

## Inulin, oligofructose and immunomodulation

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Diet is known to modulate immune functions in multiple ways and to affect host resistance to infections. Besides the essential nutrients, non-essential food constituents such as non-digestible carbohydrates may also have an impact on the immune system, especially in the area of the gut-associated lymphoid tissue (GALT). Recent data now provide first evidence that prebiotics such as inulin/oligofructose (IN/OF) modulate functions of the immune system. In animal studies IN/OF primarily activated immune cells in Peyer's patches including IL-10 production and natural killer (NK) cell cytotoxicity. Other immune functions modulated by IN/OF included the concentration of secretory IgA in ileum and caecum, splenic NK cell cytotoxicity as well as splenocyte cytokine production. In different tumour models, a lower incidence of tumours was observed, which in the case of colonic tumours was associated with enhanced NK cell cytotoxicity in the GALT. Few human studies so far have investigated the effects of IN/OF alone or in combination with other dietary supplements on immunocompetence. Supplementation of IN/OF resulted in minor changes of systemic immune functions such as decrease in phagocytic activity. No data are available on the effects of IN/OF on the GALT in man. The mechanisms of the reported effects of IN/OF on the immune system are currently investigated and include: (i) direct effects of lactic acid-producing bacteria or bacterial constituents on immune cells; (ii) the production of SCFA and binding to SCFA receptors on leucocytes. In conclusion, the current data suggest that IN/OF primarily modulate immune parameters in the GALT, but splenocytes are also activated by IN/OF. Human studies are needed to find out whether IN/OF have the potential to modulate systemic immunity in well-nourished individuals and to lower the risk of diseases such as colon cancer.

### **Inulin: Oligofructose: Prebiotic: Immune system: Gut-associated lymphoid tissue**

Diet is known to modulate immune functions in man in multiple ways and for essential nutrients a number of human studies have demonstrated a beneficial role within the immune system (Calder *et al.* 2002). In contrast, the impact of non-essential food constituents on immunocompetence has not been studied thoroughly. These constituents include phytochemicals, microbial products of food fermentation (probiotics and their metabolites) and prebiotics. For the proper functioning of the immune system, the intestinal microflora plays an important role. Its composition is largely dependent on dietary constituents including prebiotics. Besides the effects of non-digestible carbohydrates such as prebiotics on the number and composition of the intestinal microflora, their metabolites may further affect the gut-associated lymphoid tissue (GALT). Prebiotics occur naturally in plant food and have recently become a constituent of an increasing number of foods. First data now indicate that prebiotics may modulate the systemic immune system as well as the local immune system in the gut.

### **Inulin and oligofructose**

Chemically, inulin (IN) and oligofructose (OF) can be defined as polydisperse fructans. Fructans in IN are typically linked by  $\beta(2 \rightarrow 1)$ -fructofuranosyl bonds with a glucose moiety typically resident at the end of almost each fructose chain, and also contain a small branched-chain fraction (1–2% of  $\beta$ -2,6-linkages; van Loo *et al.* 1995). OF is always present in IN, a blend of fructose oligomers and polymers, and differs from IN in regard to its

degree of polymerisation (DP;  $2 < DP < 10$  for OF;  $2 < DP < 60$  for IN). OF can be produced from IN by controlled partial enzymatic hydrolysis of IN, e.g. from chicory roots (Flickinger *et al.* 2003). IN and OF are widespread plant storage carbohydrates in vegetables (chicory root, Jerusalem artichoke, onion, garlic, leek, asparagus), cereals (wheat, rye, barley) and fruit (banana; van Loo *et al.* 1995). They are added to food due to their technological benefits (fat and sugar replacement, organoleptic properties, texture improvement; Franck, 2002) or used as supplements due to their various functional properties (improvement of mineral absorption, impact on bowel habit, interaction with lipid metabolism, preventive effect against colon cancer; van Loo *et al.* 1999). A main characteristic effect of IN-type fructans is their prebiotic property. As neither mammalian pancreatic nor brush border enzymes are able to hydrolyse the  $\beta$ -2,1-linkage, IN and OF escape digestion in the small intestine and reach the colon virtually intact where they then undergo fermentation by the resident microbiota and stimulate bifidobacteria growth, resulting in an increased production of SCFA (Schneeman, 1999; Delzenne, 2003).

