

## IMMUNOLOGICAL PROPERTIES OF INULIN-TYPE FRUCTANS

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IMMUNOLOGICAL PROPERTIES OF INULIN-TYPE FRUCTANS

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### ABSTRACT

Beneficial effects of inulin-type fructans are discussed in view of studies that applied the oligosaccharides in colon cancer, chronic inflammatory diseases, vaccination efficacy, and prevention of infection and allergy. In the present paper we discuss their immunomodulating effects. It is suggested that immunomodulation is elicited through indirect and direct mechanisms. Indirect mechanisms encompass stimulation of growth and activity of lactic acid bacteria, but can also be caused by fermentation products of these bacteria i.e. short chain fatty acids (SCFAs). Evidence for direct effects on the immune system generally remains to be confirmed. It is suggested that inulin-type fructans can be detected by gut dendritic cells (DCs), through receptor ligation of Pathogen Recognition Receptors (PRRs) such as Toll Like Receptors (TLRs), nucleotide oligomerization domain containing proteins (NODs), C-type lectin receptors (CLRs), and galectins, eventually inducing pro- and anti-inflammatory cytokines. DCs may also exert antigen presenting capacity towards effector cells, such as B cells, T cells, and natural killer (NK) cells locally, or in the spleen. Inulin-type fructans may also ligate PRRs expressed on gut epithelium, which could influence its barrier function. Inulin-type fructans are potent immunomodulating food components that hold many promises for prevention of disease. However, more studies into the mechanisms, dose-effect relations, and structure-function studies are required.

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### INTRODUCTION

The scientific and industrial functional food worlds are meeting some new challenges. The consumer awareness that food is not only required to supply energy and nutrition, but also that healthy food is essential for prevention of disease and for both physical and mental well-being (Menrad. 2003, Mollet and Rowland. 2002, Rowland et al. 1998) is growing. This causes an increased demand for functional foods. A category of functional foods that has received much attention in the last decade are products containing prebiotic fibers. Inulin-type fructans belong to this family. Inulin-type fructans are naturally occurring linear plant oligo- and polysaccharides which consist of minimally two fructose-units, and at least one  $\beta(2-1)$  fructosyl-fructose glycosidic bond (Kelly. 2008). It is a family of molecules which meet the three classification criteria for being considered a prebiotic, as defined by Gibson and Roberfroid (Gibson and Roberfroid. 1995); i.e. resistance to hydrolysis or absorption in the upper gastrointestinal (GI) tract, fermentation by the intestinal microbiota, and selective stimulation of the growth and/or activity of beneficial intestinal bacteria, such as *Lactobacillus* species and *Bifidobacterium* species. Well-known effects of inulin-type fructans on the gut microbiota are the increase in numbers of these types of bacteria in the intestinal tract, and the selective fermentation of  $\beta(2-1)$  fructans by most *Bifidobacterium* species (Gibson et al. 1995), and also by some *Lactobacillus* species (Kaplan and Hutkins. 2000).

For a considerable period of time, research has mainly focussed on the prebiotic, i.e. indirect effects of inulin-type fructans (Eiwegger et al. 2010, Alles et al. 1996, Roberfroid. 2007, Roberfroid et al. 2010, Delzenne et al. 2007) Somewhat more recent came the notion that prebiotic carbohydrates such as inulin-type fructans may elicit additional direct effects such as

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immunomodulation along the gastrointestinal tract (Gibson and Roberfroid. 1995, Eiwegger et al. 2010, Roberfroid et al. 2010, Niess et al. 2005, Hapfelmeier et al. 2008). This may occur via direct contact with gut DCs which sample immune active components from the gut lumen, and intraepithelial lymphocytes (IELs) which can respond upon contact with immune active food components (Hapfelmeier et al. 2008, Chieppa et al. 2006, Murosaki et al. 1999). It is also conceivable that contact of inulin-type fructans with the gut epithelial cells modulates the innate immune barrier by modifying epithelial tight junction integrity, or alters the signals from epithelial cells to the underlying immune cells (Cummings et al. 2001). In addition, the glycosidic and non-glycosidic fermentation products produced by gut microbiota upon fiber supplementation are under investigation for their beneficial health effects as reviewed recently by Meijer et al. (Meijer et al. 2010) and Macfarlane and Macfarlane (Macfarlane and Macfarlane. 2011). The glycosidic fermentation products can be small sized oligosaccharides and the non-glycosidic fermentation products include SCFAs such as acetate, propionate, and butyrate (Kolida et al. 2002, Wong et al. 2006). Although there are some recent reviews on the immunomodulatory properties of inulin-type fructans (Vos et al. 2007b, Seifert and Watzl. 2007, Lomax and Calder. 2009), in the majority of reviews dealing with inulin-type fructans, immunomodulation was mainly discussed as an integral part of the health benefits of prebiotic fibers (Roberfroid et al. 2010, Albers et al. 2005, de Vrese and Schrezenmeir. 2008, Kelly. 2009, Rijnierse et al. 2011). As many studies demonstrate that inulin-type fructans have unique ways for immunomodulation, but the underlying mechanisms are still incompletely understood, we decided to write this review with a focus on the mechanisms of immunoregulatory properties of different types of inulin-type fructans. The present paper provides an overview of the current knowledge of the direct and indirect immunomodulatory properties of inulin-type fructans and

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the possible signalling pathways. This will be done in view of the beneficial effects in several diseases including colon cancer, (Delzenne et al. 2007,Forest et al. 2005,Rafter et al. 2007,Rafter et al. 2007,Reddy et al. 2007), chronic inflammatory diseases (Apanavicius et al. 2007,Lindsay et al. 2006,Casellas et al. 2007,Osman et al. 2006,Ito et al. 2009), vaccination efficacy (Vos et al. 2007a,Vos et al. 2006a,Vos et al. 2006b,Benyacoub et al. 2008,Bunout et al. 2002,Adogony et al. 2007,Saavedra and Tschernia. 2002,Duggan et al. 2003), and prevention of infection and allergy (Eiwegger et al. 2010,Benyacoub et al. 2008,Schouten et al. 2009,van Hoffen et al. 2009,Arslanoglu et al. 2008,Arslanoglu et al. 2007,Perez et al. 2010,Fujitani et al. 2007,Moro et al. 2006a).

### Structure and terminology of inulin-type fructans

Before discussing the immunomodulating properties of inulin-type fructans it is mandatory to discuss the structure and terminology of the family of molecules since their structure or more specifically their chain length probably determines their function in the host. Several studies have indicated that polymer chain length or degree of polymerisation (DP), is an important feature to consider, as it determines where along the GI tract fermentation occurs (Alles et al. 1996,Rumessen et al. 1990,van de Wiele et al. 2007). It appears that short chain fructans are generally fermented relatively fast in the proximal colon, whereas fructans with a relatively long chain resist fermentation until they reach the distal colon where they are metabolized (Roberfroid. 2007,Cummings et al. 2001). In addition, *Bifidobacterium* species differ along the GI tract, so the different DP can determine the types of bacteria that become enriched. This could render different outcomes in health related parameters (Alles et al. 1996).

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Fructans are denoted as Fn, with F for fructose and n representing the number of fructose subunits in the polymer. Most inulin-type fructans in nature contain a terminal glucose residue (denoted as a GFn) as biosynthesis starts with sucrose to which fructose residues are added (Kelly. 2008). Figure 1 depicts Haworth projections of these two types of fructans. When the fructan chain starts with a glucose molecule, this glucose can be removed from the chain by hydrolysing sucrase enzymes, which are produced at the tips of the small intestinal epithelial villi (Wu et al. 1992).

Based on chain length, inulin-type fructans are usually rather arbitrarily divided in subcategories with a relatively small (2 to 4), medium (5 to 10) and relatively large chain length (11 to 60 fructose units). Over the course of time the nomenclature to describe inulin-type prebiotics has been inconsistent. Historically, the term fructooligosaccharides or FOS was used for DP 3-5 material derived from sucrose which is thereby only of the GFn type (Carabin and Flamm. 1999). The term oligofructose or OF was used for DP3-10 material derived from native inulin which can be of both the GFn and the Fn type (Roberfroid. 1999). Later, FOS and OF were and are more and more used as synonyms to describe fructans with a chain length ranging between 2 and 10 subunits (Alles et al.1999). To discriminate, the term short chain FOS (sc-FOS) was used by the company producing this ingredient (Actilight®, Eridania-Beghin Say, Belgium), (Bouhnik et al.2007). Some companies use the term long chain FOS (lc-FOS) or OF (lc-OF) for the long chain inulin that is part of a specific galactooligosaccharide (GOS)/inulin mixture. The term inulin is often applied to inulin-type fructans with chain lengths above 10 subunits, however, inulin is the generic term describing all  $\beta(2,1)$ -fructans without specification of chain length (Kelly. 2008,Roberfroid et al. 2010). In this review we will apply the term FOS for inulin-type fructans of 2 to 10 subunits and we will use the term inulin for inulin-type

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fructans with chain lengths above 10 subunits. Chain length is specified where possible to render an overview of the properties of these specific compounds. Figure 3 depicts chain length profiles as an example for a FOS, a FOS-enriched inulin and a high average molecular weight inulin.

