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 β(2→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 β-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 < 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 β-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.
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⁺ T-helper (Th), CD8⁺ T-suppressor cells (Ts) or cytotoxic (CTL) and regulatory CD4⁺/CD25⁺ T-cells (Treg; Janeway et al. 2001). The Th subset is further divided into Th1 cells, which secrete cytokines such as IL-2 and interferon-gamma (IFN-γ) 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 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 Treg-cells with a cytokine profile distinct from either Th1 or Th2 cells (e.g. TGF-β and IL-10). After antigenic stimulation Treg-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 (Guiroz et al. 2002). The percentage of CD3⁺, CD4⁺ and CD8⁺ 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

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**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 control group; therefore, the possibility that the finding arose by chance cannot be excluded. A study in free-living elderly individuals 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-γ). 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 influenzae 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 probiotic 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 cellulinose-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-Quaglia 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 & Barthomeuf, 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-γ, 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+ and CD8+ 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 E2 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+ and CD8+ 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-γ 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
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 ($42.6 \pm 6$% v. $32.6 \pm 6$, $P<0.05$). In rats without AOM treatment OF-enriched IN stimulated NK cell activity in the spleen ($43.10$% v. $31.4$% 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+ and CD8+ T-lymphocytes. In AOM-exposed rats prebiotic and synbiotic supplementation enhanced IL-10 production in PP compared with controls ($331 \pm 99$ ng/l, SYN $324 \pm 147$ ng/l v. control $197 \pm 85$ 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; Kadok 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; Boullik 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 $\mu$M for...
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 (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 (Siemann et al. 2000; Cavaglieri et al. 2003; Kurita-Ochiai et al. 2003). NF-κB, 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. 2003). 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 leukocytes within the GALT.

Recently, two receptors for SCFA have been identified on leukocytes, opening up new perspectives for understanding how SCFA may activate leukocytes 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 β-glucans derived from the yeast cell wall are particularly potent stimulator of this receptor. Recently, the β-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 β-1,3-linked and β-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 (Muroski 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 colon 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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References


S54

B. Watzl et al.