### **Overview of the immune system**

The immune system guards the body against foreign substances and protects from invasion by pathogenic organisms. It can be divided into two arms: the innate or non-specific immune system and the acquired or specific immune system.

**Abbreviations:** AOM, azoxymethane; DP, degree of polymerisation; FAE, follicle-associated epithelium; GALT, gut-associated lymphoid tissue; GOS, galactooligosaccharides; IEL, intraepithelial lymphocytes; IFN- $\gamma$ , interferon-gamma; IN, inulin; LP, lamina propria; NK, natural killer; OF, oligofructose; PP, Peyer's patches; PRR, pattern recognition receptors; TLR, toll-like receptors.

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The innate immune system acts as a first line of defence by preventing the entry of infectious agents or by eliminating invaded pathogens. It comprises physical barriers such as skin or mucous membranes as well as cells in blood and tissue, e.g. phagocytes or natural killer (NK) cells, but also soluble mediators like complement proteins or cytokines. A challenge to the innate immune system often leads to the activation of the acquired immune system. This system consists of two major cell types, the T- and B-lymphocytes, which enable the specific recognition of and response to invaders. Each B-cell is programmed to produce one type of antibody matching only one specific antigen, so B-lymphocytes represent a part of the memory of the immune system. T-lymphocytes develop into functionally different cell types with specific cytokine patterns: CD4<sup>+</sup>T-helper (Th), CD8<sup>+</sup>T-suppressor cells (Ts) or cytotoxic (CTL) and regulatory CD4<sup>+</sup>/CD25<sup>+</sup>T-cells (T<sub>reg</sub>; Janeway *et al.* 2001). The Th subset is further divided into Th1 cells, which secrete cytokines such as IL-2 and interferon-gamma (IFN- $\gamma$ ) and mediate immunity to intracellular pathogens, and into Th2 cells. These cells are responsible for mediating immunity to extracellular pathogens and stimulate antibody production by the secretion of cytokines such as IL-4 and IL-13 (Janeway *et al.* 2001; Jankovic *et al.* 2001; McGuirk & Mills, 2002; Mowat, 2003). CTL provide for the direct killing of virally infected cells and are able to suppress responses of Th-lymphocytes (Janeway *et al.* 2001). Immunosuppressive functions are also attributed to T<sub>reg</sub>-cells with a cytokine profile distinct from either Th1 or Th2 cells (e.g. TGF- $\beta$  and IL-10). After antigenic stimulation T<sub>reg</sub>-lymphocytes can specifically inhibit the immune response of Th cells (Iijima *et al.* 2001; Janeway *et al.* 2001; McGuirk & Mills, 2002).

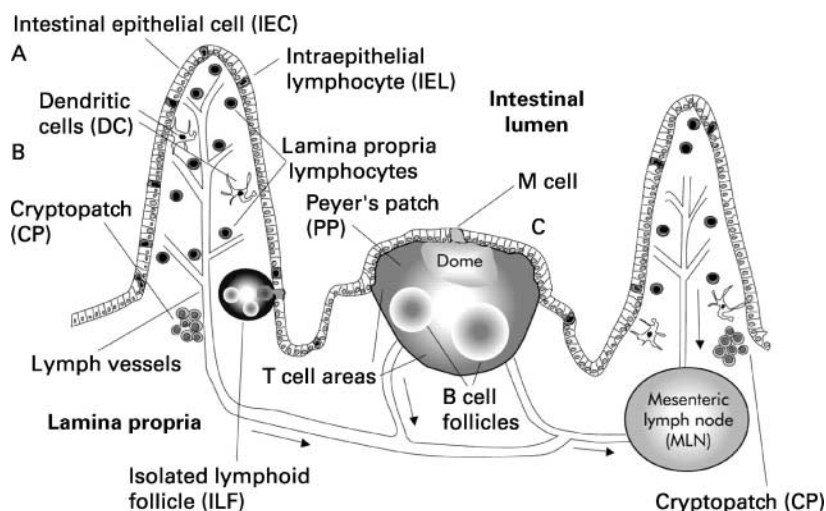
The largest immune organ is situated in the gut where continuous exposure to diverse antigens takes place. The GALT contains about 60% of all lymphocytes in the body and is compartmentalised into inductive and effector sites of aggregated (e.g. Peyer's patches (PP)) and non-aggregated cells (e.g. lamina propria (LP) and intraepithelial lymphocytes (IEL)) forming a unique immune network (Mowat & Viney, 1997; Iijima *et al.* 2001; Fig. 1). PP are lymphoid aggregates in the submucosa separated from the lumen by

