

## Review Article

# Prebiotics, immune function, infection and inflammation: a review of the evidence

Amy R. Lomax\* and Philip C. Calder

*Institute of Human Nutrition, School of Medicine, University of Southampton, Tremona Road, Southampton, SO16 6YD, UK*

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$\beta$ -2-1 Fructans are carbohydrate molecules with prebiotic properties. Through resistance to digestion in the upper gastrointestinal tract, they reach the colon intact, where they selectively stimulate the growth and/or activity of beneficial members of the gut microbiota. Through this modification of the intestinal microbiota, and by additional mechanisms,  $\beta$ -2-1 fructans may have beneficial effects upon immune function, ability to combat infection, and inflammatory processes and conditions. In this paper, we have collated, summarised and evaluated studies investigating these areas. Twenty-one studies in laboratory animals suggest that some aspects of innate and adaptive immunity of the gut and the systemic immune systems are modified by  $\beta$ -2-1 fructans. In man, two studies in children and nine studies in adults indicate that the adaptive immune system may be modified by  $\beta$ -2-1 fructans. Thirteen studies in animal models of intestinal infections conclude a beneficial effect of  $\beta$ -2-1 fructans. Ten trials involving infants and children have mostly reported benefits on infectious outcomes; in fifteen adult trials, little effect was generally seen, although in specific situations, certain  $\beta$ -2-1 fructans may be beneficial. Ten studies in animal models show benefit of  $\beta$ -2-1 fructans with regard to intestinal inflammation. Human studies report some benefits regarding inflammatory bowel disease (four positive studies) and atopic dermatitis (one positive study), but findings in irritable bowel syndrome are inconsistent. Therefore, overall the results indicate that  $\beta$ -2-1 fructans are able to modulate some aspects of immune function, to improve the host's ability to respond successfully to certain intestinal infections, and to modify some inflammatory conditions.

### $\beta$ -2-1 Fructans: Inulin: Oligofructose: Fructo-oligosaccharides: Cytokines

Prebiotics have been defined as 'non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth, and/or activity, of one or a limited number of beneficial bacteria in the colon and thus improve host health'<sup>(1)</sup>. Research on the potential health benefits of prebiotics has occurred over the last 15 years or so, with a recent interest in the effects on the immune system, the host's ability to fight infection, and inflammatory processes and conditions. These effects have been reviewed several times<sup>(2–7)</sup> but to our knowledge there are no reviews that bring together all of the available studies in all of these areas. Thus, the aim of the present article is to describe the structure and dietary sources of prebiotics, and to summarise and evaluate studies investigating the influence of prebiotics on immunity, host defence, and inflammatory processes and conditions.

### Structure of prebiotics

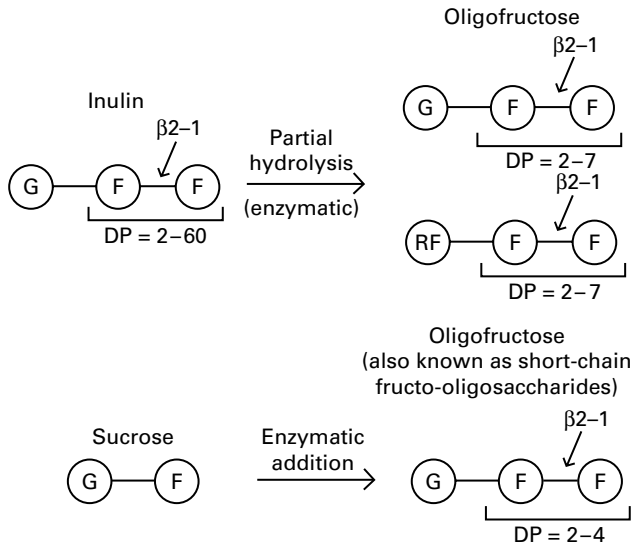
$\beta$ -2-1 Fructans, which include inulin (IN) and fructo-oligosaccharides (FOS), fulfil the criteria for prebiotics<sup>(8)</sup>. Other carbohydrates including galacto-oligosaccharides (GOS), gluco-oligosaccharides, isomalto-oligosaccharides, lactulose,

mannan-oligosaccharides (MOS), nigero-oligosaccharides, oat  $\beta$ -glucans, raffinose, soyabean oligosaccharides, transgalacto-oligosaccharides and xylo-oligosaccharides are considered as candidate prebiotics. Only studies with  $\beta$ -2-1 fructans will be considered in the present review, as these are the most widely studied with regard to potential modulation of the immune system, and relatively little information is available on the immunomodulatory properties of the other candidate prebiotics.

IN is a linear carbohydrate molecule which contains  $\beta$ -(2  $\rightarrow$  1) fructosyl–fructose linkages with a terminal glucose<sup>(9)</sup>. IN may contain between two and sixty fructose residues (Fig. 1), with an average of twelve. Partial enzymatic hydrolysis of IN yields a FOS known as oligofructose (OF), which can have a terminal glucose or fructose residue (Fig. 1). In OF there can be two to eight (average five) fructose residues with a terminal glucose residue or a chain of three to eight (average five) fructose residues<sup>(10)</sup>. Thus IN and OF differ according to degree of polymerisation (Fig. 1). Short-chain FOS may also be derived by enzymatic addition of fructose residues to sucrose (Fig. 1); the products formed contain two to four fructose residues with a terminal glucose residue (Fig. 1). Some studies have used products

**Abbreviations:** FOS, fructo-oligosaccharides; GALT, gut-associated lymphoid tissue; GOS, galacto-oligosaccharides; IFN, interferon; IN, inulin; MHC, major histocompatibility complex; MLN, mesenteric lymph nodes; MOS, mannan-oligosaccharides; NK, natural killer; OF, oligofructose; PP, Peyer's patches.

\*Corresponding author: Miss Amy R. Lomax, fax +44 2380 795255, email arl203@soton.ac.uk



**Fig. 1.** The structures of  $\beta$ -2-1 fructans. DP, degree of polymerisation; F, fructose; G, glucose; RF, reducing fructose.

containing OF-enriched IN or IN with shorter-chain FOS removed, while some studies do not specify exactly what they used, merely referring to FOS.

### Dietary sources of prebiotics

IN is found naturally in a variety of plant foods such as bananas, barley, chicory, garlic, Jerusalem artichoke, leeks, onions and wheat<sup>(11)</sup>. IN has been extracted from chicory roots, Jerusalem artichoke, artichoke, dahlias and dandelions<sup>(12)</sup>. Typical daily intakes of IN for adults are estimated to be between 3 and 11 g/d in Europe, and between 1 and 4 g/d in North America<sup>(11)</sup>.

Oligosaccharides, including some believed to be prebiotics, are present in human breast milk<sup>(13)</sup>. They can be found in concentrations of up to 12 g/l, making them the third largest component of breast milk<sup>(14)</sup>. The presence of oligosaccharides in large amounts in breast milk suggests that these compounds may play an important role in early infant development, perhaps of the gut, its microbiota and the immune system. Breast milk contains many compounds and substances that influence gut and immune maturation and consequently has a protective role against infections<sup>(15)</sup> and possibly allergy development<sup>(16)</sup>. Oligosaccharides may contribute to these protective actions. It is possible that the oligosaccharides are present in breast milk in the mix and concentrations required for optimum protection, and for the development of the immune system.

### Overview of the mechanism of action of prebiotics

$\beta$ -2-1 Fructans fulfil the three criteria which must be met in order to be classified a prebiotic, as defined by Gibson & Roberfroid<sup>(1)</sup>:

- (1) Resistance to hydrolysis or absorption in the upper gastrointestinal tract (as the  $\beta$ -(2  $\rightarrow$  1) osidic bond is not hydrolysed by mammalian digestive enzymes). This was shown in early *in vitro* tests, where  $\beta$ -2-1 fructans were incubated with rat pancreatic and small intestinal homogenates, and shown to be poorly digested<sup>(17)</sup>. Fulfilment of this

criterion has also been demonstrated in man through the study of ileostomy subjects, where 87 % of dietary IN was recovered in the ileum<sup>(18)</sup>, thus establishing the survival of IN through the upper gastrointestinal tract. The non-digestibility of  $\beta$ -2-1 fructans in the small intestine has also been demonstrated in healthy volunteers<sup>(19)</sup>.

- (2) Fermented by the intestinal microbiota. This has been demonstrated in experiments in which  $\beta$ -2-1 fructans were completely metabolised in microbial fermentation cultures<sup>(11,20)</sup>.
- (3) Selectively stimulate the growth and/or activity of beneficial intestinal bacteria, such as *Lactobacillus* species and *Bifidobacterium* species. Studies in laboratory animals and man show that prebiotics do increase the numbers of these types of bacteria in the intestinal tract<sup>(21-26)</sup>. Other experiments establish that  $\beta$ -2-1 fructans are selectively fermented by most *Bifidobacterium* species<sup>(27)</sup>, and also by some *Lactobacillus* species<sup>(28)</sup>, as these bacteria produce the intracellular fructosyl-fructofuranosidase that is needed for hydrolysis of the  $\beta$ -(2  $\rightarrow$  1) osidic bond in  $\beta$ -2-1 fructans<sup>(10)</sup>.

As a result of intestinal fermentation and promotion of growth of beneficial members of the gut microbiota, prebiotics may influence host defence (Fig. 2). Firstly, by increasing the number of bifidobacteria, there will be increased competition with pathogenic bacteria for binding sites on the intestinal epithelium and for nutrients, thus inhibiting survival of the pathogenic strains. Beneficial members of the gut microbiota bacteria may also cross the intestinal barrier into the Peyer's patches (PP), and activate immune cells there<sup>(29)</sup>. Others suggest that it is not the beneficial bacteria themselves that cross the barrier, but microbial substances such as cell wall components and cytoplasmic antigens<sup>(30)</sup>. *Bifidobacterium* species and *Lactobacillus* species are able to produce antibacterial substances that can inhibit the growth and survival of pathogens<sup>(31)</sup>.

Secondly, the fermentation of prebiotics by the *Bifidobacterium* species produces SCFA<sup>(1)</sup>, which have the following effects:

- Acidification of the colonic environment, which is detrimental to some pathogenic strains of bacteria<sup>(32)</sup> such as some pathogenic species of bacteroides, clostridia and coliforms<sup>(31)</sup>.
- Acidification of the colon favouring mucin production<sup>(33)</sup>. This is believed to improve mucosal morphology, so decreasing pathogenic bacterial colonisation and translocation.
- Binding to SCFA receptors (G protein coupled receptors 41 and 43) on immune cells within the gut-associated lymphoid tissues (GALT)<sup>(34-36)</sup>.
- Butyrate decreases the requirement of epithelial cells for glutamine, thus sparing more for GALT<sup>(37)</sup>.
- Butyrate may also alter epithelial cell gene expression, for example IL-8 and monocyte chemoattractant protein 1, and this in turn would alter the signalling of the epithelial cell to the mucosal immune system<sup>(38)</sup>.

Finally, prebiotics may also influence host immune function through alternative mechanisms to the modulation of beneficial bacteria in the gut. It is hypothesised that carbohydrate moieties on the prebiotic may interact with receptors on

Prebiotics and immunity

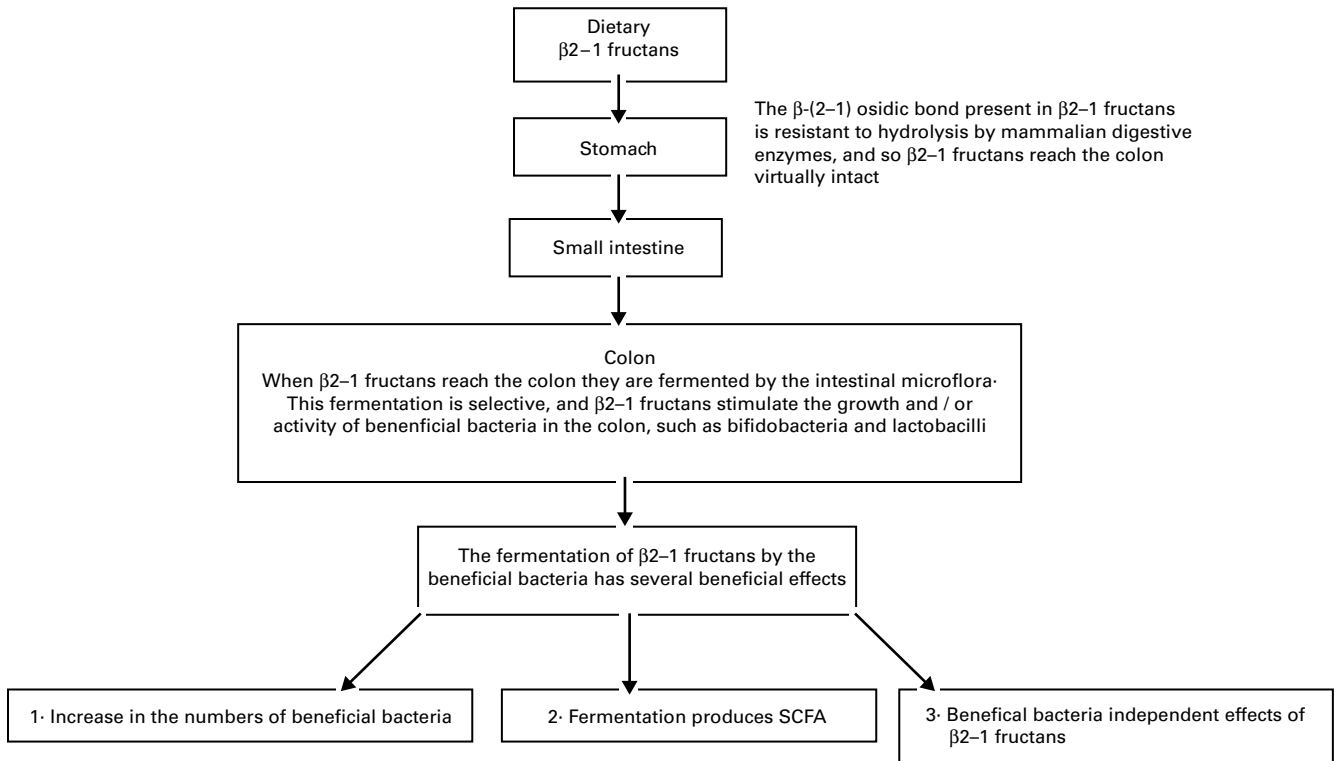


Fig. 2. Mechanisms by which beta-2-1 fructans may influence host defence.

immune cells. Although a specific fructose receptor has not yet been identified, receptors for beta-glucan<sup>(39,40)</sup> and mannose<sup>(41)</sup> have been identified on immune cells, and *in vitro*, fructose has been shown to alter non-opsonic phagocytosis<sup>(42)</sup>, suggesting that a receptor for fructose on immune cells may exist. In addition, some oligosaccharides, for example OF, can bind to receptors on pathogenic bacteria and prevent them from attaching to this same sugar on the epithelial membrane, thus preventing adherence<sup>(43)</sup>.

**Prebiotics and immune function**

This section reviews studies in experimental animals and in man that investigate the effects of increased consumption of beta-2-1 fructans on aspects of immune function.

