Introduction

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1. Introduction

Dietary fibers are carbohydrate fibers coming into the diet via consumption of cereal grains, fruits, and vegetables. These fibers have always been an integral part of the human diet and are important for maintaining health. The earliest illustration of the importance of dietary fibers was published after comparing African diets and Western diets [1]. It was found that differences in carbohydrate content in these diets may be responsible for the ever increasing frequency of typical Western diseases [2]. African diets contain higher amounts of dietary fibers and is associated with a much lower frequency of typical western diseases like inflammatory bowel disease, diabetes, and cancer in native Africans [3]. The initial health promoting effects were related to the fecal bulking effects of dietary fibers [1]. The water retention property of these fibers delays the passage of food in intestine, supporting nutrition absorption, and also dilutes the toxic agents in the food [4]. Other than bulking effects, dietary fibers are digested by commensal bacteria and thus support their growth and contribute to microbial diversity in the gut [5]. Due to lower amounts of dietary fibers in the Western diet it was assumed that the above mentioned effects of dietary fibers were less adequate in the Western society with disease development as a consequence [3]. However during recent years other explanations for health benefits of dietary fibers have emerged.

One of the mechanism for beneficial effects of dietary fibers is through promotion of growth of beneficial bacteria in the gut. These kind of dietary fibers are known as pre-biotics [6]. Examples of beneficial commensal bacteria are Bifidobacteria and Lactobacteria [7]. Some dietary fibers can be added to infant formula as pre-biotics to support Bifidobacteria. The pre-biotics mimic the function of breast milk oligosaccharides that also promotes growth of Bifidobacteria [7]. In infants, it has been shown that pre-biotic administration can reduce bouts of infections [8]. This could have been caused by Bifidobacteria spp enhancing effects of the carbohydrate pre-biotics as the spp are known to have barrier protective, anti-microbial, and a protective role in inflammatory bowel disease [9]. Inulin, a dietary fibers has a well-established bifidogenic effect in adults [10] as well as in infants [11] and has in this context been studied for use in infant formulas. Many new health promoting effects of dietary fibers have been discovered with inulin as model molecule [12].

Apart from the prebiotic effects, dietary fibers also act as fermentation substrate for commensal bacteria. The fermentation of dietary fibers in the gut can enhance the production of short chain fatty acids (SCFAs). The most abundant SCFAs in the intestine are butyrate, propionate, and acetate [5]. These SCFAs play several roles in metabolism [13, 14] as well as in immunity [15, 16]. Butyrate is one
of the best studied SCFA produced in the gut, which is associated with reduced inflammations [15], energy expenditure [17] and changes the gut motility [18].

The current theories for health effects of dietary fibers are thus: (i) effects on the intestinal immune system through supporting growth of commensal bacteria and (ii) support of production of their fermentation products like SCFA. These, however are the events that mainly occur in the large intestine. As most of the intestinal immune system lies in the small intestine this cannot explain all beneficial effects of dietary fibers in immune disorders [19]. Another argument for involvement of more processes is the fact that effects of SCFA on immunity are mainly attenuating while supportive and immune activating effects of dietary fibers have been described [20, 21]. In this thesis, we have focused on a new mechanism explaining immunomodulatory effects of dietary fibers. This is through direct interaction with immune receptors in the intestine. In order to understand further the role of these immune receptors in the intestine, we will discuss some essential features of the intestinal immune system which may be influenced by dietary fibers and the role of innate immune receptors such as Toll-like receptors (TLR) and Dectin-1 receptors in maintaining immune homeostasis.

1.1 The intestinal immune system

Dietary fibers come into contact with the mucosal immune system in the small intestine as soon as they leave the stomach. The intestine is containing approximately 70% of all human immune cells [19]. The majority of these immune cells are located in the small intestine and involved in keeping a strict balance between acceptance of beneficial food ingredients and microbes and reacting strongly to undesired toxins and pathogenic bacteria. The immune cells are integrated in a well-organized network of intestinal cells that form a barrier between the immune system and the luminal content. As represented in Figure 1, this barrier is formed by mucus and the closely connected epithelial cells [22]. The epithelial cells in the intestine form finger like structures known as villi. At the base of the villi there are concave intrusions known as the crypts. The crypts contain stem cells at the base that produce new intestinal epithelial cells. These stem cell-derived epithelial cells continuously migrate towards the tip of the villi to replace damaged or dying epithelial cells [23]. At the base of the crypts, along the stem cells, paneth cells are present. Paneth cells produce anti-microbial peptides to keep the pathogenic load under control [24]. A subpopulation of the paneth cells, the so called secretory goblet cells, also produce anti-microbial peptides and are mainly responsible for mucus production [25]. The mucus layer on the epithelial lining in the intestine is an important physical barrier between the
commensal bacteria, the epithelial cells, and underlying immune cells [26]. Apart from above mentioned specialized cells, most of the epithelial lining of intestine is made of absorptive enterocytes responsible for nutrient uptake (Figure 1).

**Figure 1: Representative image of intestinal immune system.** The intestinal immune cells are separated from the intestinal lumen by a layer of intestinal epithelial cells and mucus. This separation is important to maintain intestinal homeostasis in presence of commensal microbiota in the intestinal lumen. Varieties of specialized cells, such as M-cells (antigen capture cells), paneth cells (produce anti-microbial peptides) and goblet cells (produce mucus) also contribute to maintain the barrier and balances in the intestinal immune system. The immune cells lie in specialized centers such as peyer's patches and are also present in the lamina propria. The intestinal lumen also contains dietary fibers, which can contribute to maintain the immune homeostasis.

Underneath the epithelial layer is the largest immune surveillance zone of the body named the lamina propria [19]. The lamina propria is composed of loosely packed muscle cells interspersed with capillaries and nerve cells. The lamina propria contains numerous B cells, T cells, and innate immune cells like dendritic cells, macrophages and eosinophils [19] (Figure 1). The immune effects occurring in the lamina propria are communicated with the immune cells present in the mesenteric lymph nodes (MLNs) which can activate a regulatory immune response. This transfer of immune signals is mainly done by a subpopulation of dendritic cells expressing CD103 which is a adhesion molecule facilitating migration of these cells to the MLNs [27]. The MLN are the largest drainage lymph nodes of the body and specifically drain the intestine [28]. The MLNs is the specific site where regulatory T cells (Treg)
are generated that regulate and attenuate proinflammatory immune responses in the intestine [29].