the 'follicle-associated epithelium' (FAE). The FAE contains specialised epithelial cells, M-cells, which are responsible for the uptake and transport of antigen into the underlying lymphatic tissue where it is presented to dendritic, T- and B-cells. Lymphocytes activated within the inductor region of PP disseminate via mesenteric lymph nodes, the crossroads between peripheral and mucosal recirculation, migrate into the bloodstream through the thoracic duct and finally return to mucosal sites (Mowat, 2003). The layer of connective tissue between the epithelium and the *muscularis mucosae* forms the LP and comprises B-cells (memory cells as well as IgA-producing plasma cells), mast cells, dendritic cells, macrophages and T-cells of mainly Th function (MacDonald, 2003). IEL, another arm of the effector site of the GALT, are located between epithelial cells along the small and large intestine and therefore directly facing the bowel lumen – they represent the first component of the mucosal immune system to encounter bacterial and food antigens. In contrast to LP leucocytes, IEL are a population of mainly CTL and suppressor-type T-lymphocytes. They help to eliminate infected or transformed epithelial cells and play a crucial role in the maintenance of oral tolerance, the unresponsiveness of systemic lymphoid organs to harmless foreign antigens (for example, food-borne), while sustaining protection against pathogens (Abreu-Martin & Targan, 1996; Iijima *et al.* 2001; MacDonald, 2003).

### Immunomodulatory effects of inulin and oligofructose

#### Human studies

The direct effects of prebiotics on the human immune system have so far only been investigated in a few studies (Schley & Field, 2002). In a study with elderly people living in a nursing home, 3 weeks of OF supplementation at a dose of twice 4 g/d increased faecal bacterial counts of bifidobacteria (Guigoz *et al.* 2002). The percentage of CD3<sup>+</sup>, CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes was raised compared to controls. In contrast, phagocytic activity of peripheral blood granulocytes and monocytes as well as the expression of IL-6 mRNA in monocytes was decreased. The authors speculate that



**Fig. 1.** Schematic overview of the lymphoid elements of the gut-associated lymphatic system. Peyer's patches (PP) and mesenteric lymph nodes (MLN) are organised intestinal lymphoid follicles. (A–C) Pathways of intestinal antigen uptake: luminal antigen can be taken up by (A) intestinal epithelial cells, (B) interdigitating lamina propria dendritic cells, and by (C) M cells. The lymphatic drainage of PP and villus lamina propria goes to the MLN (direction of lymph flow indicated by arrows). (From Spahn & Kucharzik, 2004.)

due to a possible reduction in pathogenic bacteria induced by OF supplementation, inflammatory processes such as phagocytosis and IL-6 production were decreased. However, the study did not include a time control; therefore, the possibility that the finding arose by chance cannot be excluded. A study in free-living elderly receiving a nutritional supplement with placebo or with OF (6 g/d) for a period of 28 weeks investigated the immune response to vaccination with influenza and pneumococcal vaccines (Bunout *et al.* 2002). No differences in serum antibodies between placebo and OF + nutrient supplement were observed after vaccination. *Ex vivo*, mononuclear cells showed similar lymphocyte proliferative responses and cytokine secretion capacities (IL-4, IFN- $\gamma$ ). Since there was no study group which received the OF alone, it is difficult to separate the effects of the nutrient supplement that provided 50 % of vitamin daily reference values from the effects of OF. Elderly subjects with adequate nutrition are known to have appropriate immune functions that cannot be further stimulated by dietary supplements (Watzl *et al.* 2000). In a study with infants (aged 6–12 months) OF (0.67 g/d) in combination with a cereal supplement had no effect on diarrhoea prevalence and antibody titres to *Haemophilus influenza* compared to the cereal supplement alone (Duggan *et al.* 2003). Since 87 % of the children were breast-fed, human milk may have provided adequate amounts of oligosaccharides to exert prebiotic effects in the gut. Two studies have looked at the effect of the combined application of probiotics with a galactooligosaccharide (GOS; Chiang *et al.* 2000; Sheih *et al.* 2001). While GOS in combination with *Bifidobacterium lactis* enhanced NK cell activity compared to the probiotic alone, GOS in combination with *Lactobacillus rhamnosus* HN001 was not significantly different from the probiotic alone. Neither study included a prebiotic group alone or was controlled for time effects, so conclusions regarding the immunomodulatory potential of GOS cannot be drawn. In summary, only one human study so far has investigated the effects of OF alone on the immune system. More human studies including dose–response studies with prebiotics such as IN/OF are needed, since the demonstrated change in microflora composition associated with IN/OF intake (Kaur & Gupta, 2002) is expected to modulate the activity of the GALT.