### **The gastrointestinal immune barrier and inulin-type fructans**

Many of the studies addressing immunomodulating effects of inulin-type fructans have focused on the Gut Associated Lymphoid Tissue (GALT, Figure 2). Constituents of this tissue are the lamina propria, Peyer's patches with follicles containing B and T lymphocytes, isolated lymph nodes, mesenteric lymph nodes, and the appendix (Mowat. 2003). Important players in this system are follicle associated Microfold cells (M cells), which are part of the epithelial layer covering the Peyer's patches, and are specialised in transporting antigens from the lumen to the GALT (Ramiro-Puig et al. 2008). DCs and IELs lie in between and just below the epithelial surface. The DCs are capable of sampling and sensing the events in the gut lumen and are strong antigen presenting cells (APCs) (MacPherson et al. 2004). Lamina propria DCs can respond to antigens which have penetrated gut tissue beyond the epithelial barrier and are also strong APCs (MacPherson et al. 2004). Depending on the cytokine environment, APCs can determine whether the T cells they encounter and present their antigen to, differentiate into regulatory T lymphocytes (Tr1 or Th3) or into effector (helper) T lymphocytes (Th1, Th2, or Th17) (Ramiro-Puig et al. 2008). Antigen presentation can occur in the lamina propria or specifically in Peyer's patches or mesenteric lymph nodes (Ramiro-Puig et al. 2008). The immunoglobulin (Ig)M+ B lymphocytes in Peyer's patch follicles are plasma-cell precursors that produce IgA. Memory IgA+ B lymphocytes are generated in the germinal centers of these follicles (Ramiro-Puig et al. 2008,Guilliano et al. 2001,Newberry and Lorenz. 2005). IgA is mainly synthesized in response

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to T lymphocyte activation and the production is again regulated by the cytokine environment. Interleukin (IL)-5, IL-6, and IL-10 stimulate final differentiation of B lymphocytes into IgA-secreting plasma cells (Chin et al. 2003). IgA is the most abundant immunoglobulin in the intestinal mucosa (80-90%) and forms the first line of defence against colonisation and invasion by pathogens, and against damaging toxins (Trushina et al. 2005). T lymphocyte subtypes can be characterised by the cytokines they produce. Th1 lymphocytes typically secrete Interferon (IFN)- $\gamma$ , IL-2, and Tumor Necrosis Factor (TNF)- $\beta$ , and their main function is phagocyte-mediated defence against viral, bacterial, and protozoic infections. Th2 lymphocytes typically secrete IL-4, IL-5, and IL-13, and act as allergic response mediators and defenders against infections produced by helminths and arthropods (Chin et al. 2003, Brandtzaeg and Johansen. 2005). Although it is becoming clear that the Th1/Th2 model is too simplistic, the Th model has still played an important part in developing our understanding of the roles and behavior of Th cells and the cytokines they produce during an immune response. Therefore, other subtypes to discuss are Th3 cells, Th5 cells, Th9 cells, Th17 cells, and Th22 cells. Th3 cells produce the cytokine Transforming Growth Factor-beta (TGF- $\beta$ ) and IL-10. Both cytokines are inhibitory to Th cells; TGF- $\beta$  suppresses the activity of most of the immune system. Th5 cells constitute a subpopulation of Th cells described by Kurowska-Stolarska *et al.* (Kurowska-Stolarska et al. 2008). These cells produce mainly IL-5, but not IL-4, both of which are characteristic type 2 cytokines produced by Th2 cells. Recent studies by Veldhoen *et al.* (Veldhoen et al. 2008) revealed that another Th subset may exist. Th9 cells are claimed to be an IL-9 producing T cell subset focused on defending helminth infections. These cells have been identified as a unique subset of Th cells and constitute a subset of cells known as neutrophil-regulatory T-cells. They are CD4(+) T-cells that are defined by the production of IL-17. Th17 cells develop from naive T-

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cells along a pathway that is distinct from the differentiation pathways that give rise to the Th cell populations known as Th1 cells and Th2 cells (Harrington et al. 2005, Harrington et al. 2006). Th22 cells are IL-22-producing cells which coexpress the chemokine receptor CCR6 and the skin-homing receptors CCR4 and CCR10. This subset of IL-22-producing cells is suggested to be important in skin homeostasis and pathology (Trifari et al. 2009). All these different arms of the gastrointestinal immune barrier can be modulated either indirectly, i.e. via microbiota or directly upon consumption of inulin-type fructans.

### **Indirect mechanism of immunomodulation: Bifidobacteria and SCFAs**

The prebiotic effects of inulin-type fructans are often referred to as bifidogenic effects. These bifidogenic effects were shown in infants (Moro et al. 2006a, Brunser et al. 2006, Kapiki et al. 2007, Kim et al. 2007, Scholtens et al. 2008), adults (Buddington et al. 1996, Bouhnik et al. 1999, Menne et al. 2000, Langlands et al. 2004, Bouhnik et al. 2006), and elderly (Kleessen et al. 1997, Guigoz et al. 2002, Bouhnik et al. 2007). Classically the beneficial effect of inulin-type fructans was assumed to be determined by the effects on the commensal microbiota that formed a barrier for pathogens to enter the host. However, its beneficial effect on commensals probably also has an effect on prevention of inflammation in the systemic circulation. The fermentation products of inulin-type fructans are carbondioxide, hydrogen, lactate, and SCFAs, including acetate, propionate, and butyrate (Kelly. 2008). These products have been studied in relation to beneficial effects and have been reported to protect from colonisation by pathogens or non-commensals by acidification of the colonic content (Alles et al. 1996, Rumessen et al. 1990, Bruhwiler et al. 2008). In addition, they are rapidly adsorbed by the human body (Sembries et al. 2003) and exert their effect on immune cells by binding to activating G protein-

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coupled receptors (GPR) (Covington et al. 2006) GPR41 and GPR43 (Covington et al. 2006, Brown et al. 2003, Le Poul et al. 2003). GPR43 is highly expressed in polymorphonuclear cells (PMNs, e.g. neutrophils) and at lower levels in Peripheral Blood Mononuclear Cells (PBMCs) and purified monocytes. GPR41 is similarly expressed in PBMCs but not in PMNs, monocytes and DCs (Le Poul et al. 2003). Both receptors are equally expressed in bone marrow and spleen. The possible immunomodulatory functions of SCFA are highlighted by a recent study in GPR43<sup>-/-</sup> mice (Maslowski et al. 2009). These mice suffer more from inflammation due to lack of GPR43 binding by SCFA, which normally results in anti-inflammatory effects (Meijer et al. 2010). In these mice, production of inflammatory mediators and immune cell recruitment are increased. These results suggest an immunoregulatory effect for SCFA-mediated GPR43 signalling. More studies are required to confirm whether inulin-type fructan supplementation and subsequent SCFA production actually affects these receptors, but as it has a strong effect on SCFA producing bacteria it is very likely a mechanism by which inulin-type fructans exert their immunomodulatory effect (Stewart et al. 2008).

The intraindividual bifidogenic effect can differ in outcome depending on the initial level of *Bifidobacteria*, and may also differ between individual *Bifidobacterium* species (Alles et al. 1996). Although in general this prebiotic effect is present (Kim et al. 2007, Buddington et al. 1996, Bouhnik et al. 1999, Bouhnik et al. 2006, Bouhnik et al. 1996) there are inconsistencies in prebiotic properties of inulin-type fructans throughout literature (Kelly. 2008). These should probably be explained by differences in the applied type i.e. chain length of fructan, the dose, the study population, the duration of supplementation, and the time intervals for microbiological analysis (Kelly. 2008, Brunser et al. 2006, Kim et al. 2007, Bouhnik et al. 1999, Bouhnik et al. 2006). The digestible mono-, and dimers of fructose or glucose which are present in most

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prebiotics may also influence the bacterial composition upon supplementation. Minor data are available on possible differences in effects of chain length of inulin-type fructans on prebiotic effects, besides the fact that short chain fructans are fermented by more *Bifidobacterium* species compared to long chain fructans (Rossi et al. 2005). Future studies addressing the immunological effects of inulin-type fructan supplementation should take into account the microbiota as the composition of the immune system and the microbiota composition are closely related. Therefore future studies should screen broader and also include microbiota analysis and SCFA measurements in order to allow clear interpretation of what are direct and what are indirect effects.

### **Direct mechanisms of immunomodulation: ligation of PRRs**

To be able to distinguish the good from the bad, the gut immune system is equipped with PRRs. These PRRs recognize molecular structures that are highly conserved and broadly shared by pathogens, known as pathogen-associated molecular patterns (PAMPs) (Janeway. 1989). PRRs include the well-known TLRs, the membrane-bound CLRs, the cytosolic proteins such as NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs), and still to be discovered PRRs that mediate sensing of cytosolic DNA or retrovirus infection (Osorio and Reis e Sousa. 2011, Loo and Gale. 2011, Elinav et al. 2011). Upon PAMP recognition, PRRs initiate signaling processes that may lead to cytokine release, inflammation with clearance of the pathogen as a final goal.

Possible direct effects of inulin-type fructans are thought to entail ligation of PRRs on the surface of gut DCs which continuously sample the gut content and are strong APCs (MacPherson et al. 2004). Potential receptors involved are the TLRs (de Kivit et al. 2011). TLRs are involved in epithelial cell proliferation, secretion of IgA into the gut lumen and expression of

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antimicrobial peptides, which are crucial factors for maintaining a healthy epithelial barrier (Abreu. 2010, Hooper and Macpherson. 2010). They are typically known to possess carbohydrate binding properties and upon ligation will instigate several immune responses. For the same reasons, CLRs, NLRs, and galectins are also potentially involved in inulin-type fructan signalling (de Kivit et al. 2011). Besides DCs, many cell types express TLRs, including epithelial cells (Abreu. 2010). It is conceivable that as polysaccharides, inulin-type fructans could ligate TLRs on the gut epithelial cells and thereby modulate barrier function by promoting tight junction stability, similar to the mechanism reported by Karczewski *et al.* (Karczewski et al. 2010). In addition, the activation of epithelial TLRs could alter their interactions with or signals towards surrounding immune cells such as DCs or IELs (Murosaki et al. 1999). Finally, inulin-type fructans may possess the capacity of interacting with cell membrane lipids or even inserting in the membrane (Figdor and van Spriel. 2010). Vereyken et al. (Vereyken et al. 2003a, Vereyken et al. 2003b, Vereyken et al. 2003c) found that there was a chain length-dependent interaction of inulin with lipids. Inulin-type fructans may interact with or even insert into membrane lipid bilayers (Vereyken et al. 2003a, Vereyken et al. 2003b, Vereyken et al. 2003c). This may have a consequence for stimulating events; if insertion renders the membrane more fluid and more dynamic this may facilitate or enhance receptor clustering and subsequent signal transduction.

Notably, this may be more or only relevant for sites where the mucus layer is relatively thin i.e. the small bowel (Johansson et al. 2011), so fructans can reach the cells relatively easily as compared to the large bowel, considering the thickness of the mucus. It should be noted that most hypotheses on direct ligation of inulin-type fructans or their direct contact with epithelial structures remain to be investigated and are at this point still only speculative.