*Studies in laboratory animals*

Studies conducted in laboratory animals are useful because they can be highly controlled, thus eliminating sources of variation in diet and in immune response. Twenty-two studies of beta-2-1 fructans reporting immune outcomes were identified in mice, rats, pigs and dogs, and are summarised in Table 1. Many of these studies show benefits of beta-2-1 fructans to some aspects of immune function, while showing no effect on other aspects. Thus, beta-2-1 fructans may have specific effects upon different components of the immune system. Here, the studies are separated into those which investigate the GALT and those which investigate the systemic immune system. The GALT is made up of the mucosa-associated lymphoid tissues of the gut, and is located underneath a columnar epithelial layer and mucus layer. Within the epithelial

layer, M (microfold) cells are distributed. These are antigen-presenting cells and are capable of transporting antigen from the gut lumen into the PP of the GALT. It is in the PP that antigen-presenting cells process and present the antigen to lymphocytes, which subsequently become activated. These lymphocytes then travel via the lymph to the mesenteric lymph nodes (MLN), through the thoracic duct and into the blood, where they become re-localised to the lamina propria of the intestine. Thus, the antigen-specific activated lymphocytes become distributed throughout the intestine.

*Gut-associated lymphoid tissue. Innate immune system:* The effect of beta-2-1 fructans upon macrophage number and function has been studied, with the results suggesting that macrophage functions are enhanced by the addition of beta-2-1 fructans to the diet. In *Clostridia difficile*-challenged mice, caecal macrophage and granulocyte numbers were increased in response to antibiotic treatment when a short course of FOS was given<sup>(44)</sup>. Peritoneal macrophage phagocytic activity was also increased in rodents given IN or OF for varying periods of time<sup>(45,46)</sup> and in mice vaccinated with *Salmonella typhimurium*<sup>(47)</sup> and respiratory burst was also increased<sup>(45)</sup>. Major histocompatibility complex (MHC) II molecule expression was also shown to increase on antigen-presenting cells in the MLN of rats upon OF and IN supplementation<sup>(45)</sup>. However, natural killer (NK) cell cytotoxicity in intra-epithelial lymphocytes of adult dogs was not affected by supplementation of FOS with other fermentable fibres<sup>(48)</sup>, and NK cell activity in MLN or PP of rats was not affected by OF-enriched IN<sup>(49)</sup>.

Thus, from the limited number of animal studies available, it appears that the innate immune system of the gut may be improved by beta-2-1 fructan intake, which could result in a

**Table 1.** Effects of  $\beta$ 2-1 fructans on immune function in laboratory animals

Reference no.	Prebiotic(s) used	Animal studied (sex and age where given)	Findings
44	Fructo-oligosaccharides (3 % w/v drinking water)	Mice (male, 12 weeks old); half were treated with antibiotics, half were not; all challenged with <i>Clostridium difficile</i>	<ul style="list-style-type: none"> <li>• <math>\uparrow</math> Caecal macrophages and granulocytes in antibiotic-treated mice</li> <li>• Non-significant <math>\uparrow</math> in macrophage numbers in colon</li> <li>• Fructo-oligosaccharide treatment prevented the following effects seen with antibiotics: <ul style="list-style-type: none"> <li>• Increased dendritic cells and <math>\gamma\delta</math> T cells in lamina propria of the caecum</li> <li>• Decreased PGE<sub>2</sub> and LTB<sub>4</sub> in small intestine, colon and caecum</li> </ul> </li> <li>• Stimulation of the gut-associated lymphoid tissue and development of PP, as shown by an increase in small intestinal lymphoid nodule count</li> </ul>
128	Short-chain fructo-oligosaccharides (5.8 % w/w diet)	Mice (male and female, 6–7 weeks old); C57BL/6J <i>min/+</i> mice (model of familial adenomatous polyposis and sporadic intestinal cancers, lesions mainly affect the small intestine)	<ul style="list-style-type: none"> <li>• <math>\uparrow</math> CD4<sup>+</sup>/CD8<sup>+</sup> ratio</li> <li>• <math>\downarrow</math> Proportion of B (Ig<sup>+</sup> cells (IgG<sup>+</sup>, IgA<sup>+</sup>, IgM<sup>+</sup>))</li> <li>• No change in NK cytotoxicity</li> <li>• No change in mitogen responses of peripheral blood mononuclear cells</li> </ul> <p>In gut-associated lymphoid tissue:</p> <ul style="list-style-type: none"> <li>• <math>\uparrow</math> Proportion of CD4<sup>+</sup> cells (expressing CD45R<sup>+</sup>) and CD5<sup>+</sup> cells (total T cells) in MLN cells</li> <li>• <math>\uparrow</math> Proportion of CD8<sup>+</sup> cells in intra-epithelial, PP and lamina propria cells</li> <li>• <math>\downarrow</math> CD4<sup>+</sup>/CD8<sup>+</sup> ratio in lamina propria</li> <li>• <math>\uparrow</math> T cell mitogen responses in intra-epithelial lymphocytes (to phytohaemagglutinin) and MLN (to all mitogens)</li> <li>• <math>\downarrow</math> T cell mitogen responses in PP (to concanavalin A and phytohaemagglutinin) and lamina propria cells (to concanavalin A)</li> <li>• No change in NK cytotoxicity in intraepithelial lymphocytes</li> </ul>
48	Fermentable fibre, including fructo-oligosaccharides, beet pulp and gum arabic (0.87 % w/w diet)	Dogs (adult)	<p>In peripheral blood:</p> <ul style="list-style-type: none"> <li>• <math>\uparrow</math> CD4<sup>+</sup>/CD8<sup>+</sup> ratio</li> <li>• <math>\downarrow</math> Proportion of B (Ig<sup>+</sup> cells (IgG<sup>+</sup>, IgA<sup>+</sup>, IgM<sup>+</sup>))</li> <li>• No change in NK cytotoxicity</li> <li>• No change in mitogen responses of peripheral blood mononuclear cells</li> </ul> <p>In gut-associated lymphoid tissue:</p> <ul style="list-style-type: none"> <li>• <math>\uparrow</math> Proportion of CD4<sup>+</sup> cells (expressing CD45R<sup>+</sup>) and CD5<sup>+</sup> cells (total T cells) in MLN cells</li> <li>• <math>\uparrow</math> Proportion of CD8<sup>+</sup> cells in intra-epithelial, PP and lamina propria cells</li> <li>• <math>\downarrow</math> CD4<sup>+</sup>/CD8<sup>+</sup> ratio in lamina propria</li> <li>• <math>\uparrow</math> T cell mitogen responses in intra-epithelial lymphocytes (to phytohaemagglutinin) and MLN (to all mitogens)</li> <li>• <math>\downarrow</math> T cell mitogen responses in PP (to concanavalin A and phytohaemagglutinin) and lamina propria cells (to concanavalin A)</li> <li>• No change in NK cytotoxicity in intraepithelial lymphocytes</li> </ul>
56	Fructo-oligosaccharides (1 % w/w diet), with/without probiotic mix of <i>Lactobacillus acidophilus</i> , <i>L. rhamnosus</i> , <i>Enterococcus faecium</i> , <i>Streptococcus thermophilus</i> , <i>L. bulgaricus</i>	Piglets (21 d old, <i>Salmonella</i> -free); infected with <i>S. typhimurium</i>	<p>No effect of fructo-oligosaccharides on:</p> <ul style="list-style-type: none"> <li>• Whole blood phagocyte activation level</li> <li>• Recovery of IgA in small intestine</li> </ul>
129	Oligofructose (3 g/d) plus <i>Lactobacillus paracasei</i> <i>L. paracasei</i> was also given alone	Piglets (newborn)	<p>10 d after birth the following were observed:</p> <ul style="list-style-type: none"> <li>• Significant differences between the <i>L. paracasei</i> group and the synbiotic group regarding counts of leucocytes, lymphocytes, neutrophils, CD2<sup>+</sup>T cells, CD4<sup>+</sup>T cells, B cells and macrophages in blood</li> <li>• <math>\downarrow</math> % phagocytic activity of leucocytes and neutrophils in blood in the synbiotic group compared to the control group</li> <li>• No effect of <i>L. paracasei</i> or the synbiotic upon total Ig concentrations in the sera</li> </ul> <p>10 d after weaning the following were observed:</p> <ul style="list-style-type: none"> <li>• In the synbiotic group, counts of leucocytes, lymphocytes, neutrophils, CD2<sup>+</sup>T cells, CD4<sup>+</sup>T cells, CD8<sup>+</sup>T cells, B cells and macrophages in blood had increased compared to the previous measurements</li> <li>• The synbiotic group had significantly greater CD4<sup>+</sup>T cell and B cell counts in blood compared to the <i>L. paracasei</i> group</li> <li>• <math>\uparrow</math> % phagocytic activity of leucocytes and neutrophils in blood in the synbiotic group, but not in the <i>L. paracasei</i> group</li> <li>• No effect of <i>L. paracasei</i> or the synbiotic upon total Ig concentrations in the sera</li> </ul>

Table 1. Continued

Reference no.	Prebiotic(s) used	Animal studied (sex and age where given)	Findings
53	Fructo-oligosaccharides and/or mannanoligosaccharides (2 g/d)	Dogs (female, adult)	<ul style="list-style-type: none"> <li>• ↑ Ileal IgA concentration with fructo-oligosaccharides plus mannanoligosaccharides</li> </ul> No effect of fructo-oligosaccharides alone or in combination with mannanoligosaccharides on: <ul style="list-style-type: none"> <li>• Total leucocyte, neutrophil or lymphocyte numbers</li> <li>• Serum IgA, IgG or IgM concentrations</li> <li>• Faecal IgA concentrations</li> </ul>
54	Fructo-oligosaccharides (4 g/d) and mannanoligosaccharides (2 g/d)	Dogs (adult)	<ul style="list-style-type: none"> <li>• ↓ Blood neutrophils</li> <li>• ↑ Blood lymphocytes</li> </ul> No effect upon serum, faecal or ileal Ig concentrations
88	Oligofructose (1.25 g/l drinking water) in combination <i>Bifidobacterium bifidum</i> and <i>B. infantis</i>	Mice (pups); infected with Rhesus rotavirus	Oligofructose did not have any additional benefit compared to when the probiotic was given alone, but both treatments: <ul style="list-style-type: none"> <li>• ↑ Rotavirus-specific IgA levels in serum 28 d post-infection</li> <li>• ↓ Duration of a strong rotavirus-specific IgA response in faeces</li> <li>• ↓ IgA and IgG positive B cell percentages in the PP at 28 d post-infection</li> </ul> No effect of bifidobacteria or bifidobacteria plus oligofructose upon: <ul style="list-style-type: none"> <li>• Serum rotavirus-specific IgG</li> <li>• Rhesus rotavirus antigen concentration in stools</li> </ul>
51	Fructo-oligosaccharides (2.5 or 7.5 % w/w diet)	Mice (female, 6 weeks old)	<ul style="list-style-type: none"> <li>• ↑ Total faecal IgA with 2.5 % but not 7.5 % fructo-oligosaccharides</li> <li>• ↑ Size of PP with 7.5 % fructooligosaccharides</li> <li>• ↑ Total IgA secretion by PP cells with 2.5 and 7.5 % fructo-oligosaccharides</li> <li>• ↑ IL-10 and IFN-<math>\gamma</math> production (dose dependent) from PP CD4<sup>+</sup>T cells stimulated with sonicated components from <i>B. pseudocatenulatum</i> from Gram-positive bacteria, but not by lipopolysaccharide or concanavalin A stimulation</li> <li>• High levels of IL-5 secretion from PP CD4<sup>+</sup>T cells maintained with both doses fructo-oligosaccharides, and IL-6 secretion was maintained with 7.5 % fructo-oligosaccharides</li> <li>• ↑ IFN-<math>\gamma</math> production from spleen CD4<sup>+</sup>T cells (dose dependent)</li> <li>• ↓ IL-5 and IL-6 production from spleen CD4<sup>+</sup>T cells</li> <li>• ↓ Serum IgG1 with both doses of fructo-oligosaccharides</li> <li>• No effect on serum IgG2a</li> </ul>
46	Inulin with shorter-chain fructo-oligosaccharides removed (10 % w/w diet) or oligofructose (either 2.5 or 10 % w/w diet)	Mice (female, 5–6 weeks old)	<ul style="list-style-type: none"> <li>• ↓ Leucocyte counts with 10 % inulin or 2.5 or 10 % oligofructose</li> <li>• ↑ NK activity of splenocytes with 10 % inulin or oligofructose</li> <li>• ↑ Peritoneal macrophage phagocytosis of <i>Listeria monocytogenes</i> with 10 % inulin or oligofructose</li> </ul> No effect on: <ul style="list-style-type: none"> <li>• Lymphocyte subsets in spleen and thymus (% of CD4<sup>+</sup>, CD8<sup>+</sup>, CD4<sup>+</sup>/CD8<sup>+</sup>, T and B cells)</li> <li>• Faecal IgA concentrations</li> </ul>
50	Fructo-oligosaccharides (10 % w/w diet)	Mice (female, 6–8 weeks old); healthy or endotoxaemic (induced by lipopolysaccharide from <i>Escherichia coli</i> )	<ul style="list-style-type: none"> <li>• ↑ Total number of immune cells in PP in healthy and endotoxaemic mice</li> <li>• ↑ B lymphocytes in PP in both healthy and endotoxaemic mice</li> <li>• ↑ T lymphocytes and CD4<sup>+</sup>/CD8<sup>+</sup> ratio in PP in endotoxaemic mice only</li> </ul>
58	Chicory (1 % w/w diet) and/or mannanoligosaccharides (1 % w/w diet)	Dogs (male and female, seniors, 8–11 years old)	<ul style="list-style-type: none"> <li>• Chicory in combination with mannanoligosaccharides decreased peripheral blood lymphocyte concentration</li> <li>• Chicory alone and in combination with mannanoligosaccharides non-significantly increased neutrophil concentrations in blood</li> </ul> No effect of chicory alone or in combination with mannanoligosaccharides on: <ul style="list-style-type: none"> <li>• Total peripheral leucocyte concentration</li> <li>• Serum concentrations of IgA, IgG or IgM</li> <li>• Concentrations of monocytes or eosinophils in blood</li> </ul>