#### Animal studies

OF (3 g/d) in combination with *Lactobacillus paracasei* was given to piglets either 10 d after birth or 10 d after weaning (Herich *et al.* 2002). After birth, the combined treatment (synbiotic) resulted in reduced phagocytic activity compared with control. When compared to the probiotic group, the synbiotic supplement resulted in lower numbers of leucocytes, lymphocytes and monocytes in the blood. After weaning, no significant differences between groups were observed. Since there was no pure OF group, it is speculative to discuss whether OF modulated phagocytic activity and leucocyte numbers in these piglets. A study with rotavirus-challenged mice treated with a combination of bifidobacteria and an OF supplement also found no differences in diarrhoea incidence and faecal IgA when compared to the probiotic alone. Again, a pure OF group was not included in the study (Qiao *et al.* 2002). Buddington *et al.* (2002) exposed mice supplemented with OF (10 %, w/w) or IN (10 %, w/w) to enteric and systemic pathogens or to different tumour inducers. The incidence of lung tumours after injection of B16F10 tumour cells was not affected by the prebiotic supplements. However, carcinogen-induced aberrant crypt foci in the distal colon were reduced in rats supplemented with OF or IN.

Pathogen exposure in OF- and IN-supplemented rats resulted in reduced mortality compared to cellulose-supplemented controls (10 %, w/w). Taken together, these data suggest that OF and IN enhance immunity against these pathogens and against aberrant cells in the colon. In a follow-up study these authors investigated the modulatory effects of these prebiotics at a similar dose on immune functions in mice (Kelly-Quagliana *et al.* 2003). After a period of 6 weeks with OF and IN supplementation, both prebiotics increased NK cell activity compared to the cellulose group. Lymphocyte subpopulations and faecal IgA did not differ between groups, while phagocytic activity of peritoneal macrophages was stimulated in mice fed either prebiotic. Since control mice received cellulose, and intestinal cellulose degradation differs from intestinal IN/OF fermentation, it is difficult to ascribe the observed changes to a decrease in cellulose intake or an increase in prebiotic intake.

OF obtained by enzymatic synthesis from sucrose has also been classified as prebiotic (Gibson & Roberfroid, 1995; Grizard & Barthelemy, 1999). This OF given to mice for a period of 4 weeks (2.5 and 7.5 %) resulted in minor effects on the immune system (Hosono *et al.* 2003). However, *ex vivo* culture of immune cells isolated from PP of these OF-fed mice together with sonicated *Bifidobacterium pseudocatenulatum* resulted in increased concentrations of total IgA, IFN- $\gamma$ , IL-5, IL-6 and IL-10 compared to mice without OF supplementation. Furthermore, the size of PP in the small intestine was increased in mice supplemented with 2.5 or 7.5 % OF without changing the cell number (Hosono *et al.* 2003). Several other studies also observed a change in PP cellularity. In the *Min* mouse model OF supplementation (5.8 %) resulted in significantly higher numbers of macroscopically detectable lymphoid nodules in the small intestine (Pierre *et al.* 1997). In another mouse study 16 d of OF supplementation (10 %) elevated the total cell number in PP (Manhart *et al.* 2003). While the number of CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocytes was not affected by OF, a significantly higher number of B-lymphocytes was detected in PP compared to controls. These studies suggest that OF fermentation in the large intestine induces changes in distant compartments of the GALT. Bacteria in the terminal ileum may also contribute to OF fermentation, but this has not been proven so far.