### Experimental evidence for immunomodulation

Many supplementation studies with inulin-type fructans were performed in healthy experimental animal models. Some studies apply inulin-type fructans only in a synbiotic treatment, i.e. in combination with a prebiotic, limiting the possibility to evaluate the actual fructan effects. The features reported most frequently in healthy experimental animals are increased IgA secretion in serum and fecal samples, and increased IL-10 and IFN- $\gamma$  production in two specific structures of the GALT; the mesenteric lymph nodes and the Peyer's patches (Swanson et al. 2002b, Hosono et al. 2003, Nakamura et al. 2004, Roller et al. 2004b) (Table 1). Inconsistency in results are present for IgA in ileum, serum, and feces (Swanson et al. 2002b, Hosono et al. 2003, Nakamura et al. 2004, Kelly-Quagliana et al. 2003, Verlinden et al. 2006). The IgA production was increased or not affected (Swanson et al. 2002b, Hosono et al. 2003, Nakamura et al. 2004, Kelly-Quagliana et al. 2003, Verlinden et al. 2006), the number of lymphocytes in the blood was increased or unaltered (Trushina et al. 2005, Nakamura et al. 2004, Roller et al. 2004b, Shim et al. 2005, Janardhana et al. 2009), and the number of lymphocytes or subsets in the spleen and thymus were increased or unaltered (Rumessen et al. 1990, Trushina et al. 2005, Kapiki et al. 2007, Waligora-Dupriet et al. 2007). In a single study in sea bream, inulin-type fructan supplementation significantly inhibited phagocytosis and respiratory burst in leukocytes (Cerezuela et al. 2008). However, in a study in salmon, supplementation with 7,5% inulin did not protect against soybean meal-induced colitis (Bakke-McKellep et al. 2007). Evidence for immunomodulation on a genetic level was reported by Yasuda et al. (Yasuda et al. 2009) in a 7 week supplementation study in pigs. Inulin-type fructans were added to the basal corn and soybean meal, which significantly decreased the expression of inflammation related genes, especially in lower gut mucosa. These different reports might be attributed to differences in the

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administered type of fructan (-mixtures) and other differences in experimental set up such as animal species or feeding protocol (Roberfroid. 2005). More and better designed studies in healthy experimental animals and humans are required to determine the specific immunomodulating effects of different inulin-type fructans. Although inulin-type fructan supplementation studies in healthy adult humans have been performed, immunological parameters were unfortunately often not measured. Immunological effects of inulin-type fructans have been studied in infants and elderly, but taking into account their immune status, these groups are to be categorized as immunocompromised, because the microbiota and immune system of infants is not fully developed and the microbiota composition and immune function decreases qualitatively with age (Delzenne et al. 2005). The only conclusions we can draw from the current supplementation studies in healthy human adults, studies in healthy experimental animals, as well as studies in infants and elderly, is that inulin-type fructan supplementation in healthy human adults will generally result in increased *Bifidobacteria* numbers in the gut (Buddington et al. 1996, Bouhnik et al. 1999, Menne et al. 2000, Langlands et al. 2004, Bouhnik et al. 2006), increased levels of fecal sIgA (Swanson et al. 2002, Hosono et al. 2003, Nakamura et al. 2004), increased levels of IL-10 and IFN- $\gamma$  in Peyer's patches (Swanson et al. 2002b, Hosono et al. 2003, Nakamura et al. 2004, Roller et al. 2004b) and increased activity of different immune cells in the spleen (Benyacoub et al. 2008, Trushina et al. 2005, Roller et al. 2004b, Kelly-Quagliana et al. 2003, Stillie et al. 2005), as these are the parameters which are most often reported to have been changed upon supplementation. The evidence for immunomodulation on a systemic level may be somewhat less strong than locally in the gut, however, the local cytokine levels in the gut may have more impact on an immune parameter such as prevention of

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infections, since the gut is the largest organ of the human body to be in such close contact with the outside world.

In the following sections we will review the effects of inulin-type fructans on immune structures in the context of the different disease models which have been studied up to now.

### Cancer models

Two studies using experimental animal cancer models focused on immune parameters involved in anti-tumorigenic reactions. In a study by Roller *et al.* (Roller et al. 2004a), the effects of probiotic *Lactobacillus* LGG and *Bifidobacterium lactis* Bb12, and FOS (“Raftilose,” chain length range 2-10, average 4, 100 g/kg of diet) synbiotic treatment on the immune system of rats were investigated in an azoxymethane (AOM)-induced colon cancer model. Synbiotic supplementation significantly restored Natural Killer cell-like cytotoxicity ( $p < 0.01$ ) and suppressed proliferative responsiveness of lymphocytes in Peyer’s patches of AOM-treated rats. It should be noted that no normal diet or placebo diet group was included in this study and that this alteration of responsiveness may be related to the background of the high fat diet. FOS supplementation significantly stimulated IL-10 production in Peyer’s patches and mesenteric lymph nodes of rats not treated with AOM ( $p < 0.05$ ). In pro- and synbiotic groups, IFN- $\gamma$  production in Peyer’s patches was significantly decreased independent of AOM treatment ( $p < 0.05$ ). A study by Forest *et al.* (Forest et al. 2005) showed that short chain fructans (chain length range 1-4, mostly 3”) reduced colon tumor incidence in intestinal neoplasia prone “adenomatous polyposis coli /multiple intestinal neoplasia” (*Apc*<sup>+/Min</sup>) mice via a functional local immune response. *Apc* is a tumor suppressor gene involved in development of colorectal

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cancer (Kartheuser et al. 1995). In the colons of *Apc+/Min* mice, FOS treatment restored large intestine-intraepithelial lymphocytes (LI-IELs) surface expression of anti-tumorigenic IL-15/IL-15R $\alpha$ . In addition, FOS specifically induced a decrease in the proportion of CD4<sup>+</sup> CD25<sup>+</sup> LI-IELs which are considered to be tumor facilitating cells (Forest et al. 2005). Publications on fiber supplementation in human cancer patients are rare, and mostly discuss FOS, inulin, or FOS-enriched inulin in synbiotic mixtures using *Lactobacillus* LGG and *Bifidobacterium lactis* Bb12 (Rafter et al. 2007, Roller et al. 2007); or *Lactobacillus acidophilus* La5, *Lactobacillus bulgaricus*, *Bifidobacterium lactis* BB-12, and *Streptococcus thermophilus* (Roller et al. 2007). These studies demonstrated that supplementation induced secretion of IL-2 and IFN- $\gamma$  by PBMCs and decreased bacterial translocation (Rafter et al. 2007, Reddy et al. 2007, Roller et al. 2007). Results for immunological parameters in experimental animal cancer models and in human colon cancer patients upon inulin-type fructan supplementation are summarized in Table 2. Studies regarding tumor growth and outcome of disease upon supplementation with inulin-type fructans have demonstrated anti-carcinogenic properties in multiple experimental animal models (Taper et al. 1997, Taper et al. 1998) and cell lines (Yeh et al. 2007, Klinder et al. 2004). As previously reviewed by Taper *et al.* (Taper and Roberfroid. 2005) dietary treatment with inulin and/or FOS incorporated in the basal diet for experimental animals: (i) reduced the incidence of mammary tumors induced in Sprague-Dawley rats by methylnitrosourea; (ii) inhibited the growth of transplantable malignant tumors in mice; (iii) decreased the incidence of lung metastases of a malignant tumor implanted intramuscularly in mice. Moreover, dietary treatment with inulin and/or FOS (iv) significantly potentiated the effects of cytotoxic drugs and potentiated the effects of radiotherapy on solid form of transplantable lymphoid tumor. Especially the fermentation products of FOS-enriched inulin (“Synergyl”) i.e. SCFA and

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Deoxycholic Acid appear to limit tumor growth (Munjal et al. 2009). The most consistent findings were reductions in aberrant crypt foci, tumor incidence and metastasis in models which make use of chemically induced pre-neoplastic lesions or tumors in the colon of rats and mice (Femia et al. 2002, Poulsen et al. 2002, Verghese et al. 2002, Buddington et al. 2002, Taper and Roberfroid. 2002). Only one (preliminary) study in patients with colorectal adenomas was performed so far. This study was an open multicenter study on the effects of FOS. No beneficial effect was found on proliferation at the rectal crypts (Boutron-Ruault et al. 2005). However, from experimental use of human ex vivo cells, significant anti-carcinogenic effects were reported (Burns and Rowland. 2004, Reddy et al. 1997, Reddy. 1998, Rao et al. 1998, Pool-Zobel et al. 2002). Moreover, when applied in a synbiotic protocol, inulin has already shown beneficial effects on inhibition of carcinogenic processes (Misikangas et al. 2005).

The prescription of inulin-type fructans to colon/colorectal cancer patients should be applied with some caution, as there are reports that under certain circumstances, inulin-type fructans can actually enhance proliferation of adenomas (Misikangas et al. 2005, Misikangas et al. 2008, Pajari et al. 2003). It should be noted that these reports involve mice studies and results so far do not show these effects in human colon cancer. The mechanisms behind these effects are not clear and require further investigation. The production and balance of anti-inflammatory and pro-inflammatory/anti-tumorigenic cytokines such as IL-10 and IL-12 respectively may play a role in these observations because anti-inflammatory cytokines could confer an inhibitory effect on the anti-tumorigenic properties of proinflammatory cytokines. On the other hand, inulin-type fructan supplementation has shown promising anti-tumorigenic properties (Forest et al. 2005, Roller et al. 2004b) and further investigation into the underlying mechanisms of these processes, which may involve immunomodulation, is warranted.

