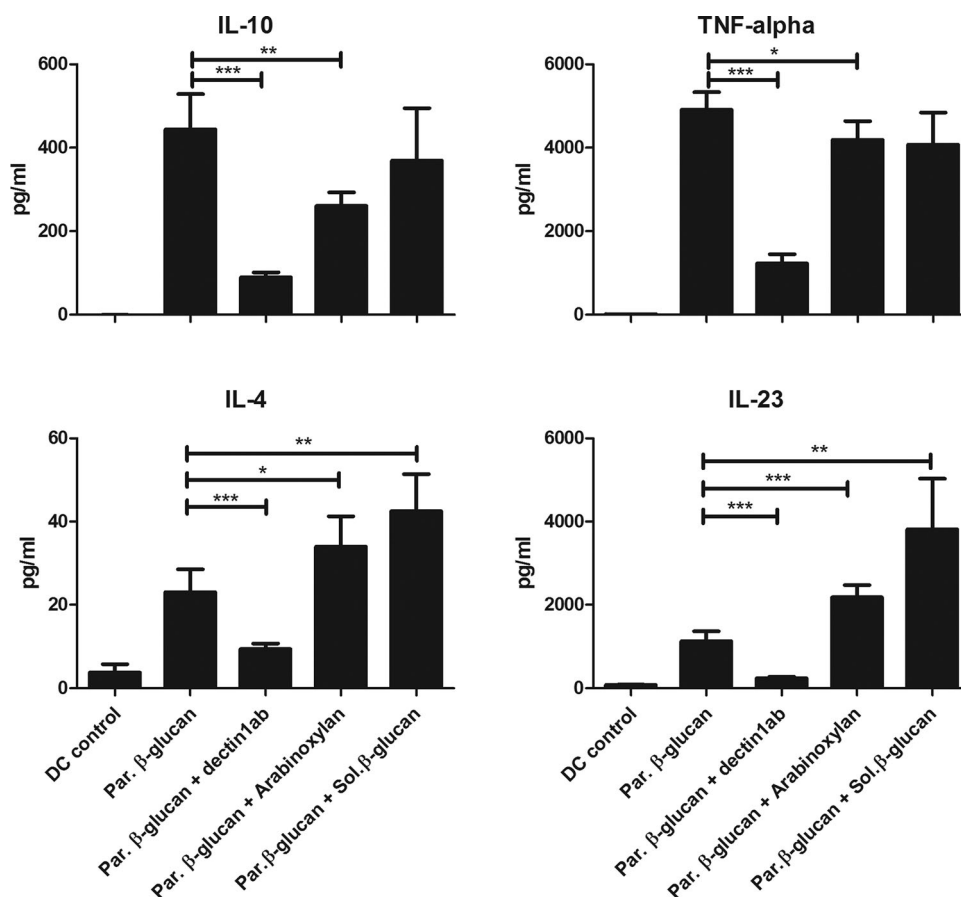


Figure 6. Arabinosylylan inhibits IL-10, TNF- α and enhances IL-4, IL-23 expression in Dectin-10-stimulated human dendritic cells. Human dendritic cells were pretreated with arabinosylylan and soluble (Sol.) β -glucan laminarin at 500 μ g/mL followed by treatment with particulate (Par.) β -glucan at 100 μ g/mL for 24 h. The Dectin-1 specificity of activation by particulate (Par.) β -glucan was assessed by Dectin-1 blocking antibody treatment at 10 μ g/mL. Untreated dendritic cells (DC control) served as control. Expression of IL-10, TNF- α , MCP-1, RANTES, IL-4, and IL-23 was measured in the media supernatant of the stimulated cells with multiplex analysis. The statistical differences were measured with unpaired *t*-test with five replicates (**p* < 0.05; ***p* < 0.01; ****p* < 0.001).

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