In mice infected with *Clostridium difficile* and treated with an antibiotic, OF supplementation (30 g/l drinking-water) increased macrophage numbers in caecum and colon without affecting prostaglandin E<sub>2</sub> production of the macrophages (Gaskin *et al.* 1996). In the *Min* mouse model with tumours occurring primarily in the small intestine, OF reduced the incidence of colon tumours (Pierre *et al.* 1997). Interestingly, mice depleted in CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes had significantly more tumours than immunocompetent mice (Pierre *et al.* 1999) suggesting that reduction of colonic tumour incidence after OF feeding requires a normal functioning immune system. In a follow-up study, the effect of OF on cytokine mRNA expression in *Min* mouse colons was studied (Bassonga *et al.* 2001). mRNA expression of IL-4, IL-5, IL-13 and IFN- $\gamma$  was not affected by OF supplementation, while IL-15 mRNA expression was significantly enhanced.

#### Immunomodulatory effects of oligofructose-enriched inulin

Short- and long-term studies from our laboratory with F344 rats indicate that OF-enriched IN primarily modulates immune functions in the GALT. In both studies, a high-fat, low-fibre

diet was used as the control diet. The sources of carbohydrates in the control diet were sucrose and maltodextrins. The prebiotic group was fed the control diet supplemented with 10% OF-enriched IN (which is a 1:1 mixture of long- and short-chain fractions of IN, extracted from chicory roots). The synbiotic group was fed a similar diet as the prebiotic group diet supplemented with *L. rhamnosus* GG and *B. lactis* Bb12 to provide  $\sim 5 \times 10^{11}$  cfu of each strain per kg of diet. After a feeding period of 4 weeks, functions of immune cells isolated from the major immune organs including blood, spleen, mesenteric lymph nodes and PP were investigated. In addition, secretory immunoglobulin A (sIgA) in ileum and in caecum was quantified. No significant differences in the proportion of CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocytes (all tissues), lymphocyte proliferation (all tissues), NK cell cytotoxicity (all tissues), neutrophil and monocyte phagocytosis (blood and spleen) as well as in neutrophil oxidative burst activity (blood) were observed between groups (Roller *et al.* 2004a). In contrast, OF-enriched IN supplementation significantly increased the production of IL-10 in PP compared with controls fed the high-fat, low-fibre control diet ( $0.56 \pm 0.38 \mu\text{g/l}$  v.  $0.31 \pm 0.13 \mu\text{g/l}$ ,  $P < 0.05$ ). The production of IL-10 was highly correlated with the production of IFN- $\gamma$  ( $r = 0.90$ ;  $P < 0.0001$ ) suggesting that the prebiotic supplement simultaneously activated different T-lymphocyte subpopulations and/or dendritic cells of the GALT. The synbiotic treatment significantly enhanced the production of sIgA in the ileum ( $1.30 \pm 0.75 \text{ OD}_{450}$  v.  $0.78 \pm 0.36 \text{ OD}_{450}$ ,  $P < 0.05$ ), while the prebiotic treatment increased sIgA in the caecum ( $1.15 \pm 0.77 \text{ OD}_{450}$  v.  $0.66 \pm 0.42 \text{ OD}_{450}$ ,  $P < 0.05$ ). Probably, the availability of prebiotics in the ileum supported the growth of the simultaneously supplemented prebiotics and consequently stimulated sIgA synthesis. In contrast, in caecum with a high quantity of endogenous micro-organisms, prebiotic if applied alone supported growth of these bacteria which also stimulated sIgA production. In synbiotic-treated rats, however, prebiotics may have already been metabolised in the ileum and therefore could not further support bacterial growth in the caecum. Our results are in line with findings of a recent study demonstrating that feeding of OF to mice increased faecal IgA concentration (Hosono *et al.* 2003).

In the long-term study (33 weeks) we focused on the effects of prebiotics and synbiotics on the incidence of colonic tumours and on immune parameters within the same animals in a rat model of colon carcinogenesis (Roller *et al.* 2004b). The basic diet as well as the prebiotic- and synbiotic-supplemented diets were the same as in the short-term study. Ten days after beginning feeding the experimental diets, a number of the rats were administered the colon carcinogen azoxymethane (AOM). In all groups four or five animals were treated with saline instead of AOM and served as control for the carcinogen treatment. Supplementation of the control diet with the OF-enriched IN reduced the incidence of colonic tumours (Femia *et al.* 2002). The AOM treatment by itself reduced NK cell activity in all tissues investigated. Prebiotic (PP) and synbiotic (PP and spleen) supplementation prevented this AOM-associated suppression of NK cell activity, indicating a stimulatory potential of OF-enriched IN under these conditions. In addition, PP of synbiotic-supplemented rats exposed to AOM showed significantly higher NK cell activity compared with controls ( $42 \pm 6\%$  v.  $32 \pm 6\%$ ,  $P < 0.05$ ). In rats without AOM treatment OF-enriched IN stimulated NK cell activity in the spleen ( $43 \pm 10\%$  v.  $31 \pm 4\%$  in controls,  $P < 0.05$ ). In rats without AOM treatment prebiotic reduced the CD4:CD8 ratio in spleen

