

## Prebiotics in inflammatory bowel diseases

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In genetically susceptible individuals, an altered mucosal immune response against some commensal bacteria of the gut ecosystem appears to be the principal mechanism leading to intestinal lesions in inflammatory bowel disease (IBD). The information currently available does not provide an exact explanation about the origin of this important dysfunction of the interaction between host and commensal bacteria, but an altered microbial composition has been detected in the gut ecosystem of patients with Crohn's disease or ulcerative colitis. Prebiotics are food ingredients not digested nor absorbed in the upper intestinal tract that are fermented by intestinal bacteria in a selective way promoting changes in the gut ecosystem. Experimental and human studies have shown that inulin and oligofructose stimulate saccharolysis in the colonic lumen and favour the growth of indigenous lactobacilli and bifidobacteria. These effects are associated with reduced mucosal inflammation in animal models of IBD. Strong experimental evidence supports the hypothesis that inulin and oligofructose can offer an opportunity to prevent or mitigate intestinal inflammatory lesions in human Crohn's disease, ulcerative colitis, and pouchitis. Encouraging results have been obtained in preliminary clinical trials.

**ulcerative colitis: Crohn's disease: pouchitis: inulin: oligofructose: lactulose: animal models of IBD: microbiota**

The term 'inflammatory bowel disease' (IBD) refers mainly to three separate clinical entities: Crohn's disease, ulcerative colitis and pouchitis. These diseases are characterized by persistent mucosal inflammation at different levels of the gastrointestinal tract. Typically, IBDs exhibit undulating activity with bouts of uncontrolled, chronic mucosal inflammation, followed by remodelling processes that occur during periods of remission. Incidence of such diseases has been growing steadily during the past 5 decades in Western Europe, and is now expanding dramatically in Asian and Eastern European countries<sup>1</sup>. IBDs are becoming an important burden also in young populations<sup>2</sup>.

The precise aetiologies of these chronic inflammatory conditions remain to be elucidated, but the most important pathophysiological mechanisms that lead to the mucosal inflammatory lesions are being unveiled. These mechanisms result from complex interaction of environmental, genetic and immunoregulatory factors. Abnormal communication between gut microbial communities and the mucosal immune system has been suggested as the core defect leading to IBD in genetically susceptible individuals<sup>3</sup>. Within the gastrointestinal tract, the inflammatory capacity of commensal bacteria is varied. Some resident bacteria are proinflammatory, whereas others attenuate inflammatory responses<sup>4–6</sup>. Prebiotics such as inulin and oligofructose can improve the microbial balance in the human gut microbiota by increasing the number and activity of bacteria associated with health benefits<sup>7</sup>. This article reviews experimental and clinical evidence supporting the use of prebiotics for the prevention and control of IBD.

### The gut microbiota

The term "microflora" or "microbiota" refers to the community of living micro-organisms assembled in a particular ecological niche of a host individual. The human gut is the natural habitat for a large, diverse and dynamic population of micro-organisms which over millennia have adapted to live on the mucosal surfaces or in the lumen<sup>8</sup>. The number of resident bacteria increases along the small bowel, from approximately  $10^4$  in the jejunum to  $10^7$  colony-forming units per gram of luminal content in the distal ileum. The large intestine is the most heavily populated region of intestine, where several hundred grams of bacteria are harboured at densities around  $10^{12}$  colony forming units per gram of luminal content.

Our current knowledge about the microbial composition of the intestinal ecosystem in health and disease is still very limited. Studies using classical techniques of microbiological culture can only recover a minor fraction of faecal bacteria. Over 50% of bacteria cells that are observed by microscopic examination of faecal specimens cannot be grown in culture<sup>9</sup>. Molecular biological techniques based on the sequence diversity of the bacterial genome are being used to characterize non-cultivable bacteria<sup>10</sup>. Molecular studies on the faecal microbiota have highlighted that only 7 of the 55 known divisions or superkingdoms of the domain 'bacteria' are detected in the human gut ecosystem, and of these, 3 bacterial divisions dominate, i.e. Bacteroidetes, Firmicutes and Actinobacteria. However, at species and strain level, microbial diversity between individuals is highly remarkable up to the point

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that each individual harbours his or her own distinctive pattern of bacterial composition<sup>10</sup>.

On the other hand, studies comparing animals bred under germ-free conditions with their conventionally raised counterparts have clearly demonstrated the important impact of resident bacteria on host physiology. The interaction between gut bacteria and their host is a symbiotic relationship mutually beneficial for both partners. The host provides a nutrient-rich habitat and the bacteria confer important benefits to the host<sup>8</sup>. Functions of the microbiota include **nutrition** (fermentation of nondigestible substrates that results in production of short chain fatty acids, absorption of ions, production of aminoacids and vitamins), **protection** (the barrier effect that prevents invasion by alien microbes), and **trophic effects** on the intestinal epithelium and the immune system (development and homeostasis of local and systemic immunity).