The adaptive immune response center in the intestine, is present in specialized centers called the Peyer’s patches (Figure 1). Peyer’s patches are globule like structures distributed throughout the small intestine and are found in higher numbers near the ileum i.e. the distal part of small intestine [30]. Peyer’s patches are lined by specialized cells called microfold cells (M cells) for sampling particulate ingredients in the intestinal lumen and passing on to the dendritic cells in Peyer’s patches. The dendritic cells in the Peyer patches are of a special form and present the particulate food antigens to adaptive immune cells in the mesenteric lymph nodes. However, M cells are also one of the main entry points for pathogens in the intestine [31]. Peyer’s patches are equipped to react instantly to these pathogens [31]. The Peyer patches contain B cell lymphoid follicles surrounded by smaller T-cell regions. These B cells from Peyer’s patches produce secretory IgA in the intestine, assisting in binding and clearing pathogens or toxins. M-cells are however not the only sampling cells in the intestine. Some subpopulation of dendritic cells are known to directly protrude their dendrites in the intestinal lumen and induce an immune response in case of pathogenic stimulus [32].

1.2 Pattern recognition receptors in intestine

The microbiota, food antigens, and pathogens in the intestine are recognized in the mucosal immune system by specialized receptors. The receptors are called pattern recognition receptors (PRRs). PRRs can recognize various antigenic patterns on pathogenic microbes known as pathogen associated molecular patterns (PAMP) or intracellular ligands from stressed cells known as damage associate molecular patterns (DAMP). Different families of PRRs are expressed in the intestinal lumen [33]. Herein, we will discuss the two main PRRs, which have shown to interact with dietary fibers i.e. Toll-like receptors and Dectin-1 receptors [20, 21].

1.2.1 Toll-Like receptors

The best studied family of PRRs are the Toll-like receptors (TLRs). TLRs are having 13 members in humans. TLRs can recognize various PAMPs and DAMPs. Each TLR has an extracellular leucine rich repeat (LRR) domain, transmembrane and intracellular domain [34]. TLRs can be expressed on cell surface (TLR1, TLR2, TLR4, and TLR5), or intracellularly in endosomes (TLR3, TLR4, TLR7, TLR8, and TLR9). TLR4 has a unique property of being present both on the cell surface and in the endosomes [34]. TLR11 to TLR13 have not been completely characterized yet and probably there are
more TLRs [35]. Both TLR11 and TLR13 have been characterized to be expressed intracellularly, but the ligands are not known yet [34, 36].

Typically, different TLRs recognize different PAMPs and DAMPs depending on the ligand binding site in the LRR domain. However, all the TLRs signal through cytoplasmic Toll/interleukin-1 receptor domain that activate downstream pathway [37]. After binding of ligand, single or combinations of adapter proteins having TIR domains (MyD88, TRIF, TIRAP or TRAM) bind to TLR cytoplasmic domains [34]. MyD88 adapter protein is used by all the TLR activation pathways except for TLR3 which acts through TRIF. TLR4, for its endosomal activation pathway utilizes TRIF as well. Thus TLR signaling pathways can be MyD88 dependent or independent [34]. After binding of the adapter molecules, the activation pathway leads to phosphorylation and ubiquitination of inhibitor of NF-κB (IκB) leading to transport of NF-κB transcription factor to the nucleus and thus activation of pro-inflammatory genes against the TLR stimulus [38].

Microbes, food antigens, or pathogens that stimulate pattern recognition receptors can elicit different protective responses in the mucosal immune barrier. For example, activation or inhibition of certain TLRs can influence tight junctions in the intestinal epithelial cells [33]. But also other immune processes, still to be discovered, processes are under tight control of TLRs. Rakoff-Nahoum et.al. [39], showed that MyD88 deficient and TLR deficient mice were more susceptible to chemically induced colitis model in mice. To avoid spurious activation of inflammation through TLRs, specialized regulation mechanisms are known to be present in intestine. The polarized nature of intestinal epithelial cells allow for simultaneous activation of different pathways by TLR on the apical or basolateral membrane of the intestine. For example, basolateral activation of TLR9 leads to NF-κB activation whereas apical activation leads to stabilization of IκB and thus inhibition of NF-κB [40]. Under non-diseased conditions intestinal epithelial cells also express regulators for PRR to prevent uncontrolled inflammation [41, 42].

Overexpression or inadequate regulation of TLRs is implicated in diseases. For example, TLR4 expression in the healthy adult colon is very low, but in intestinal epithelial cells from Crohn’s disease patient, high levels of TLR4 expression was detected on the apical side of the cells [43]. TLR2 is also an important receptor in the regulation of immune responses as TLR2 knockout mice have reduced symptoms for chemotherapy induced intestinal inflammations [44]. Thus, differential roles and spatial distribution of TLRs play an important role in maintaining intestinal homeostasis.
1.2.2 Dectin-1 receptors

Dectin-1 is a carbohydrate binding receptors which is known to recognize dietary fiber β-glucan [45]. It is considered to be the major β-glucan receptor present on the immune cells [45]. β-glucan is present in cereal grains like oats and barley, and it is a major component of fungal cell walls [21, 45]. Dectin-1 was initially described to be present only on dendritic cells but was later found to be expressed on myeloid cells like neutrophils, macrophages, and B and T cells [46]. The receptor is composed of an extracellular carbohydrate binding domain also known as C-type lectin-like domain (CTLD), a transmembrane domain and cytoplasmic immunoreceptor tyrosine-based activation (ITAM)-like motif, similar to those present in T-cell receptors (TCR) [47]. The signaling pathway of Dectin-1 starts with ligand binding to the CTLD followed by phosphorylation of ITAM-like motif by Src kinases and the subsequent binding by Syk kinases through SH2 domains. This is followed by signaling through CARD9-Bcl-10-Malt1 scaffold. This stimulation leads to activation of the transcription factors NFAT, IRF1, IRF5, and both canonical and non-canonical NF-κB [48].

The stimulation of Dectin-1 has been shown to depend on particulate nature of β-glucan. It is known that particulate β-glucans can cluster Dectin-1 receptors and enhance immune responses [46]. The clustering of Dectin-1 receptors leads to expulsion of negative regulator tyrosine phosphatases CD45 and CD148. The mechanism of clustering allows immune cells to differentiate between particulate β-glucan from adjacent fungal cells and soluble β-glucan in the circulation [46]. Thus this mechanism allows cells to activate anti-fungal response in a localized manner to the fungal cells without causing erroneous inflammations against the soluble ligand.