Table 1. Continued

Reference no.	Prebiotic(s) used	Animal studied (sex and age where given)	Findings
52	Fructo-oligosaccharides (5 % w/w diet)	Mice (newborn)	<ul style="list-style-type: none"> <li>• ↑ Total intestinal IgA (ileum, jejunum and colon)</li> <li>• ↑ Ileal and colonic polymeric Ig receptor expression at 36 d</li> <li>• ↑ Ileal IgA secretion rate at 37 d</li> <li>• ↑ IgA response of PP cells</li> <li>• ↑ % of B220<sup>+</sup> IgA<sup>+</sup> cells (IgA committed cells, thus promoted isotype switching from IgM to IgA in PP B cells)</li> </ul>
49	Oligofructose-enriched inulin (10 % w/w diet) Also studied probiotics ( <i>L. rhamnosus</i> GG and <i>B. lactis</i> Bb12) and a synbiotic (combination of Synergy1 <sup>®</sup> and the probiotics)	Rats (male, 12–13 weeks old)	<p>No effect on % of B220<sup>+</sup> cells or % of B220<sup>+</sup> IgM<sup>+</sup> cells in PP</p> <ul style="list-style-type: none"> <li>• ↓ Oxidative burst activity in blood neutrophils in synbiotic group compared to probiotic group</li> <li>• ↑ IL-10 and IFN-γ production in PP in oligofructose-enriched inulin group</li> <li>• ↑ Secretory IgA concentration in ileum in synbiotic group</li> <li>• ↑ Secretory IgA concentration in caecum in oligofructose-enriched inulin group</li> </ul> <p>No effect of oligofructose-enriched inulin on:</p> <ul style="list-style-type: none"> <li>• CD4<sup>+</sup> and CD8<sup>+</sup>T lymphocytes in spleen, MLN and blood</li> <li>• Neutrophil and monocytes phagocytosis of <i>E. coli</i> in blood and spleen</li> <li>• NK activity in spleen, MLN, PP (non-significant tendency to increase this in peripheral blood mononuclear cells)</li> <li>• Lymphocyte proliferation in spleen, MLN, PP</li> <li>• Cytokine production in spleen or MLN</li> </ul>
57	Oligofructose-enriched inulin (10 % w/w diet) Also studied probiotics ( <i>L. rhamnosus</i> GG and <i>B. lactis</i> Bb12) and a synbiotic (combination of Synergy 1 <sup>®</sup> and the probiotics)	Rats (male, 4-5 weeks old); model of colon cancer	<p>Oligofructose-enriched inulin:</p> <ul style="list-style-type: none"> <li>• ↑ NK cell-like cytotoxic function in the spleen of rats without colon cancer</li> <li>• Prevented the ↓ in NK cell-like cytotoxic function seen in controls, in rats with colon cancer</li> <li>• ↑ IL-10 production in MLN of rats without colon cancer</li> <li>• ↑ IL-10 production by PP in rats with colon cancer</li> <li>• ↓ CD4<sup>+</sup>/CD8<sup>+</sup> ratio in spleen without significantly changing the % of CD4<sup>+</sup> or CD8<sup>+</sup>T-lymphocytes in rats without colon cancer</li> </ul> <p>• No effect on IL-10 production by splenocytes in rats with or without colon cancer</p>
130	Inulin (4-8 % w/w diet)	Rats (21 d old, male and female); diabetes resistant or diabetes prone	<p>In diabetes-resistant rats:</p> <ul style="list-style-type: none"> <li>• ↑ Small intestine length</li> <li>• ↓ Number of splenocytes</li> <li>• ↑ CD8<sup>+</sup> lymphocytes in PP</li> <li>• ↑ Proliferation of splenocytes and MLN cells to mitogens</li> <li>• ↓ Production of IL-4 and</li> <li>• ↑ production of IL-10 by stimulated splenocytes</li> </ul> <p>No effect on:</p> <ul style="list-style-type: none"> <li>• Spleen weight</li> <li>• Immune cell numbers in PP</li> <li>• IgA<sup>+</sup> cells in jejunal lamina propria</li> <li>• Immune cell phenotypes in spleen and MLN</li> <li>• Splenocyte production of IL-2, IFN-γ or TGF-β</li> <li>• MLN cell production of IFN-γ, TNF-α or TGF-β</li> <li>• PP cell production of TGF-β</li> </ul> <p>In diabetes-prone rats:</p> <ul style="list-style-type: none"> <li>• ↓ Number of splenocytes</li> <li>• ↑ IgA<sup>+</sup> cells in jejunal lamina propria</li> <li>• ↑ B lymphocytes in PP</li> <li>• ↓ Production of IL-4 and</li> <li>• ↑ production of IL-10 by stimulated splenocytes</li> </ul>



Table 1. Continued

Reference no.	Prebiotic(s) used	Animal studied (sex and age where given)	Findings
			<p>No effect on:</p> <ul style="list-style-type: none"> <li>• Small intestine length</li> <li>• Spleen weight</li> <li>• Immune cell numbers in PP</li> <li>• Immune cell phenotypes in spleen and MLN</li> <li>• Proliferation of splenocytes and MLN cells in response to mitogens</li> <li>• Splenocyte production of IL-2, IFN-<math>\gamma</math> or TGF-<math>\beta</math></li> <li>• MLN cell production of IFN-<math>\gamma</math>, TNF-<math>\alpha</math> or TGF-<math>\beta</math></li> <li>• PP cell production of TGF-<math>\beta</math></li> </ul>
45	Oligofructose or inulin (10 % w/w diet)	Rats (male)	<p>Oligofructose and inulin:</p> <ul style="list-style-type: none"> <li>• <math>\uparrow</math> number of T cells in spleen, MLN and thymus</li> <li>• <math>\uparrow</math> MHCII on antigen-presenting cells in spleen, MLN and thymus</li> <li>• <math>\uparrow</math> IL-2 and IL-4 in blood</li> <li>• <math>\uparrow</math> peritoneal macrophage phagocytosis</li> <li>• <math>\uparrow</math> peritoneal macrophage superoxide production</li> </ul> <p>No effect on:</p> <ul style="list-style-type: none"> <li>• Apoptosis of immunocytes</li> </ul>
55	Inulin (3 % w/w diet)	Dogs (adult, 2–11 years old); hypoallergenic	<p>No effect on:</p> <ul style="list-style-type: none"> <li>• Total serum IgA, IgE, IgG or IgM concentrations</li> <li>• Total faecal IgA concentration</li> </ul>
23	Galacto-oligosaccharides and inulin with the shorter-chain fructooligosaccharides removed (9:1 ratio) at different doses (1–10 % w/w diet)	Mice (female, 6–8 weeks old); vaccinated with influenza vaccine; DTH to influenza vaccine	<ul style="list-style-type: none"> <li>• <math>\uparrow</math> DTH response in a dose-dependent manner (5 % w/w of diet was the optimal dose)</li> </ul> <p>No effect on:</p> <ul style="list-style-type: none"> <li>• Vaccine-specific serum antibody (total IgG, IgG1 and IgG2a) concentration (humoral response)</li> <li>• Vaccine-induced splenocyte proliferation (T cell activation)</li> <li>• Other oligosaccharides were also tested (inulin/FOS; short-chain FOS; inulin) but these did not significantly affect the DTH response</li> </ul>
131	Oligofructose (0.91 % w/w diet)	Dogs (pregnant female + pups to 49 d of age); pups vaccinated with <i>Bordetella bronchiseptica</i> vaccine at day 21	<p>In pregnant females and pups no effect on:</p> <ul style="list-style-type: none"> <li>• Serum IgG1, IgG2, IgA or IgM</li> </ul> <p>In colostrum and milk:</p> <ul style="list-style-type: none"> <li>• <math>\uparrow</math> IgM, but no effect on IgG1, IgG2 or IgA</li> </ul> <p>In pups no effect on:</p> <ul style="list-style-type: none"> <li>• Post-vaccination Ig nasal secretions</li> </ul>
78	Short-chain oligofructose or inulin (1 % w/w diet)	Puppies (12 weeks old, male and female); infected with <i>S. typhimurium</i>	<ul style="list-style-type: none"> <li>• Tendency for inulin to <math>\downarrow</math> serum IgG (due to epithelial damage)</li> </ul> <p>Both short-chain oligofructose and inulin:</p> <ul style="list-style-type: none"> <li>• No change in serum IgA or IgM concentrations</li> <li>• <math>\uparrow</math> Vaccine-specific faecal IgA and plasma IgG levels</li> <li>• <math>\uparrow</math> Peritoneal macrophage activity 1 week post-immunisation</li> <li>• <math>\uparrow</math> Mean fluorescence intensity of MHCII<sup>+</sup> cells in spleen</li> <li>• <math>\uparrow</math> IL-12 and IFN-<math>\gamma</math> production by splenocytes</li> <li>• <math>\uparrow</math> Survival from <i>Salmonella</i> infection when given vaccine</li> </ul>
47	Oligofructose and inulin with the shorter-chain fructo-oligosaccharides removed (70:30 ratio; 5 % w/w diet)	Mice (female, 6 weeks old); immunised with <i>S. typhimurium</i> vaccine	<p>No effect on:</p> <ul style="list-style-type: none"> <li>• Total serum IgG or total faecal IgA</li> <li>• % of cell subsets (CD4<sup>+</sup>, CD8<sup>+</sup>, B220<sup>+</sup>, MHCII<sup>+</sup>, CD11b<sup>+</sup>, CD11c<sup>+</sup>) in spleen</li> <li>• Splenocyte proliferation rates</li> <li>• TNF-<math>\alpha</math> production by splenocytes</li> <li>• <i>Salmonella</i> translocation to lymphoid organs (PP, MLN, liver and spleen)</li> </ul>

DTH, delayed type hypersensitivity; IFN, interferon; LTB<sub>4</sub>, leukotriene B<sub>4</sub>; MHC, major histocompatibility complex; MLN, mesenteric lymph nodes; NK, natural killer; PP, Peyer's patches; TGF, transforming growth factor;  $\uparrow$ , increase/increased;  $\downarrow$ , decrease/decreased.

beneficial effect on the host's primary response to infection. However, studies measuring NK cell activity did not find any effect upon this component of the innate immune system, which plays a major role in the anti-tumour immunity and destruction of virus-infected cells. Future studies should build upon those reported here, to create a more complete picture of how  $\beta$ -1 fructans affect the innate immune system.

**Adaptive immune system:** in healthy and endotoxaemic mice supplemented for a short time with FOS, B cell numbers were increased in the PP<sup>(50)</sup>. Several studies report an increase in intestinal or faecal IgA levels upon supplementation with various  $\beta$ -1 fructan preparations<sup>(47,49,51–53)</sup>. FOS supplementation increased total faecal IgA and IgA secretion by PP cells in young mice<sup>(51)</sup>, and increased various intestinal measures of IgA production in newborn mice, but did not alter B220<sup>+</sup> IgM<sup>+</sup> cell percentages in PP<sup>(52)</sup>. In rats, OF-enriched IN increased caecal secretory IgA concentrations<sup>(49)</sup>. FOS in combination with MOS increased ileal IgA in adult dogs<sup>(53)</sup>, but there was no effect upon faecal IgA concentrations in this same study<sup>(53)</sup>. Vaccine-specific faecal IgA was increased in mice supplemented with a combination of OF and IN with shorter-chain FOS removed and vaccinated with *Salmonella typhimurium*, but total faecal IgA was not<sup>(47)</sup>. As IgA antibodies present at the mucosal surface of the gut prevent adherence of pathogens to the gut mucosa, these findings would indicate improved health of the host upon  $\beta$ -1 fructan supplementation. However, several other studies do not show an effect of  $\beta$ -1 fructan supplementation on intestinal or faecal IgA levels. Faecal and ileal Ig concentrations were not altered in adult dogs fed FOS in combination with MOS<sup>(54)</sup>. No effect of IN with shorter-chain FOS removed or OF on faecal IgA concentrations was observed in mice or hypoallergenic dogs<sup>(46,55)</sup>, and there was no effect of FOS on IgA in the small intestine of piglets<sup>(56)</sup>. Thus, there is some disagreement about the effects of  $\beta$ -1 fructans on IgA levels in the gastrointestinal tract, with three out of the four mouse models showing an enhancement<sup>(47,51,52)</sup> and a single study reporting no effect<sup>(46)</sup>. These studies were all in young mice. None of the three studies that were conducted in adult dogs showed an effect upon faecal IgA concentrations<sup>(53–55)</sup>, but one did show an effect upon ileal IgA concentration<sup>(53)</sup>. Thus it seems that the animal used and age may be important in determining whether or not prebiotic supplementation is beneficial on this aspect of immune function. There may be a greater effect in younger animals as their gut immune system is still developing and may therefore be more susceptible to modulation. Other explanations for why there is disparity in the results reported could include (1) that faecal IgA may not be an accurate marker of what is happening inside the gut, and (2) that the level of IgA that is reported would depend, perhaps, on the site of the gut at which IgA is measured. If  $\beta$ -1 fructans enhance the immune system through promotion of the growth of beneficial members of the gut microbiota, and if a prebiotic, by definition, is specific with respect to the beneficial bacteria it stimulates, then there will be parts of the gut where these beneficial bacteria are most abundant and therefore where the largest effect upon the immune response would be observed. This may partly explain why results reporting IgA at different locations vary.

$\beta$ -1 Fructan supplementation has been reported to have effects upon T cell subsets and function, but these effects

vary depending upon the anatomical site of origin of the cells, and the animal model used. The number of T cells in the MLN of rats was increased upon OF or IN supplementation<sup>(45)</sup>. The proportions of CD4<sup>+</sup> cells (expressing CD45R<sup>+</sup>) and CD5<sup>+</sup> cells in MLN were increased in adult dogs supplemented with FOS combined with other fermentable fibres, but the proportion of intra-epithelial, PP and lamina propria CD8<sup>+</sup> cells was increased<sup>(48)</sup>. Thus a decrease in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio in the lamina propria cells was observed<sup>(48)</sup>. In contrast, the CD4<sup>+</sup>/CD8<sup>+</sup> ratio was increased in PP of endotoxaemic mice fed FOS<sup>(50)</sup>, and there was no effect on CD4<sup>+</sup> or CD8<sup>+</sup>T cells in the MLN of rats supplemented with OF-enriched IN<sup>(49)</sup>. Responses of T cells to mitogens were increased for intra-epithelial lymphocytes and MLN, but decreased for PP and lamina propria cells in dogs supplemented with FOS plus fermentable fibres<sup>(48)</sup>, and no effect of OF-enriched IN was seen on MLN or PP lymphocyte proliferation in rats<sup>(49)</sup>. Enhancement of T cell cytokine production has been reported, with an increase in IL-10 and interferon (IFN)- $\gamma$  production from stimulated PP CD4<sup>+</sup>T cells seen in FOS-supplemented female mice, and high levels of IL-5 and IL-6 secretion from these cells was also maintained<sup>(51)</sup>. IL-10 production from PP and MLN, and IFN- $\gamma$  production from PP, was also increased in rats with OF-enriched IN supplementation<sup>(49,57)</sup>; however, cytokine production in MLN was not altered<sup>(49)</sup>. Taken together these findings do not present a clear picture of the effects of  $\beta$ -1 fructans on T cell numbers in GALT or on T cell responses. It is possible that the effects of prebiotic supplementation upon cell-mediated immunity in the GALT are dependent upon the site of origin of the cells and the animal model used.

**Systemic immune system. Innate immune system:** the systemic immune system has been more widely studied in the context of prebiotic supplementation than the GALT. As observed in the GALT, after OF or IN supplementation, MHCII expression was increased in antigen-presenting cells in the spleen and thymus of male rats<sup>(45)</sup> and mean fluorescence intensity of MHCII<sup>+</sup> cells in spleen of mice also increased, although percentage of MHCII<sup>+</sup> cells did not change here<sup>(47)</sup>. No measures of macrophage activity in the systemic immune system have been recorded with  $\beta$ -1 fructan supplementation. No effect upon monocyte or eosinophil numbers in the blood was reported when aged dogs were supplemented with chicory alone or in combination with MOS, although a non-significant increase in neutrophil concentrations was seen<sup>(58)</sup>, and phagocytic activity of monocytes and neutrophils in the blood or spleen was not altered upon OF-enriched IN supplementation in rats<sup>(49)</sup>. FOS did not affect whole blood phagocyte activation in piglets infected with *Salmonella typhimurium*, although when given in a synbiotic (a mixture of a probiotic and a prebiotic), this marker was increased<sup>(56)</sup>. FOS in combination with MOS did not affect blood neutrophil numbers of adult dogs in one study<sup>(53)</sup> but this supplement decreased neutrophil numbers in another study<sup>(54)</sup>. This could be due to the use of different doses of FOS: in the later study a higher dose of FOS was given. Thus, in the systemic immune system, there seems to be little effect of  $\beta$ -1 fructans upon phagocytic function.

NK cytotoxicity in the peripheral blood was not altered by FOS supplementation in adult dogs<sup>(48)</sup>, similar to the effects seen in the GALT. However, in the spleen, a preparation of



IN, with shorter-chain FOS removed, in combination with OF increased NK activity of splenocytes in female mice<sup>(46)</sup>, and OF-enriched IN increased NK cell-like cytotoxic function in the spleen of male rats<sup>(57)</sup>, and the same supplement non-significantly increased this function in blood mononuclear cells in another study by the same group<sup>(49)</sup>. Thus, in the spleen, at least, NK cell function may be enhanced by OF or IN supplementation.