Animals bred in a germ-free environment show low densities of lymphoid cells in the gut mucosa and low concentrations of serum immunoglobulins. Exposure to commensal microbes rapidly expands the number of mucosal lymphocytes and increases the size of germinal centres in lymphoid follicles. Immunoglobulin producing cells appear in the lamina propria, and there is a significant increase in serum immunoglobulin concentrations<sup>11</sup>. Most interestingly, recent findings suggest that some commensals play a major role in the induction of regulatory T cells in gut lymphoid follicles<sup>12</sup>. Regulatory pathways mediated by regulatory T cells are essential homeostatic mechanisms by which the host can tolerate the massive burden of innocuous antigens within the gut or on other body surfaces without responding through inflammation.

### Bacteria and inflammatory bowel disease

The mechanisms of regulation and tolerance of bacterial antigens in the gut microbiota seem to be altered in subjects with IBD. The normal mucosal defence is based mainly on the production of IgA antibodies that are secreted into the gut lumen and neutralize microbes in the lumen, thus avoiding mucosal inflammation<sup>13,14</sup>. In IBD, however, mucosal production of IgG antibodies against intestinal bacteria is highly increased, and mucosal defence relies on both IgG mediated responses within the tissue and hyper-activated lymphocytes in the lamina propria reacting against bacterial antigens<sup>13–15</sup>. These events result in inflammation and tissue injury. The altered immune response is not specifically targeted towards a single group of potential pathogens, but involves a large and undefined number of commensal species belonging to the common enteric microbiota. A microbial imbalance in the gut ecosystem could explain the abnormal reactivity of the mucosal immune system against enteric bacteria.

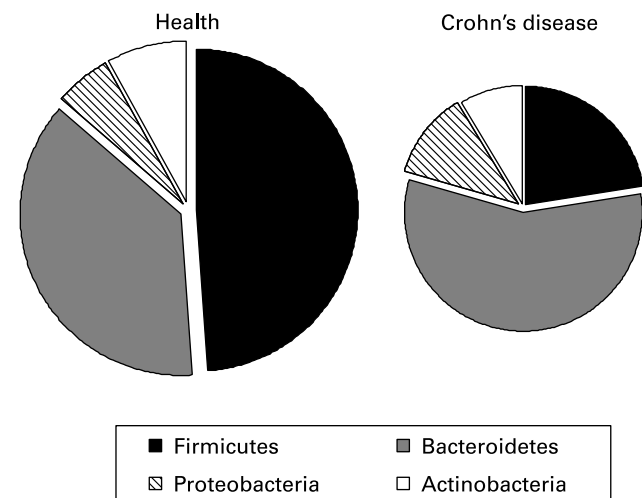
Several studies have shown that the composition of the faecal microbiota differs between subjects with IBD and healthy controls<sup>16</sup>. Molecular studies show that a substantial proportion of faecal bacteria (up to 30 to 40% of dominant species) in patients with active Crohn's disease or ulcerative colitis belong to phylogenetic groups that are unusual in healthy subjects<sup>17</sup>. These remarkable changes could be secondary to disease activity but they are not observed in patients with infectious diarrhoea. On the other hand, studies have shown reduced diversity of bacteria species in both faecal and mucosa-associated communities in patients with IBD<sup>18,19</sup>. Manichanh and coworkers<sup>18</sup> employed

a metagenomic approach for exhaustive investigation of bacterial diversity in Crohn's disease and found a striking reduction of Firmicutes in patients in remission compared with healthy controls (Fig. 1).

Studies on mucosa-associated bacteria have found high concentrations of adherent bacteria in patients with clinically active ulcerative colitis or Crohn's disease, but not in healthy controls<sup>20</sup>. The concentrations of mucosal adherent bacteria increased progressively with the severity of mucosal inflammation, and the identified bacteria were of faecal origin. The fluorescent in situ hybridization (FISH) technique demonstrated bacterial invasion of the mucosa in most mucosal specimens from ulcerative colitis and Crohn's disease patients, but not in any of the mucosal specimens from controls<sup>21</sup>. Invading bacteria belonged to a great variety of genera, including *Proteobacteria*, *Enterobacteriaceae*, *Bacteroides/Prevotella* cluster, *Clostridium*, and sulphate-reducing bacteria. However, mucosal invasion by *Bifidobacterium* or *Lactobacillus* species was not detected<sup>21</sup>. Moreover, Macfarlane and coworkers<sup>22</sup> observed that numbers of adherent non-invading bifidobacteria were lower in rectal biopsies from ulcerative colitis patients than controls.

### Prebiotics

A healthy or 'balanced' microbiota has been considered to be one that is predominantly saccharolytic and comprises significant numbers of bifidobacteria and lactobacilli<sup>23</sup>. Inulin and oligofructose are prebiotic carbohydrates that resist digestion by intestinal and pancreatic enzymes in the human gastrointestinal tract and are fermented by bacteria living in the intestinal ecosystem<sup>24</sup>. When administered in adequate amounts, these prebiotics increase saccharolytic activity within the gut and promote selectively the growth of bifidobacteria. Numerous studies have shown an increase in counts of bifidobacteria in faeces from subjects consuming inulin or oligofructoses<sup>7,25</sup>. Moreover, oral intake of inulin and oligofructoses increases



**Fig. 1.** The faecal microbiota of patients with Crohn's disease contains a reduced proportion of Firmicutes. The graph shows data from Manichanh and coworkers (ref 18) and represents number of phylotypes per division in 6 healthy persons and 6 patients in clinical remission.

