

## RESEARCH ARTICLE

# The impact of dietary fibers on dendritic cell responses in vitro is dependent on the differential effects of the fibers on intestinal epithelial cells

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**Scope:** In the present study, the direct interaction of commonly consumed fibers with epithelial or dendritic cells (DCs) was studied.

**Methods and results:** The fibers were characterized for their sugar composition and chain length profile. When in direct contact, fibers activate DCs only mildly. This was different when DCs and fibers were co-cultured together with supernatants from human epithelial cells (Caco spent medium). Caco spent medium enhanced the production of IL-12, IL-1Ra, IL-6, IL-8, TNF- $\alpha$ , MCP-1 (monocyte chemotactic protein), and MIP-1 $\alpha$  but this was strongly attenuated by the dietary fibers. This attenuating effect on proinflammatory cytokines was dependent on the interaction of the fibers with Toll-like receptors as it was reduced by Pepinhyd88. The interaction of galacto-oligosaccharides, chicory inulin, wheat arabinoxylan, barley  $\beta$ -glucan with epithelial cells and DCs led to changes in the production of the Th1 cytokines in autologous T cells, while chicory inulin, and barley  $\beta$ -glucan reduced the Th2 cytokine IL-6. The Treg-promoting cytokine IL-10 was induced by galacto-oligosaccharides whereas chicory inulin decreased the IL-10 production.

**Conclusions:** Our results suggest that dietary fibers can modulate the host immune system not only by the recognized mechanism of effects on microbiota but also by direct interaction with the consumer's mucosa. This modulation is dietary fiber type dependent.

**Keywords:**

Dendritic cells / Epithelial cells / Fibers / Transwell

## 1 Introduction

Consumption of high amounts of dietary fibers is associated with lower mortality in subjects suffering from circulatory, digestive, and noncardiovascular noncancerous inflammatory diseases [1–3]. The mechanisms by which fibers contribute

to lower mortality remain to be elucidated but have been suggested to be of both metabolic and immunological nature [4, 5]. Cereal fibers have been associated with lower concentrations of inflammatory markers, but there is still an urgent need for immunological studies addressing specific fibers to obtain a better understanding of which cellular processes are involved in immunomodulating effects of dietary fibers [6].

Classically, health effects of dietary fibers are considered to be caused by beneficial effects on the activity of gastrointestinal microbiota [7], i.e. prebiotic effects or by improving fermentability. However, evidence is accumulating that dietary fibers can also interact directly with the immune system [8]. For example, in a recent paper, we demonstrated that  $\beta$ 2 $\rightarrow$ 1-fructans bind to so-called pattern recognition receptors (PRRs).  $\beta$ 2 $\rightarrow$ 1-fructans were shown to mainly stimulate

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**Abbreviations:** Caco-SM, Caco spent medium; DCs, dendritic cells; GOS, galacto-oligosaccharides; HLA-DR, anti-human leukocyte antigen-DR; IECs, intestinal epithelial cells; PRRs, pattern recognition receptors; TLRs, Toll-like receptors

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Toll-like receptor (TLR)-2 resulting in NF- $\kappa$ B/AP-1 activation and cytokine release from human peripheral blood mononuclear cells [5]. Similar effects of other dietary fibers can be expected. Other dietary fibers of which immunological health benefits have been described are galacto-oligosaccharides (GOS) [9], pectin [10], arabinoxylans [11], and  $\beta$ -glucans [12]. These are all applied in consumer products but have not been studied in detail for their direct effects on inflammatory cells.

The intestinal epithelial cells (IECs) are the first cells that encounter dietary fibers. Here, the dietary fibers will also meet dendritic cells (DCs) that extrude their dendrites between IECs [13]. As oligosaccharides and some dietary fibers have been shown to be able to pass the epithelial barrier and can even be found in the systemic circulation [14–16], it is likely that also DCs in the lamina propria encounter dietary fibers by direct contact. In the present study, the effects of the dietary fibers GOS, chicory inulin, sugar beet pectin, wheat arabinoxylan, barley  $\beta$ -glucan on cytokine production and maturation of DCs either in single or in co-culture with epithelial cells have been studied. Fibers were characterized for their chain length profile and sugar composition. Finally, the effects of T-cell polarization were analyzed.

## 2 Materials and methods

### 2.1 Characterization of dietary fibers

We studied the Vivinal GOS (FrieslandCampina, Wageningen, The Netherlands), chicory inulin (Royal Cosun, Roosendaal, The Netherlands), sugar beet pectin (Danone, Wageningen, The Netherlands), wheat arabinoxylan (Kellogs, Kalamazo, USA), barley  $\beta$ -glucan (Kellogs). Fibers were characterized for their chain length profile and sugar composition.

#### 2.1.1 Constituent monosaccharide composition

The monosaccharide composition was determined using a prehydrolysis step with 72% w/w sulfuric acid at 30°C for 1 h. A hydrolysis with 1 M sulfuric acid at 100°C for 3 h followed. The originated monosaccharides were derivatized to alditol acetates and analyzed by GC using inositol as an internal standard [17]. The colorimetric m-hydroxydiphenyl assay [18] was used to determine the total uronic acid content with an automated method as described by Thibault [19].

The content of inulin was determined according to Stöber et al. [20]. For the determination of the total fructose and glucose content, 500  $\mu$ g milliQ water and 290  $\mu$ L of 0.1 M NaOAc buffer pH 4.7 were added to 200  $\mu$ L of the 5 mg/mL sample solution and incubated with 10  $\mu$ L Fructozyme L (Novozyme, Bagsvaerd, Denmark) at 50°C for 18 h. Free fructose, glucose, and sucrose were determined using non-

incubated reference samples. The samples were analyzed using the high performance anion exchange chromatography (HPAEC; Dionex Corporation, Sunnyvale, CA, USA) as described below. The gradient was 0–400 mM NaOAc in 100 mM NaOH during 40 min, followed by a 5 min washing step (1 M NaOAc in 100 mM NaOH) and 15 min equilibration of the column (100 mM NaOH). For quantification, standard calibration curves of fructose, glucose, and sucrose were used (0.001–0.005 mg/mL in NaOAc buffer).

#### 2.1.2 High performance size exclusion chromatography

High performance size exclusion chromatography was used to determine the molecular weight distribution of wheat arabinoxylan, barley  $\beta$ -glucan, and sugar beet pectin. A Dionex Ultimate 3000 HPLC (Dionex) with a RI-101 refractive index detector (Shodex RI 101; Showa Denko K.K., Kawasaki, Japan), equipped with three TSK-Gel columns (Tosoh Bioscience, Tokyo, Japan) connected in series (4000-3000-2500 Super AW; 150  $\times$  6 mm) was used. Additionally, a TSK Super AW-L guard column (35  $\times$  4.6 mm; Tosoh Bioscience) was applied. The polysaccharides were eluted with 0.2 M NaNO<sub>3</sub> at 40°C with a flow rate of 0.6 mL/min. Pullulan molecular-mass standards (Polymer Laboratories, Palo Alto, CA, USA) were used for calibration.

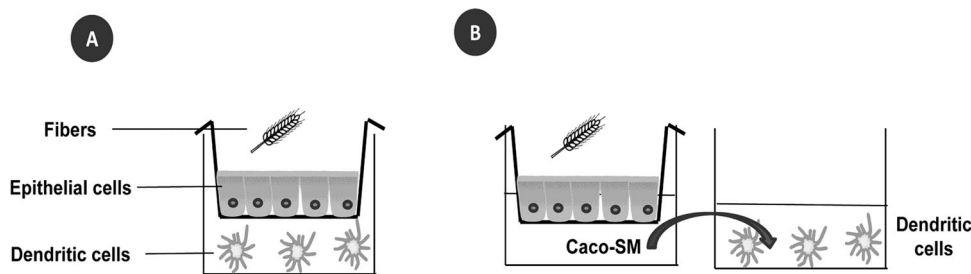
#### 2.1.3 High performance anion exchange chromatography

In order to characterize chicory inulin and GOS, HPAEC on a ICS5000 system (Dionex Corporation) with a Dionex CarboPac PA-1 column (2  $\times$  250 mm) in combination with a CarboPac PA-1 guard column (2  $\times$  50 mm) was used. The flow rate was 0.3 mL/min with a gradient starting with 0–40% 1 M NaOAc in 0.1 M NaOH within 40 min, followed by 6 min isocratic 100% 1 M NaOAc in 0.1 M NaOH. Subsequently, the column was washed for 19 min with 0.1 M NaOH. An ICS5000 ED (Dionex) pulsed amperometric detector and the software Chromeleon version 7 were used.