### Intestinal inflammation models

Inflammatory Bowel Disease (IBD) is a group of inflammatory conditions of the GI tract. IBD is thought to be caused by a combination of genetic, environmental, and immunological factors (Sartor. 2004) The current paradigm is that these diseases result from a lack of tolerance to resident intestinal bacteria in genetically susceptible hosts (Podolsky. 2002, Strober et al. 2007, Xavier and Podolsky. 2007, Hata et al. 2001, Rath et al. 2001). The major types of IBD are Crohn's Disease (CD) and Ulcerative Colitis (UC) (Crohn et al. 1932). CD and UC share similar symptoms but also differ in substantial features. CD can occur along the entire tract, from mouth to anus, whereas UC specifically affects the large intestine or colon. Another difference is that UC occurs more superficially in the gut lining while CD can also affect deeper layers of the intestine. Another affliction of the intestine is Irritable Bowel Syndrome (IBS); a functional bowel disorder characterized by chronic abdominal pain, discomfort, bloating, and alteration of bowel habits in the absence of any detectable organic cause (van der Horst et al. 2010). Evidence is slowly increasing that inulin-type fructan supplements exert beneficial effects on both bowel movements (Kleessen et al. 1997, Lopez Roman et al. 2008, Gruenwald et al. 2009, Marteau et al. 2011) as well as on GI immune parameters (Lindsay et al. 2006, Bakker-Zierikzee et al. 2006). Supplementation studies have been performed in several animal colitis models as well as in patients with IBD or IBS (Table 3 and 4). Leenen and Dieleman (Leenen and Dieleman. 2007) recently reviewed the effects of pre-, and synbiotics on IBD/IBS. Few studies using prebiotics alone have been performed so far, however results look promising with regard to therapeutic use in treatment of IBD/IBS. FOS-enriched inulin supplementation lowered disease activity scores (Lindsay et al. 2006), fecal calprotectin (a gut inflammation marker) (Casellas et al. 2007), and inulin supplementation lowered pouchitis disease index (Welters et al. 2002). Sigmoidoscopy

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scores (i.e. inflammation scores of endoscopy of the distal colon) were reduced, and endoscopically and histologically verified reductions in inflammation of the mucosa of the ileal reservoir were observed (Lindsay et al. 2006, Casellas et al. 2007). mRNA levels of beta defensins 2, 3, and 4 (i.e. antimicrobial proteins) were significantly reduced by treatment while these markers are normally upregulated in active UC (Macfarlane et al. 2005). When applied in synbiotic set up, increased amounts of *Bifidobacteria* in rectal mucosa were reported, and significant reductions in the expression of molecules that control inflammation in active UC (Macfarlane et al. 2005). TNF $\alpha$ , and IL-1 $\alpha$  mRNA levels in mucosal tissue were significantly reduced (p=0.0175 and p=0.0379) but also, a significant increase in IL-10 positive CD11+ DCs and expression of TLR2 and TLR4 was reported (Macfarlane et al. 2005). Beneficial effects in UC patients have been reported (Welters et al. 2002, Macfarlane et al. 2005, Furrie et al. 2005) as supplementation resulted in improvement of the full clinical appearance of chronic inflammation in patients receiving this therapy. In addition to reduction of intestinal inflammation, regeneration of epithelial tissue was observed. To our knowledge no trials have been conducted as yet to determine whether chronic supplementation with inulin-type fructans might ameliorate disease progression, to prevent disease recurrence, or sustain periods of clinical remission. Currently available data concerns trials aiming at investigating the immediate effects (Welters et al. 2002, Macfarlane et al. 2005, Furrie et al. 2005), but it may be worthwhile in future studies to include longer trial periods.

Regarding IBD, studies on treatment of chronic intestinal inflammation using inulin-type fructans have shown major benefits in experimental animal models of colitis (Osman et al. 2006, Ito et al. 2009, Catala et al. 1999, Butel et al. 2002, Videla et al. 2001, Cherbut et al. 2003, Schultz et al. 2004, Hoentjen et al. 2005, Daddaoua et al. 2006, Winkler et al. 2007, Smith et

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al. 2008) (Table 3). Several types of experimental animal models exist to mimic IBD; dextran sodium sulfate (DSS)-induced colitis (Osman et al. 2006, Videla et al. 2001, Winkler et al. 2007, Moreau et al. 2003), trinitrobenzene sulphonic acid (TNBS)-induced colitis (Ito et al. 2009, Cherbut et al. 2003, Daddaoua et al. 2006, Holma et al. 2002), and a HLA-B27 transgenic colitis model (Schultz et al. 2004, Hoentjen et al. 2005) were studied in relation to inulin-type prebiotics. In most of these experimental animal models, supplementation rendered statistically significant beneficial effects by reduction of mucosal damage and reduced release of inflammatory mediators such as IL-1 $\beta$  (Osman et al. 2006, Hoentjen et al. 2005, Daddaoua et al. 2006), inducible nitric oxide synthase (Daddaoua et al. 2006), myeloperoxidase activity (Cherbut et al. 2003, Smith et al. 2008, Lara-Villoslada et al. 2006) cyclooxygenase 2, and mucin 3 (Daddaoua et al. 2006). In conclusion, inulin-type fructans are promising agents to modulate the immune parameters involved in colitis. Underlying mechanisms of these effects are however still unclear and warrant more studies on the effects of inulin-type fructans in experimental animal colitis models. Moreover, in IBD patients, long term intervention studies and follow up are required to determine whether inulin-type fructans might have long term beneficial effects in treatment of these diseases.

IBS is a common disorder of the GI tract and there is increasing evidence to support the role for immune activation in IBS (Hunter et al. 1999, Astegiano et al. 2006, Kennedy et al. 2011, Clarke et al. 2010, Wouters and Boeckxstaens. 2011). In a number of patients the onset is triggered by acute gastroenteritis (Spiller et al. 2000, Cumberland et al. 2003, Dunlop et al. 2003, Neal et al. 1997). Evidence of sustained immune activation has been found in these cases (Gwee et al. 1999). However low-grade immune activation without previous acute gastroenteritis can also induce IBS symptoms (Chadwick et al. 2002, Tornblom et al. 2002). The immune

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parameters most often increased in IBS, are IL-6 and IL-8 levels (Dinan et al. 2006, Dinan et al. 2010) and baseline and lipopolysaccharide (LPS) induced TNF- $\alpha$ , IL-1 $\beta$  and IL-6 levels in IBS patients PBMCs (Liebregts et al. 2007). In experimental IBS rat models, increased TLR expression was found in the colonic mucosa of these animals (McKernan et al. 2009, O'Malley et al. 2011). These altered TLR responses may play a significant role in the enhanced immune activity in IBS (McKernan et al. 2011). The increased risk of developing IBS following gastroenteritis and the co-existence of a disturbed composition of the microbiota, elevated luminal gas production and immune activation, indicate that the gastrointestinal microbiota may be a therapeutic target in IBS. There are no recent clinical trials aimed at studying possible immunological benefits of inulin-type fructans in IBS, although previous prebiotic studies indicate potential health benefit at lower doses, i.e. an intake of 3.5-5g/day (Whelan. 2011). In the studies of Hunter et al., and Astegiano et al. (Hunter et al. 1999, Astegiano et al. 2006), only FOS was applied so possible chain length effects have yet to be evaluated. Two other studies incorporated inulin-type fructans in a synbiotic mixtures (FOS "Actilight" and *Bifidobacterium longum* W11) / (IBS Active; inulin with *Lactobacillus sporogenes*, *Lactobacillus acidophilus*, *Streptococcus thermophilus* and other additives) (Colecchia et al. 2006, Paineau et al. 2008). In these synbiotic combinations with inulin, significant reduction in IBS pain symptoms, abdominal distension, and regulation of bowel movement occurred. Moreover, increased stool frequency, reduced abdominal pain and reduced bloating were reported. Olesen et al. (Olesen and Gudmand-Hoyer. 2000) reported no beneficial effects in a study with IBS patients who were given chicory derived FOS. Concluding from these results, FOS is a promising agent in IBS therapies when combined with the appropriate probiotics and other cofactors. Future IBS studies including inulin-type fructan supplementation should include measurements of the immune

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parameters mentioned above to evaluate whether based on immunology, inulin-type fructans can provide therapeutic options.

### **Systemic immune benefits of inulin type fructan supplementation**

There are several clear physiological links between symptoms of rheumatoid arthritis (RA) and IBD, such as a shared inflammatory cytokine expression pattern and the success of several therapies in both diseases (Videla et al. 2001, Whelan. 2011, Travis. 2006, Lories. 2006, Macfarlane et al. 2008). This may indicate that where inulin-type fructans might alleviate IBD symptoms, RA patients may equally benefit from such supplementation. Experimental data on the benefits of inulin-type fructans in RA are scarce but studies in an HLA-B27 rat models demonstrated reduced severity of colitis as well as reduced severity of arthritis (Schultz et al. 2004, Hoentjen et al. 2005). After inulin-type fructan supplementation a significant reduction in inflammatory scores and pro-inflammatory cytokines was observed (Hoentjen et al. 2005, Whelan. 2011). In adjuvant-induced arthritis in Wistar rats, and type II collagen-induced arthritis in DBA/1 J mice,  $\alpha$ -GOS supplementation decreased erythema and swelling of limbs, as well as decreased histopathological findings in the hind paw joints (Abe et al. 2004). In conclusion, supplementation with inulin-type fructans and similar prebiotics such as  $\alpha$ -GOS as mentioned above, seem to be a promising therapeutic strategy to reduce disease symptoms in experimental animal colitis models and may prove useful in human inflammatory diseases such as IBD and RA (Table 3 and 4). (Schultz et al. 2004, Hoentjen et al. 2005, Macfarlane et al. 2008). However, more experimental animal studies are first required to confirm these beneficial effects and their underlying physiological mechanisms (Macfarlane et al. 2008).

### Allergy, infection, and immunization models

The effects of inulin-type fructans on allergies and immunization have been studied extensively in experimental animal models, mostly in combination with GOS administration, but protocols without GOS already show substantial immunological effects, which are summarized in Table 5. Many studies have been performed in pigs, where supplementation with inulin-type fructans mostly shows significant protective effects in infection models (Gomez-Conde et al. ,Middelbos et al. 2007,McGlone and Fullwood. 2001,Petkevicius et al. 2007,Petkevicius et al. 2003). In one study by Milo et al. supplementation of piglets with 1% of inulin for 1 week did not affect immune parameters or infection symptoms upon inoculation with *Salmonella typhimurium*. In a study in dogs, inoculated with *Salmonella typhimurium* DT104, a 14 day supplementation with inulin or FOS (1%) improved food intake and enterocyte sloughing and attenuated fever (Apanavicius et al. 2007). Manhart et al. reported that a 16 day supplementation trial with 10% FOS induced an increased CD4+/CD8+ ratio in an experimental mouse model for LPS-induced endotoxemia (Manhart et al. 2003). Inulin-type fructans have been administered to infants and children because of their potential to modulate the intestinal microbiota and to benefit the development of an adequate innate and adaptive immune response (Table 6). In healthy infants, the most obvious effect upon supplementation was increased levels of IgA in fecal samples, which can protect against pathogens in the gut lumen (van Hoffen et al. 2009,Scholten et al. 2008,Bakker-Zierikzee et al. 2006,Halas et al. 2009,Raes et al. 2010). Saavedra et al. (Saavedra and Tschernia. 2002) demonstrated an increase in blood IgG levels after measles vaccination in a 10 week supplementation study with OF/inulin (7/3, 0,2 g/kg BW/d) in 7-9 months old infants. However in a study by Duggan et al.(Duggan et al. 2003) in which 6-12 month old infants were supplemented with OF (0.7g/d), no effect was observed on antibody response after vaccination

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with *H. influenza* type B vaccine. Results from these studies may be related to the specific pathogen, or the type of fructans used in the vaccine but further studies are required to investigate these differences.