*Adaptive immune system:* in adult dogs, the proportion of B cells in the peripheral blood was decreased when a high fermentable fibre diet including FOS was fed<sup>(48)</sup>. The majority of studies measuring the effect of  $\beta$ -1 fructans on serum Ig show no effect. This was observed in murine<sup>(23,51)</sup> and canine<sup>(53–55,58)</sup> models, with supplementation of FOS alone, FOS in combination with MOS, IN alone or IN in combination with GOS or MOS. Antibodies measured included total serum Ig, IgA, IgE, IgG, IgG2a, IgM and vaccine-specific antibodies to influenza vaccination (total IgG, IgG1, IgG2a). Two studies report a decrease in serum antibody concentrations upon OF supplementation. A study in dogs showed that the proportion of B cells in the peripheral blood was decreased with a high fermentable fibre diet<sup>(48)</sup>, and a study in mice demonstrated that FOS supplementation was associated with a decrease in serum IgG<sub>1</sub><sup>(51)</sup>. Just one study reports an increase in vaccine-specific plasma IgG levels in mice vaccinated with *Salmonella typhimurium*, although no effect on total serum IgG was observed<sup>(47)</sup>. Thus, there seems to be little effect on systemic humoral immunity by  $\beta$ -1 fructan supplementation, and the studies which have shown an effect have mostly shown a suppressive effect, in contrast to the GALT where the results suggest that this aspect of immune function may be enhanced.

T cell subpopulations may be altered with  $\beta$ -1 fructan supplementation. T cell numbers were increased in the spleen and thymus of rats<sup>(45)</sup>. In the blood of adult dogs supplemented with FOS, the CD4<sup>+</sup>/CD8<sup>+</sup> ratio was increased<sup>(48)</sup>. In contrast, supplementation with OF-enriched IN decreased the spleen CD4<sup>+</sup>/CD8<sup>+</sup> ratio in rats<sup>(57)</sup>. In mice, IN with shorter-chain FOS removed or OF had no effect on lymphocyte subsets (CD4<sup>+</sup> and CD8<sup>+</sup> percentages and or CD4<sup>+</sup>/CD8<sup>+</sup> ratio) in the spleen or thymus<sup>(46)</sup>. Neither was there any effect of OF-enriched IN on numbers of CD4<sup>+</sup> or CD8<sup>+</sup>T cells in the spleen and blood of rats<sup>(49)</sup>, or of OF in combination with IN with shorter-chain FOS removed on the percentage of spleen cell subsets (CD4<sup>+</sup>, CD8<sup>+</sup>, B220<sup>+</sup>, CD11b<sup>+</sup> or CD11c<sup>+</sup>) in mice<sup>(47)</sup>. Thus, although some studies show that  $\beta$ -1 fructans may alter T cell subpopulations in the blood and spleen, other studies report no effect on these measurements in the spleen, thymus and blood.

Vaccine-induced splenocyte proliferation was not altered in mice supplemented with a combination of GOS and IN with shorter-chain FOS removed<sup>(23)</sup>. Neither was lymphocyte proliferation altered in the spleen of rats supplemented with OF-enriched IN<sup>(49)</sup> nor splenocyte proliferation in mice vaccinated against *Salmonella typhimurium* and supplemented with OF in combination with IN with shorter-chain FOS removed<sup>(47)</sup>. Thus, lymphocyte proliferation in the spleen appears not to be susceptible to modification by  $\beta$ -1 fructans.

As in the GALT, T cell cytokine production may be altered with  $\beta$ -1 fructan supplementation: IFN- $\gamma$  production from spleen CD4<sup>+</sup>T cells was increased in mice supplemented with FOS, although IL-5 and IL-6 production were decreased<sup>(51)</sup>,

and IL-12 and IFN- $\gamma$  production from splenocytes was increased upon supplementation with a combination of OF and IN with shorter-chain FOS removed in mice, although TNF- $\alpha$  production was not altered<sup>(47)</sup>. There was no effect of OF-enriched IN supplementation in rats upon IL-10 production by splenocytes<sup>(57)</sup>, or upon cytokine production in the spleen<sup>(49)</sup>. Blood IL-2 and IL-4 concentrations were increased upon IN or OF supplementation in rats<sup>(45)</sup>. Thus, the limited number of studies reporting T cell-derived cytokine production in animals receiving  $\beta$ -1 fructans suggest that some modification occurs. Why T cell cytokine production should be altered when T cell proliferation is not affected is not clear. The delayed type hypersensitivity response represents the summation of a cell-mediated immune response to an antigenic challenge, largely representing antigen-presenting cell and T cell function. Therefore the observation that the delayed type hypersensitivity response to influenza vaccine was increased when GOS and IN with shorter-chain FOS removed were supplemented to mice<sup>(23)</sup> supports the findings of improved T cell cytokine production with prebiotics.

#### Studies in man

Twelve studies that included supplementation with  $\beta$ -1 fructans, either alone or in combination with other components, on the human immune system were identified; these have mainly measured aspects of the systemic immune system, via blood immune markers and immune cell responses, and are summarised in Table 2. Four of these studies investigated the effects of  $\beta$ -1 fructans alone<sup>(24,59–61)</sup> and five investigated supplements that contain  $\beta$ -1 fructans combined with antioxidants, vitamins, minerals, other prebiotics and fats<sup>(62–66)</sup>. Thus, it is difficult to determine whether the effects that were observed were due to  $\beta$ -1 fructans, or to another component of the supplement. The remaining three studies investigated synbiotics<sup>(67–69)</sup>, but did not include a prebiotic alone group, and so will be considered separately.

*Innate immune system.* A decrease in monocyte and granulocyte phagocytosis of *Escherichia coli* was observed when elderly adults resident in a nursing home were supplemented with OF for 3 weeks, although no control group was included in this study making it difficult to interpret the findings<sup>(24)</sup>. However, the finding of decreased phagocytosis is in contrast to what was observed in senior dogs and adult rats, where no modification of blood monocyte concentrations or phagocytosis was seen<sup>(49,58)</sup> and to the findings of Seidel *et al.*<sup>(64)</sup> of no effect on phagocytosis of *E. coli* by granulocytes taken from young adult males consuming bread containing IN.

Neither OF<sup>(24)</sup> nor a bread containing IN<sup>(64)</sup> affected NK cell numbers in human blood. To our knowledge there have been no reports of the effect of  $\beta$ -1 fructans on human NK cell activity.

*Adaptive immune system.* The percentage of blood B cells (defined as CD19<sup>+</sup>) was increased in young male adults after consumption of a bread containing IN<sup>(64)</sup>. B cell number was increased in elderly residents of a long-term care facility supplemented with FOS<sup>(63)</sup>. Thus there is some consistency in findings in man regarding the effect of  $\beta$ -1 fructans on B cell numbers (an increase), but this is in contrast to observations in adult dogs, where B cell numbers in the blood were decreased<sup>(48)</sup>.

**Table 2.** Effect of  $\beta$ 2-1 fructans on immune function in man

Reference no.	Prebiotic(s) used	Subjects studied	Findings
59	Oligofructose and inulin (0.2 g/kg body wt/d for 10 weeks)	Healthy infants; age 8 months; immunised with measles vaccine 4 weeks into the trial; <i>n</i> 24 in prebiotic group; <i>n</i> 25 in control group	<ul style="list-style-type: none"> <li>• <math>\uparrow</math> Post-vaccination total IgG levels in blood</li> </ul>
24	Oligofructose (8 g/d for 3 weeks)	Elderly adults in a care home; mean age 85 years; male and female; <i>n</i> 19; no control group	<ul style="list-style-type: none"> <li>• <math>\uparrow</math> % of peripheral blood T, CD4<sup>+</sup>T and CD8<sup>+</sup>T cells</li> <li>• <math>\downarrow</math> Monocyte and granulocyte phagocytosis of <i>Escherichia coli</i></li> <li>• <math>\downarrow</math> IL-6 mRNA expression in peripheral blood mononuclear cells</li> </ul> <p>No effect on:</p> <ul style="list-style-type: none"> <li>• Total number of leucocytes, activated T lymphocytes or NK cells in blood</li> </ul>
60	Oligofructose and inulin (6 g/d for 28 weeks)	Elderly free-living adults; age $\geq$ 70 years; immunised with influenza and pneumococcal vaccines 2 weeks into the trial; <i>n</i> 20 in prebiotic group; <i>n</i> 23 in control group	<ul style="list-style-type: none"> <li>• <math>\uparrow</math> Antibody response to influenza B virus and <i>Streptococcus pneumoniae</i> in both prebiotic and control groups</li> </ul> <p>No effect on:</p> <ul style="list-style-type: none"> <li>• Serum proteins, albumin, C-reactive protein, Ig (IgA, IgG, IgM), salivary secretory IgA</li> <li>• Serum antibody titres against influenza A virus</li> <li>• IL-4 and IFN-<math>\gamma</math> production by cultured peripheral blood mononuclear cells</li> <li>• Stimulated lymphocyte proliferation</li> </ul> <p>No effect on:</p> <ul style="list-style-type: none"> <li>• Post-vaccination antibody response to <i>H. influenzae</i> type B (measured at 1 month post-vaccination)</li> </ul>
61	Oligofructose (in cereal supplemented with 3.6 % w/w oligofructose = 0.55 g/d for 6 months)	Peruvian infants; aged 6–12 months; immunised with <i>Haemophilus influenzae</i> type B vaccine 5 months into the trial; <i>n</i> 129 in prebiotic group; <i>n</i> 122 in control group	<ul style="list-style-type: none"> <li>• Post-vaccination antibody response to <i>H. influenzae</i> type B (measured at 1 month post-vaccination)</li> </ul>
67	Oligofructose and inulin (2:1 ratio; 6 g/d for 1 year) Included in the formula were also protein, fat, carbohydrate, vitamin E, vitamin B <sub>12</sub> , thiamine, folic acid, riboflavin, pyridoxine, other nutrients and <i>L. paracasei</i>	Elderly free-living adults; age $\geq$ 70 years; immunised with influenza and pneumococcal vaccines 4 months into the trial; <i>n</i> 30 in control group; <i>n</i> 30 in experimental group	<p>At 4 months follow up:</p> <ul style="list-style-type: none"> <li>• <math>\uparrow</math> NK activity</li> <li>• Prevention of the <math>\downarrow</math> in proportion of T cells with NK activity that was seen in the control group</li> <li>• No effect on any other lymphocyte subpopulation</li> <li>• No significant effect on lipopolysaccharide-stimulated mononuclear cell production of IL-1, TNF-<math>\alpha</math> or IL-6</li> </ul> <p>After vaccination:</p> <ul style="list-style-type: none"> <li>• Prevention of the <math>\downarrow</math> in IL-2 production by mononuclear cells seen in the control group</li> </ul> <p>No effect on:</p> <ul style="list-style-type: none"> <li>• IFN-<math>\gamma</math> production after influenza antigen stimulation</li> <li>• Delayed type hypersensitivity</li> <li>• Magnitude of the rise in influenza antibodies or pneumococcus antibodies</li> </ul>
62	Fructo-oligosaccharides (4.95 % of the energy intake of the 226.8 g (8 oz) formula/d for 26 weeks) The formula also contained antioxidants (vitamins C, E and $\beta$ -carotene), B vitamins, zinc, selenium, other vitamins and minerals, and structured TAG	Adults aged $\geq$ 65 years; assisted-living and independent-living; immunised with influenza vaccine 2 weeks into the trial; <i>n</i> 18 in control group; <i>n</i> 16 in experimental group	<ul style="list-style-type: none"> <li>• Improved response to some vaccine components</li> <li>• <math>\uparrow</math> Lymphocyte proliferation to influenza vaccine components</li> </ul>
65	Galacto-oligosaccharides and inulin with shorter-chain fructo-oligosaccharides removed (ratio 9:1); 0.6 g/100 ml formula for 32 weeks Also looked at <i>B. animalis</i>	Newborn infants; non-breast-fed; <i>n</i> 19 in prebiotic group; <i>n</i> 19 in probiotic group; <i>n</i> 19 in control group (standard infant formula)	<ul style="list-style-type: none"> <li>• Trend for <math>\uparrow</math> faecal secretory IgA (statistically significant at 16 weeks)</li> </ul>

Table 2. Continued

Reference no.	Prebiotic(s) used	Subjects studied	Findings
63	Fructo-oligosaccharides (4.95 % of the energy intake of the 240 ml formula/d for 10 weeks) The formula also contained antioxidants (vitamins C, E and $\beta$ -carotene), B vitamins, zinc, selenium, other vitamins and minerals, and structured TAG	Frail adults aged $\geq 65$ years; living in long-term care facilities; immunised with influenza vaccine 4 weeks into the trial; <i>n</i> 40 in control group; <i>n</i> 52 in experimental group	<ul style="list-style-type: none"> <li>• Improved response to some vaccine components</li> <li>• <math>\uparrow</math> B cells</li> <li>• <math>\downarrow</math> Memory cytotoxic T cells</li> <li>• <math>\uparrow</math> Influenza-activated lymphocytes (CD69 and CD25) (this was due to changes in CD2<sup>-</sup> T cells and CD2<sup>-</sup>CD8<sup>-</sup> Th cell populations)</li> <li>• <math>\downarrow</math> IL-6 and a trend for <math>\downarrow</math> IL-10 production by phytohaemagglutinin-stimulated mononuclear cells at week 6 post-vaccination</li> <li>• <math>\downarrow</math> Fever</li> <li>• Non-significant <math>\uparrow</math> in NK T cells (expressing CD56<sup>+</sup>/CD57<sup>+</sup>, CD28<sup>+</sup> and CD3<sup>+</sup>)</li> <li>• Non-significant <math>\uparrow</math> in naïve Th cells</li> </ul> <p>No effect on:</p> <ul style="list-style-type: none"> <li>• Geometric mean antibody titres</li> <li>• Leucocyte counts, percentage of lymphocytes and T cell subsets (CD4<sup>+</sup> or CD8<sup>+</sup>)</li> </ul>
68	Synbiotic containing oligofructose-enriched inulin (12 g/d for 12 weeks) plus <i>Lactobacillus delbreuckii</i> subsp. <i>rhamnosus</i> GG and <i>Bifidobacterium lactis</i> Bb12	Adults; colon cancer and polypectomised patients; <i>n</i> 38 in test group; <i>n</i> 36 in placebo group	<ul style="list-style-type: none"> <li>• Prevented increased IL-2 secretion from stimulated peripheral blood mononuclear cells in polypectomised patients, but not in cancer patients</li> <li>• <math>\uparrow</math> IFN-<math>\gamma</math> production from stimulated peripheral blood mononuclear cells in colon cancer patients, but not in polypectomised patients</li> </ul>
69	Synbiotic containing oligofructose-enriched inulin (10 g/d for 12 weeks) plus <i>L. rhamnosus</i> GG and <i>B. lactis</i> Bb12	Adults; colon cancer and polypectomised patients; <i>n</i> 38 in test group; <i>n</i> 36 in placebo group	<ul style="list-style-type: none"> <li>• <math>\uparrow</math> IL-2 secretion by activated mononuclear cells</li> <li>• <math>\uparrow</math> IFN-<math>\gamma</math> producing capacity of peripheral blood mononuclear cells at 12 weeks compared to 6 weeks in cancer patients</li> </ul> <p>No effect on:</p> <ul style="list-style-type: none"> <li>• % of phagocytically active neutrophils and monocytes and their phagocytic intensity in cancer and polypectomised patients</li> <li>• % of neutrophils that produced reactive oxygen species and the intensity of this production in cancer or polypectomised patients</li> <li>• Lytic activity of NK cells in cancer or polypectomised patients</li> <li>• Production of IL-10, IL-12 and TNF-<math>\alpha</math> from activated peripheral blood mononuclear cells in cancer or polypectomised patients</li> <li>• IFN-<math>\gamma</math> production by mononuclear cells in polypectomised patients</li> <li>• IL-2 secretion by activated peripheral blood mononuclear cells in cancer patients</li> <li>• PGE<sub>2</sub> and TGF-<math>\beta</math>1 concentrations in faecal water in cancer or polypectomised patients</li> </ul>
64	Inulin (4 % w/w of a bread, which also contained other prebiotics with/without antioxidants; approximate intake 9 g inulin/d for 5 weeks)	Adults; male; mean age 27 years; smokers and non-smokers; <i>n</i> 19 in prebiotic group; <i>n</i> 19 in prebiotic plus antioxidant group	<p>Twenty-three immunological parameters were measured in the peripheral blood, most of which were unchanged by either the prebiotic or the prebiotic plus antioxidant bread. However:</p> <ul style="list-style-type: none"> <li>• <math>\uparrow</math> % CD19 (B) cells after prebiotic bread</li> <li>• <math>\downarrow</math> % ICAM-1 bearing lymphocytes after prebiotic bread</li> <li>• <math>\downarrow</math> % CD3<sup>+</sup> NK<sup>+</sup> cells after prebiotic and prebiotic plus antioxidant bread</li> <li>• <math>\uparrow</math> CD3<sup>+</sup> HLA-DR<sup>+</sup> (activated T cells) after prebiotic bread</li> </ul> <p>No effect upon:</p> <ul style="list-style-type: none"> <li>• Numbers of leucocytes</li> <li>• Phagocytosis of <i>E. coli</i> by granulocytes</li> <li>• Numbers of CD3, CD4, CD8, CD4:CD8, CD57, CD8<sup>+</sup>, CD4<sup>+</sup>CD25<sup>+</sup>, CD122, CD4<sup>+</sup>CD54<sup>+</sup></li> <li>• Numbers of NK cells</li> <li>• Numbers of activated T cells (CD25)</li> </ul>