Endotoxin levels as tested by a Limulus amoebocyte lysate assay of all used dietary fibers samples were always below  $0.3 \times 10^{-3} \mu\text{g}^{-1}$  which has no effect on the responsiveness of the cells.

## 2.2 In vitro cell culture methods

First, we analyzed the effects of the fibers on human DCs in order to investigate if dietary fibers can activate immune cells by direct contact. The next step was to determine whether the DCs cytokine responses are affected by IECs. To this end, an in vitro transwell co-culture model was used [21–23]. IECs



**Figure 1.** Design of the coculture studies. DCs were cocultured with the fibers with in between an epithelial layer (A) or with medium released from epithelial cells after fiber stimulation (B).

were cultured in transwell inserts and incubated with DCs in the lower chamber (Fig. 1A). After fiber stimulation, cytokine production was measured and used as a measure for DC responses.

In order to identify if IECs, after contact with fibers, can confer signals to immune cells on the basolateral side in the intestine, such as DCs, supernatants of IECs stimulated with the fibers (Caco spent medium, i.e. Caco-SM) were incubated with DCs (Fig. 1B). These latter studies were done in the absence and presence of the MyD88 inhibitor Pepinh-myd88 to study TLRs dependency of the DC activation.

Finally, T cells were exposed to fiber-stimulated IECs-DCs supernatant to study the effects of humeral factors released by IECs and DCs exposed to fibers on naïve-T-cell activation and their role in the induction of immune response.

### 2.2.1 DCs and T cells

DCs, generated from umbilical cord blood CD[34]<sup>+</sup> progenitor cells (hematopoietic stem cells), and autologous T cells were supplied by Mattek Corporation (Ashland, MA, USA) [21–23]. The T cells are obtained from umbilical cord blood by negative selection (Dynal beads) and characterized by FACS analysis for purity using antibodies against CD3, CD4, and CD8. The T cells are CD3<sup>+</sup> (97–99%). Both DCs and autologous T cells were cultured according to the manufacturer's instructions.

### 2.2.2 Generation of an epithelial cell monolayer

Human colon carcinoma Caco-2 cells were grown in flasks (T-25/75 cm<sup>2</sup>) in DMEM (Gibco, Life technologies, Bleiswijk, The Netherlands) supplemented with 10% fetal calf serum (Hyclone, Thermo Scientific, Breda, The Netherlands), 2 mM L-glutamine, 1% nonessential aminoacids (Gibco), and 50 µg/mL Penicillin-Streptomycin Solution (ATCC®, The Netherlands). Thereafter, Caco-2 cells were seeded in the upper chamber of a transwell filter (3 µm pore size, 6.5 mm diameter; Corning, NY, USA) for 15–21 days. The cells were grown to confluence until the trans-epithelial resistance reached 300 Ωcm<sup>2</sup>.

### 2.2.3 Cells stimulation

In all the experiments, the fibers were dissolved in culture medium at a final concentration of 400 µg/mL and added to the cells for a period of 24 h (37°C, 5% CO<sub>2</sub>).

First, 3 × 10<sup>5</sup> DCs were exposed directly to dietary fibers for 24 h. Supernatants were collected for cytokine measurements. DCs were analyzed phenotypically for expression of surface activation markers (CD83, CD86, and anti-human leukocyte antigen-DR (HLA-DR)).

The second step was co-culturing IECs and DCs (Fig. 1A). Briefly, IEC-containing filters were turned upside down and 5 × 10<sup>4</sup> DCs were seeded on the filter facing the basolateral membrane of epithelial cells for 4 h to allow the cells to attach to the filters. Then, filters were turned again into a 24-well plate, containing 3 × 10<sup>5</sup> DCs/well at the lower chamber [21–23]. The apical surface of IECs monolayers was challenged by dietary fibers in the upper chamber for 24 h. Supernatants were collected from the lower chamber for cytokine analysis and DCs were analyzed by flow cytometry.

The third step was to study whether IECs produce soluble signals that may affect DCs. Therefore, DCs were exposed to supernatants collected from IECs after fiber stimulation. In the subsequent sections, this supernatant will be referred to as Caco-SM (Fig. 1B). IECs monolayers were first incubated in the upper chamber with dietary fibers. The fibers were added on the apical surface (upper chamber) for 24 h. Afterward, culture supernatants, i.e. the Caco-SM, were collected by aspiration. The Caco-SM was added to DC cultures for 24 h. The supernatant was collected for measurement of cytokines and chemokines. The DCs were also analyzed for activation markers by flow cytometry.

The fourth step was to study whether the fiber-stimulated DCs-IECs supernatant, thus the DC-SM, stimulate or inhibit T cells. Therefore, the medium of DCs exposed to the fibers (DC-SM) and the Caco-SM was added to autologous T cells to study possible T-cell polarization. T-cell polarization is defined as change in producing either Th1 or Th2, Th17 or Treg patterns of cytokines [24]. Supernatant were analyzed before and after addition to the T cells to confirm that the cytokines were derived from the T cells.

DC-SM from the different dietary fibers was added to T-cell cultures. After 24-h stimulation, supernatants were collected for cytokine measurements. T cells were analyzed by flow

cytometry for T-cell subset specific surface markers. Negative controls were nonstimulated Caco-SM.

### 2.2.4 Assessment of TLR dependency

To study the involvement of TLRs on DC activation upon fibers stimulation, DCs were exposed either to fibers or to Caco-SM in the presence and absence of MyD88 inhibitor Pepinh-myd88 (InvivoGen, Toulouse, France). MyD88 is an adapter protein that links TLRs and IL-1 receptors with downstream signaling molecules. Only TLR-3 is an exception as this receptor is signaling in a Myd88-independent fashion [25]. Briefly, DCs were pretreated with 25  $\mu$ M Pepinh-myd88 for 6 h at 37°C. Then, the dietary fibers or Caco-SM were added and incubated for 24 h. Afterward culture supernatants were collected for cytokine quantification.

### 2.2.5 DC staining

Three-color fluorescence staining was performed using rat or mouse anti-human MAb and their isotype controls: HLA-DR (FITC), anti-CD86 (PE/Cy7), and anti-CD83 (APC; all purchased from Biolegend, San Diego, CA, USA). In short,  $3 \times 10^5$  cells were centrifuged at 1200 rpm for 7 min and then incubated in FACS buffer containing 10% v/v human serum for 30 min to prevent nonspecific antibody staining. Subsequently, the cells were incubated with the appropriate fluorescein-conjugated anti-human antibody for 30 min, in the dark. Afterward, cells were washed three times with FACS buffer (PBS containing 2% heat-inactivated fetal calf serum). The whole procedure was performed on ice.

### 2.3 Flow cytometric analysis

Flow cytometry was performed using the FACSCalibur Flow Cytometer (BD Bioscience, San Jose, CA, USA). Data analysis was performed using Flowjo 7.6.5 software. During analysis, 20 000 events, gated on an FSC versus SSC dot plot, based on viability, were recorded. CD83, CD86, or HLA-DR isotype controls were used to set the gate to 99% negative cells (Fig. 2).