In both experimental animal studies and human studies, the use of inulin-type fructans has demonstrated beneficial effects on Th1 as well as on Th2 responses upon vaccination or sensitization protocols. Th1 cells normally drive the cellular immunity pathway to fight viruses and other intracellular pathogens, eliminate cancerous cells, and stimulate delayed-type hypersensitivity (DTH) skin reactions (Perez et al. 2010). Th2 cells drive the humoral immunity pathway and up-regulate antibody production to fight extracellular organisms. In a study by Vos et al. (Vos et al. 2006) supplementation of mice which were vaccinated with influenza virus, a 9:1 mixture of GOS/FOS enhanced DTH responses dose-dependently, but a mixture of FOS/inulin did not. Fujitani et al. (Fujitani et al. 2007) describe anti-allergic effects of FOS in Nc/jic mice upon supplementation with 5% FOS. Schouten et al. (Schouten et al. 2009) demonstrated that a mixture of GOS/FOS inhibited sensitization to orally supplemented whey in mice, but this was only effective when used in synbiotic combination with *Bifidobacterium breve*. Inulin-type fructans appear to modulate both reactions; stimulating the adaptive immune response in a Th1-direction upon vaccination or sensitization, inhibiting infections (Vos et al. 2006) or Th-2 related immune disorders such as allergies (Vos et al. 2007a, Schouten et al. 2009, Fujitani et al. 2007, Schouten et al. 2011), although in these experimental animal studies, inulin-type fructan effectiveness was most pronounced when used in combination with either GOS or *Bifidobacterium breve*. On the other hand they can induce increased antibody production (IgA) as part of a Th2 response, increasing clearance of luminal pathogens and reducing the chance of pathogen tissue entry.

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Evidence for prevention of incidence of allergies or atopic symptoms in infants was reported by Moro et al. (Moro et al. 2006b), and Arslanoglu et al (Arslanoglu et al. 2008,Arslanoglu et al. 2007) but in these studies inulin-type fructans were only supplemented in combination with GOS. In a study by Raes et al. (Raes et al. 2010) in which infants received breast milk, formula or formula supplemented with GOS/FOS, no clear differences were observed in the investigated immune parameters, but a trend was observed that GOS/FOS supplementation tended to increase blood IgG levels.

In conclusion, reduction of incidence of allergic symptoms or protective effects on development of allergy upon supplementation with inulin-type fructans have been shown in infants (van Hoffen et al. 2009,Scholtens et al. 2008,Bakker-Zierikzee et al. 2006,Halas et al. 2009,Raes et al. 2010). For elderly, promising results have been shown for supporting immune function, including for defense against respiratory infections (Vulevic et al. 2008,Amati et al. 2010,Langkamp-Henken et al. 2004,Langkamp-Henken et al. 2006,Schiffirin et al. 2007) (Table 6). Other groups of patients which may benefit from inulin-type fructan supplementation by means of Th-1/Th-2 modulation are pregnant women (Shadid et al. 2007), or burn patients (Olguin et al. 2005) but the small number of supplementation studies performed with these groups show no beneficial effects as yet. In a study in adult male smokers and non-smokers, 4 out of 23 immunological parameters were changed upon supplementation (Seidel et al. 2007). However, in the experimental set up of this study, inulin was incorporated in prebiotic bread, which also contained linseed and soy fiber so observed effects cannot solely be attributed to inulin intake. More studies in healthy human subjects are required to assess the immunomodulatory potential of inulin-type fructans in healthy conditions.

### Possible explanations for the inconsistencies

Some of the inconsistencies in the studies focusing on the immunomodulating effects of inulin-type fructans are caused by pertinent differences in study design and the application of different types of inulin-type fructans. In many studies the type of fructan has not been clearly documented. In part this can be explained by the inconsistent use of nomenclature regarding chain length. The chain length should always be included as it has been shown that chain length is a determining factor for the beneficial effects (Roberfroid et al. 2010, Poulsen et al. 2002, Buddington et al. 2002, Reddy et al. 1997). The mechanisms behind this might be that specifically long chain fructans bind to typical receptors in the membrane and cluster them into membrane microdomains (Figdor and van Spriel. 2010) or influence other membrane lipid dynamics (Vereyken et al. 2003a, Vereyken et al. 2003b, Vereyken et al. 2003c) whereas the shorter chain fructans may lack these properties and exert their effects via different routes. These findings suggest that the DP of fructans influences the efficacy in modulating immune functions and warrant further investigation (Roberfroid et al. 2010).

Many of the studies mentioned in this review report altered cytokine levels upon supplementation with inulin-type fructans. Nowadays a lot is known about the effects that cytokines or chemokines have on humans. The connection between inulin ingestion, the production of cytokines or chemokines, and the observed downstream health effects is gradually becoming clearer. On a chemical and cellular level inulin-type fructans can exert several effects and most pronounced effects are observed in the GALT. Several studies report modulation of IgG (Benyacoub et al. 2008, Hosono et al. 2003, Janardhana et al. 2009, Reddy et al. 1997, Raes et al. 2010), and IgA (Benyacoub et al. 2008, Fujitani et al. 2007, Nakamura et al. 2004, Smith et al. 2008, Thomsen et al. 2005) levels in serum and/or feces, changes in cytokine expression, mainly

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IFN- $\gamma$  (Benyacoub et al. 2008, Hosono et al. 2003, Roller et al. 2004b, Roller et al. 2004a, Roller et al. 2007), IL-4 (Trushina et al. 2005, Taper and Roberfroid. 2000), IL-10 (Hosono et al. 2003, Roller et al. 2004b, Roller et al. 2004a, Taper and Roberfroid. 2000, Kidd. 2003) and, IL-12 (Benyacoub et al. 2008), and altered activity of spleen NK cells (Shim et al. 2005, Roller et al. 2004a, Rao et al. 1998). IFN- $\gamma$  and IL-12 are factors responsible for Th1 differentiation, whereas IL-4 promotes Th2 subpopulations (Perez et al. 2010, Newberry and Lorenz. 2005). Regulatory T lymphocytes (Treg) are expanded in response to IL-10 and IL-10 skews the Th1/Th2 balance to Th2 *in vivo* by selectively blocking IL-12 synthesis by the antigen-presenting cells such as DCs (Newberry and Lorenz. 2005, Whelan. 2011). In addition, IL-4 induces IgA synthesis by follicular B lymphocytes in the Peyer's patches (Guilliano et al. 2001). These B lymphocytes, plasma-cell precursors, will migrate, mature, and undergo clonal expansion, and finally migrate to the intestinal lamina propria, where they finalize their maturation into IgA-secreting cells (Chin et al. 2003). A great variety of factors can influence this migration, including the cytokines (Brandtzaeg and Johansen. 2005) induced by the inulin-type fructans. From this expression pattern of cytokines it appears that several types of T cell responses are induced by inulin-type fructans. Because of this complex interplay, and depending on the experimental set up, different outcomes may be observed.

### **Future studies with inulin-type fructans**

There are many biomarkers to quantify immunomodulation in human nutrition intervention studies, but the repercussions of variations in these markers are still unclear, especially in healthy people. A review by Albers *et al.* (Albers et al. 2005) discusses the suitability of a large panel of biomarkers for the evaluation of nutritional intervention. However, the choice of immune

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markers needs to be correlated with the particular condition that is being assessed, the relevant clinical end-points, and whether any immune markers are differentially expressed in disease and control populations (Macfarlane et al. 2008). Concluding from this review, recommended biomarkers, typically suitable for inulin-type fructan supplementation studies are IgG and IgA levels in serum and feces, cytokine expression patterns in the GALT, and NK cell activity in the spleen. Measurements of these markers may be affected by age and gender, and they might vary because of other external confounding factors such as stress, smoking, and alcohol intake (Macfarlane et al. 2008). This necessitates careful selection of the control subjects (Macfarlane et al. 2008). Patient populations which are likely to benefit from inulin-type fructan supplementation include allergic individuals, IBD patients, RA patients and likely also patients suffering from other chronic inflammatory afflictions. Well designed, randomized placebo controlled trials are useful to evaluate possible benefit these patient groups may derive from inulin-type prebiotic supplementation.

The physiological effects of ingested inulin-type fructans are likely the combined effects of circumstances including an altered gut microbiota, the presence of produced fermentation products in the gut, and direct effects on gut epithelium and GALT, which complicates the analysis of the induced health effects. To investigate the direct effects, germ free experimental animal models or finely designed SPF experimental animal models and in vitro assays with suitable cell lines may shed more light on the induced physiological processes. Specific molecular or cellular effects which are reported repeatedly throughout literature are important to consider in unraveling the mechanisms behind the observed health benefits and can form a basis for further explorative research. It appears that ingestion of inulin type fructans affects a spectrum of immune reactions in the body including Th1 (Rafter et al. 2007, Benyacoub et al.

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2008, Roller et al. 2004a, Roller et al. 2007) Th2 (Trushina et al. 2005, Stillie et al. 2005), anti-inflammatory reactions (Hosono et al. 2003, Roller et al. 2004b, Stillie et al. 2005, Roller et al. 2004a, Langkamp-Henken et al. 2004), B cell activity (Ito et al. 2009, van Hoffen et al. 2009, Janardhana et al. 2009, Stillie et al. 2005, Pasare and Medzhitov. 2005) and NK cell activity (Le Poul et al. 2003, Shim et al. 2005, Langkamp-Henken et al. 2004). Depending on the research question, in vitro studies may provide useful information about the typical processes which are induced upon ingestion. In vitro studies into the signalling capacity of inulin-type fructans could entail the use of NK cell activity reporter assays. Another signalling target could be B cell activation; Peyer's patch DCs are able to induce B cell maturation and IgA production under influence of intestinal bacteria, via Peyer's patch DC derived cytokines such as B-cell activating factor (BAFF) and A Proliferation- Inducing Ligand (APRIL) (Heer et al. 2007, Pedersen et al. 1997). It is possible that inulin-type fructans could exert the same effect. Another possibility of promoted IgA production under influence of inulin-type fructans could be due to the traditional activation of T cells in the Peyer's patch follicles by DCs which have sampled the intestinal lumen and have encountered inulin molecules, followed by T helper cell mediated B cell maturation and IgA production. Finally, it would be interesting to see whether direct ligation of B cell TLRs (Heer et al. 2007, Hijova et al. 2009, Parnell and Reimer. 2010) by inulin-type fructans could result in IgA production. Regarding the altered cytokine levels in inulin-type fructan supplementation studies, it would be interesting to investigate which immune cell types are capable of recognizing inulin-type fructans and of mounting a subsequent cytokine response. This may be a wide range of cell types because many cell types express TLRs and CLR. From there, results can be translated to the relevant populations which may actually come in contact with fructan molecules after their ingestion. Moreover, the capacity of APCs to report

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the presence of inulin-type fructans or parts of the molecules to effector cells is still an uncharted area of research which deserves further exploration.

### CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The European Food Safety Authority (EFSA) panel aims to create guidance documents on the scientific requirements for the substantiation of nutritional health claims, including claims related to gut and immune function. They strive to include input from all stakeholders such as applicants for health claims, non-governmental organizations, industry organizations, and academia. This is an ongoing process of gathering input from the scientific community and all interested parties in order to provide clear answers on how to make a certified health claim about a product or ingredient. The status quo of inulin-type fructans is certainly that it a functional food, but that for more specific claims require more scientific evidence. The immunomodulatory properties are convincing, but to unravel the impact this modulation has on more specific components of the immune system such as resistance against intestinal infections or potentiation of vaccination programs, more studies are highly recommended. There are still missing links to fully comprehend the effects of inulin type fructans and the underlying signalling mechanisms. From the results of this literature study, it can be concluded that immunological effects are elicited by ingestion of inulin type fructans, in experimental animals as well as in humans. This could be caused by several factors or a combination of factors, such as the effect of increased *Bifidobacteria* numbers per se (Kelly. 2008, Kelly. 2009), and/or the increase of fermentation products such as SCFAs. These could bind to GPRs and other immune receptors (Delzenne et al.

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2005, Spahn and Kucharzik. 2004). Moreover, it is possible that the inulin itself ligates specific immune receptors such as TLRs or CLRs (Adib-Conquy et al. 2003, Chermesh et al. 2007). The extent to which each of these factors is individually responsible for the reported health effects remains to be determined. To study any direct effects, in vitro studies with different relevant cell types such as gut epithelium or intestinal DCs or lymphocytes can be useful to first study signal transduction upon contact of these cell types. Follow up could include supplementation studies with inulin-type fructans in a germ free experimental animal model. This can render new information about the fate of inulin after ingestion. However, ultimately this does not represent the natural situation of the subject and results should be confirmed in the context of a healthy individual.

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**Table 1. Effects of inulin–type fructans from chicory on immune function in healthy experimental animal models.**

REF.	Fructans used (concentration )	Study design, duration, number of subjects	Results
Swanson et al. 2002a.	FOS + MOS (2/1 g/d)	Dogs, 14d, n=8, via gelatin capsule	Lowered blood neutrophils, increased blood lymphocytes vs placebo
Swanson et al. 2002b.	FOS and/or Mannose oligosaccharide (MOS, 1/1 g/d)	Dogs, 14d, n=4, ileal canulas	Ileal & serum IgA increased, increased % blood lymphocytes
Hosono et al. 2003.	FOS (0-7.5%)	BALB/c Mice, 6wk, n=7, oral admin.	fecal IgA increased, IgA secretion by Peyer's patch (PP) cells upregulated, CD4+ T cells from PP showed increased IL-10 and IFN- $\gamma$ , IL-5 and IL-6. Suppressed serum IgG1
Kelly-Quagliana et al. 2003.	Cellulose, cellulose/OF, OF or inulin (10%)	Mice, 6wk, n=8, diet ad lib.	Decreased leukocyte counts with inulin and OF, increased macrophage phagocytosis, increased NK activity of spleen cells. No effects on fecal IgA or on lymphocyte subsets in spleen and thymus
Nakamura et al. 2004.	FOS (5%)	Infant BALB/c mice, 38d, n=4	IgA in gut tissue extracts and ileal IgA secretion rate increased, increased polymeric immunoglobulin receptor (pIgR) expression, increased % of B220(+)IgA+ cells in PP
Roller et al. 2004b.	OF/inulin (1/1, 10%)	Rats, 4 wk, n=80, suppl to high fat diet	Higher production of IL-10 and IFN- $\gamma$ in PP, no effect on NK activity, lymphocyte proliferation and cytokine production in spleen MLN and PP
Shim et al. 2005.	OF (0,2%), syn: OF-PRO (0,2/0,3%)	Suckling piglets, 21d, n=50, diet ad lib.	% Lymphocytes and neutrophils in blood unaltered
Trushina et al. 2005.	FOS, inulin (10%)	Rats, 4mo, n=nd, isocaloric purified diets containing supplement of OF or inulin	Increased number of T cells in spleen, MLN and thymus, level of IL-4 in blood. No effect on immune apoptosis
Verlinden et al. 2006.	Inulin 3%	Dogs, 26d, n=4, supplementation to basal diet	No effect on serum IgA, IgE, IgG and IgM, or on fecal IgA
Cerezuela et al. 2008.	Inulin (0,5 or 1%)	Seabream, 1wk, n=45, suppl. to commercial diet	Inhibition of phagocytosis and respiratory burst in leucocytes

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Janardhana et al. 2009.	FOS or MOS (5 g/kg)	Chickens, 25d, n=125, add.to basal diet	Reduced proportion of B cells and mitogen responsiveness of lymphocytes in Cecal Tonsil, enhanced plasma IgM and IgG
Yasuda et al. 2009.	FOS, inulin, FOS/inulin (4%)	Pigs, 7wk, suppl. To corn/soybean basal meal	Decreased expression of inflammation related genes especially in lower gut mucosa

Abbreviations: FOS – Fructooligosaccharides, MOS - Mannose oligosaccharide, Ig – Immunoglobulin, OF – Oligofructose, PP – Peyer’s patches, IL – Interleukin, IFN – Interferon, SYN – Synbiotic, PRO – Probiotic, Ad lib – Ad libitum, MLN – Mesenteric lymph nodes

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**Table 2. Immunological parameters in experimental animal cancer models and human cancer patients.**

REF.	Fructans used (concentration )	Study design, duration, number of subjects	Results
<i>Studies in experimental animals</i>			
Roller et al. 2004b.	PRE,; inulin enriched with FOS, 100 g/kg PRO: LGG + BB12 SYN: PRE + PRO	Cancer model rats, 33wk, n=32,33,32	Restored NK cell-like cytotoxicity in PP, Increased IL-10 production in GALT Decreased PP IFN- $\gamma$ production Suppression of lymphocytes PP proliferative responsiveness
Forest et al. 2005.	FOS (sc-FOS, 5,8%)	Apc+/Min mice Cancer model , n=12, diet ad lib.	Decreased nr of CD25+ LI-IELs, Increased nrs of IL- 15+/IL-15R $\alpha$ + IELS Increase in CD69+cells Decreased CD4+ CD25+ LI-IELs
<i>Studies in humans</i>			
Rafter et al. 2007.	SYN: FOS-enriched inulin (10g) (SYN1) + (LGG and BB12	Human cancer, polyp-ectomized patients P,RCT,DB, n=80, 12 wk	SYN prevented an increased secretion of IL- 2 by PBMCs in the polypectomized patients and increased the production of IFN-gamma in the cancer patients. Other immunity-related parameters were not affected by SYN treatment
Reddy et al. 2007.	SYN: FOS + <i>B.</i> longatum	Human Colonectomy patients n=64	Significantly lower incidence of translocation, no change in intestinal permeability, inflammatory response or septic morbidity
Roller et al. 2007.	SYN: FOS-enriched inulin (10g) (SYN1) + LGG and BB12	Colon cancer patients and polyp-ectomized patients 12- wk, P, RCT, DB, n=74	IL-2 secretion by activated PBMC from the polyp group increased. In the cancer group, SYN treatment resulted in an increased capacity of PBMC to produce IFN-gamma. Other immunity-related parameters were not affected by SYN treatment.

Abbreviations: FOS – Fructooligosaccharides, PP – Peyer’s patches, IL – Interleukin, IFN – Interferon, SYN – Synbiotic, PRO – Probiotic, Ad lib – Ad libitum, PRE – Prebiotic, GALT – Gut associated lymphoid tissue, NK – Natural killer, Sc – Short chain, Apc+/Min - adenomatous polyposis coli /multiple intestinal neoplasia, LI-IEL – Large intestinal intraepithelial lymphocytes, LGG – *Lactobacillus rhamnosus* GG, BB12 - *Bifidobacterium lactis*

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Bb12, P or PC – Placebo controlled, RCT – Randomized controlled trial, DB - Double blind, PBMC – Peripheral blood mononuclear cell.

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**Table 3. Effects of inulin-type fructans on gastrointestinal inflammation in experimental animals.**

REF.	Fructans used (concentration)	Animals	Results
Catala et al. 1999, Butel et al. 2002	OF (3%)	Quails / inoculation with clostridia to induce necrotizing enterocolitis	less lesions , less severity of disease index
Videla et al. 2001.	Inulin (1% in drinking water or 400 mg/day)	Rats DSS colitis	Reduced mucosal damage, less inflammatory mediators released
Holma et al. 2002.	GOS (4g/kg.day)	Rats TNBS colitis	No reduction of colitis
Cherbut et al. 2003.	1 g/d FOS	Rats TNBS colitis	Reduced the gross score for inflammation, myeloperoxidase (MPO) activity
Moreau et al. 2003.	FOS (unspecified)	Rats DSS colitis	No reduction of colitis
Schultz et al. 2004.	Synbiotic: inulin (unspecified) and L. acidophilus La-5 and Bifidobacterium lactis Bb	Transgenic rats HLA-B27 colitis	Colitis reduction (histological score of Inflammatory cells, ulcer, Goblet cells, crypt abscess, Mucosa thickening, Submucosa cell infiltration, destruction of architecture)
Hoentjen et al. 2005.	FOS/inulinHP (1/1, 5 g/kg bodyweight via drinking water)	Transgenic Rats HLA-B27 colitis	Reduction of colitis scores, inflammation in gut mucosa, reduced IL-1 $\beta$ but increased TGF- $\beta$ in cecum
Daddaoua et al. 2006.	Goat milk oligosaccharides 500 mg/(kg.d)	Rats TNBS colitis	Colitis reduction: reduced bowel wall thickening and longitudinal extension of necrotic lesions; downregulated colonic expression of IL1 $\beta$ , inducible nitric oxide synthase, cox2, and mucin 3; increased trefoil factor 3.
Lara-Villoslada et al. 2006.	Goat milk oligosaccharides 5%	Rats DSS colitis	Colitis reduction (less colonic myeloperoxidase activity/ clinical symptoms, mucin-3 expression return to basal)
Osman et al. 2006.	OF/inulin (1/1, 0,5 g/d)	Rats DSS colitis	Lower disease index, reduction colonic IL-1 $\beta$ less bacterial translocation to MLN no effect on colonic TGF- $\beta$ or IL-10