Table 2. Continued

Reference no.	Prebiotic(s) used	Subjects studied	Findings
66	Inulin (0.88 g/d) + galacto-oligo saccharides (8.18 g/d for 15 weeks; last trimester of pregnancy)	Pregnant women; n 17 in test group, median age 33 years; n 16 in control group, median age 35 years	<p>No effect on:</p> <ul style="list-style-type: none"> <li>• Ratio of CD4<sup>+</sup> to CD8<sup>+</sup> cells in cord blood</li> <li>• % CD4<sup>+</sup>CD45RA<sup>+</sup>, CD8<sup>+</sup>CD45RA<sup>+</sup>, CD4<sup>+</sup>CD45RO<sup>+</sup>, CD4<sup>+</sup>CD45RO<sup>+</sup>, CD4<sup>+</sup>CD45RO<sup>+</sup>, CD4<sup>+</sup>CD45RO<sup>+</sup>, CD4<sup>+</sup>CD45RO<sup>+</sup>, CD4<sup>+</sup>CD45RA<sup>+</sup>CD8<sup>+</sup>CD45RA<sup>+</sup>CKR<sup>+</sup> cells in cord blood</li> <li>• CD69 and CD25 expression on CD4<sup>+</sup> subsets in cord blood (non-stimulated)</li> <li>• Frequency of CD4<sup>+</sup>CD25<sup>high</sup> regulatory T cells</li> <li>• Production of TNF-<math>\alpha</math>, IL-1, IL-6, IL-8, MCP-1, MIP<math>\beta</math>, IFN-<math>\gamma</math>, IL-2, IL-4 or IL-10 by cultured cord blood</li> </ul>

MCP-1, monocyte chemoattractant protein 1; NK, natural killer; TGF, transforming growth factor;  $\uparrow$ , increase/increased;  $\downarrow$ , decrease/decreased; MIP, macrophage inhibitory protein.

In a study of healthy free-living elderly adults, there was no effect of a combination of OF and IN upon serum Ig concentrations (IgA, IgG, IgM)<sup>(60)</sup>, which is agreement with several studies in laboratory animals which show no effect of  $\beta$ 2-1 fructans on these<sup>(23,51,53–55,58)</sup>. In the same study, there was no effect of the combination of OF and IN upon salivary secretory IgA levels<sup>(60)</sup>. In newborn infants supplemented with a mixture of GOS and IN with shorter-chain FOS removed in their formula, an increase in faecal secretory IgA levels was seen<sup>(65)</sup>, which fits with the findings from animal studies which show an enhancement of the antibody response in the GALT by prebiotics<sup>(47,49,51–53)</sup>.

The antibody response to vaccination is considered to be the gold standard for measuring the functioning of the immune system *in vivo*, based on its biological relevance, sensitivity and practical feasibility<sup>(70)</sup>. Thus several human studies use this marker, but these have generated mixed results. In healthy, free-living elderly adults, OF plus IN increased the antibody response to influenza B virus and *Streptococcus pneumoniae* after vaccination, yet this was also seen in the control group<sup>(60)</sup>. In the same study, there was no effect of the supplementation upon antibody titres against influenza A virus. In a study of elderly adults, FOS supplementation increased the number of subjects with a four-fold or greater increase in serum antibody titre, and an antibody titre of forty or more, to the A/Beijing component of the influenza vaccine 6 weeks after the vaccine was administered<sup>(62)</sup>, but this was not seen for the other components of the vaccine. In a group of elderly adults resident in long-term care facilities and vaccinated with influenza, FOS supplementation did not alter the geometric mean antibody titre, but did increase the number of subjects with an antibody titre greater than 100 to the H1N1 component 6 weeks after the vaccine was given<sup>(63)</sup>. A study in 8-month-old infants supplemented with OF plus IN reported an increase in post-vaccination measles IgG levels in the blood<sup>(59)</sup>, but in Peruvian infants given OF-enriched cereal, and immunized against influenza, there was no effect upon post-vaccination antibody titres to *Haemophilus influenzae* type B<sup>(61)</sup>. Taken together, these studies suggest that  $\beta$ 2-1 fructans may increase the response to some vaccines or vaccine components but not all. This conclusion is consistent with that from animal studies<sup>(23,47)</sup>.

The percentages of peripheral blood T cells, CD4<sup>+</sup>T cells and CD8<sup>+</sup>T cells were increased in elderly nursing home residents upon OF supplementation, although there was no effect on the number of activated T cells<sup>(24)</sup>. No effect of FOS was seen on percentages of lymphocytes, or CD4<sup>+</sup> or CD8<sup>+</sup>T cells in elderly adults resident in long-term care facilities, although influenza-activated T cells were increased and memory cytotoxic T cells were decreased, and NK T cells were non-significantly increased<sup>(63)</sup>. Likewise, there was no effect of consumption of a bread containing IN on the percentage of T, activated T, CD4<sup>+</sup> or CD8<sup>+</sup> cells in the blood of young male adults<sup>(64)</sup>. However, the percentage of activated T cells (defined as CD3<sup>+</sup>HLA-DR<sup>+</sup>) increased while that of NK T cells (defined as CD3<sup>+</sup>NK<sup>+</sup>) decreased<sup>(64)</sup>. This mixed picture of effects of  $\beta$ 2-1 fructans on blood lymphocyte subsets is similar to that seen in laboratory animals<sup>(48,49)</sup>.

Lymphocyte proliferation to influenza vaccine components was increased in elderly adults supplemented with FOS<sup>(62)</sup>, but in another study in elderly adults, there was no effect of

OF plus IN upon stimulated lymphocyte proliferation<sup>(60)</sup>. Regarding cytokine expression, in elderly nursing home residents, OF supplementation decreased IL-6 mRNA expression in blood mononuclear cells<sup>(24)</sup>, and in elderly adults resident in long-term care facilities, IL-6 production by stimulated blood mononuclear cells was decreased<sup>(63)</sup>. As an increase in IL-6 is associated with the pro-inflammatory state associated with ageing<sup>(71)</sup>, this could be considered a beneficial effect. In healthy free-living elderly adults, there was no effect of OF plus IN upon IFN- $\gamma$  and IL-4 secretion by cultured mononuclear cells<sup>(60)</sup>. This is in contrast to a study in rats that showed an increase in blood measurements of IL-4<sup>(45)</sup>. A trend for a reduced IL-10 production from stimulated blood mononuclear cells was observed upon FOS supplementation in the elderly<sup>(63)</sup>.

*Synbiotics and the immune system in man.* In a study of adult colon cancer or polypectomised patients, OF-enriched IN was given in combination with *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb12. The synbiotic prevented the increase in IL-2 secretion by mononuclear cells from polypectomised patients that was seen in control patients, and also increased IFN- $\gamma$  production by mononuclear cells in colon cancer patients<sup>(68,69)</sup>. However, the synbiotic had no effect on several other immune markers in either cancer or polypectomised patients, including percentages of phagocytically active neutrophils and monocytes and their phagocytic intensity, percentage of neutrophils producing reactive oxygen species and the intensity of production, lytic activity of NK cells or production of IL-10, IL-12 and TNF- $\alpha$  by activated blood mononuclear cells. It is interesting to note that in another population, the healthy elderly immunised with influenza and pneumococcal vaccines, some of these findings are replicated. OF and IN in combination with *Lactobacillus paracasei* prevented the decrease in IL-2 production by mononuclear cells seen in controls after vaccination, and there was no effect upon TNF- $\alpha$  production<sup>(67)</sup>. However, other findings differ, such as an increase in NK activity and no effect on IFN- $\gamma$  production with influenza virus antigen stimulation. There was no effect on IL-6 or IL-1 production by stimulated blood mononuclear cells, or on lymphocyte subpopulations<sup>(67)</sup>. The decrease in numbers of T cells with NK activity that was seen in the control group was prevented with the supplementation<sup>(67)</sup>. After the vaccinations were given there was no effect of the synbiotic on the magnitude of the increase in anti-influenza vaccine or anti-pneumococcus vaccine antibodies, or on the delayed type hypersensitivity response<sup>(67)</sup>.

### Prebiotics and infection

If prebiotics improve host immune defences, then it would be expected that they decrease susceptibility to and/or severity of infection. This section will review studies in experimental animals and in man that investigate the effect of increased consumption of  $\beta$ 2-1 fructans on infectious outcomes.

#### *Studies in laboratory animals*

Seventeen animal studies of infection were identified (two of which used synbiotics), and  $\beta$ 2-1 fructan supplementation generally appears to be beneficial in the models used (Table 3).

A series of studies using piglets infected with *Oesophagostomum dentatum* or *Trichuris suis* showed decreases in *Oesophagostomum dentatum* and *Trichuris suis* faecal egg counts, intestinal worm recovery, size of worms and the female worm's ability to reproduce after IN supplementation<sup>(72–77)</sup>. Another study, in *Salmonella typhimurium*-infected puppies, showed that OF or IN decrease the severity of enterocyte sloughing, suggesting a reduction in epithelial damage compared to controls<sup>(78)</sup>. FOS supplementation increased survival in a hamster model of *Clostridium difficile* infection<sup>(79)</sup> and in murine models of *Listeria monocytogenes* and *Salmonella typhimurium* infection both IN and OF increased survival<sup>(80)</sup>. In the latter study, IN was more effective than OF at decreasing mortality. FOS in drinking water decreased the shedding of *Salmonella typhimurium* in the faeces of piglets infected with *Salmonella typhimurium*, although the effect was not significant<sup>(81)</sup>, and FOS prevented diarrhoea induced by *Salmonella typhimurium* in piglets<sup>(82)</sup>. FOS decreased diarrhoea and increased survival rates in piglets infected with *E. coli*<sup>(83)</sup>. These studies provide a consistent picture that  $\beta$ 2-1 fructans do improve host resistance to bacterial infections.

In contrast to the studies described earlier, a series of studies investigating OF supplementation in calcium-deficient rats suggest increased *Salmonella typhimurium* colonisation and translocation, and increased mucosal irritation<sup>(84–87)</sup>. These findings may be explained by the calcium-deficient state of the rats used, since a direct comparison of OF in rats fed calcium-deficient and calcium-sufficient diets showed different effects<sup>(86)</sup>. While the calcium-deficient animals displayed increased susceptibility to *S. typhimurium*, calcium-sufficient animals did not. Thus, the relevance of the findings to animals or man that are not calcium-deficient is limited.

Two studies have investigated the use of synbiotics in animal models of infection. A study in mice pups infected with rhesus rotavirus demonstrated that OF in combination with *Bifidobacterium bifidum* and *Bifidobacterium infantis* reduced the duration of diarrhoea, although the synbiotic was no more effective than the probiotic alone<sup>(88)</sup>. Rotavirus infects the enterocytes of the small intestine, but prebiotics and probiotics have their effects mainly in the large intestine. Thus, although improving the health of the large intestine is likely to be useful in diarrhoea, prebiotics may not be more helpful than a probiotic alone because of limited effects in the small intestine. Although piglets infected with *Salmonella typhimurium* were shown to have decreased shedding of *Salmonella typhimurium* in faeces when supplemented with FOS, FOS given as part of a synbiotic had no effect on *Salmonella typhimurium* infection<sup>(81)</sup>.

#### *Studies in man*

*Infants and children.* Several studies have shown some benefit from  $\beta$ 2-1 fructans on common childhood and acute diarrhoea (Table 4). Although OF-enriched cereal had no effect upon frequency or duration of common childhood diarrhoea in non-breast-fed American infants, it reduced the severity<sup>(89,90)</sup>. In another study episodes of common childhood diarrhoea were reported to be reduced in healthy infants supplemented with OF<sup>(91)</sup>. In Indonesian children aged 1–14