compared with animals in the control group ( $7.9 \pm 1.8$  v.  $13.5 \pm 2.4$ ;  $P < 0.01$ ) without significantly changing the percentage of CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocytes. In AOM-exposed rats prebiotic and synbiotic supplementation enhanced IL-10 production in PP compared with controls (PRE  $331 \pm 99 \text{ ng/l}$ , SYN  $324 \pm 147 \text{ ng/l}$  v. control  $197 \pm 85 \text{ ng/l}$ ,  $P < 0.01$ ). Taken together, short- and long-term (AOM-exposed rats) supplementation with OF-enriched IN affected primarily immune functions of the GALT. Long-term supplementation of this prebiotic in rats not exposed to AOM basically affected the spleen. The differences between the systemic and the local immunomodulatory effects of OF-enriched IN in the gut may be related to the underlying mechanisms such as the production of SCFA in the large intestine.

#### Mechanisms for the effects of inulin/oligofructose on the immune system

Data from animal studies with different types of dietary fibres suggest that these non-digestible constituents exert distinct immunological effects in the GALT (Lim *et al.* 1997; Kudoh *et al.* 1998, 1999; Field *et al.* 1999). Based on the outcome of the studies described earlier, non-digestible oligosaccharides that classified as prebiotics also affect the GALT. A major outcome of these studies is that OF supplementation increases cell number and cell composition in PP. The underlying mechanism of prebiotic-induced alterations of the cellular structures of PP is not yet known. Substantial experimental data suggest at least three different types of mechanisms of prebiotics that mediate these and other immunological effects (Table 1).

First, IN/OF are known to increase the amount of lactic acid-producing bacteria, especially bifidobacteria (Gibson *et al.* 1995; Howard *et al.* 1995; Bounnik *et al.* 1999). The IN/OF-induced shift in the intestinal microflora towards bifidobacteria and other SCFA-producing bacteria changes the presence of pathogen-associated molecular patterns in the intestinal lumen including endotoxin or lipopolysaccharides, teichoic acids and unmethylated CpG motifs of DNA (Akira *et al.* 2001). Immune cells as well as epithelial cells respond via pattern recognition receptors (PRR) such as the toll-like receptors (TLR) to these molecular motifs. TLR signalling results in the activation of NF- $\kappa$ B and the secretion of pro-inflammatory cytokines (Abreu, 2003; Cherayil, 2003). Ingestion of bifidobacteria is associated with increased IgA levels in the small intestine and faeces and *ex vivo* IgA production by PP B-lymphocytes (Takahashi *et al.* 1998; Fukushima *et al.* 1999; Qiao *et al.* 2002). One study with dogs supplementing a low dose of OF (2 g/d) could not find significant effects on the number of bifidobacteria or on immunological markers (Swanson *et al.* 2002). This outcome supports the hypothesis that changes in numbers of bifidobacteria induced by OF supplementation are a prerequisite for changes of immunological functions such as IgA production. In addition to the increase in bacterial cell number, prebiotics promote the increase in bacterial cell wall components as well as DNA derived from luminal bacteria that in turn may stimulate mucosal immune cells.

A second potential mechanism relates to the enhanced production of SCFA in IN/OF-supplemented animals. SCFA are produced by microbial fermentation in the colon with total concentrations ranging from 70 to 140 mM (Engelhardt *et al.* 1991) and are rapidly transferred to the bloodstream. Usual SCFA concentrations in the bloodstream are 100–150  $\mu\text{M}$  for

**Table 1.** Potential mechanisms of prebiotic-induced immune alterations in the gut

Selective increase/decrease in specific bacteria which modulate cytokine and antibody production
Increase in intestinal SCFA production and enhanced binding of SCFA to G-coupled protein receptors on leucocytes
Interaction with carbohydrate receptors on leucocytes

acetate, 4–5  $\mu\text{M}$  for propionate and 1–3  $\mu\text{M}$  for butyrate (Wolver *et al.* 1997). Colonic infusion of butyrate or SCFA resulted in enhanced epithelial proliferation in distant intestinal segments (Kripke *et al.* 1989; Ichikawa *et al.* 2002) suggesting that the production of SCFA in the colon induces physiological changes throughout the intestinal tract.