Dectin-1 is expressed not only by immune cells but also in intestinal epithelial cells [49]. Dectin-1 is recognized to keep the fungal infection under control in the intestine [50, 51]. Dectin-1 receptors are also involved in soluble IgA uptake by M-cells, thus contributing to the antigen transport capacity of M-cells [52]. Recently, it was shown that Dectin-1 inhibition by soluble β-glucan could ameliorate colitis symptoms in mice by increase in Treg population with increase in Lactobacillus murinis [53]. Thus, in addition to response to fungal and dietary β-glucans, Dectin-1 receptor is also involved in maintaining intestinal homeostasis.

1.2.3 Collaborative effect of PRRs

As explained above, PRRs can recognize a variety of ligands and guide immune responses. In order to fine tune the immune responses, PRRs can also collaborate with each other depending on the ligands presented to them. One the best known example for such collaboration is between TLR2 and TLR1 or TLR6. TLR2-TLR1 heterodimer can recognize tri-acyl lipopeptides (three lipid chains attached to a
peptide chain) wherein two lipid chains of lipopeptide are bound by TLR1 and one lipid chain by the TLR2 ligand binding site [54]. On the other hand TLR2-TLR6 can recognize di-acyl lipopeptide (two lipid chains attached to a peptide chain) [55]. The TLR6 ligand binding site can accommodate only one of the lipid chains of lipopeptide while the other lipid chain is recognized by TLR2 [55]. The immune response initiated by TLR2 also differs depending on the collaboration with TLR1 or TLR6 [56, 57]. TLR2-TLR1 heterodimer has been shown to induce pro-inflammatory Th17 skewed immune response [57], whereas, TLR2-TLR6 has been described to induce an IL-10 stimulating regulatory immune response [56]. Thus, TLR2 can recognize a variety of ligands by collaborating with TLR1 or TLR6 and also TLR2 can stimulate different immune responses depending on the collaboration with TLR1 or TLR6.

Besides TLR2, the β-glucan receptor Dectin-1 can also collaborate with different TLRs but this has not been studied in the detail as for TLRs. Dectin-1 has been shown to collaborate with TLR2 and TLR4 to induce an exponential cytokine response in human monocytes and macrophages [58]. Stimulation of peripheral blood mononuclear cells (PBMCs) and specifically of human monocyte derived macrophages with β-glucan (Dectin-1 agonist) along with P3CSK4 (TLR2 agonist) or LPS (TLR4 agonist) lead to increased immune responses by PBMCs and macrophages compared to stimulation by either of the agonists alone [58]. Thus, it was suggested that Dectin-1 can collaborate with TLR2 and TLR4 to enhance immune response in dendritic cells [58]. Also it has been shown in that susceptibility for pathogenic fungal infections such as Candida albicans in increased in TLR4 knock out mice [59]. However, a real collaboration between Dectin-1 and TLR2 or TLR4 by a ligand such as β-glucan has not been described yet but might occur. Thus, PRRs collaborate with each other to enhance or differentiate immune responses for different agonists.

1.2.4 Commensal bacteria manipulate the mucosal immune system to prevent immune response

Pattern recognition receptors are not only involved in recognition of undesired antigens or microorganisms in the lumen but also in the prevention of immune responses against commensal bacteria. The human adult microbiome consists mainly of Bacteroidetes and Firmicutes phyla [60]. The adult microbiota is rather stable with around 60% of all the bacterial strains in an individual persisting for a period of 5 years [61]. Crosstalk between the immune system and microbiota in the intestine are responsible for this tolerance to gut bacteria. For example, Bacteroides fragilis has been shown to induce a specific regulatory immune response by directing the development of immune-regulatory Foxp3+ Treg cells in the intestine [62]. Polysaccharide A from Bacteroides fragilis which is a ligand for the PPR TLR2 was
responsible for this inducible regulatory response [62, 63].

Commensal bacteria can also prevent their own eradication by the immune system through fermentation of dietary fibers and producing immune attenuating metabolites such as SCFA. Butyrate is recognized as an example for its anti-inflammatory effect through induction of Treg cell formation and also suppresses the growth of colonic cancer cells [64, 65]. Butyrate and propionate are also involved in epigenetic changes in the colon lining through histone deacetylase. Histone deacetylase action of butyrate results in induction of Foxp3 transcription factor and thus induction of Tregs [64].

1.3 Direct immunomodulatory effects of dietary fibers

As mentioned above dietary fibers can indirectly induce a regulatory immune response through production of SCFAs [64]. Dietary fibers can also modulate the immune system by changing the composition of microbiota by selective enrichment of beneficial immune active bacteria [66] or by increase in diversity of microbial composition in the gut [67]. In addition, as dietary fibers pass form the intestine, they might as well be recognized by PRRs on epithelial and immune cells in intestine. However, this possibility of interaction between dietary fibers and the immune system has not been explored in depth. Some well-known pattern recognition receptor such as Dectin-1 are known to recognize β-glucans from the fungal cell walls [45] as well as dietary β-glucan from cereal grains [21]. Similarly, inulin type fructans were shown to stimulate TLR2 in intestinal epithelial cells to induce epithelial cell tight junctions in vitro [20].

The immunomodulatory capacity of inulin type fructans was shown to be dependent on the chain length i.e. polymerization levels of fructans [20, 68]. The longer chain length fructans induced higher TLR2 stimulation than short chain fructans, whereas shorter chain length fructans showed higher regulatory immune response in PBMCs than long chain fructans [68]. Dietary inulin type fructans in intestine can also be digested by commensal bacteria to generate different chain lengths of fructans [69, 70]. Thus, it can be suggested that the direct immunomodulatory effects of inulin type fructans in vivo can also differ depending on the commensal microbiota digestion of the inulin type fructans to different chain length of fructans. Similarly microbiota might enhance bioactivity of dietary fibers. For example, some β-glucan sources such as oat β-glucan has been shown to have some efficacy in immunomodulation in vivo [71, 72] but do not have this potency in in vitro assays [73]. The numerous microbiota derived enzymes might facilitate the exposure of specific Dectin-1 binding groups in the intestine and enhance bioactivity.
This hypothesis will be tested in this thesis.

Current research efforts are concentrating on both direct (through immune receptors) and indirect (through microbiota dependent effects) immunomodulatory effects of dietary fibers in the intestine. In the present thesis a number of commonly consumed immune active dietary fibers have been studied for interaction with one or more PRRs. These are the dietary fibers β-glucan, arabinoxylans and pectins. In the next sections we will discuss the known structural features of these dietary fibers and in brief we review the beneficial effects of these fibers in metabolic or immune disorders.