**Table 3.** Effects of  $\beta$ 2-1 fructans on infectious outcomes in animal models

Reference no.	Prebiotic(s) used	Animal studied (sex and age where given)	Findings
83	Fructo-oligosaccharides (3 g/d)	Piglets (7 d old); infected with <i>Escherichia coli</i>	<ul style="list-style-type: none"> <li>• ↓ Symptoms of anorexia, pyrexia, dehydration and diarrhoea</li> <li>• ↑ Survival from <i>E. coli</i> infection</li> </ul>
72	Inulin (7 % w/w diet)	Pigs (male, 12 weeks old); pathogen-free; infected with <i>Oesophagostomum dentatum</i> and <i>Ascaris suum</i>	<ul style="list-style-type: none"> <li>• ↓ <i>O. dentatum</i> faecal egg count</li> <li>• ↓ Total intestinal <i>O. dentatum</i> worm recovery, but when different parts of the large intestine were investigated, changes were not always significant</li> <li>• No effect upon <i>A. suum</i> prevalence or fecundity</li> <li>• ↓ Mortality</li> </ul>
79	Fructo-oligosaccharides (30 g/l drinking water)	Hamsters (female); infected with <i>Clostridium difficile</i>	<ul style="list-style-type: none"> <li>• Fructo-oligosaccharides in drinking water non-significantly ↓ shedding of <i>S. typhimurium</i> in faeces</li> </ul>
81	Fructo-oligosaccharides (1 % w/w of diet in water/feed), with/without probiotic mix (Ferlac2) of <i>Lactobacillus acidophilus</i> , <i>L. rhamnosus</i> , <i>Enterococcus faecium</i> , <i>Streptococcus thermophilus</i> and <i>L. bulgaricus</i>	Piglets (21 d old); infected with <i>S. typhimurium</i>	<p>No effect:</p> <ul style="list-style-type: none"> <li>• On the number of <i>S. typhimurium</i> in the mesenteric lymph nodes</li> <li>• Of the synbiotic on <i>S. typhimurium</i> infection</li> </ul>
73	Inulin with shorter-chain fructo-oligosaccharides removed (6 % w/w diet) in combination with sugarbeet fibre	Pigs (pathogen-free, 16 weeks old); infected with <i>O. dentatum</i>	<ul style="list-style-type: none"> <li>• ↓ <i>O. dentatum</i> faecal egg count</li> <li>• At 3 and 12 weeks post-infection ↓ total <i>O. dentatum</i> worm numbers in large intestine</li> <li>• At 3 and 12 weeks post-infection ↓ size of worms</li> <li>• ↓ Female worm fecundity</li> <li>• ↓ Density of <i>C. albicans</i> in small intestine with oligofructose or inulin</li> <li>• ↓ Mortality from <i>L. monocytogenes</i> with oligofructose or inulin (0 % mortality with inulin; 12 % mortality with oligofructose; 28 % mortality with control)</li> <li>• ↓ Mortality from <i>S. typhimurium</i> with inulin (60 % mortality with inulin; 75 % mortality with oligofructose; 85 % mortality with control)</li> </ul>
80	Oligofructose or inulin (10 % w/w diet)	Mice (female, 5 weeks old); infected with <i>Candida albicans</i> , or <i>Listeria monocytogenes</i> or <i>S. typhimurium</i>	<ul style="list-style-type: none"> <li>• ↓ Mortality from <i>S. typhimurium</i> with inulin (60 % mortality with inulin; 75 % mortality with oligofructose; 85 % mortality with control)</li> </ul>
88	Oligofructose (1.25 g/l drinking water) in combination <i>Bifidobacterium bifidum</i> and <i>B. infantis</i>	Mice (pups); infected with Rhesus rotavirus	<p>Oligofructose did not have any additional benefit compared to when the probiotic was given alone, but both treatments:</p> <ul style="list-style-type: none"> <li>• ↓ Duration of diarrhoea</li> <li>• ↓ Diarrhoea induced by <i>S. typhimurium</i></li> </ul>
82	Fructo-oligosaccharides (7.5 g/l drinking water)	Piglets (2 d old); infected with <i>S. typhimurium</i>	<ul style="list-style-type: none"> <li>• ↓ Faecal shedding of <i>S. enteritidis</i> (thus improved colonisation resistance to <i>S. enteritidis</i>)</li> </ul>
85	Oligofructose (4 % w/w diet)	Rats (male, 8 weeks old); pathogen-free; infected with <i>S. enteritidis</i> ; calcium deficient	<ul style="list-style-type: none"> <li>• ↑ Translocation (↑ <i>Salmonella</i> counts in the liver and spleen)</li> <li>• ↑ Faecal mucin excretion</li> <li>• ↑ Myeloperoxidase activity in caecum and colon, but not in the ileum</li> <li>• ↓ Worm numbers in anterior and middle parts of large intestine</li> </ul>
74	Inulin (6 % w/w diet) in combination with sugarbeet fibre	Pigs (13 weeks old); pathogen-free; infected with <i>O. dentatum</i>	<ul style="list-style-type: none"> <li>• ↓ Worm numbers in anterior and middle parts of large intestine</li> </ul>
75	Inulin alone (16 % w/w diet) or in combination with sugarbeet fibre (6 % w/w diet)	Pigs (10 weeks old); pathogen-free; infected with <i>O. dentatum</i>	<ul style="list-style-type: none"> <li>• At 13 weeks post-infection, pigs fed the inulin alone diet had a 99 % ↓ in <i>O. dentatum</i> faecal egg count</li> <li>• Total colonic worm recovery was ↓ by 97 % in inulin alone group and by 86 % in inulin plus sugarbeet fibre group</li> </ul>
84	Oligofructose (3 or 6 % w/w diet)	Rats (male, 8 weeks old); specific pathogen-free; infected with <i>S. enteritidis</i> ; calcium deficient	<ul style="list-style-type: none"> <li>• ↑ <i>Salmonella</i> number in caecal contents and ↑ caecal mucosal colonisation by <i>Salmonella</i> (dose-dependent)</li> <li>• ↑ Translocation</li> <li>• ↓ Animal growth</li> <li>• ↑ Faecal mucin excretion</li> </ul>
86	Oligofructose or inulin (6 % w/w diet)	Rats (male, 8 weeks old); specific pathogen-free; infected with <i>S. enteritidis</i> ; calcium deficient	<p>Oligofructose and inulin:</p> <ul style="list-style-type: none"> <li>• ↑ Caecal colonisation of <i>Salmonella</i></li> <li>• ↑ Translocation</li> </ul>



Table 3. Continued

Reference no.	Prebiotic(s) used	Animal studied (sex and age where given)	Findings
87	Oligofructose (6 % w/w diet)	Rats (male, 8 weeks old); specific pathogen-free; infected with <i>S. enteritidis</i> ; calcium deficient	<ul style="list-style-type: none"> <li>• ↑ Faecal mucin concentration</li> <li>• ↓ Intestinal permeability before and after infection</li> <li>• ↑ Translocation to extra-intestinal sites</li> <li>• In a separate experiment where rats were not infected with <i>S. enteritidis</i>, oligofructose ↑ mucin concentrations in caecal and colonic contents and in the caecal mucosa, but not in ileal contents or in the ileal or colonic mucosa</li> <li>• ↓ Faecal egg counts</li> <li>• ↓ Worm size</li> <li>• No effect at 8 weeks post-infection on worm counts, but at 12 weeks post-infection, worm counts were ↓</li> </ul>
77	Inulin with shorter-chain fructo-oligosaccharides removed (6 % w/w diet)	Pigs (10 weeks old); pathogen-free; infected with <i>Trichuris suis</i>	<ul style="list-style-type: none"> <li>• Both short-chain oligofructose and inulin: <ul style="list-style-type: none"> <li>• ↓ Severity of enterocyte sloughing (thus ↓ epithelial damage)</li> </ul> </li> <li>• Prevented the ↓ in ileal Na<sup>+</sup>-dependent glucose transport seen in controls</li> <li>• ↓ Faecal egg counts at 7 weeks post-infection</li> <li>• If pigs were switched from the control diet to the inulin diet, faecal egg counts ↓</li> </ul>
78	Short-chain oligofructose or inulin (1 % w/w diet)	Puppies (12 weeks old, male and female); infected with <i>S. typhimurium</i>	<ul style="list-style-type: none"> <li>• ↓ Number of worms recovered in large intestine at 7 and 9 weeks post-infection</li> <li>• ↓ Fecundity of female worms at 7 and 9 weeks post-infection</li> </ul>
76	Inulin (16 % w/w diet)	Pigs (10 weeks old); infected with <i>T. suis</i>	<ul style="list-style-type: none"> <li>• ↓ Fecundity of female worms at 7 and 9 weeks post-infection</li> </ul>

↑, increase/increased; ↓, decrease/decreased.

years, the duration of acute diarrhoea was reduced with FOS supplementation<sup>(92)</sup>, and incidence of acute diarrhoea was also reduced in infants who received an infant formula containing GOS and FOS<sup>(93)</sup>. Incidence of upper respiratory tract infections was also reduced in the group consuming this formula<sup>(93)</sup>. Intestinal permeability was improved in infants fed with a formula containing GOS and IN as compared to a control formula<sup>(94)</sup>. A trial carried out in Peruvian infants found no effect of OF on the occurrence or prevalence of diarrhoea<sup>(61)</sup>, and a trial in infants with diarrhoea showed no benefit of including IN and FOS in the rehydration solution on duration of diarrhoea<sup>(95)</sup>.

In infants, there was a non-significant trend for a reduction in respiratory tract infections in those receiving a formula containing a synbiotic that included a GOS–FOS mixture compared to those receiving the control formula<sup>(96)</sup>. A study in Chilean children colonised with *Helicobacter pylori* showed a reduction in the number colonised in both synbiotic and probiotic groups, but there was no difference between these two groups<sup>(97)</sup>.

Taken together data from studies using prebiotics in infants and children are suggestive of a reduction in incidence or duration of some infections.

**Adults.** Three out of the six studies identified show significant benefit of β2-1 fructans on infections in adult human subjects (Table 4). These studies showed a decrease in relapse rate of in-patients with *Clostridium difficile*-associated diarrhoea<sup>(98)</sup>, decreased upper respiratory tract infections in older adults post-influenza vaccine<sup>(62)</sup>, and decreased respiratory, skin, gastrointestinal and genitourinary infections in older adults immunised with influenza and pneumococcal vaccines<sup>(67)</sup>. No effect was seen when OF was supplemented to in-patients receiving broad-spectrum antibiotics on antibiotic-associated diarrhoea caused by *Clostridium difficile* or other causes<sup>(99)</sup>. There was no significant effect of FOS supplementation upon travellers' diarrhoea in people holidaying to areas of medium/high risk of diarrhoea, although there was a non-significant decrease in diarrhoea and an increase in feelings of well-being<sup>(100)</sup>. In burns patients, OF did not improve a variety of measures of the severity or duration of infections<sup>(101)</sup>. In healthy adults, OF had no effect upon faecal mucin excretion<sup>(102)</sup>. In men consuming a diet with limited calcium, intestinal permeability did not differ between the control and OF supplement periods, although faecal mucin excretion was increased with the addition of OF to the diet<sup>(103)</sup>. In a study of patients on an enteral diet, IN had no effect upon intestinal permeability<sup>(104)</sup>.

Most trials of synbiotics and infection in human adults have been carried out in patients admitted to intensive care or surgery wards (Table 4). Synbiotics appear to exert some beneficial effects on infections in these patients<sup>(105–107)</sup>. In studies where synbiotics have been compared with prebiotics alone, synbiotics have been shown to be more beneficial in terms of reducing length of antibiotic therapy and bacterial infections in patients undergoing duodenal surgery<sup>(108)</sup> and liver transplantation<sup>(109)</sup>. However, some of these studies report no effects of synbiotics on other outcomes measured<sup>(105,107)</sup>. No effect of a synbiotic was seen regarding bacterial colonisation to lymph nodes or terminal ileal serosa, gastric colonisation or septic complications in adult patients undergoing elective abdominal surgery<sup>(110)</sup>.

**Table 4.** Effects of  $\beta$ 2-1 fructans on infections in man

Reference no.	Prebiotic used	Subjects studied	Findings
104	Inulin (30–37.5 g/d, for 1 week, in enteral nutrition formula)	Patients receiving enteral nutrition for a variety of reasons; aged 19–76 years; <i>n</i> 9; no control	<ul style="list-style-type: none"> <li>• No effect upon intestinal permeability measured by <sup>51</sup>Cr-EDTA absorption</li> </ul>
89, 90	Oligofructose (in cereal supplemented with 3.6 % w/w oligofructose = 1.1 g/d for 6 months)	American infants; non-breast-fed; aged 4–24 months; <i>n</i> 63 in prebiotic group; <i>n</i> 60 in control group	<ul style="list-style-type: none"> <li>• ↓ Occurrence of fever during diarrhoea</li> <li>• ↓ Seeking of medical attention during fever</li> <li>• ↓ Number of days of day-care missed because of diarrhoea</li> <li>• ↓ Occurrence of fever with cold symptoms</li> <li>• ↓ Antibiotic use during respiratory illness</li> <li>• ↓ Uncomfortable bowel movements</li> </ul> <p>No effect on:</p> <ul style="list-style-type: none"> <li>• Diarrhoea frequency</li> <li>• Diarrhoea duration</li> <li>• ↑ Feeling of well-being</li> <li>• Non-significant decrease in diarrhoea</li> </ul>
100	Fructo-oligosaccharides (10 g/d for 2 weeks prior to travel and 2 weeks whilst travelling)	Adult travellers to medium/high-risk areas of diarrhoea; mean age 50 years; <i>n</i> 117 in prebiotic group; <i>n</i> 127 in control group	<ul style="list-style-type: none"> <li>• ↓ Duration of diarrhoea in all ages</li> </ul>
92	Fructo-oligosaccharides (2.5– 5 g/d, depending on age of child, for an unspecified period)	Indonesian children; aged 1–14 years; with acute diarrhoea; <i>n</i> 93 in prebiotic group; <i>n</i> 25 in control group	<ul style="list-style-type: none"> <li>• ↓ Duration of diarrhoea in all ages</li> </ul>
61	Oligofructose (in cereal supplemented with 3.6 % w/w oligofructose = 0.55 g/d for 6 months)	Peruvian infants; immunised with <i>Haemophilus influenzae</i> type B vaccine 5 months into the trial; aged 6–12 months; <i>n</i> 129 in prebiotic group; <i>n</i> 122 in control group	<p>No effect on:</p> <ul style="list-style-type: none"> <li>• Occurrence of diarrhoea</li> <li>• Prevalence of diarrhoea</li> </ul>
110	Oligofructose (32 g/d for 1–2 weeks pre-surgery, and until discharge from hospital) plus probiotics (Trevis capsules, containing <i>Lactobacillus acidophilus</i> La5, <i>L. bulgaricus</i> , <i>B. lactis</i> Bb12 and <i>S. thermophilus</i> )	Patients undergoing elective abdominal surgery; median age 71 years; <i>n</i> 72 in test group; <i>n</i> 65 in control group	<p>No effect on:</p> <ul style="list-style-type: none"> <li>• Bacterial translocation to lymph nodes or terminal ileal serosa</li> <li>• Gastric colonisation</li> <li>• Systemic inflammation (measured by serum C-reactive protein, IL-6 or IgM serial antiendotoxin core antibody)</li> <li>• Septic complications</li> <li>• Length of hospital stay</li> </ul>
95	Fructo-oligosaccharides and inulin (0.185 and 0.215 %, respectively, of an oral re-hydration solution, also containing other non-digestible carbohydrates, until cessation of diarrhoea)	Infants with diarrhoea and mild to moderate dehydration; aged 1–36 months; male; <i>n</i> 70 in test group; <i>n</i> 74 in control group	<p>No effect on:</p> <ul style="list-style-type: none"> <li>• Duration of diarrhoea</li> <li>• Duration of hospital stay</li> <li>• Risk of vomiting</li> </ul>
105	Oligofructose (15 g/d for the duration of hospital stay) plus probiotics ( <i>L. acidophilus</i> La5, <i>B. lactis</i> Bb12, <i>S. thermophilus</i> and <i>L. bulgaricus</i> )	Adult patients admitted to intensive care units; aged over 62–80 years; <i>n</i> 45 in test group; <i>n</i> 45 in control group	<ul style="list-style-type: none"> <li>• After 8 d, patients receiving the synbiotic had significantly reduced incidence of potentially pathogenic bacteria and multiple organisms in nasogastric aspirates</li> </ul> <p>No effect on:</p> <ul style="list-style-type: none"> <li>• Intestinal permeability</li> <li>• C-reactive protein levels</li> <li>• Septic complications</li> <li>• Mortality</li> <li>• Length of hospital stay</li> </ul>
67	Oligofructose and inulin (2:1 ratio; 6 g/d for 1 year) Included in the formula were also protein, fat, carbohydrate, vitamin E, vitamin B <sub>12</sub> , thiamine, folic acid, riboflavin, pyridoxine, other nutrients and <i>L. paracasei</i>	Elderly free-living adults; age $\geq$ 70 years; immunised with influenza and pneumococcal vaccines 4 months into the trial; <i>n</i> 30 in control group; <i>n</i> 30 in experimental group	<p>After vaccination:</p> <ul style="list-style-type: none"> <li>• ↓ infections (respiratory, skin, gastrointestinal and genitourinary)</li> </ul>
97	Inulin (5 g/d, given in a synbiotic with <i>S. boulardi</i> , for 8 weeks)	Children in Chile colonised by <i>Helicobacter pylori</i> ; age 5–12 years; <i>n</i> 62 in synbiotic group; <i>n</i> 63 in probiotic group ( <i>L. acidophilus</i> LB); <i>n</i> 57 in antibiotic group; <i>n</i> 71 asymptomatic children with no treatment	<ul style="list-style-type: none"> <li>• <i>H. pylori</i> was eradicated in 66 % of the antibiotic group; 12 % of the synbiotic group and 6.5 % of the probiotic group. The difference between the synbiotic and probiotic groups was not significant</li> </ul>