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Apanavicius et al. 2007.	Inulin or FOS, 1%	Dogs, 14d, n=10, Salmonella inoculation	Improved food intake, attenuated enterocyte sloughing,
Bakke-McKellep et al. 2007.	Inulin (7,5%)	Salmon soybean induced colitis	No effects
Winkler et al. 2007.	FOS 1.5 g/mL	Mouse DSS colitis	Increased crypt depth and area , faster recovery from damage
Smith et al. 2008.	FOS (3%), FOS (6%), or synbiotic (BR11/FOS).	Rats 5-fluorouracil mucositis	Histological damage increased for every group, reduced jejunal inflammation, no effect on mucositis
Ito et al. 2009.	scFOS (DP4-8), 60 g DP4 or DP8 per kilogram for 7 days	Rats TNBS colitis	Significantly reduced colonic injuries, bacterial translocation, increased cecal IgA

Abbreviations: FOS – Fructooligosaccharides, Ig – Immunoglobulin, OF – Oligofructose, IL – Interleukin, IFN – Interferon, SYN – Synbiotic, MLN – Mesenteric lymph nodes, Sc – Short chain, DSS – Dextran sodium sulfate, TNBS – Trinitrobenzenesulphonic acid, GOS – Galactooligosaccharides, DP – Degree of polymerization, GMOS – Goat milk oligosaccharides, TGF – Transforming growth factor, BR11 - *Lactobacillus fermentum* BR11.

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**Table 4. Effects of inulin type fructans in human irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD).**

REF.	Fructans used (concentration)	Study design, duration, number of subjects	Target group / Condition	Results
Studies in adult patients with IBS or constipation				
Hunter et al. 1999.	FOS (6g/d)	DB, CO, 4 wk, n=21	Adults/IBS	No effects on symptom scores
Olesen and Gudmand-Hoyer. 2000.	FOS (20g/d)	RCT, DB, parallel, 12 wk, n=98	Adults/IBS	Greater improvement in placebo group; no difference for symptoms at end of treatment
Astegiano et al. 2006.	Dietary integrator (IBS Active), L-tryptophan, inulin, angelica, vegetal charcoal, vitamin PP, group B vitamins (B1, B2, B6), ( <i>L. sporogenes</i> , <i>L.acidophilus</i> , <i>S.thermophilus</i> , syn	6 mo, n=37, control group without supplementation on normal treatment	Adults/IBS	Significant reduction in pain symptoms, abdominal distension and regulation of bowel movement in IBS patients
Colecchia et al. 2006.	SYN: <i>B. longum</i> and FOS Actilight 3g/d	N=636, 43 centers, 36 d	adults / constipation-IBS	Increased stool frequency and reduced abdominal pain and bloating
Paineau et al. 2008.	FOS (5g/d)	Questionnaire digestive disorders, 6wk	Minor IBS	Decreased intensity and incidence of digestive discomfort
Studies in adult patients with pouchitis and/or IBD				
Welters et al. 2002.	Inulin (24 g/d)	RCT, DB, CO, 3 wk, n=24	Patients/Pouchitis	No effect on clinical symptoms, lower pouchitis disease index
Furrie et al. 2005.	SYN: 2x1 <i>B. longum</i> 16 g/d	DB, RCT, n=18, 1 mo	Adults/ IBD (UC)	Sigmoidoscopy scores reduced, mRNA levels beta defensins 2, 3, and 4, upregulated in

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				active UC, significantly reduced in treatment, TNF $\alpha$ and IL1 $\alpha$ , which induce defensin expression, reduced after treatment Reduction of intestinal Inflammation and regeneration of epithelial tissue
Macfarlane et al. 2005.	SYN: <i>B.longum</i> combined with FOS-enriched inulin - Synergy 1	RCT n=18, 1 mo	Adults/ IBD (UC)	Increased bifidobacteria in rectal mucosa, Significant reductions in the expression of molecules that control inflammation in active UC: TNF $\alpha$ and IL-1 $\alpha$ , that induce defensin expression, were reduced
Lindsay et al. 2006.	FOS/Inulin (7/3, 15 g/d)	3 wk, n= 10	Adults/IBD (CD)	Lower disease activity scores
Casellas et al. 2007.	FOS/Inulin (1/1, 12 g/d)	RCT, DB, parallel, 2 wk, n=19	Adults/IBD (UC)	Lower disease activity, no difference with placebo. Lower fecal calprotectin
Paineau et al. 2008,Chermesh et al. 2007.	SYN 2000: cocktail containing 4 probiotic species and 4 prebiotics	MC, RCT, n=30,	Adults/ IBD (CD)	No effect on postoperative recurrence

Abbreviations: FOS – Fructooligosaccharides, IL – Interleukin, SYN – Synbiotic, RCT – Randomized controlled trial, DB – Double blind, CO – Cross over, IBS – Irritable bowel syndrome, IBD – Inflammatory bowel disease, UC – Ulcerative colitis CD – Crohn’s disease, mRNA – Messenger ribonucleic acid, TNF – Tumor necrosis factor.

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**Table 5. Inulin-type fructan effects in experimental animal models for allergy, diabetes, and immunization.**

REF.	Fructans used (concentration)	Study design, duration, number of subjects	Target group / Condition	Results
Buddington et al. 2002.	10% OF or inulin	45d trial. Exposure to <i>Candida albicans</i> (enterically), or <i>Listeria monocytogenes</i> or <i>Salmonella typhimurium</i> . N=25 per group.	Mice 1,2- dimethylhydr azine colon cancer model	Decreased <i>C. albicans</i> numbers, inulin abolished mortality upon <i>Listeria</i> infection, mortality by infection of <i>S. typhimurium</i> was decreased compared to control (60% vs 80%)
Manhart et al. 2003	10% FOS	16d trial, LPS challenge to induce endotoxemia, n=8 per group	Balb/c mice	Increased total cell yield. B lymphocytes were increased in both groups. T lymphocytes increased in LPS-challenged mice after FOS enrichment The increase of CD4(+) cells was more pronounced than that of CD8(+) cells, increasing the CD4:CD8 ratio
Milo et al. 2004.	Inulin (1%)	1wk supplementation, <i>Salmonella typhimurium</i> infection at d7. Blood sampling at d14. N=4-6 per group.	Piglets	No effect on blood phagocyte activation level or small intestinal IgA upon <i>Salmonella typhimurium</i> infection
Stillie et al. 2005.	Inulin (4,8%)	35d trial. N=30-38 per dietary group.	Diabetes prone /resistant rats	In diabetes prone rats: higher level of B cells in PP, higher number of IgA+ cells in jejunum. In diabetes resistant rats: higher level of CD8+ lymphocytes in PP. Both types: lower number of splenocytes, decreased production of IL-4 and increased production of IL-10 by stimulated spleen cells, no effect on production of IFN- $\gamma$ or TGF- $\beta$ by

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				splenocytes or MLN cells
Vos et al. 2006.	OF/inulin (1/1, 1-10%)	Influenza vaccination model, n=10 per group. Trial of 31d. Dietary intervention of 14d and 20d.	Mice Influenza vaccination study	Increased DTH response to influenza vaccine. No effect on vaccine specific serum IgG. No effect on DTH response with inulin/OF, OF or inulin
Adogony et al. 2007.	scFOS (nd)	Supplementation to the mother from d35 of gestation to weaning, (n=16), vaccination of pups.	Dogs	Mammary secretions in dogs increased in IgM content (no effect on IgG1, IgG2 and IgA) and concomitantly increased IgM immune response to vaccination of pups
Fujitani et al. 2007.	5% FOS	Effect on food allergy, 8 wk. N=10 OVA nonsensitized, n=6 OVA sensitized, n=7 OVA sensitized + FOS diet.	Nc/jic mice	Reduced number of CCR4+cells, mast cells and edema formation rate in the duodenum (antiallergic activity for food allergy)
Vos et al. 2007a.	1-5% (w/w) acidic oligosaccharides (AOS), combinations of AOS and 9/1 LC GOS/FOS	6 wk, vaccination trial, n=10 per group	Mice Influenza vaccination study	AOS enhanced vaccine-specific DTH responses and reduced in T-helper2 (Th2) cytokine production by splenocytes in vitro. (systemic immune response was Th1-skewed). GOS/FOS and AOS were more effective in enhancing DTH responses than either of the oligosaccharides alone
Barrat et al. 2008.	GOS/inulin, 88/12; 5.6 g/L	40d trial, 14d formula supplemented with prebiotics. D18: n=20 CTL, n=22 GOS/inulin and n=7	Rats	Most parameters unaltered but increased bacterial translocation (BT) toward spleen

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		mother-reared rats; d40: n =14 CTL and GOS/inulin)		
Benyacoub et al. 2008.	5% FOS:inulin	Salmonella typhimurium infection, n=20 per group. Trial of 5 wk, 1 wk of dietary intervention before immunization.	Balb/c mice	Specific Salmonella serum IgG and fecal IgA significantly increased Peritoneal macrophage phagocytic activity increased Production of IFN $\gamma$ , IL-12, and TNF- $\alpha$ increased in spleen upon stimulation Survival rate upon challenge with virulent Salmonella improved
Schouten et al. 2009.	GOS/lc inulin (9/1, 2%)	Allergic model, n=6 per group. Dietary intervention 2 wk prior to sensitization and during trial duration of 8wk	Mice Whey sensitization protocol	Prebiotics or probiotic B. breve alone were less effective for reducing anaphylactic reaction as compared to the combination; allergic skin response reduced with prebiotics; further enhancement by B. breve. Acute allergic skin reaction is diminished+ ; whey specific Treg cells may be involved

Abbreviations: FOS – Fructooligosaccharides, Ig – Immunoglobulin, OF – Oligofructose, PP – Peyer’s patches, IL – Interleukin, IFN – Interferon, SYN – Synbiotic, MLN – Mesenteric lymph nodes, Sc – Short chain, GOS – Galactooligosaccharides, TGF – Transforming growth factor, TNF – Tumor necrosis factor, LPS – Lipopolysaccharide, DTH – Delayed type hypersensitivity, OVA – Ovalbumin, CCR4 – C-C chemokine receptor type 4, AOS – Acidic oligosaccharides, Lc – Long chain, Th – T helper.