1.4 β-glucan

1.4.1 β-glucan structure

β-glucans are polymers of D-glucose monomeric units bound by β-glucosidic linkages such as β(1-3), β(1-4) and/or β(1-6). Cellulose is the insoluble and most common β-glucan in nature. Cellulose has continuous β(1-4) linkages and is found in all the terrestrial plants [74]. Cellulose forms a nonflexible secondary cell wall in plant cells, supporting structure of the wall. This is accomplished by unsubstituted and unbranched structures of cellulose [74]. Other common dietary β-glucans are β(1-3) (1-4) linked glucans from grains like oats, barley, rye, and wheat (Figure 2A) [21]. These (1-3, 1-4) linked β-glucans do not form a compact structure like cellulose and thus provide a certain amount of flexibility to the structure and also contribute more to palatability than cellulosic structure. Other than grains, β-glucan is also present in fungal cell walls in the form of β(1-3) and/or β(1-6) linked glucans (Figure 2B). These, fungal β-glucans are present in diets consisting of mushrooms and yeast cell walls [75]. The solubility of β-glucans, as can be gauged from above examples, depends on the source and the linkages present in the molecule. Cellulose is insoluble, whereas β-glucans from grains and fungal sources show some level of solubility.

1.4.2 β-glucan receptors

Fungal β-glucans were the first dietary fibers to be recognized by an immune receptors such as Dectin-1 receptor, complement receptor 3 (CR3) and lactosylceramide [76]. Among these receptors, CR3 was the first characterized receptor for β-glucan, also known as CD11b/CD18 [77]. CR3 is expressed on macrophages, neutrophils, NK cells, dendritic cells and monocytes [78]. The CR3 receptor contains two distinct ligand binding sites as 1-domain and lectin-like site. 1-domain recognizes complement component iC3b, extracellular matrix and intercellular adhesion molecules like...
ICAM1 (Intercellular adhesion molecule 1). The lectin-like domain recognizes β-glucan and also other polysaccharides containing mannose and glucose sugars [79]. Lactosylceramide is a glycosphingolipid which was shown to bind soluble β(1-3, 1-6) glucan [80], but the signaling pathway is not completely known yet.

Although CR3 and lactosylceramide are shown to be β-glucan receptors, Dectin-1 is considered to be the major β-glucan receptor present on the immune cells [45]. As described in previous section, only particulate β-glucan can efficiently activate Dectin-1 receptors through receptor clustering. While soluble β-glucan fails to cluster the Dectin-1 and stimulates a weak immune response [46]. CR3 receptors, on the other hand is able to bind and activate signaling with both particulate [81] and soluble β-glucans [82]. While Lactosylceramide can bind only soluble β-glucan wherein binding to the receptor could only be inhibited by high molecular weight β-glucan and not low molecular weight β-glucan [80].

1.4.3 Structural features of β-glucan for Dectin-1 binding

The structural features and carbohydrate backbone composition of β-glucan that interacts with murine Dectin-1 was studied by Adams et.al. [83]. Adams et.al suggested that the minimal structural feature required in β-glucan to interact with Dectin-1 is seven β(1-3) linked glucose backbone with one β(1-6) branched glucose on the backbone. The helical conformation of β-glucan was suggested to fit in the Dectin-1 ligand binding groove, wherein larger β-glucan structures with multiple linked glucose units were shown to have higher binding affinity to Dectin-1 receptors. It was demonstrated that linear β-glucan could bind Dectin-1 receptor with lower
efficiency compared to a single branched subunit with similar sugar units in the molecule. Thus both β(1-3) and β(1-6) linkages in β-glucan molecule are important for interaction with Dectin-1 receptors [83].

Another study by Hanashima et al. [84] also confirmed that the larger fungal β-glucan chain length activates Dectin-1 better than short chain oligomers [84]. Adachi et al. [85] studied the ligand binding site of Dectin-1 receptor and found out that Trp221 and His223 are critical for interaction of β-glucan with Dectin-1 [85]. Thus, it might be that the branched structure of β-glucan fits better in the Dectin-1 groove with Trp221 and His223, facilitating stronger activation. Several natural immune-active β-glucan from sources like *Aureobasidium pullulans*, *Saccharomyces cerevisiae* and several Basidiomycetes also have β(1-3) backbone with β(1-6) branching, justifying the requirement of branching for immune stimulation [86-88]. Further structural insight into interaction of β-glucan with Dectin-1 can be obtained from studies in blastospore and hyphal forms of *Candida albicans*. The hyphal *C. albicans* shows higher immune activation potential in PBMCs (peripheral blood mononuclear cells) than blastospore form and the activation is Dectin-1 dependent [89]. The hyphal region β-glucan was shown to have double frequency of branching than blastospore and in closed chain cyclical structure. These structural differences in β-glucan might be responsible for stronger immune activation by hyphal *C. albicans* [89].

1.4.4 Immunomodulatory effects of β-glucan

Dietary β-glucans can come into the human diet by consumption of mushrooms, yeast, or from plant sources like barley, oats and rye [90]. The immunomodulatory properties of β-glucan is mainly studied for fungal β-glucan. Fungal β-glucan has been studied with respect to its anti-tumor effects, against infections [91, 92], as adjuvants in vaccination, as vaccinating agents against fungal infections [93], as dietary intervention against inflammations [94], against upper respiratory tract infections [95] and against type-1 diabetes [96]. As mentioned above, β-glucan can induce immune stimulation through receptors like Dectin-1, CR3, and lactosylceramide. Dectin-1 activator, curdlan a β(1-3) glucan when given intra-peritoneally could stimulate Th17 immune response *in vivo* and also could induce antibody response in mice [93]. Curdlan could also be used as adjuvants to induce Th17 response against ovalbumin. In addition to Th17 responses, Dectin-1 activation by curdlan in dendritic cells was also shown to induce cytotoxic T cell response [97]. Thus, Dectin-1 receptor acts as bridge between innate and adaptive immune response. Soluble β-glucan activation of CR3 receptor could prevent trans-endothelial movement of activated neutrophils, without affecting the binding of neutrophils to extracellular matrix through I-domain in CR3 [98]. This might be one of the mechanisms by which β-glucan plays a protective role in auto-immune diseases [99].
The immunomodulatory effects of β-glucan are well characterized for application as adjuvant in cancer therapies. The proposed mechanism suggests a role for CR3 in recognition of β-glucans as well as for recognition by complement iC3b. Intra-venous β-glucans administration increased the immune targeting capacity of the naturally induced anti-tumor antibodies in mice by targeting them to iC3b coated tumor cells. This binding with tumor cells resulted in complement driven cytotoxic activity and reduction in tumor size [100]. The anti-tumor activity of β-glucans could be observed by both intra-venous and oral route suggesting that β-glucans may come into the circulation by direct passage through the intestinal barrier [101, 102]. Hong et.al. therefore studied the possible uptake of barley and yeast β-glucan from diet into the circulation. They showed that dietary β-glucans can be taken up by macrophages and passed on to lymph nodes, spleen, and bone marrow. In bone marrow, these β-glucan molecules were degraded and passed on to granulocytes which could direct and activate the cells to kill the complement coated tumor cells [103].