### 2.4 Cytokine expression

After 24 h of incubation cytokine levels in the supernatant were measured using a MilliPlex™ premixed cytokine assay, according to the manufacturer's instructions (Linco Research Inc, MO, USA). This customized kit measures simultaneously several of the following molecules: human IFN- $\gamma$ , IL-12p40, IL-10, IL-17, IL-1 $\beta$ , IL-1Ra, IL-2, IL-4, IL-6, IL-8, MCP-1/ CCL2, MIP-1 $\alpha$ / CCL3, RANTES/ CCL5, and TNF- $\alpha$ . Concentration series of cytokine standards were prepared for the appropriate concentration range, and coupled beads were

diluted ten times, resuspended, and added to a prewetted filter plate. After washing the plate twice, standards, negative controls and samples (all in duplicate) were transferred into the plate (50  $\mu$ L per well), and the plate was sealed and incubated on a shaker at 4°C overnight (16–18 h) in the dark. After incubation, the plate was washed three times. Detection antibodies were resuspended and diluted ten times and 25  $\mu$ L was added to each well. The plate was incubated on a shaker at room temperature for 1 h in the dark. After washing three times, 50  $\mu$ L of streptavidin-phycoerythrin was added to each well and the plate was incubated on a shaker at room temperature for 30 min in the dark. After washing the plate three times, 125  $\mu$ L of assay buffer was added per well. The plate was incubated on a shaker for 5 min and fluorescence was measured using a Luminex 100 System and StarStation software.

To evaluate whether the dietary fibers induce a more anti-inflammatory or proinflammatory effect in the DCs, the IL-10/IL-12 ratio was calculated for each dietary fiber. A higher IL-10/IL-12 ratio is representative for a regulatory or anti-inflammatory effect [5].

### 2.5 Statistical analysis

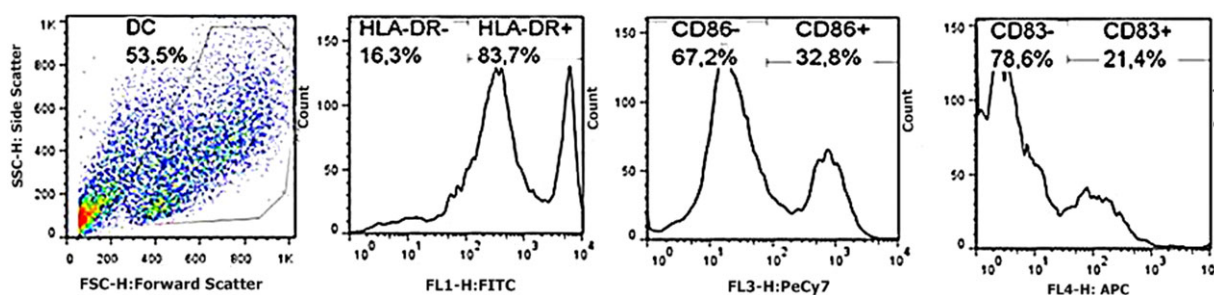
Differences between treatments compared to controls (non-stimulated DCs) were assessed by Kruskal–Wallis test. Posthoc analysis was performed using Bonferroni multiple comparison test. Results are expressed as mean  $\pm$  SD, respectively. Analyses were performed using NCSS 2007 software (Kaysville, UT, USA). A *p*-value < 0.05 was considered statistically significant.

## 3 Results

### 3.1 Characterization of GOS, chicory inulin, sugar beet pectin, wheat arabinoxylan, barley $\beta$ -glucan

The GOS has been enzymatically produced from lactose and consisted of galactose (73%) and glucose (27%). HPAEC (Fig. 3A) demonstrated the presence of small amounts of monomeric glucose and galactose (G) and lactose (L), next to a broad range of oligosaccharides ranging from DP 2 to 7 as has been described before [26]. The sugar composition of the inulin was analyzed after enzymatic hydrolysis into glucose and fructose, followed by HPLC analysis of these sugars. The chicory inulin tested consisted of 98% fructose and only 2% glucose. HPAEC analysis of individual oligomers (Fig. 3B) showed the presence of mainly higher GF<sub>n</sub> type fructan-oligomers (5). The sugar composition of the other fibers after acid hydrolysis to monomers is presented in Table 1.

The barley  $\beta$ -glucan consists solely of glucose connected within the polymer through either 1–3 or 1–4 linkages [27]. The wheat arabinoxylan consists of xylose (65%) organized in a  $\beta$ -1-4-linked xylan backbone, substituted with arabinose



**Figure 2.** Dendritic cells (DCs) were gated in the forward side scatter plot, based on viability. Within the DC population, CD83, CD86, or HLA-DR isotype controls were used to set the gate to 99% negative cells. This gate was copied to the samples stained for CD80, CD86, and CD103 and the frequency of positive cells was determined.

(35%) as single unit substituents linked to O-2 and/or O-3 of the xylose residues [28]. Both  $\beta$ -glucan and wheat arabinoxylan have a similar homogeneous molecular weight distribution with a molecular mass between  $10^5$  and  $10^6$  Da (Fig. 4). The sugar beet pectin is the most complex dietary fiber in this study. The main building block is galacturonic acid present in a  $\alpha$ -1-4-linked galacturonan backbone, while these galacturonan segments are interrupted by rhamnogalacturonan I structural elements consisting of alternating rhamnose and galacturonic acid moieties with arabinose- and galactose-rich side chains linked to the rhamnose [29]. The molecular weight distribution of sugar beet pectin is also between  $10^5$  and  $10^6$  Da, but the range is broader in comparison to the other two polysaccharides and there are also molecules with a molecular mass between  $10^4$  and  $10^5$  Da present.

### 3.2 Dietary fibers induce a regulatory phenotype in human DCs

First, we investigated the effects of GOS, chicory inulin, sugar beet pectin, wheat arabinoxylan, barley  $\beta$ -glucan on human IFN- $\gamma$ , IL-10, IL-12p40, IL-1 $\beta$ , IL-1Ra, IL-6, IL-8, MCP-1, MIP-1 $\alpha$ , RANTES, and TNF- $\alpha$  production by DCs (Table 2). To this end, DCs were incubated with one of the fibers.

There were no statistical significant effects of the dietary fibers on the proinflammatory cytokine IL-12 although some reduction was found with chicory inulin, wheat arabinoxylan, and barley  $\beta$ -glucan. However, the regulatory cytokine IL-10 was reduced by wheat arabinoxylan ( $p < 0.05$ ). The other

fibers had no effect on IL-10 production. The production of IL-8 was increased by GOS, sugar beet pectin, and barley  $\beta$ -glucan ( $p < 0.05$ ). IL-6 production was reduced after wheat arabinoxylan stimulation. The production of MCP-1 was decreased by wheat arabinoxylan (Table 2).

The activation state of the DCs was also studied by quantifying the expression of the activation markers CD83, CD86 as well as HLA-DR on the DCs. As shown in Table 3, only barley  $\beta$ -glucan elevated the expression of CD83 ( $p < 0.05$ ).