Table 4. Continued

Reference no.	Prebiotic used	Subjects studied	Findings
62	Fructo-oligosaccharides (4.95 % of the energy intake of the 226.8 g (8 oz) formula/d for 26 weeks) The formula also contained antioxidants (vitamins C, E and $\beta$ -carotene), B vitamins, zinc, selenium, other vitamins and minerals, and structured TAG	Adults aged $\geq 65$ years; assisted-living and independent-living; immunised with influenza vaccine 2 weeks into the trial; <i>n</i> 18 in control group; <i>n</i> 16 in experimental group	<ul style="list-style-type: none"> <li>• <math>\downarrow</math> Median days of upper respiratory tract infections</li> </ul>
98	Oligofructose (12 g/d during diarrhoea and for 30 d after cessation)	Adult in-patients with <i>C. difficile</i> -associated diarrhoea; <i>n</i> 72 in test group; <i>n</i> 70 in control group	<ul style="list-style-type: none"> <li>• <math>\downarrow</math> Relapse of diarrhoea (8.3 % prebiotic group relapsed compared to 34.3 % of placebo)</li> </ul>
99	Oligofructose (12 g/d until 7 d after ceasing antibiotic use)	Adult in-patients receiving broad-spectrum antibiotics; aged over 65 years; <i>n</i> 215 in prebiotic group; <i>n</i> 220 in control group	<ul style="list-style-type: none"> <li>• No effect on antibiotic-associated diarrhoea caused by <i>C. difficile</i> or other causes</li> </ul>
101	Oligofructose (6 g/d for 15 d)	Second and third degree burns patients; <i>n</i> 15 in control group, mean age 41.2 years; <i>n</i> 16 in test group, mean age 38.6 years	<p>No differences between groups regarding:</p> <ul style="list-style-type: none"> <li>• Measures of intestinal permeability</li> <li>• Length of hospitalisation</li> <li>• Number of surgical interventions</li> <li>• Rates of burn infection</li> <li>• Number of complications</li> </ul>
109	Inulin (5 g/d for 14 d), given in combination with three other fibres, or with the three other fibres plus <i>Pediococcus pentoseceus</i> , <i>L. mesenteroides</i> , <i>L. paracasei</i> and <i>L. plantarum</i>	Adult patients undergoing liver transplantation; <i>n</i> 33 in fibre plus probiotic group, mean age 53 years; <i>n</i> 33 in fibre only group, mean age 50 years	<p>In those receiving fibres plus probiotics, as opposed to those receiving fibres alone, there was:</p> <ul style="list-style-type: none"> <li>• <math>\downarrow</math> Duration of antibiotic therapy</li> <li>• <math>\downarrow</math> Incidence of post-operative infections (mostly upper and lower urinary tract infections)</li> </ul> <p>No difference between the groups regarding:</p> <ul style="list-style-type: none"> <li>• Length of hospital stay</li> <li>• CD4<sup>+</sup>, CD8<sup>+</sup>, CD19<sup>+</sup>, or NK cells, CD4<sup>+</sup>:CD8<sup>+</sup> ratio or IgA</li> </ul>
93	Galacto-oligosaccharides/fructo-oligosaccharides (9:1 ratio) in infant formula (for 12 months)	Infants aged 15–120 d; <i>n</i> 136 in test group; <i>n</i> 145 in control group	<ul style="list-style-type: none"> <li>• <math>\downarrow</math> Incidence of acute diarrhoea</li> <li>• <math>\downarrow</math> Number of children with more than three episodes of upper respiratory tract infections/year</li> <li>• <math>\downarrow</math> Number of children receiving more than two antibiotic courses/year</li> </ul>
94	Galacto-oligosaccharides and inulin	Full-term infants; formula fed; <i>n</i> 15 in test group; <i>n</i> 15 in control group; <i>n</i> 30 in breast-fed group	<ul style="list-style-type: none"> <li>• Those receiving the GOS/inulin formula showed an intestinal permeability similar to that of the breast fed infants, and significantly different to that of the control formula fed infants</li> </ul>
106	Inulin (2.5 g/d for 15 d) plus three other fibres and <i>Pediococcus pentoseceus</i> , <i>L. mesenteroides</i> , <i>L. paracasei</i> and <i>L. plantarum</i>	Adult multiple trauma victims admitted to surgical intensive care units; <i>n</i> 35 in test group, mean age 52.9 years; <i>n</i> 30 in control group, mean age 55.9 years	<ul style="list-style-type: none"> <li>• <math>\downarrow</math> Systemic infection rate</li> <li>• <math>\downarrow</math> Septic complications</li> <li>• <math>\downarrow</math> Septic inflammatory response syndrome (procalcitonin, C-reactive protein, malondialdehyde, TNF-<math>\alpha</math> and IL-6)</li> <li>• <math>\downarrow</math> Severe sepsis</li> <li>• <math>\downarrow</math> Number of days of stay in the intensive care unit</li> <li>• <math>\downarrow</math> Number of days under mechanical ventilation</li> </ul>
107	Oligofructose (30 g/d; duration not specified) plus <i>L. acidophilus</i> La5, <i>L. bulgaricus</i> , <i>B. lactis</i> Bb12 and <i>S. thermophilus</i>	Adult elective colorectal surgery patients; aged 53–81 years; <i>n</i> 20 in synbiotic + neomycin + MBP group; <i>n</i> 22 in synbiotic + neomycin group; <i>n</i> 22 in neomycin + MBP group; <i>n</i> 24 in MBP group	<ul style="list-style-type: none"> <li>• The synbiotic plus neomycin plus MBP group had <math>\downarrow</math> incidence of bacterial translocation to MLN after bowel mobilisation</li> </ul> <p>No effect of the synbiotic upon:</p> <ul style="list-style-type: none"> <li>• Intestinal permeability</li> <li>• Inflammatory response (C-reactive protein or IL-6 levels)</li> <li>• Septic morbidity</li> <li>• Wound infections</li> <li>• Incidence of lower respiratory tract infections</li> </ul>

Table 4. Continued

Reference no.	Prebiotic used	Subjects studied	Findings
102	Oligofructose (25–30 g/d for 2 weeks)	Adults; mean age 21.4 years; n 11, crossover design	• No effect on faecal mucin excretion
103	Oligofructose (20 g/d for 2 weeks)	Men; Ca-restricted diet; mean age 27.7 years; n 34, crossover design	• ↑ Faecal mucin excretion No effect on intestinal permeability
132	Inulin (1.5 g/l in formula) plus <i>L. rhamnosus</i> , zinc, iron and soyabean polysaccharides	Indonesian infants with acute diarrhoea; aged 3–12 months; male; moderately dehydrated and subsequently re-hydrated; n 58 across two groups	• ↓ Duration of diarrhoea
96	Galacto-oligosaccharide/fructooligosaccharide mixture at 9:1 ratio (4 g/l in formula for 112 d) plus <i>B. longum</i> BL999	Infants; full-term newborns; not breast-fed after 14th day of life; n 42 in test group, n 55 in control group	• Non-significant trend for infants receiving the test formula to have fewer respiratory tract infections in those receiving fibre plus probiotics, as opposed to those receiving fibre alone, there was: • ↓ Duration of antibiotic therapy • ↓ Bacterial infections (wound, peritonitis, pneumonia)
108	Inulin (5 g/d for 8 d) plus three other fibres, with or without <i>Pediococcus pentoseus</i> , <i>L. mesenteroides</i> , <i>L. paracasei</i> and <i>L. plantarum</i> (Synbiotic2000)	Adults; patients undergoing duodenal surgery; n 40 in fibre group; n 40 in synbiotic group	No effect on length of hospital stay
91	Oligofructose (2 g/d for 21 d)	Infants; aged 7–19 months; n 10 in prebiotic group; n 10 in control group	• ↓ Clostridial colonisation measured in faeces • ↓ Diarrhoea episodes • ↓ Vomiting • ↓ Fever • ↓ Number of infectious diseases requiring antibiotic treatment

MBP, mechanical bowel preparation; MLN, mesenteric lymph nodes; NK, natural killer; ↑, increase/increased; ↓, decrease/decreased.

Overall, studies of  $\beta$ 2-1 fructans in adults are less convincing of a benefit with respect to infections compared with studies in infants and children. However, it appears that  $\beta$ 2-1 fructans are useful in this regard in some situations<sup>(98)</sup> and as a component of a synbiotic.

**Prebiotics and inflammation**

This section will review studies in experimental animals and in man that investigate the effect of increased consumption of  $\beta$ 2-1 fructans on inflammatory outcomes.

*Studies in laboratory animals*

Ten studies were identified (two using synbiotics), with mostly consistent results, showing positive effects of  $\beta$ 2-1 fructans on inflammation in animal models (Table 5).

Considering the reported effects of  $\beta$ 2-1 fructans on T cell numbers and function in animal models described previously<sup>(45,48–51,57)</sup> it is not surprising that they are effective in colitis: four out of five rodent studies of colitis report a decrease in inflammatory markers and the severity of disease when animals were supplemented with  $\beta$ 2-1 fructans. Three studies using IN, FOS or OF-enriched IN in colitis models in rats report reductions in mucosal damage, release of a range of inflammatory mediators (such as PGE<sub>2</sub>, thromboxane B<sub>2</sub>, leukotriene B<sub>4</sub> and pro-inflammatory cytokines) in different parts of the gut, and various other markers of inflammation<sup>(21,25,111)</sup>. A model of colitis in female mice showed that FOS decreased disease activity index and damage to the distal colon<sup>(112)</sup>. However, OF did not have an effect on total macroscopic scores, or on caecal, proximal and distal colon histological scores in a colitis model in male rats<sup>(113)</sup>.  $\beta$ 2-1 Fructans given as part of a synbiotic have also been shown to have beneficial effects in rat models of colitis: when IN was given along with *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12, colonic inflammation was reduced<sup>(114)</sup>, and OF-enriched IN given alone or in combination with *Bifidobacterium infantis* reduced bacterial translocation to MLN, colonic myeloperoxidase activity (an indicator of inflammatory granulocyte infiltration) and disease activity index<sup>(26)</sup>.

Neonatal necrotising enterocolitis may be in part caused by interactions between intestinal immaturity, inappropriate bacterial colonisation and infections<sup>(115)</sup>. Thus, from the known beneficial microbiota-stimulating effects, and previously described effects of  $\beta$ 2-1 fructans on the immune system, it is possible that  $\beta$ 2-1 fructans could be of benefit in this disease. Indeed, in a quail model of neonatal necrotising enterocolitis, OF decreased the occurrence and severity of intestinal lesions, although the clostridial species that was used to induce the colitis had an effect on the magnitude of this effect<sup>(22,116)</sup>.

In a rat model of allergic airway eosinophilia, FOS provided no benefit, regarding total cell, eosinophil, macrophage or lymphocyte numbers in bronchoalveolar lavage fluid, or IL-4, IL-5 or IFN- $\gamma$  mRNA levels in lung tissue<sup>(117)</sup>. The lack of effect of FOS on airway inflammation may be due to the distance of this compartment from the gastrointestinal tract.

**Table 5.** Effects of  $\beta$ -2-1 fructans on inflammation in laboratory animal models

Reference no.	Prebiotic(s) used	Animal studied (sex and age where given)	Findings
25	Inulin (1 % w/v in drinking water, or oral gavage 400 mg/d in tap water, or rectal enema of 400 mg/d in sterile saline)	Rats (male); colitis induced by dextran sodium sulphate	<ul style="list-style-type: none"> <li>• <math>\downarrow</math> Mucosal damage with inulin in drinking water or by oral gavage</li> <li>• <math>\downarrow</math> Release of inflammatory mediators into colonic lumen (PGE<sub>2</sub>, thromboxane B<sub>2</sub>, LTB<sub>4</sub>) with inulin in drinking water or by oral gavage</li> <li>• <math>\downarrow</math> Tissue myeloperoxidase activity with inulin in drinking water or by oral gavage</li> </ul>
22, 116	Oligofructose (replacing 3 % w/w diet)	Quails (2 weeks old); germ-free; inoculated with different clostridial species to induce neonatal necrotising enterocolitis)	<ul style="list-style-type: none"> <li>• Occurrence and severity of intestinal lesions, but the effects varied according to the clostridial species used to induce neonatal necrotising enterocolitis:</li> <li>• With <i>Clostridium perfringens</i> + <i>Klebsiella pneumoniae</i>, oligofructose completely inhibited development of neonatal necrotising enterocolitis-like lesions</li> <li>• With <i>C. perfringens</i> + <i>Clostridium difficile</i>, oligofructose significantly <math>\downarrow</math> severity of disease</li> <li>• With <i>C. perfringens</i> + <i>C. difficile</i> + <i>C. paraputrificum</i> oligofructose <math>\downarrow</math> occurrence of neonatal necrotising enterocolitis-like lesions</li> </ul>
111	Fructo-oligosaccharides (1 g/d)	Rats (male); colitis induced by trinitrobenzene sulphonic acid	<ul style="list-style-type: none"> <li>• <math>\downarrow</math> Gut mucosa inflammation shown by <math>\downarrow</math> myeloperoxidase activity and <math>\downarrow</math> macroscopic damage</li> </ul>
113	Oligofructose (6.3 % w/w diet) also looked at resistant starch	Rats (male); colitis induced by dextran sodium sulphate	<ul style="list-style-type: none"> <li>• At day 14, total macroscopic scores were <math>\downarrow</math> in the resistant starch supplemented rats compared to both control and fructo-oligosaccharide treated rats</li> <li>• At days 7 and 14 histological scores in the caecum and distal colon were <math>\downarrow</math> in resistant starch treated rats compared to controls, but there was no significant effect of fructo-oligosaccharides</li> <li>• No effect on histological scores in the proximal colon</li> <li>• After 2 weeks, colonic inflammation (measured histologically in the colonic tissues) in the synbiotic group was <math>\downarrow</math> compared to controls</li> </ul>
114	Inulin (in a synbiotic: also containing <i>Lactobacillus acidophilus</i> La5 and <i>Bifidobacterium lactis</i> Bb12)	Rats (8 weeks old); colitis induced (HLA-B27-B2-microglobulin transgenic)	<ul style="list-style-type: none"> <li>• <math>\downarrow</math> Development of colitis shown by <math>\downarrow</math> gross caecal scores, <math>\downarrow</math> inflammatory histological scores in caecum and colon, and <math>\downarrow</math> inflammation of gut mucosa</li> <li>• <math>\downarrow</math> IL-1<math>\beta</math> and <math>\uparrow</math> TGF-<math>\beta</math> in caecum; no effect on IL-10</li> <li>• <math>\downarrow</math> Interferon-<math>\gamma</math> production from MLN cells; no effect on IL-10</li> </ul>
21	Oligofructose-enriched inulin (5 g/kg body wt)	Rats (7 weeks old); specific pathogen free; colitis induced (HLA-B27 transgenic)	<ul style="list-style-type: none"> <li>• No effect on allergic airway eosinophilia, with no differences in total cell, eosinophil, lymphocyte or macrophage numbers in bronchoalveolar lavage fluid, or on IL-4, IL-5 or interferon-<math>\gamma</math> mRNA levels in lung tissue</li> </ul>
117	Fructo-oligosaccharides (5 % w/w diet)	Rats (male, 5 weeks old); with allergic airway eosinophilia	<ul style="list-style-type: none"> <li>• <math>\downarrow</math> Disease activity index</li> <li>• <math>\downarrow</math> Colonic myeloperoxidase activity</li> <li>• <math>\downarrow</math> Colonic tissue IL-1<math>\beta</math></li> <li>• <math>\downarrow</math> Bacterial translocation to MLN</li> </ul>
26	Oligofructose-enriched inulin (1 g/d) with/without <i>B. infantis</i>	Rats; colitis induced by dextran sodium sulphate	<ul style="list-style-type: none"> <li>• No effect on:</li> <li>• Colonic TGF-<math>\beta</math> or IL-10</li> <li>• Detection of colonic TNF-<math>\alpha</math></li> <li>• <math>\downarrow</math> Number of mast cells in the duodenum</li> <li>• <math>\downarrow</math> Duodenal villus oedema formation rate</li> <li>• Non-significant <math>\downarrow</math> in number of CCR4<sup>+</sup> cells in duodenal mucosa</li> </ul>
133	Fructo-oligosaccharides (5 % w/w diet)	Mice (male, 6 weeks old); pathogen-free conditions; ovalbumin sensitised	<ul style="list-style-type: none"> <li>• No effect on:</li> <li>• Serum total IgE and ovalbumin-specific IgE</li> <li>• <math>\downarrow</math> Disease activity index whether fructo-oligosaccharides given before and during, during, or during and after colitis induction</li> <li>• <math>\downarrow</math> Damage to distal colon when fructo-oligosaccharides given before and during, and during colitis induction, as shown by an increased colon length</li> <li>• <math>\uparrow</math> Crypt depth and crypt area in distal colon when fructo-oligosaccharides given during colitis induction</li> <li>• Faster recovery from damage (shown by crypt area and crypt depth in the distal colon) when fructo-oligosaccharides given during and after colitis induction</li> </ul>
112	Fructo-oligosaccharides (1.15 g/ml twice a day, administered at different stages of colitis induction)	Mice (female, greater than 8 weeks old); colitis induced by dextran sodium sulphate	

LTB<sub>4</sub>, leukotriene B<sub>4</sub>; MLN, mesenteric lymph nodes; TGF, transforming growth factor;  $\uparrow$ , increase/increased;  $\downarrow$ , decrease/decreased.