Apart from their well-defined adjuvant effect in the antibody mediated cancer immunotherapy, β-glucans were also shown to stimulate hematopoiesis in mice in a concentration dependent manner [104]. Particulate β-glucan when administered intravenously was able to induce hematopoiesis in irradiated mice [105], suggesting that β-glucans could be used as a support therapy in cancer patients receiving radiation therapy. The hematopoietic efficacy of β-glucans was recently further confirmed in breast cancer patients undergoing chemotherapy [106]. The patients receiving β-glucan showed increased blood IL-12 levels and white blood cell counts were less reduced compared to the placebo group [106]. In Japan, treatment with lentinan from Shiitake mushroom (Lentinula edodes) has been approved for clinical cancer therapy trials for more than a decade and has been used for gastric [107], colorectal, prostate [108] and breast cancer [109]. However, apart from CR3, the effect of β-glucan stimulation on Dectin-1 receptor in cancer therapy has not been studied yet.

1.4.5 Dietary metabolic effects of β-glucan

Many studies have shown that dietary β-glucan have beneficial effects against hyperglycemia and hyperlipidemia. Initial reports suggested that the viscosity of β-glucan rich oat and barley diet were the reasons for better glucose tolerance and insulin sensitivity [110]. A study with obese women showed that consumption of 10g of β-glucan is able to reduce postprandial insulin response wherein the efficiency was concentration dependent and reduced with lower concentrations of β-glucan [111]. Similar studies were performed in diabetic patients in which β-glucan inclusion in diet showed beneficial effects insulin resistance [112, 113]. Chronic
consumption of high β-glucan barley diet in Zucker diabetic fatty rats was able to reduce hyperglycemia and fatty liver syndrome [114]. Although the role of enhanced viscosity in intestine and resulting enhanced uptake of nutrients cannot be excluded as causal factor, similar beneficial effects have been reported for intra-peritoneal administration of β-glucan where viscosity of β-glucan does not directly affect the nutrition absorption process in intestine. A study has shown that intra-peritoneal injection of β-glucan from *S cerevisiae* was able induce regulatory immune response in Type-I diabetes NOD mice [96].

A recent study in rats examined the effects of higher β-glucan content in barley for their anti-inflammatory and hypocholesterolemic effects in rats on high fat diet [94]. This study compared two barley varieties with high and low β-glucan contents. The higher amount of β-glucan in barley supplemented high fat diets in rats was associated with hypocholesterolemic effects [94]. In their study model in rats, high fat diet reduced the butyrate concentrations in caecum SCFA, which were counteracted by higher β-glucan content in barley [94]. Butyrate as mentioned earlier is known to stimulate Treg cell production [64, 65] and thus is known as anti-inflammatory agent.

In another study in rats fed with high fat diet which were also fed with barley with high (3%) and low (0%) β-glucan did not show any improvement in glucose tolerance. β-glucan content on the other hand improved total SCFA concentration and butyrate concentration in cecum compared to the low β-glucan barley diet [115]. The hypocholesterolemic effect of β-glucan is generally accepted however from above example, we can conclude that the efficacy might differ with extraction process, branching, solubility and also mode of administration of the β-glucan molecule. Although the immune effects are known to be affected by structural differences in β-glucan, it is not studied conclusively for their effects on insulin sensitivity and hypoglycemic effects.

### 1.4.6 Dietary β-glucan effects in production animals

β-glucan is also used in feed as immune-stimulant to reduce infection load and to reduce antibiotic use in livestock [116]. Inclusion of 50mg/kg of β-glucan from *Saccharomyces cerevisiae* in diets of weaned piglets was protective against LPS induced inflammatory cytokines IL-6 and TNF-α, and also increased the levels of immune-regulatory IL-10 [117]. Dietary β-glucan also lead to increased antibody responses to ovalbumin in only the first week after ovalbumin stimulation compared to piglets on conventional diets [117]. Similarly, effects of live yeast supplemented diet was studied in *E coli* infection in weaned piglets. The diarrhea scores were reduced and IgA secretion was improved in these live yeast supplemented piglets [118]. Broilers,
fed with *S. cerevisiae* supplemented diet, showed increased macrophage phagocytic activity and increased humoral response [119]. Similarly, β-glucan addition to diet reduced the lesions of Eimeria infection in one day old broiler chicks [120]. On other hand, another study using *S. cerevisiae* β-glucan supplemented diet showed minimal growth performance improvement with no immune beneficial effects against porcine reproductive and respiratory syndrome (PRRS) vaccination [121]. The reason for these opposing observations could be due to different extraction process of β-glucan in above studies. Otherwise, the contrasting observations might be due to different hygiene conditions and living conditions in above mentioned studies.

### 1.5 Arabinoxylan

#### 1.5.1 Arabinoxylan structure

Arabinoxylan is one of the most common dietary fibers. It comes into the diet by eating cereal grains like wheat, corn, oat and barley [122]. It is a hemicellulose and the basic structure consists of a xylan backbone consisting of β(1-4) linked xylopyranose residues. The xylan linear chain can be substituted with arabinose residues at O-2 and/or O-3 positions (Figure 3). The arabinoxylan thus can have four kinds of structural features in its chain; mono-substituted xylans at O-2 position or at O-3 position, di-substitution at both O-2 and O-3 positions, and unsubstituted xylose residues in the xylan chain. The structural features of arabinoxylan differs according to the source and depends on the arabinose to xylose ratio in the arabinoxylan chain [123]. The level of substitution in different sources of arabinoxylan determines the solubility of the polymer. In wheat arabinoxylan the substitution is mainly at O-3 position (21%) or di-substitutions at O-2 and O-3 positions wherein 66% of the xylose residues are unsubstituted. Whereas in barley and corn the substitution levels are lower making the arabinoxylan less soluble [122]. However the solubility is not only dependent on the level of substitution, but also on level of chain-chain interaction and molecular weight. Increased chain-chain interaction reduce interaction with water and also high molecular weight chains can form gel structures making them insoluble [124].