### 3.3 Modulation of responses by DCs following direct contact with IECs

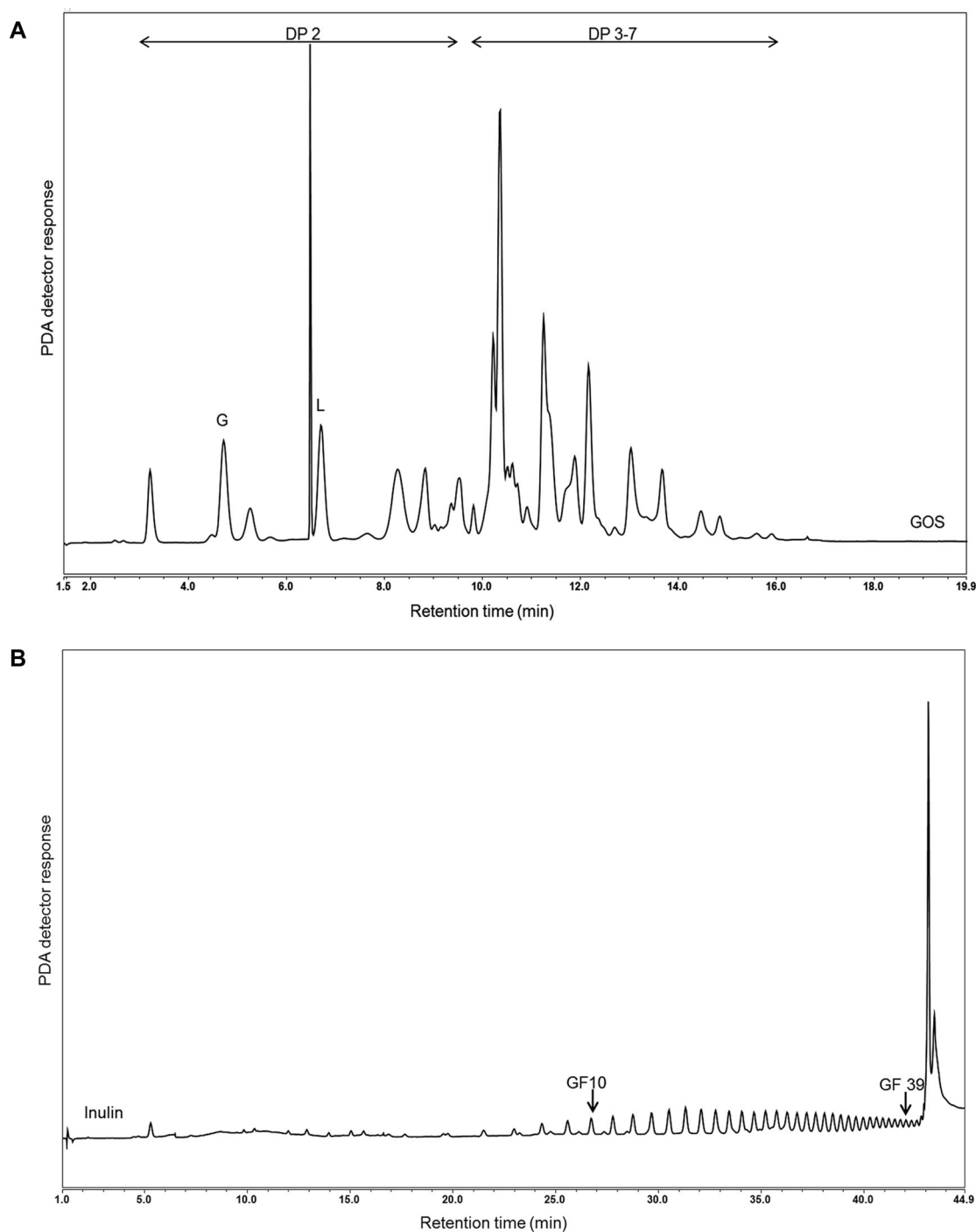
It is well recognized that the crosstalk between DCs and IECs is essential for maintaining gut homeostasis and DC phenotype [13]. For this reason, we investigated how IECs, stimulated with the fibers, can impact DC behavior across an epithelial barrier. This was done by using an in vitro transwell co-culture system (Fig. 1A). The effects of GOS, chicory inulin, sugar beet pectin, wheat arabinoxylan, barley  $\beta$ -glucan on IFN- $\gamma$ , IL-10, IL-12p40, IL-1 $\beta$ , IL-1Ra, IL-6, IL-8, MCP-1, MIP-1 $\alpha$ , RANTES, and TNF- $\alpha$  production by DCs and IECs were analyzed.

As shown in Table 4, the proinflammatory cytokine IL-12 was decreased by GOS, chicory inulin, sugar beet pectin, and wheat arabinoxylan and barley  $\beta$ -glucan (after which exposure it was undetectable;  $p < 0.05$ ). In addition, the production of the proinflammatory cytokines IL-6 and IL-8 was decreased by all the fibers tested ( $p < 0.05$ ). Moreover, all the fibers tested

**Table 1.** Analyzed constituent monosaccharide composition (mol%) of the fibers

	(mol%)								(w/w%)
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronic acid	Total
Oat $\beta$ -glucan	0	0	0	0	0	0	99	1	88
Wheat arabinoxylan	0	0	35	67	0	0	0	0	82
Sugar beet pectin	2	0	6	0	0	11	1	80	71
GOS	0	0	0	0	0	61	23	1	85



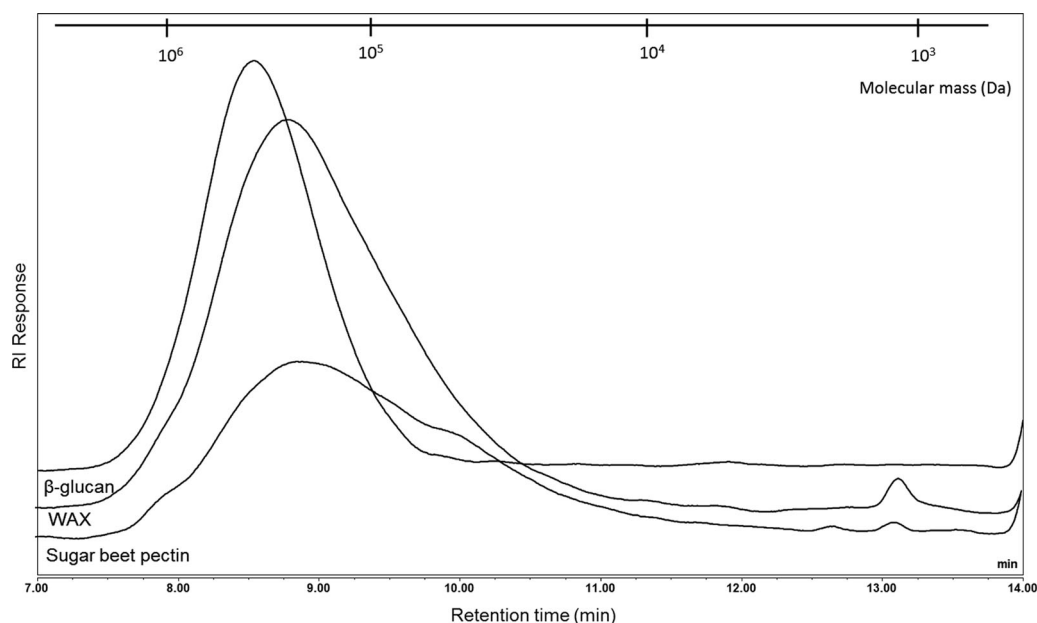


**Figure 3.** High performance anion exchange chromatography (HPAEC) of the fibers GOS (A) and Inuline (B). G, galactose/glucose; L, lactose. GF10 indicates a decamer of nine fructoses and one glucose.

reduced the release of IL-1Ra, a natural inhibitor of proinflammatory IL-1 $\beta$ . No statistical differences were observed for the chemokines MCP-1, MIP-1 $\alpha$ , and RANTES after dietary fiber stimulation. The chemokine MCP-1 was slightly increased by GOS and pectin.

### 3.4 IECs derived factors induce proinflammatory responses in DCs

As IECs have been described to be producers of many bioactive factors involved in both proinflammatory and



**Figure 4.** High performance size exclusion chromatography (HPSEC) of  $\beta$ -glucan, wheat arabinoxylan, and sugar beet pectin. Calibration of the system with pullulan is indicated on top of the graph.

regulatory responses, we decided to repeat the above experiment in medium obtained from Caco-2 culture (Caco-SM; Fig. 1B).

As shown in Table 5 cytokine production of DCs was indeed modulated by Caco-SM from untreated Caco cells. Caco-SM enhanced the production of the proinflammatory cytokines IFN- $\gamma$ , IL-12p40, IL-6, IL-8, MCP-1, MIP-1 $\alpha$ , RANTES, and TNF- $\alpha$  when compared to unstimulated DCs ( $p < 0.005$ ). The anti-inflammatory cytokine IL-10 was also enhanced.