Long-term supplementation of rats with OF-enriched IN increased caecal SCFA concentrations in rats and especially enhanced butyrate levels (Femia *et al.* 2002). Butyrate is known to suppress lymphocyte proliferation, to inhibit cytokine production of Th1-lymphocytes, to induce T-lymphocyte apoptosis and to upregulate IL-10 production (Säemann *et al.* 2000; Cavaglieri *et al.* 2003; Kurita-Ochiai *et al.* 2003). NF- $\kappa\text{B}$ , a central pro-inflammatory transcription factor regulating cytokine mRNA expression, is influenced by butyrate (Inan *et al.* 2000). In the rat total parenteral nutrition supplemented with SCFA significantly stimulated NK cell cytotoxicity compared to total parenteral nutrition without SCFA (Pratt *et al.* 1996). Intravenous application of pharmacological doses of acetate also enhanced NK cell cytotoxicity (Ishizaka *et al.* 1993). Taken together, these data suggest that SCFA as fermentation products of IN/OF may affect leucocytes within the GALT.

Recently, two receptors for SCFA have been identified on leucocytes, opening up new perspectives for understanding how SCFA may activate leucocytes and induce signal transduction. For the G-protein-coupled receptor GPR43, acetate and propionate have been found to be the most potent ligands (Brown *et al.* 2003; Nilsson *et al.* 2003). Butyrate and isobutyrate are more active on the receptor GPR41 (Le Poul *et al.* 2003). While GPR41 is expressed in a wide range of tissues including neutrophils, GPR43 is highly expressed in immune cells (Brown *et al.* 2003; Le Poul *et al.* 2003).

The average concentrations of propionate and butyrate in blood are too low to activate GPR41 or GPR43. However, the blood concentrations reached by acetate are well within the active range for GPR43 (Le Poul *et al.* 2003). Enhanced SCFA production in the gut after prebiotic supplementation may increase SCFA supply to immune cells located along the GALT (Bach Knudsen *et al.* 2003) and activate these cells via the SCFA receptors. Such local effects of SCFA may explain in part the observed differences between the systemic and the local immune effects in the gut in IN/OF-supplemented animals and in dogs supplemented with different types of fermentable dietary fibres (Field *et al.* 1999).

The third mechanism points to interactions of prebiotic carbohydrates with carbohydrate receptors on immune cells. Phagocytic cells, minor subsets of T- and B-lymphocytes and NK cells express the complement receptor 3 (CD11b/CD18; Ross & Vetvicka, 1993). This receptor mediates cellular cytotoxic reactions against target cells bearing specific carbohydrate structures. Soluble  $\beta$ -glucans derived from the yeast cell wall are particularly potent stimulators of this receptor. Recently,

the  $\beta$ -glucan receptor dectin-1 on neutrophils, monocytes and macrophages has been identified (Brown & Gordon, 2001; Herre *et al.* 2004). This C-type lectin receptor belongs to the PRR, is widely expressed in thymus, spleen and the small intestine, and recognises a variety of  $\beta$ -1,3-linked and  $\beta$ -1,6-linked glucans (DP > 7) from fungi and plants. *In vitro*, the non-digestible oligosaccharides nigerooligosaccharides stimulated NK cell cytotoxicity pointing to a direct effect of this oligosaccharide on NK cells via specific lectin-type receptors (Murosaki *et al.* 1999). While mannose receptors have also been identified on immune cells (Herre *et al.* 2004), it is presently not known whether specific fructose receptors exist on immune cells. Fructose *in vitro* is known to modulate non-opsonic phagocytosis and reactive oxygen species production of phagocytes (Speert *et al.* 1984; Sehgal *et al.* 1993).

## Conclusions

Although data from human studies are still scarce, the results from recent animal studies clearly suggest that IN/OF have a strong impact on the immune system, immune cells of the PP being primarily activated by such prebiotics. Data from tumour models further demonstrated that a reduced number of colonic tumours in IN/OF-supplemented animals coincided with enhanced NK cell cytotoxicity. Whether humans with a daily intake of prebiotics also benefit in regard to improved host resistance and reduced colon cancer risk remains to be studied.

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