![Arabinoxylan structure with β(1-4) linked xylose backbone. Xylose mono- or di-substituted with arabinose.](image)

*Figure 3: Arabinoxylan structure with β(1-4) linked xylose backbone. Xylose mono- or di-substituted with arabinose.*
1.5.2 Immune receptors for arabinoxylan

The immunomodulatory effects of arabinoxylan in vivo have been known [125]. The immunomodulatory effects of arabinoxylan have also been described in vitro, thus independent of SCFA and microbiota dependent effects [126]. However, arabinoxylan has not been described as yet to interact with any immune receptor. Thus, further studies are required to understand the direct immunomodulatory effects of arabinoxylan with respect to pattern recognition receptors.

1.5.3 Immunomodulatory effects of arabinoxylan

The structure to function relationship of arabinoxylan and immunity was studied in finger millet (Eleusine coracana) arabinoxylan. Finger millet arabinoxylan was studied with different arabinoxylan molecular weights, arabinose to xylose ratio, and phenolic acid content [125]. The immune stimulatory effect was studied using murine lymphocytes and peritoneal macrophages. It was suggested that the immune-stimulatory activity of arabinoxylan directly depends on and correlates with ferulic acid content but not on molecular weight and arabinose to xylose ratio [125]. Another article tested both water and alkali extractible wheat arabinoxylan to study whether they differ in their protective action against DNA damage, in which both the arabinoxylans could protect the colon cells against H2O2 induced DNA damage [127].

The immunomodulatory effects of MGN3, a rice bran arabinoxylan were studied in clinical trials for irritable bowel syndrome patients. MGN3 administration led to reduced scores for diarrhea and constipation in irritable bowel syndrome patients [128]. The immunomodulatory effect of wheat arabinoxylan was studied in dendritic cells co-cultured with colon carcinoma cell lines [126]. Arabinoxylan reduced IL-6 and MCP-1 levels in dendritic cells and was able to decrease IL-12 levels in co-culture systems [126]. These immunomodulatory effects were studied in vitro thus were independent of microbiota or SCFA effects. Therefore, it could be concluded that arabinoxylan also can impart their immunomodulatory effects through interaction with immune receptors.

Rice bran arabinoxylan MGN3 has been extensively studied for its anti-carcinogenic effects [129]. The initial reports of immune effects of MGN3 were realized when NK cell activity was increased in individuals that were given MGN3 orally [130]. Later, the same group showed that MGN-3 treatment of NK cells results in production of TNF-α and IFN-γ, and MGN3 also sensitized leukemic T-cells for CD-95 induced apoptosis, further making MGN3 an attractive immunomodulatory agent for cancer treatments [129, 131]. The anti-tumor properties of MGN3 have been shown in animal models for ehrlich carcinoma [132, 133], colon carcinogenesis
and neuroblastoma [135]. Clinical trials against hepatocellular carcinoma [136] and multiple myeloma [137] also support the notion of anti-carcinogenic effects of MGN3. Thus, from these animal models and clinical trials MGN3 looks as a promising immune stimulating agent in cancer therapy.

### 1.5.4 Dietary metabolic and pre-biotic effects of arabinoxylan

Arabinoxylan has also been studied for its effect on cholesterol levels [138], and effects on microbiota and SCFA levels [139] in both animal models [140] and clinical trials [141, 142]. Wheat arabinoxylan supplementation in healthy volunteers reduced postprandial insulin and glucose responses [143]. In type 2 diabetes and metabolic syndrome patients the arabinoxylan supplement helped in reducing insulin and glucose response with increase in fecal butyrate and acetate concentrations [141, 142]. A study with high fat diet fed mice supplemented with wheat arabinoxylan showed that arabinoxylan decreased high fat induced adiposity, body weight, cholesterol accumulation in serum and liver, and insulin resistance [139]. The beneficial effects were accompanied by marked increases in *Bifidobacteria* frequencies and improved gut barrier with reduced inflammatory markers IL-6 and MCP-1 in serum [139]. Another study showed beneficial effects of wheat arabinoxylan oligosaccharides (5900 Da) in high fat fed mice wherein they observed a bifidogenic effects [140].

Wheat arabinoxylan and arabinoxylan oligosaccharides differ considerably in their molecular weights. Thus, to determine the role of polymerization levels in their pre-biotic effects van Craeyveld et.al. [144] performed study with rats fed with western life style low fiber diet supplemented with wheat arabinoxylans differing in their degree of polymerization (DP) (molecular weight) and arabinose to xylose ratio (DAS). They showed that the effect of DP was much more pronounced than the effect of DAS [144]. Higher DP arabinoxylan reduced protein fermentation in the intestine. Whereas, low DP arabinoxylan increased acetate and butyrate in colon with increased *Bifidobacteria* [144]. Effects of arabinoxylan in feed of production animals like pigs showed similar beneficial effects with increased butyrate and SCFA levels, induced growth of *Bifidobacteria* and *Lactobacteria* spp [145]. Aarabinoxylan thus appears to be a promising natural dietary intervention.
1.6 Pectin

1.6.1 Pectin structure

Pectin is a plant cell wall polysaccharide mainly composed of homogalacturonic acid polysaccharide units. The galacturonic acid units can be methyl esterified at the C6 carboxyl group or it can be acetylated at O-2 or O-3. Homogalacturonic units make up to 70% of the pectin structure (Figure 4A). Apart from homogalacturonan, pectin contains substituted galacturonic acid units with xylose called xylogalacturonan, rhamnogalacturonan-I and branched regions of rhamnogalacturonan-II [146]. The branched region of rhamnogalacturonan-II is the most complex structure in pectin and may comprise 12 different sugar molecules linked with different linkages (Figure 4C). Rhamnogalacturonan-I is unique in its disaccharide backbone of galacturonic acid and rhamnose wherein the branched regions with arabinose and galactose sugar residues adorn the backbone (Figure 4B). The galacturonic acid in rhamnogalacturonan-I is hardly ever seen to have esterification and branching is always on the rhamnose residue of the disaccharide backbone. Other known structures of pectin are xylogalacturonan having xylose residue on the homogalacturonan backbone. Also apiogalacturonan having mono or disaccharide apioduranosyl attached to the main homogalacturonan chain [147].

1.6.2 Pectin receptors

Modified citrus pectin (MCP) is the most well studied pectin and has been reported to bind to the PRR Galectin-3 [148]. Galectin-3 has a lectin domain which is able to bind to galactose residues. Galectin-3 is expressed intracellularly and also on the membrane [149]. It is mainly involved in cell-cell binding and also in interaction of cells with extracellular matrix [149]. Galectin-3 has been shown to be over expressed in many cancer cells and thus provides an attractive target for anti-cancer therapies [148]. MCP is mainly studied for its anti-carcinogenic effects, as outlined later.