Incubation of Caco cells with the dietary fibers attenuated the proinflammatory phenotype induced by Caco-SM

(Table 5). When Cacos were pretreated with dietary fibers, a statistical significantly diminished release of IL-12, IL-6 and IL-1Ra, TNF- $\alpha$ , MIP-1 $\alpha$ , RANTES, MCP-1 ( $p < 0.05$ ) was observed in DCs exposed to dietary-treated Caco-SM. However, also the regulatory cytokine IL-10 was reduced compared to nontreated Caco-SM, but not compared to nonstimulated DCs (Table 5). Overall, however, despite the IL-10 decrease, the dietary fibers increased the IL-10/IL-12 ratio of Caco-SM-treated DCs (Fig. 5). This reached statistical significance for chicory inulin, sugar beet pectin, wheat arabinoxylan, barley  $\beta$ -glucan, and GOS ( $p < 0.005$ ). These findings clearly

**Table 2.** Effects of GOS, chicory inulin, sugar beet pectin, wheat arabinoxylan, barley  $\beta$ -glucan on human IFN- $\gamma$ , IL-10, IL-12p40, IL-1 $\beta$ , IL-1Ra, IL-6, IL-8, MCP-1, MIP-1 $\alpha$ , RANTES, and TNF- $\alpha$  production by DCs

	Control	GOS	Inulin	Pectin	Arabinoxylan	$\beta$ -Glucan
IFN- $\gamma$	0.06 $\pm$ 0.00	0.06 $\pm$ 0.00	0.06 $\pm$ 0.00	0.09 $\pm$ 0.02	0.06 $\pm$ 0.00	0.09 $\pm$ 0.02
IL-10	2.13 $\pm$ 0.17	1.61 $\pm$ 0.13	1.61 $\pm$ 0.07	1.97 $\pm$ 0.25	1.39 $\pm$ 0.04*	1.61 $\pm$ 0.04
IL-12p40	54.31 $\pm$ 3.45	48.6 $\pm$ 7.11	37.16 $\pm$ 2.33	55.27 $\pm$ 12.05	33.35 $\pm$ 1.49	41.93 $\pm$ 1.55
IL-1 $\beta$	37.78 $\pm$ 1.50	36.31 $\pm$ 1.71	35.44 $\pm$ 1.56	37.18 $\pm$ 1.24	39.87 $\pm$ 0.86	37.96 $\pm$ 2.19
IL-1Ra	8896.33 $\pm$ 30.62	9289.56 $\pm$ 285.06	9356.82 $\pm$ 188.45	8544.49 $\pm$ 254.65	8108.83 $\pm$ 199.42*	8207.14 $\pm$ 219.92
IL-6	9.52 $\pm$ 0.22	9.52 $\pm$ 0.39	9.04 $\pm$ 0.32	8.33 $\pm$ 0.49	7.14 $\pm$ 0.32*	8.09 $\pm$ 0.32
IL-8	310.85 $\pm$ 5.94	346.14 $\pm$ 5.32*	331.87 $\pm$ 5.81	340.31 $\pm$ 9.55	283.83 $\pm$ 11.77	339.37 $\pm$ 5.06
MCP-1	167.86 $\pm$ 6.10	183.23 $\pm$ 2.43	156.04 $\pm$ 4.60	161.95 $\pm$ 6.57*	141.85 $\pm$ 4.02*	157.22 $\pm$ 1.85*
MIP-1 $\alpha$	35.03 $\pm$ 1.49	40.47 $\pm$ 13.86	25.11 $\pm$ 1.17	41.87 $\pm$ 14.86	16.19 $\pm$ 1.54	37.12 $\pm$ 3.28
RANTES	4.68 $\pm$ 0.25	7.9 $\pm$ 1.31	7.9 $\pm$ 0.25	7.58 $\pm$ 1.80	3.55 $\pm$ 0.22	5.81 $\pm$ 0.26
TNF- $\alpha$	46.17 $\pm$ 0.99	47.66 $\pm$ 0.99	36.49 $\pm$ 8.57	68.51 $\pm$ 22.43	36.49 $\pm$ 2.30	62.55 $\pm$ 6.40

The data shown are the means and SD of four different experiments. Differences between treatments compared to controls (nonstimulated DCs) were assessed by Kruskal–Wallis test. Posthoc analysis was performed using Bonferroni multiple comparison test.  $p$ -Values  $< 0.05$  are denoted with asterisk (\*).

**Table 3.** Expression of the activation markers CD83, CD86, and HLA-DR on the DCs, stimulated with GOS, inulin, pectin, arabinosyloxylan, or  $\beta$ -glucan

	Control	GOS	Inulin	Pectin	Arabinosyloxylan	$\beta$ -Glucan
CD83	23.58 $\pm$ 3.45	21.68 $\pm$ 0.94	24.1 $\pm$ 0.44	23.6 $\pm$ 1.10	22.28 $\pm$ 2.16	28.55 $\pm$ 1.82*
CD86	29.85 $\pm$ 3.24	29.9 $\pm$ 0.78	29.32 $\pm$ 0.38	32.7 $\pm$ 1.17	31.4 $\pm$ 2.44	35.83 $\pm$ 1.78
HLA-DR	66.7 $\pm$ 3.02	67.92 $\pm$ 2.31	69.12 $\pm$ 0.49	67.9 $\pm$ 1.18	72.42 $\pm$ 2.34	70.98 $\pm$ 1.27

Numbers indicate the mean percentage of positive cells in the gate and SD of four different experiments. Differences between treatments compared to controls (nonstimulated DCs) were assessed by Kruskal–Wallis test. Posthoc analysis was performed using Bonferroni multiple comparison test. *p*-Values < 0.05 are denoted with asterisk (\*).

demonstrate the essence of combined presence of tolerogenic signals from IECs and the dietary fibers on the generation of tolerogenic DCs. This was further strengthened by the FACS analysis on DCs where we observed a reduction of CD83 and CD86 by Caco-SM of dietary fibers (*p* < 0.005; Table 6).

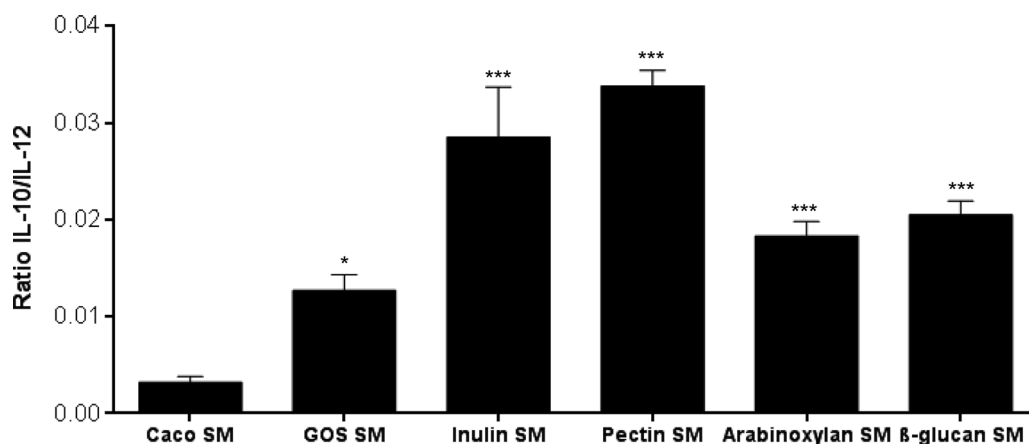
### 3.5 Attenuation of the proinflammatory responses is MyD88 dependent

Next, we studied the involvement of TLR adaptor MyD88 in the attenuation of IFN- $\gamma$ , IL-12p40, IL-6, IL-8, MCP-1, MIP-1 $\alpha$ , RANTES, and TNF- $\alpha$  production by DCs. To this end we

**Table 4.** Production of IFN- $\gamma$ , IL-10, IL-12p40, IL-1 $\beta$ , IL-1Ra, IL-6, IL-8, MCP-1/ CCL2, MIP-1 $\alpha$ / CCL3, RANTES/ CCL5, and TNF- $\alpha$  by DCs co-cultured with IECs in transwell and stimulated with dietary fibers for 24 h