Thus, animal studies provide fairly strong evidence of a protective effect of  $\beta$ 2-1 fructans on colitis and necrotising enterocolitis. The consistent findings may relate to the action of prebiotics directly at the site of pathology. Effects of prebiotics on inflammatory processes distant from the intestinal tract (e.g. the lung) may not be expected or may be much smaller in magnitude.

#### Studies in man

Eleven studies of  $\beta$ 2-1 fructans in human inflammatory conditions were identified (four where synbiotics were used), of which ten were conducted in adults (Table 6).

In accordance with findings from animal experiments,  $\beta$ 2-1 fructans supplementation was shown to be beneficial in ulcerative colitis patients. OF-enriched IN supplementation in such patients decreased faecal calprotectin (a marker of intestinal inflammation) and perception of abdominal pain, although there was no change in the inflammatory mediators measured ( $\text{PGE}_2$  and IL-8) or on faecal excretion of human DNA (a result of the mucosal inflammation seen in ulcerative colitis)<sup>(118)</sup>. Although IN supplementation in patients with ileal pouch–anal anastomosis did not produce any effects on clinical symptom scores, there were reductions in total endoscopic scores, mucous exudates, total histological scores and total Pouchitis Disease Activity Index<sup>(119)</sup>. A trial into OF supplementation to patients with ileo-colonic Crohn's disease reported positive results: disease activity scores were reduced, and expression of toll-like receptor 4 on dendritic cells in the lamina propria was increased, while there were non-significant improvements in several other outcomes<sup>(120)</sup>.

Most trials in irritable bowel syndrome do not report beneficial effects of  $\beta$ 2-1 fructans on symptom scores<sup>(121,122)</sup>, perhaps due to the nature of the disease regarding the relapse and remission pattern, although there is one positive study in this disorder<sup>(123)</sup>.

In contrast to the single animal study that reported no benefit of FOS on allergic airway eosinophilia<sup>(117)</sup>, a study in infants at risk from atopy found a reduction in the development of atopic dermatitis in the group supplemented with FOS in combination with GOS<sup>(124)</sup>. Several reasons could be given to explain this inconsistency in results. In the human study, the composition of this FOS–GOS supplement was designed to closely resemble the composition of oligosaccharides of the mother's milk, but as this was not the case in the animal study it may be that the amount of prebiotic given was not appropriate. Also, the rats were at a later stage of development than the infants in the human study, and so as their immune systems would have been more developed, the prebiotics may have had less of an effect upon their immune system. Finally, the infants were at risk from allergy because of parental allergy (i.e. genetics was most likely an important factor), while in the animal model, the allergy was induced in the affected animals.

Synbiotic therapy for inflammatory bowel diseases produces mixed results. OF-enriched IN in combination with *Bifidobacterium longum* improved markers of inflammation in patients with active ulcerative colitis, such as decreases in TNF- $\alpha$ , IL-1 $\alpha$  mRNA levels in mucosal tissue and decreased C-reactive protein levels in the blood<sup>(125)</sup>. Mucosal tissue mRNA levels of the  $\beta$ -defensins that are up-regulated in ulcerative

colitis were also reduced in this study<sup>(125)</sup>. IN given in combination with other fermentable fibres and four lactic acid bacteria had no effect on relapse rates (either endoscopic or clinical) in Crohn's disease patients undergoing resection<sup>(126)</sup>. In a study of patients with acute pancreatitis, a synbiotic supplement was found to be more beneficial than when the prebiotics were given alone regarding outcomes such as systemic inflammatory response and multi-organ failure<sup>(127)</sup>. Regarding irritable bowel syndrome, a formula containing IN as well as *Lactobacillus acidophilus*, *Lactobacillus sporogenes* (this bacterium is actually *Bacillus sporogenes*, a soil micro-organism claimed to have probiotic properties) and *Streptococcus thermophilus*, amino acids and vitamins resulted in significant reductions in abdominal pain, distension and constipation<sup>(123)</sup>.

#### Conclusions

This paper has presented and evaluated results from all of the studies available, to our knowledge, of the effects of  $\beta$ 2-1 fructans upon immune function, the host's ability to fight infection, and inflammatory processes and conditions. The results of these studies are often difficult to compare, due to inconsistencies in methodology and the heterogeneity of the subjects used. Despite this, much evidence suggests that  $\beta$ 2-1 fructans do influence some aspects of host immunity. In laboratory animals, the innate and adaptive immune systems of both the GALT and the systemic immune system have been shown to be modified by  $\beta$ 2-1 fructans. In man, most studies have investigated the effects of  $\beta$ 2-1 fructans upon the systemic immune system, with little effect observed on innate immune function, but with many mixed results reported regarding the adaptive immune system, suggesting modification by  $\beta$ 2-1 fructans on this aspect of immunity. In animal models of infections, findings are conclusive regarding the benefits of  $\beta$ 2-1 fructans upon improving host resistance. In man there is convincing evidence that  $\beta$ 2-1 fructans may reduce the incidence and duration of certain infections in infants and children.  $\beta$ 2-1 Fructan supplementation in adults has not, generally, produced beneficial results, but when given as a synbiotic to critically ill or surgical patients,  $\beta$ 2-1 fructans were shown to reduce infections. Taken together these results suggest that  $\beta$ 2-1 fructans, especially IN and OF, may be most beneficial in those who are particularly susceptible to modifications of their immune system. In animal models of inflammation,  $\beta$ 2-1 fructans have shown benefits in models of colitis and necrotising enterocolitis, perhaps due to the pathological site of these conditions being the same as the site of action of prebiotics. This theory is supported by the observation of the lack of effect of  $\beta$ 2-1 fructans upon a model of allergic airway eosinophilia, an inflammatory condition distant from the gut. However, in human infants, an improvement in atopic dermatitis was observed in one study. Inflammatory bowel conditions in human adults are improved upon  $\beta$ 2-1 fructan supplementation, but findings in irritable bowel syndrome are mixed. It is important that future studies build upon the findings of the studies reported here, in order that a more complete picture of the effects of  $\beta$ 2-1 fructans upon immune function, infections and inflammation is formed. The funding of these future studies needs to be considered carefully. The majority of studies conducted to date



**Table 6.** Effects of  $\beta$ 2-1 fructans on inflammation in human disease

Reference no.	Prebiotic used	Subjects studied	Findings
121	Oligofructose (6 g/d for 4 weeks)	Patients with irritable bowel syndrome patients; aged 18–65 years; <i>n</i> 21; cross-over design	No effect on: <ul style="list-style-type: none"> <li>• Symptom scores</li> <li>• Whole gut transit time</li> </ul>
122	Fructo-oligosaccharides (10 g/d for 2 weeks then 20 g/d for 10 weeks)	Irritable bowel syndrome patients; mean age 45.1 years; <i>n</i> 52 in test group; <i>n</i> 46 in placebo group	<ul style="list-style-type: none"> <li>• Improvements in symptoms were seen in both groups, but greater improvement in placebo group</li> <li>• No significant differences between symptoms of irritable bowel syndrome at the end of the study between the groups</li> </ul>
119	Inulin (24 g/d for 3 weeks)	Patients with ileal pouch–anal anastomosis; mean age 37 years; <i>n</i> 19; cross-over design	<ul style="list-style-type: none"> <li>• <math>\downarrow</math> Total histological and endoscopic scores, and <math>\downarrow</math> mucous exudates, resulting in <math>\downarrow</math> Pouchitis Disease Activity Index</li> </ul>
125	Oligofructose-enriched inulin (12 g/d for 4 weeks), plus <i>Bifidobacterium longum</i>	Patients with active ulcerative colitis; age 24–67 years; <i>n</i> 8 in test group, <i>n</i> 8 in control	<p>No effect on clinical symptoms</p> <ul style="list-style-type: none"> <li>• <math>\downarrow</math> Human <math>\beta</math> defensin 2, 3 and 4 mRNA levels in mucosal tissue</li> <li>• <math>\downarrow</math> TNF-<math>\alpha</math> and IL-1<math>\alpha</math> mRNA levels in mucosal tissue</li> <li>• <math>\downarrow</math> C-reactive protein levels in blood</li> </ul>
120	Oligofructose and inulin (15 g/d for 3 weeks)	Ileocolonic Crohn's disease patients; age 29–56 years; <i>n</i> 10; no placebo group	<p>No effect on IL-10 mRNA levels in mucosal tissue</p> <ul style="list-style-type: none"> <li>• <math>\downarrow</math> Disease activity scores</li> <li>• <math>\uparrow</math> Percentage of CD11c<sup>+</sup> dendritic cells expressing TLR-4 in lamina propria</li> <li>• Non-significant <math>\uparrow</math> in percentage of CD11c<sup>+</sup> dendritic cells expressing TLR-2</li> <li>• Non-significant <math>\uparrow</math> in percentage of IL-10 positive intestinal CD11c<sup>+</sup> dendritic cells from lamina propria mononuclear cells</li> </ul>
123	Inulin plus <i>Lactobacillus sporogenes</i> , <i>L. acidophilus</i> , <i>Streptococcus thermophilus</i> , vitamins B <sub>1</sub> , B <sub>2</sub> , B <sub>6</sub> , vitamin PP, vegetal charcoal, <i>L. tryptophan</i> and angelica for 6 months	Irritable bowel syndrome patients; <i>n</i> 37 in treatment group, mean age 44.3 years; <i>n</i> 28 in control group, mean age 48.6 years	<p>No effect on:</p> <ul style="list-style-type: none"> <li>• Number of IL-6 positive intestinal CD11c<sup>+</sup> dendritic cells</li> <li>• Number of IL-12 positive intestinal CD11c<sup>+</sup> dendritic cells</li> </ul>
124	Galacto-oligosaccharides and fructo-oligosaccharides (9:1 ratio, 0.8 g/100 ml, for 6 months)	Infants at risk from atrophy; bottle-fed; <i>n</i> 102 in prebiotic group, <i>n</i> 104 in placebo	<ul style="list-style-type: none"> <li>• <math>\downarrow</math> Abdominal pain</li> <li>• <math>\downarrow</math> Abdominal distension</li> <li>• <math>\downarrow</math> Constipation</li> <li>• <math>\downarrow</math> Diarrhoea (non-significant)</li> <li>• <math>\downarrow</math> Alternating constipation/diarrhoea (non-significant)</li> <li>• <math>\downarrow</math> Development of atopic dermatitis</li> </ul>
118	Oligofructose-enriched inulin (12 g/d for 2 weeks)	Ulcerative colitis patients with mild to moderate disease; <i>n</i> 9 in test group, mean age 37 years; <i>n</i> 9 in placebo (maltodextrin), mean age 36 years	<p>No effect on severity of dermatitis</p> <ul style="list-style-type: none"> <li>• <math>\downarrow</math> Faecal calprotectin (marker of intestinal inflammation, marker of the presence of leucocytes) at day 7 in test group</li> <li>• <math>\downarrow</math> Dyspepsia-related health scores</li> <li>• <math>\downarrow</math> Disease activity (but so did placebo)</li> </ul>
126	Inulin (2.5 g/d for 24 months) Given in Synbiotic2000 (also contains $\beta$ -glucan, resistant starch and pectin and <i>Pediococcus pentoseceus</i> , <i>L. raffinolactis</i> , <i>L. paracasei</i> subsp. <i>paracasei</i> and <i>L. plantarum</i> )	Crohn's disease patients undergoing resection; <i>n</i> 20 in test group, mean age 36.1 years; <i>n</i> 10 in control group, mean age 34.7 years	<p>No effect on:</p> <ul style="list-style-type: none"> <li>• Faecal excretion of human DNA (a result of the mucosal inflammation in ulcerative colitis, a marker of leucocyte and epithelial cell desquamation)</li> <li>• IL-8, PGE<sub>2</sub> or <math>\beta</math>-globulin gene (inflammatory mediators)</li> </ul>

Table 6. Continued

Reference no.	Prebiotic used	Subjects studied	Findings
127	Inulin (2.5 g/d for $\geq 7$ d) Given in combination with $\beta$ -glucan, resistant starch and pectin. Another group was given these fibres plus <i>Pediococcus pentoseceus</i> , <i>L. mesenteroides</i> , <i>L. paracasei</i> subsp. <i>paracasei</i> , <i>L. plantarum</i>	Patients with severe acute pancreatitis; <i>n</i> 33 in synbiotic group, mean age 47.5 years; <i>n</i> 29 in fibre only (control) group, mean age years 46.0 years	Compared to the fibre-only group, the synbiotic group had significantly: <ul style="list-style-type: none"> <li>• <math>\downarrow</math> Total incidence of systemic inflammatory response syndrome and multi-organ failure</li> <li>• <math>\downarrow</math> Number of patients developing complications during recovery</li> <li>• <math>\downarrow</math> Rate of late organ failure</li> </ul>
134	Oligofructose (5 g/d for 6 weeks)	Adults with minor bowel disorders (abdominal bloating, rumbling, transit disorders, abdominal pains, flatulence); majority female; <i>n</i> 54 in test group, mean age 39.5 years; <i>n</i> 51 in control group, mean age 37.6 years	<ul style="list-style-type: none"> <li>• <math>\downarrow</math> Symptom (abdominal pain) intensity</li> <li>• <math>\uparrow</math> Discomfort item scores in Quality of Life Assessment</li> <li>• When expressed as a change in quality of life, daily activities were improved</li> <li>• Non-significant <math>\downarrow</math> in frequency of symptoms</li> </ul>

$\uparrow$ , Increase/increased;  $\downarrow$ , decrease/decreased.

have been funded directly from industry or have involved academic collaboration of some sort with industry. It is not possible to identify whether studies funded by industry yield different findings than those not funded by industry, quite simply because there are so many of the former and so few of the latter and also because the extent of industry support and funding for some published studies is not readily apparent.

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