Apart from Galectin-3, pectin was shown to inhibit LPS induced inflammatory pathways [150]. It was shown by Chen et.al. that pectin was able to bind to LPS to reduce the interaction of LPS with TLR4 [150]. Pectin with a degree of esterification (DE) of 90 percent (DE90) had the highest TLR4 inhibition efficacy compared to DE30 and DE60 pectin as shown by reduced iNOS and COX2 production in monocytes. DE90 was able to inhibit MAPK signaling along with reduction in activation of transcription factors NF-kB and AP-1 [150].

1.6.3 Immunomodulatory effect of pectin

As mentioned above, pectin could reduce LPS induced inflammations through inhibition of TLR4 [150]. Similarly, protective effects of apple oligogalactan composed
of five galacturonic acids were through inhibition of TLR4 in a colitis associated colon cancer model [151]. In vivo the apple oligogalactan reduced expression of TLR4 and levels of TNF-α in mice with colitis. In vitro however, pectin was effective in protection only after LPS stimulation of human colon carcinoma cell line HT-29 cells [151]. Liu et.al. suggest that this inhibition by pectin was due to membrane internalization of TLR4, thus reducing activation through TLR4 [151].

Effect of pectin acidic oligosaccharides (AOS) was studied in presence of other dietary fibers, galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS) in support of influenza vaccination in mice. Presence of AOS showed improved vaccination responses in Th1 skewed immune activation [152]. A similar combination of AOS, GOS, and FOS however, did not improve antibody responses against haemophilus influenza type-B and tetanus vaccinations in infants [153] and remained ineffective in enteral dosage route as well [154]. In highly active antiretroviral therapy (HAART)-naive HIV-1-infected patients, combination of
AOS, GOS, and FOS showed bifidogenic effects, reduction of sCD14, CD4 + T-cell activation (CD25), and improved NK cell activity [155]. Thus, it can be argued that the combination of dietary fibers AOS, GOS, and FOS is not effective in vaccination in infants, but does show beneficial effects in adults, as seen with HIV patients [155].

Combinations of GOS, FOS, and AOS also reduced allergic asthma in ovalbumin induced allergic asthma model in mice [156]. Vos et.al. suggest that this combination of prebiotics enhance Th1 responses and suppresses Th2 responses [156]. Respiratory syncytial virus (RSV) infection was reduced in mice fed with GOS, FOS and AOS combination with increased CD4+ T cells producing IFN-γ, thus increasing Th1 response [157]. Sherry et.al. [158] compared effects of insoluble fiber cellulose and soluble fiber pectin in diets of mice and showed that mice fed with pectin recovered faster from endotoxin induced sickness than cellulose fed mice by increasing levels of IL-4 in ileum and spleen and Th2 polarization [158]. The origin of pectin however was not mentioned in the study [158]. From above examples, we can say that the combination of GOS, FOS, and AOS induces Th1 skewed response but pectin alone can stimulate Th2 skewed response.

In IL-10 knockout inflammation model in mice inclusion of apple pectin in diet reduced levels of proinflammatory cytokine TNF-α and expression of transcription factor GATA-3. Pectin could counteract the reduced CD4+ and CD8+ cells in mesenteric lymph nodes and payer’s patches [159]. Mice fed with pectin after endotoxin challenge in peritoneum showed higher levels of IL-1RA, reduced levels of IL-1β and TNF-α in brain. The effect was not observed in MyD88 knockout mouse suggesting that the effect might be due to interaction of pectin with TLR4 signaling [158]. Salman et.al. [160] studied in vitro the effect of DE of citrus pectin with human peripheral blood cells (PBMC) [160]. DE60 and DE90 were able to reduce pro-inflammatory IL-1β and increase expression of anti-inflammatory IL-10 and IL-1RA, but this was not observed with DE30 pectin [160]. Thus, pectin in general can be termed as anti-inflammatory dietary fiber. Although the effects have been noted, the mechanism of interaction with immune system has not been studied in many of these article.

As mentioned in previous section, MCP is known as the anti-tumorigenic agent. The mechanism for anti-tumorigenic effect is one of the most well characterized therapeutic effects of pectin. MCP is shown to have anti-metastatic, anti-angiogenic and pro-apoptotic properties. It is shown to be effective in vivo or in vitro against prostate cancer [161], breast cancer [162], melanoma [163] and angiosarcoma [164] through inhibition of PRR Galectin-3 receptor [148]. A phase II pilot study with prostate cancer patients showed that MCP increases prostate specific antigen doubling time (PSADT) [161]. Increased PSADT is associated with reduced systemic
progression. PSADT increased in 7 out of 10 patients taking MCP for 1 year than before [161]. Although this beneficial effect does not guarantee lower tumor load, but is a strong predictive measure for survival rate and metastasis progression after treatment.

1.6.4 Dietary metabolic effects of pectin

Pectin being a gel forming dietary fiber, it has been tested for its effects on satiety and glucose tolerance. An initial study by Jenkins et.al. in 1975 suggested that fruit and vegetable gels like guar and pectin show more anti-cholesterolaemic effects than wheat fibers [165]. The effectiveness was suggested to be because of its gel forming property which slows down digestion and can induce satiety in patients [166]. A study with moderately hypercholesterolaemic heathy men and women receiving psyllium, pectin, guar gum, and locust bean gum mix dietary fibers showed reduction in low density lipoproteins. However, mean plasma high density lipoproteins and triglycerides remained unaltered [167]. Inclusion of acacia gum and pectin in diets of metabolic syndrome patients showed improvement in fasting endogenous glucose turnover, but did not affect lipolysis and insulin resistance in these patients [168].

The gel forming property of pectin was also beneficial in clinical trials related to digestibility problems [169, 170]. Inclusion of pectin in diet was shown to reduce persistent diarrhea in children of less than 12 months of age in a study performed in Bangladesh. Pectin inclusion in diet of these children reduced stool frequency, vomiting frequency and diarrhea duration [169]. Pectin as thickener was also studied with children having cerebral palsy. A high pectin diet reduced gastroesophageal reflux and improved vomiting symptoms in children with cerebral palsy [170].