	Control	GOS	Inulin	Pectin	Arabinosyloxylan	$\beta$ -Glucan
IFN- $\gamma$	0.06 $\pm$ 0.00	0.06 $\pm$ 0.00	0.06 $\pm$ 0.00	0.06 $\pm$ 0.00	0.06 $\pm$ 0.00	0.06 $\pm$ 0.00
IL-10	1.34 $\pm$ 0.00	0.99 $\pm$ 0.10	1.03 $\pm$ 0.10	0.94 $\pm$ 0.04	0.9 $\pm$ 0.04	0.94 $\pm$ 0.09
IL-12p40	26.68 $\pm$ 0.90	20.01 $\pm$ 1.96*	19.06 $\pm$ 2.70*	20.01 $\pm$ 1.49*	17.15 $\pm$ 0.00*	0
IL-1 $\beta$	37.09 $\pm$ 5.63	45.25 $\pm$ 1.73	43.52 $\pm$ 2.36	39.87 $\pm$ 4.47	33.79 $\pm$ 3.01	31.96 $\pm$ 1.78
IL-1Ra	8293.03 $\pm$ 345.80	6705.62 $\pm$ 271.22*	6866.01 $\pm$ 438.71*	6749.08 $\pm$ 187.40*	6832.9 $\pm$ 227.72*	7216.82 $\pm$ 143.75*
IL-6	7.14 $\pm$ 0.00	5.23 $\pm$ 0.63*	5.47 $\pm$ 0.66*	5.00 $\pm$ 0.19*	4.76 $\pm$ 0.22*	5.00 $\pm$ 0.58*
IL-8	327.31 $\pm$ 2.53	250.15 $\pm$ 13.65*	248.98 $\pm$ 25.34*	244.32 $\pm$ 5.79*	239.4 $\pm$ 9.21*	251.97 $\pm$ 10.64*
MCP-1	230.51 $\pm$ 10.39	290.8 $\pm$ 22.51	242.33 $\pm$ 33.78	257.70 $\pm$ 15.84	234.06 $\pm$ 4.17	234.06 $\pm$ 28.32
MIP-1 $\alpha$	22.85 $\pm$ 0.67	19.37 $\pm$ 1.42	16.93 $\pm$ 2.31	18.54 $\pm$ 1.34	16.45 $\pm$ 0.62	17.41 $\pm$ 2.77
RANTES	4.19 $\pm$ 0.22	4.35 $\pm$ 0.54	3.23 $\pm$ 0.46	3.71 $\pm$ 0.25	3.23 $\pm$ 0.15	3.39 $\pm$ 0.54
TNF- $\alpha$	26.06 $\pm$ 2.08	27.55 $\pm$ 3.04	29.04 $\pm$ 4.59	29.79 $\pm$ 1.21	28.3 $\pm$ 0.99	28.3 $\pm$ 2.62

The data shown are the means and SD of three different experiments, in duplicates. Differences between treatments compared to controls (nonstimulated DCs) were assessed by Kruskal–Wallis test. Posthoc analysis was performed using Bonferroni multiple comparison test. *p*-Values < 0.05 are denoted with asterisk (\*).

**Figure 5.** IL-10/IL-12 ratio increased upon fibers stimulation. DCs were stimulated with fibers stimulated IECs supernatants for 24 h. Statistical significance levels were determined with Kruskal–Wallis test followed by Bonferroni multiple comparison test. Mean and SD of the IL-10/IL-12 ratio is plotted for the different dietary fibers-IECs supernatant. \**p*-Values < 0.05, \*\**p*-values < 0.01, and \*\*\**p*-values < 0.005.



**Table 5.** The production of IFN- $\gamma$ , IL-10, IL-12p40, IL-1 $\beta$ , IL-1Ra, IL-6, IL-8, MCP-1/CCL2, MIP-1 $\alpha$ /CCL3, RANTES/CCL5, and TNF- $\alpha$  by DCs incubated with Caco-2 SM taken after 24 h of stimulation with the respective fiber was measured

	Control	Caco-SM	GOS SM	Inulin SM	Pectin SM	Arabinoxylan SM	$\beta$ -Glucan SM
IFN- $\gamma$	0.06 $\pm$ 0.00	1.50 $\pm$ 0.05***	0.73 $\pm$ 0.07***	0.66 $\pm$ 0.00***	0.66 $\pm$ 0.00***	0.66 $\pm$ 0.00***	0.66 $\pm$ 0.00***
IL-10	2.13 $\pm$ 2.16	29.47 $\pm$ 1.69***	4.58 $\pm$ 0.70#	2.45 $\pm$ 0.06#	2.51 $\pm$ 0.06#	4.35 $\pm$ 0.73#	3.85 $\pm$ 0.98#
IL-12p40	447.67 $\pm$ 47.36	9081.6 $\pm$ 1584.66	360.26 $\pm$ 89.58#	85.85 $\pm$ 17.73#	74.37 $\pm$ 5.28#	237.13 $\pm$ 23.26#	188.08 $\pm$ 46.69#
IL-1 $\beta$	52.37 $\pm$ 0.18	41.29 $\pm$ 1.87	28.96 $\pm$ 2.67*	25.25 $\pm$ 1.59***	27.91 $\pm$ 0.33*	39.98 $\pm$ 2.05	39.61 $\pm$ 1.34
IL-1Ra	8896.33 $\pm$ 30.62	10 938.72 $\pm$ 1882.80#	4503.03 $\pm$ 723.31*	3543.89 $\pm$ 213.43**	4205.37 $\pm$ 210.04**	4069.19 $\pm$ 254.55**	3795.16 $\pm$ 348.46**
IL-6	34.12 $\pm$ 2.44	975.28 $\pm$ 44.78***	12.97 $\pm$ 2.23#	6.78 $\pm$ 0.14#	7.19 $\pm$ 0.28#	10.44 $\pm$ 0.64#	9.31 $\pm$ 1.32#
IL-8	991.98 $\pm$ 18.38	1252.05 $\pm$ 51.67***	476.41 $\pm$ 49.23**	277.9 $\pm$ 12.25**	307.64 $\pm$ 9.95**	394.74 $\pm$ 13.25**	350.56 $\pm$ 29.18**
MCP-1	826.33 $\pm$ 45.81	2306.12 $\pm$ 103.21***	376.68 $\pm$ 49.77**	307.58 $\pm$ 20.55***	345.22 $\pm$ 10.93***	415.39 $\pm$ 17.30***	358.71 $\pm$ 6.10***
MIP-1 $\alpha$	242.84 $\pm$ 13.40	1268.43 $\pm$ 50.05***	125.59 $\pm$ 23.12#	58.78 $\pm$ 6.20***	59.92 $\pm$ 4.51***	114.74 $\pm$ 6.89**	92.56 $\pm$ 10.63**
RANTES	33.13 $\pm$ 1.90	126.81 $\pm$ 3.69***	13.74 $\pm$ 2.11***	6.35 $\pm$ 0.45**	6.50 $\pm$ 0.07***	8.79 $\pm$ 0.54***	7.95 $\pm$ 0.84***
TNF- $\alpha$	185.79 $\pm$ 3.32	1432.31 $\pm$ 51.92***	105.86 $\pm$ 21.66#	53.2 $\pm$ 2.98*	52.28 $\pm$ 1.05*	71.01 $\pm$ 4.82#	62.32 $\pm$ 4.88#

The data shown are the means and SD of three different experiments, in duplicates. The # symbol indicates differences between DCs treated with Caco-SM and DCs treated with the supernatant of IECs stimulated with dietary fibers  $p < 0.05$ .

\* $p$ -Values  $< 0.05$ .

\*\* $p$ -Values  $< 0.01$ .

\*\*\* $p$ -Values  $< 0.005$ .

repeated the foregoing experiment in which Caco-SM was incubated with  $1 \times 10^6$  DCs in the presence and absence of a MyD88 inhibitor. The attenuating effect of Caco-SM after dietary fiber exposure was diminished by the MyD88 inhibitor (not shown). The role of MyD88 in the attenuating cytokine production was most illustrative with IL-8 production, which was significantly enhanced when the inhibitor was applied (Fig. 6).