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**Table 6. Inulin-type fructan induced immune effects in infants, adults, and elderly, burn patients, and pregnant women.**

REF.	Fructans used (concentration)	Study design, duration, number of subjects	Target group / Condition	Results
Studies in infants:				
Saavedra and Tschernia. 2002.	OF/inulin (7/3, 0,2 g/kg BW/d)	RCT, DB, 10 wk, n=55	Infants (7-9mo)	Higher blood IgG levels after measles vaccination
Duggan et al. 2003.	OF (0,7g/d)	RCT, 6mo, n=282	Infants (6-12mo)	No effect on antibody response after vaccination with <i>H. influenza</i> type B vaccine
Bakker-Zierikzee et al. 2006.	GOS/inulin (9/1 0,6g/dl formula)	RCT, DB, parallel, 32 wk, n=57	Infants (3d)	Higher fecal IgA (no effect of probiotic <i>B.animalis</i> )
Moro et al. 2006a	GOS/lc inulin 9/1, 0.8 g/100 ml formula	RCT, DB, parallel, 6 mo, n= 242	Infants at risk for atopy	Decreased incidence of development of atopic dermatitis, no change in severity
Arslanoglu et al. 2008, Arslanoglu et al. 2007	8 g/L scGOS/lcFOS	RCT, DB, n=152	Healthy term infants up to 2 yrs	Lower incidence of allergic manifestations, incidences for AD, recurrent wheezing, and allergic urticaria, upper respiratory tract infections, fever episodes, antibiotic prescriptions, effect lasting beyond the intervention period
Scholtens et al. 2008.	GOS/inulin (9/1 0,6g/dl formula)	RCT, DB, 26 wk, n=187	Term infants	Higher fecal sIgA levels
van Hoffen et al. 2009.	8 g/l GOS/FOS 9/1 (IMMUNOFORTIS)	Children were vaccinated with Hexavac against a.o. DTP.	Healthy infants (3 mo)	Significant reduction in plasma total IgE, IgG1, IgG2 and IgG3, no effect on IgG4. CMP-specific IgG1 significantly decreased. DTP-specific Ig levels not affected.

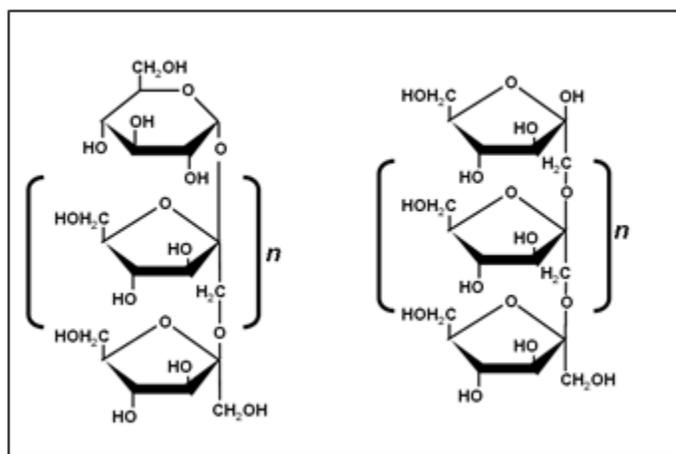
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Perez et al. 2010.	<i>L.acidophilus</i> , FOS and inulin	DB,PCT, n=162	Children with a high index of natural exposure to microorganisms	Rate of Ig and isoagglutinin acquisition was similar in both groups. No difference between groups in antibody levels neither before nor after vaccination. Days of fever and number of episodes of infection were not statistically different in either group
Raes et al. 2010.	scGOS/lcFOS, ratio 9:1	26 wk, n=215	Term infants	No changes observed
Studies in elderly people:				
Bunout et al. 2002.	OF/inulin (70/30%, 6g/d)	RCT, parallel, 28 wk, n=66	Elderly >70y	No difference between groups for antibody response to influenza and <i>S. pneumoniae</i> vaccination
Guigoz et al. 2002.	FOS 8 g/d 2x4	N=19	Elderly people	Decreased phagocytic activity of granulocytes and monocytes, as well as a decreased expression of interleukin-6 mRNA in peripheral blood monocytes
Langkamp-Henken et al. 2004.	240 mL/d FOS from sucrose	P, RCT, DB, 5mo, n=157	Elderly people >65y	Enhanced immune function indicated by increased influenza vaccine response and lymphocyte activation, less fever, fewer antibiotics
Langkamp-Henken et al. 2006.	8 oz/d of an experimental formula containing antioxidants, zinc, selenium, fermentable oligosaccharides, and structured triacylglycerol or an isoenergetic, isonitrogenous control formula	Prospective RCT DB,183d, n=66	Free living elderly >65y	Enhanced immune function and fewer days of URTI symptoms

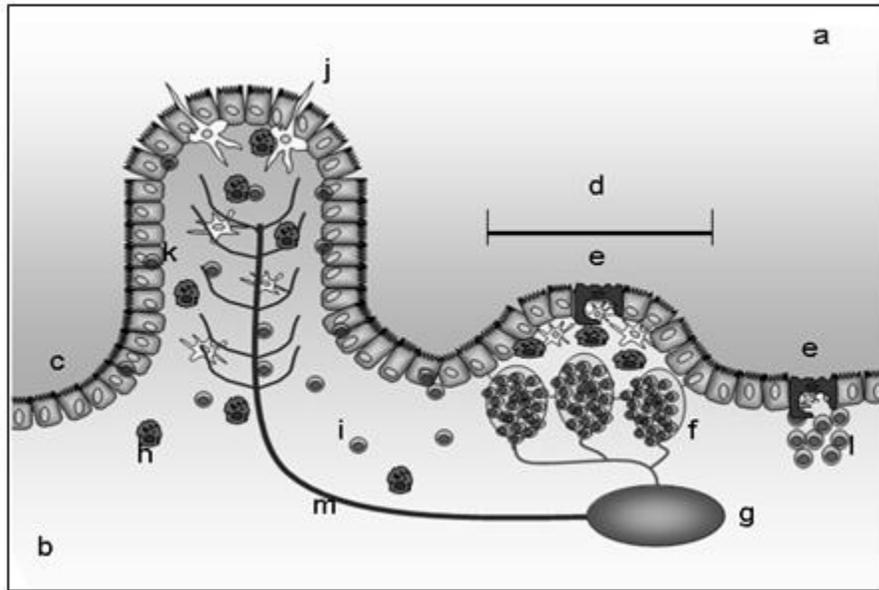
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Schiffirin et al. 2007.	1.3 g/250 ml FOS	P,RCT,DB, n= 74, 12 wk	Elderly people >70 y	Specific mRNA extracted from blood leucocytes: TNF- $\alpha$ mRNA and IL-6 mRNA diminished. Serum sCD14, a product shed by activated macrophages, decrease. No significant differences were detected in the fecal gut flora or nutritional parameters.
Vulevic et al. 2008.	B-GOS 5,5g/d	DB, PCT, CO, n=44, 24 wk	Healthy elderly people	Significant increases in phagocytosis, NK cell activity, and the production of IL-10, and significant reduction in production of IL-6, IL-1beta, and TNF- $\alpha$
Amati et al. 2010.	SYN: LGG and OF	1 mo, N=10	Elderly people	Increase age-depressed values of IL-1, IL-6 and IL-8 with a trend to a modest increase for the restant cytokines
Studies in pregnant women and burn patients:				
Shadid et al. 2007.	3 times/d with 3 g GOS/lcFOS (at a ratio of 9:1)	RCT, DB, PC, n=48. From week 25 of gestation until delivery	Pregnant women	Fetal immune parameters did not differ significantly
Olguin et al. 2005.	OF 6g/d	RCT, DB, n= 41, 15 d	Burn patients	Normalization of gastrointestinal permeability is not accelerated by prebiotic intake
Studies in healthy adult males:				
Seidel et al. 2007.	Inulin (9 g/d)	RCT, DB, parallel, 5wk, n=38	Adult males	4 of 23 immunological parameters investigated were affected

Abbreviations: FOS – Fructooligosaccharides, Ig – Immunoglobulin, OF – Oligofructose, IL – Interleukin, SYN – Synbiotic, NK – Natural killer, Sc – Short chain, LGG - *Lactobacillus rhamnosus* GG, P or PC – Placebo controlled, RCT – Randomized controlled trial, DB - Double blind, GOS – Galactooligosaccharides, CO – Cross over, mRNA – Messenger ribonucleic acid, TNF – Tumor necrosis factor, Lc – Long chain, BW – Body weight, AD – Atopic dermatitis, DTP - Diphtheria, tetanus, polio, CMP – Cow’s milk protein, URTI – Upper respiratory tract infections.



**Figure 1. Haworth projections of fructan molecules.** Left projection depicts an inulin type fructan of the GF $n$  type, right projection depicts an inulin-type fructan of the Fn type.



**Figure 2. Gut Associated Lymphoid Tissue (GALT).** Schematic representation of the GALT structure; a) Gut lumen. b) Lamina propria. c) Enterocyte lining. d) Peyer's patch. e) Microfold cell. f) Follicle with B and T lymphocytes. g) Mesenteric lymph node. h) Lamina propria mast cell. i) Lamina propria lymphocyte. j) Dendritic cell penetrating enterocyte monolayer and sampling gut lumen. k) Intraepithelial lymphocyte. l) lymphoid aggregates. m) High endothelial vessel [246]. N.B. Structural proportions were altered for illustrative purposes.

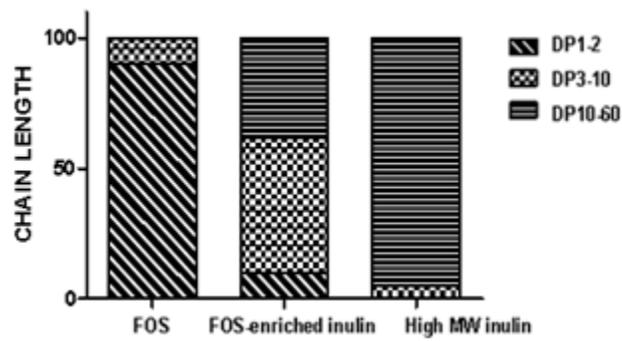


Figure 3. Chain length distribution examples of inulin-type fructans for FOS, FOS enriched inulin, and high average molecular weight inulin.