A study by Wanders et.al. [171] compared pectin with different physicochemical properties i.e. gelled pectin, viscous pectin and bulking pectin in decreasing viscosity respectively [171]. Additionally they also compared the supplementation methods of gelled pectin, capsule and liquid. Pectin was supplemented in dairy based products to healthy subjects and gelled pectin was found to reduce appetite and gastric emptying rate compared to other samples or no pectin. Gelled and viscous pectin reduced insulin responses. Among the supplementation methods, appetite was only reduced after ingestion of gelled pectin compared to capsule and liquid [171]. This study provides an important parameter to study dietary supplementation of pectin in its viscosity and also supplementation method.

However, not all studies have reported positive impact of pectin diet in metabolic control. Sugar beet pectin supplementation did not improve postprandial glucose concentrations or serum lipid profiles in patients with dysregulated glucose metabolism [172]. Also a low viscosity supplement of guar and pectin did not lower
cholesterol levels in hypercholesterolemic patients [173]. These contrasting results might be due to different sources of pectin or might be due to different model systems used in the study. These results further corroborate the importance of studying mechanisms for beneficial effects of pectin and other dietary fibers with respect to their structural features.

1.7 General aim of the studies in this thesis and clinical and industrial applicability

Cereal grains like oat, barley, and soybean are cheap sources of feed and food ingredients. Enhancing bioactivity of these dietary fibers from these sources provide a commercially valuable solution to both feed and food industry for health promoting effects. In this chapter we focused on cereal grain dietary fibers β-glucan, arabinoxylan [122] and pectin [174, 175]. We also discussed the current knowledge about immunomodulatory effects and metabolic effects of these fibers. However, the exact molecular structures responsible for direct immunomodulatory effects in these dietary fibers are not known. Furthermore, the mechanism of interaction between dietary fibers and PRRs are not known either. The studies in this thesis are designed (see next paragraph) to elucidate effector-function relationships and to enhance knowledge on receptor-dietary fibers interactions and to contribute to targeted application of dietary fibers in prevention of disease. A proof of principle study is included.
Design and rationale

As outlined in preceding sections, the immunomodulatory effects of dietary fibers are up to now considered to be predominantly microbiota dependent. Limited information is available about the direct immunomodulatory effects of dietary fibers through interaction with immune receptors. Herein, we studied the potential interaction of cereal grain dietary fibers β-glucan, arabinoxylan, and pectin with PRRs. β-glucan is accepted as a PRR interacting dietary fiber and was used as model-molecule to address key-questions. Arabinoxylan was chosen as it is a commonly consumed dietary fiber with some chemical similarities with β-glucan. Pectin was used because it is readily available in different chemical structures, cheap and therefore ideal for effector-function relationship studies.

The studies are designed to deliver a better understanding of the working mechanisms of dietary fibers which contribute to the design of strategies to enhance efficacy of these fibers in food and feed. Ultimately this should contribute to prevention of typical Western diseases that are currently associated with lowered dietary fiber intake [3]. As such, our studies suggest that ‘eating more molecular’ might contribute to healthy ageing.

Chapter 2- Oat β-glucan has been described to have limited immune-stimulation in vitro but to have immunomodulatory effects in vivo. In chapter 2 we used this discrepancy to analyze whether this can be explained by digestion by commensal microbiota in vivo with enhanced binding of the digested oat β-glucan to Dectin-1 as a consequence. This study serves as proof of principle that microbiota derived enzymes can modulate the bioactivity of dietary fibers and enhance binding capacity to the numerous PRRs in the intestine.

Chapter 3- As outlined in the introduction, arabinoxylan shows immunomodulatory effects in vivo via enhancing SCFA production by microbiota. Also, as outlined in chapter 3 arabinoxylan is the most consumed dietary fiber in the human diet and has chemical groups that are also present on β-glucan, i.e. a fiber with accepted efficacy for binding to PRRs. In chapter 3, we determined the PRRs responsible for the direct immunomodulatory effects of arabinoxylan. We demonstrate that PRRs such as Dectin-1 are more versatile in binding to dietary ligands and that relatively cheap fibers such as arabinoxylan can be used to stimulate specific responses against pathogens. It also demonstrates that competition between dietary fibers for PRR binding might exist in vivo.

Chapter 4- Dietary β-glucan is known to activate Dectin-1 receptors wherein, particulate β-glucan show enhanced Dectin-1 activation compared to soluble β-glucan. As outlined in the introduction, many PRRs work synergistically to guide
immune responses. As for Dectin-1 this has not been studied in much detail, we analyzed in chapter 4 whether the difference in immune efficacy can be explained by different synergistic effects on the Dectin-1 receptor and the PRR TLR4. This study demonstrates that even with the well-studied β-glucan-Dectin-1 interaction, processes that lead to the final immune response is much more complex than considered up to now. Efficacy of binding of β-glucan to Dectin-1 can be modulated by microbiota derived enzymes (Chapter 2), by competition for binding with other dietary fibers such as arabinoxylan (Chapter 3), and some β-glucan sources act synergistically with other TLRs.

**Chapter 5** - To further elucidate effector-function relationship between dietary fiber composition and binding to PRRs, we took lemon pectins as study subject, as pectin can be obtained in different and gradual grades of methyl acidifications. This facilitates detailed studies on molecular interactions with PRRs. In chapter 5 we analyzed the mechanism for direct immunomodulatory effects of pectin and found a degree of methyl esterification dependent binding to TLR2. As TLR2 is involved in binding to so-called danger associated molecular patterns (DAMPs) which are released by damaged cells in the intestine we hypothesized that the pectins responsible for blocking TLR2 might be instrumental in pathologies involving DAMP induced TLR2 activation. An example of this is chemotherapy induced mucositis. Therefore, the specific TLR2 blocking pectins were applied to doxorubicin treated mice that develop mucositis. We studied whether the beneficial effects can be explained by direct interactions with PRRs or is dependent on SCFA modulation by microbiota. This study demonstrates the principle applicability of our PRR screening platform and predicting efficacy of specific dietary fibers for prevention of disease.

**Chapter 6** - To gain more insight in why specific pectins bind to TLR2 while others cannot, we attempted to identify potential binding sites of pectin on the TLR2 receptor. Potential binding sites were identified by bioinformatics tools after which TLR2 mutant were generated with point mutations in the potential binding sites. This study and strategy gives to our opinion important insight in how dietary fibers have to be constructed to maximize their efficacy.

**Chapter 7** - General discussion.

**Chapter 8**
Addendum 1: Food and/or feed compositions for preventing and treating inflammatory diseases.
Addendum 2: Food compositions for managing body weight.
Addendum 3: Immune stimulating product comprising cereal β-glucan.

**Chapter 9** - General summary
References:


Introduction


122. Knudsen, K.E., Fiber and nonstarch polysaccharide content and variation in common crops