### 3.6 Fibers induce T-cell polarization in a differential fashion

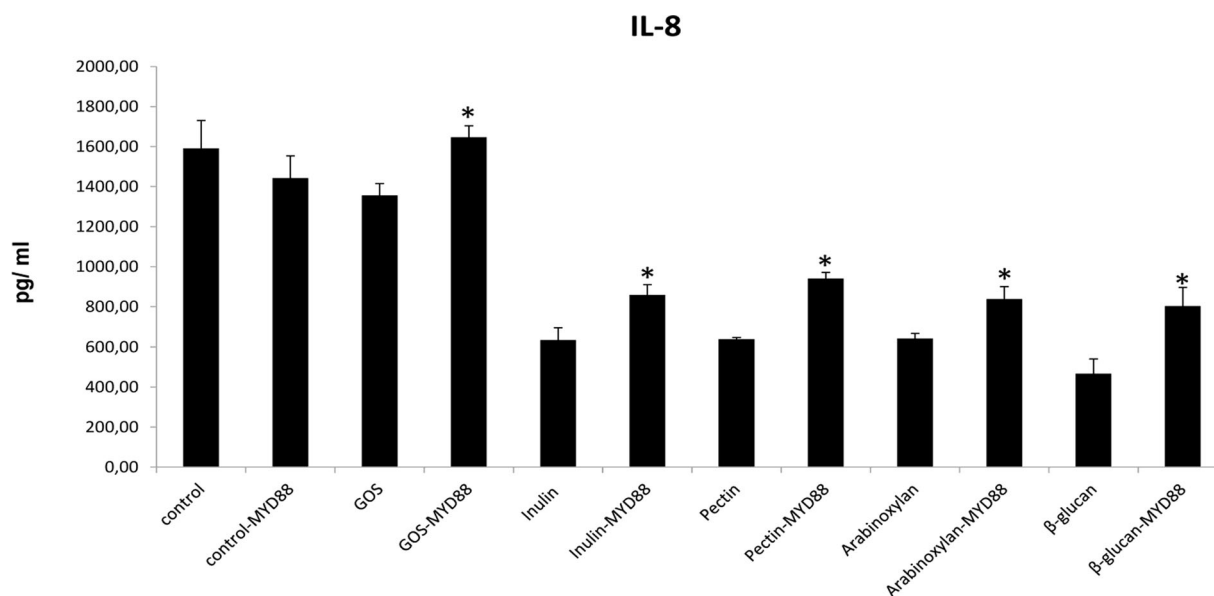
Next, we studied the effect of DCs stimulated with IECs medium (DC-SM) exposed to either GOS, chicory inulin, sugar beet pectin, wheat arabinoxylan, barley  $\beta$ -glucan on T-cell polarization. To this end, autologous naive T cells were exposed to medium of co-cultures of IECs and DCs, i.e. DC-SM. The Th1 cytokines IL-2, IFN- $\gamma$ , TNF- $\alpha$ ; the Th2 cytokines IL-4, IL-6; the Th17 cytokine IL-17; and the Treg cytokine IL-10 were studied (Table 7).

As shown in Table 7, GOS, chicory inulin, wheat arabinoxylan, and barley  $\beta$ -glucan ( $p < 0.05$ ) slightly increased the production of the Th1 cytokines IFN- $\gamma$ , while sugar beet pectin had no effect. After barley  $\beta$ -glucan exposure, the Th1 cytokine IL-2 production was also enhanced. TNF- $\alpha$ , the third Th1 cytokines tested, was decreased by GOS and wheat arabinoxylan.

The Th2 cytokine IL-6 was reduced ( $p < 0.05$ ) by chicory inulin and barley  $\beta$ -glucan. The other Th2 cytokine was not influenced by the dietary fibers. The Th17 cytokine, IL-17, was increased by GOS, chicory inulin, wheat arabinoxylan, and barley  $\beta$ -glucan but this never reached significant differences. The Treg-promoting cytokine IL-10 was increased in the T cells by GOS whereas chicory inulin decreased the IL-10 production ( $p < 0.05$ ; Table 7).

## 4 Discussion

To the best of our knowledge, this is the first study addressing the crosstalk between different cells in the mucosa after stimulation with dietary fibers in relation to its final impact on DCs responses. When studying dietary fibers, but also probiotics, there is a tendency to focus on individual cell types [30]. Most in vitro studies have focused on interactions between dietary fibers and enterocytes due to its well-known role in barrier function [31] and its expected contact with IECs in the colon and small intestine [32]. Our findings clearly demonstrate that single cultures do not really show the potential tolerogenic effects dietary fibers can have. Our data also highlight the potential of secreted bioactive factors derived from IECs upon dietary fiber stimulation to influence IEC-DC crosstalk and finally even T-cell responses.



**Figure 6.** Attenuation of the proinflammatory responses is myd88 dependent. The production of IL-8 by  $1 \times 10^6$  DCs treated with MyD88 inhibitor and fibers stimulated IECs supernatant (Caco-SM). The samples with the Myd88 inhibitor contain –MYD88 on the x-axis. The data shown are the means and SD of five different experiments. \**p*-Values < 0.05.

Our results demonstrated, just as previously suggested by Shan et al. [33], that dietary fibers may induce anti-inflammatory responses. The present study demonstrated that this might occur via several mechanisms: the first is that the fibers in general tend to suppress the production of proinflammatory cytokines [34]. The second mechanism is by changing the balance in IL-10/IL-12 ratio in favor of the regulatory cytokine IL-10. Third, by upregulating the release of neutralizing cytokines such as IL-1Ra, a natural inhibitor of proinflammatory IL-1 $\beta$ . Also, we demonstrated that dietary fibers, just like reported for probiotics [35], may contribute to shift in T-cell polarization as will be discussed in more detail below.

In the present study, we included a detailed study on the chemical composition of the fibers. This is done routinely in our group as we have shown that the structure of the fibers has an enormous impact on the immunomodulatory effects [5]. Up to now, the molecular structure has been poorly documented and is in most cases completely absent. By including the chemical structure it will be possible to interpret the data

more soundly and it will contribute to side-by-side comparison of data sets and hopefully to reproducibility of studies. This is rationally to include the complete chemical composition of the fibers applied. Also we quantified the endotoxin level and included only fibers with a low endotoxin content to avoid that effects might be caused by endotoxin contamination such as LPS.

Gut mucosal DCs, especially the CX3CR1<sup>+</sup> DCs are considered to be involved in sampling luminal antigens for priming immune activation or tolerance [13]. We decided to study direct effects on our human DCs as it has been shown that dietary fibers are able to pass the intestinal barrier and encounter DCs in the lamina propria and in the systemic circulation directly [35]. These direct effects of dietary fibers on DCs were rather minor. Only for IL-8 we observed an up-regulation induced by GOS, sugar beet pectin, and barley  $\beta$ -glucan. Supernatant of human intestinal Caco-2 cells were required to observe profound effects of dietary fibers. Our data suggest therefore that an interplay between epithelial cells, fibers and DCs is required for inducing a regulatory immune

**Table 6.** CD83, CD86, and HLA-DR surface markers staining of DCs and fibers stimulated IECs supernatant

	Caco-SM	GOS SM	Inulin SM	Pectin SM	Arabinoxylan	$\beta$ -Glucan SM
CD83	59.15 $\pm$ 0.71	19.3 $\pm$ 2.30***	20.8 $\pm$ 0.40***	22.25 $\pm$ 1.05***	21.70 $\pm$ 2.40***	20.15 $\pm$ 3.35***
CD86	58.55 $\pm$ 0.85	20.30 $\pm$ 2.60***	21.50 $\pm$ 0.10***	25.50 $\pm$ 0.00***	27.30 $\pm$ 2.40***	25.35 $\pm$ 2.75***
HLA-DR	63.25 $\pm$ 2.69	66.20 $\pm$ 2.70	68.45 $\pm$ 2.15	47.20 $\pm$ 0.00**	65.85 $\pm$ 0.35	52.95 $\pm$ 6.35

Numbers indicate the percentage of positive cells in the gate.

\**p*-Values < 0.05.

\*\**p*-Values < 0.01.

\*\*\**p*-Values < 0.005.

**Table 7.** Effects of DCs stimulated with IECs medium (DC-SM) exposed to either GOS, chicory inulin, sugar beet pectin, wheat arabinoxylan, barley  $\beta$ -glucan on T-cell polarization

	Control	GOS	Inulin	Pectin	Arabinoxylan	$\beta$ -Glucan
IFN- $\gamma$	0.13 $\pm$ 0.00	1.26 $\pm$ 0.60*	0.57 $\pm$ 0.45*	1.15 $\pm$ 0.84	0.93 $\pm$ 0.27*	0.93 $\pm$ 0.27*
IL-2	110.32 $\pm$ 0.00	235.48 $\pm$ 57.06	114.23 $\pm$ 7.67	123.2 $\pm$ 27.8	131.35 $\pm$ 2.08	320.57 $\pm$ 39.54*
TNF- $\alpha$	55.5 $\pm$ 0.00	17.04 $\pm$ 6.66*		10.38 $\pm$ 0.00	10.38 $\pm$ 0.00*	10.38 $\pm$ 0.00
IL-4	5847.85 $\pm$ 0.00	4423.51 $\pm$ 572.29	2615.78 $\pm$ 1141.31	5310.94 $\pm$ 398.41	4797.8 $\pm$ 11.41	4724.63 $\pm$ 387.20
IL-6	10.54 $\pm$ 0.45	7.25 $\pm$ 2.45	2.00 $\pm$ 0.00*		6.75 $\pm$ 1.60	2.93 $\pm$ 0.93*
IL-17	1.7 $\pm$ 0.00	3.47 $\pm$ 0.00	2.68 $\pm$ 0.20	1.40 $\pm$ 0.24	3.08 $\pm$ 0.24	2.68 $\pm$ 0.20
IL-10	14.69 $\pm$ 0.00	21.37 $\pm$ 0.77*	11.89 $\pm$ 0.47*	11.87 $\pm$ 1.62	16.47 $\pm$ 2.59	18.80 $\pm$ 2.38

The data shown are the means and SD of three different experiments. The production of Th1 cytokines IL-2, IFN- $\gamma$ , TNF- $\alpha$ ; Th2 cytokines IL-4, IL-6; Th17 cytokine IL-17; and Treg cytokine IL-10 was studied.

\**p*-Values < 0.05.

environment. Our data agree with a recent study, which reported that dietary fiber intake, especially cereal fiber, may be associated with a decreased level of proinflammatory cytokines and a more regulatory environment [36]. In this study, the authors speculate that this might be related to an action of the fibers on the gut microflora. Our data suggest that also direct effects of cereal fiber on the mucosal cells may have contributed to the more regulatory environment.

IECs medium (Caco-2-SM) not exposed to fibers induced a proinflammatory phenotype in DCs. Especially the cytokines IL-12, IL-1Ra, IL-6, IL-8, TNF- $\alpha$ , MCP-1, and MIP-1 $\alpha$  were greatly enhanced by nonstimulated IECs derived factors. This is in line with recent *in vivo* observations demonstrating that carbohydrate moieties of MUC2 are required to avoid a proinflammatory environment in the mucosa [33]. As demonstrated in the present study, also other carbohydrates may contribute to immune homeostasis. All fibers tested diminished the release of IL-12 by more than 20-fold. Also the production of the proinflammatory TNF- $\alpha$  was tenfold reduced.

Our work adds an additional level of complexity in interpreting the mechanisms by which dietary fibers contribute to beneficial effects and longevity in certain groups of consumers [1]. Up to now, it was generally assumed that dietary fibers mainly contribute to anti-inflammatory effects by enhancing production of short-chain fatty acids by the microbiota [37–39]. Here, we have shown that also direct modulation of the IECs-DCs crosstalk can induce a regulatory immune phenotype. This effect is to our opinion pronounced as fibers increase the ratio of IL-10/IL-12 more than fourfold. This argument is strengthened by our observation that the DCs profoundly decrease CD83 and CD86 expression. Interestingly, similar effects have been reported for Muc-2, *i.e.* the highly *O*-glycosylated mucin, which is secreted by goblet cells in the epithelium [40, 41]. Recently, Shan *et al.* [33] demonstrated that Muc2 provides anti-inflammatory signals to DCs in the lamina propria. This results in more tolerogenic CD103<sup>+</sup>CD11b<sup>+</sup>CX3CR1<sup>-</sup> DCs which finally prevents inflammatory responses.

In recent studies, we [5] and others [42] have demonstrated that dietary fibers can signal and modulate immune

responses via binding to PRRs [5, 43, 44]. Our data suggest that TLR receptors are also involved in the effects of the currently tested fibers as the immunomodulating capacity was reduced in the presence of a MyD88 inhibitor [5, 30, 44]. The TLR signaling pathway, except for TLR-3, involves the recruitment of MyD88, which activates the MAPK and NF $\kappa$ B signaling pathways. Our data of dietary fibers signaling via TLR are corroborated by the observation that  $\beta$ 2 $\rightarrow$ 1-fructans such as inulin can modulate immune responses and induce expedited recovery from barrier damage in human enterocytes a TLR-2-dependent fashion [44]. TLR-2 was also implicated in yeast zymosan  $\beta$ -glucan-induced cytokine production [45]. Ortega-González *et al.* [32] demonstrate that a small SP  $\beta$ 2 $\rightarrow$ 1-fructans (FOS) and other inulins and GOS can also act via TLR-4 in IECs.

The different fibers have differential effects on T-cell polarization. This was tested by co-incubating T cells with the supernatant of DCs-epithelial cell cultures stimulated with the respective dietary fiber. GOS, chicory inulin, wheat arabinoxylan, and barley  $\beta$ -glucan slightly increased the production of the Th1 cytokine IFN- $\gamma$ , whereas the Th1 cytokine TNF- $\alpha$  was decreased by GOS and wheat arabinoxylan. Overall, the most pronounced effect is that of barley  $\beta$ -glucan that increased the Th1 cytokine IL-2. This is in line with several reports that demonstrate that fungus  $\beta$ -glucan binding stimulate the polarization of Th1 or Th17 responses, after binding to PRRs such as Dectin-1 on DCs as reviewed by [46]. GOS was the only dietary fiber that increased the Treg cytokine IL-10, exhibiting a regulatory phenotype and contributing to the tolerogenic milieu in our *in vitro* system. This corroborates the findings of Eiwegger *et al.* [15] who reported that incubation of unchallenged human cord blood cells with human-milk oligosaccharides did significantly induce the production of IL-10. Interestingly, chicory inulin and barley  $\beta$ -glucan suppressed Th2 responses. As suppression of Th2 responses are associated with reduction of severity and frequency of allergy [47], our platform may be instrumental for selecting dietary fibers for future clinical trials on efficacy of dietary fibers in allergy management.

In conclusion, our findings clearly demonstrate that single cultures do not really show the potential tolerogenic effects

dietary fibers can have. To our knowledge, this is the first study that shows a differential impact of dietary fibers after fiber-induced crosstalk between IECs, DCs, and T cells. Our data demonstrate that the immunomodulating effects of the dietary fibers on immune cells, particularly DCs, depend on the previous interaction of the fibers with IECs. This action is most pronounced when IECs released factors upon fibers stimulation were incubated with DCs. The increased IL-10/IL-12 ratio suggests that dietary fibers induced a more regulatory status of DCs. Indeed, our data also highlight the potential of secreted bioactive factors derived from IECs upon dietary fiber stimulation to influence IEC-DC crosstalk and finally even T-cell responses. Finally, our results suggest that TLRs could be part of the mechanism by which dietary fibers induced anti-inflammatory and regulatory effects.

M.B.B. and P.dV. conceived and designed the experiments. M.B.B. performed the experiments. M.B.B., H.A.S., C.R. analyzed the data. N.M.S. contributed reagents/materials/analysis tools. M.B.B., H.A.S., M.M.F., P.dV. wrote the paper.

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The authors have declared no conflict of interest.

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