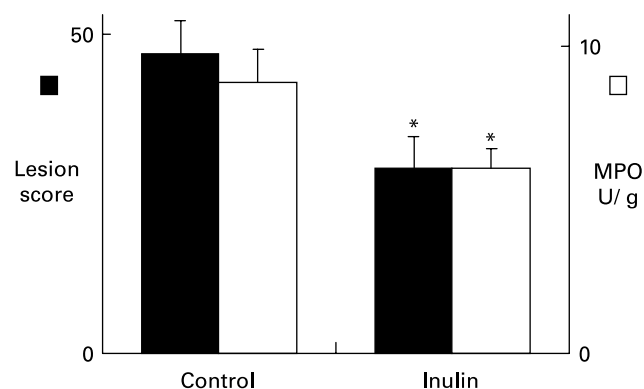
numbers of bifidobacteria and lactobacilli in the mucosa-associated communities of the human colon. Langlands et al.<sup>26</sup> showed that bifidobacteria and lactobacilli numbers could be increased more than 10-fold in biopsy mucosal specimens of the proximal and distal colons in subjects fed 15 g of a prebiotic mixture containing inulin and oligofructose for 2 weeks. Likewise, a study with ulcerative colitis patients receiving a synbiotic preparation with a *Bifidobacterium* strain and oligofructose-enriched inulin showed that counts of bifidobacteria on the rectal mucosa increased 42-fold<sup>27</sup>.

Hypothetically, by increasing the number of 'friendly' bacteria on the mucosal surface, inulin and oligofructose could improve the barrier function in IBD and prevent mucosal colonization by aerobic enterobacteria able to invade. This hypothesis has been tested in a considerable number of experimental studies using different animal models of IBD.

### Experimental models of inflammatory bowel disease

The effect of the prebiotic inulin has been tested in the rat model of colitis induced by the chemical dextran sodium sulphate (DSS)<sup>28</sup>. Oral administration of DSS over 3 to 5 days induces direct toxicity against colonic epithelial cells that results in dysfunction of the mucosal barrier with increased permeability to large size molecules<sup>29</sup>. These events are followed by crypt destruction and loss of height of the intestinal villi, with subsequent bacterial invasion and mucosal inflammation. In the rat, daily oral administration of inulin increased counts of indigenous lactobacilli in the caecal lumen and reduced the intracolonic pH. In rats exposed to DSS to induce colitis, treatment with oral inulin reduced significantly tissue myeloperoxidase activity, an index of neutrophil infiltration, and mucosal release of inflammatory mediators. Furthermore, inulin-fed rats showed a reduced extent of damaged mucosa and decreased severity of crypt destruction. Histological damage scores were significantly lower in inulin treated rats than in controls (Fig. 2). Treatment with oral inulin was equally effective whether started prior to or during exposure to DSS.

The effect of oligofructose and inulin alone or in combination with probiotic bifidobacteria was recently tested in



**Fig. 2.** Scores of colonic mucosal lesion (solid columns, left 'y' axis) and tissue content of myeloperoxidase (MPO, open columns, right 'y' axis), a marker of leukocyte infiltration, in rats with colitis induced by DDS. Daily administration of 400 mg inulin by oral gavage significantly reduced lesion scores and myeloperoxidase content in colonic tissue (see reference 28).

the same DSS model<sup>30</sup>. The prebiotic alone or in combination with *B. infantis* strains improved significantly the disease activity indexes and decreased colonic myeloperoxidase activity, as well as expression of inflammatory mediators. Interestingly, bacterial translocation to mesenteric lymph nodes and liver decreased significantly in rats treated by prebiotic, probiotic or the combination of both (synbiotic) as compared to colitis controls. The authors concluded that oligofructose and inulin as well as the *Bifidobacterium* strains tested prevented bacterial invasion and had an anti-inflammatory effect in this model.

Chronic inflammatory lesions can be induced in the distal colon by a single intracolonic administration of trinitro-benzene sulphonic acid (TNBS) diluted in ethanol (usually 20 to 50 mg TNBS in 30 to 50 % ethanol), using a rubber cannula. The effect of oligofructose has been tested in the TNBS model of colitis<sup>31</sup>. Oral administration of oligofructose significantly reduced intracolonic pH, macroscopic lesion scores, and tissue myeloperoxidase activity in TNBS treated rats. In addition, oligofructose increased the concentration of lactate and butyrate as well as counts of lactic acid bacteria in caecal contents. In subsequent ancillary experiments, these investigators demonstrated that a direct intracaecal infusion of lactic acid bacteria together with short chain fatty acids was necessary to reproduce the anti-inflammatory effects of oligofructose. They concluded that fermentation of the prebiotic by lactic acid bacteria was the principal mechanism mediating the anti-inflammatory effect.

Further experimental work evaluated the anti-inflammatory effects of inulin and oligofructose in the transgenic HLA-B27 rat model of spontaneous colitis<sup>32</sup>. Rats transgenic for the human HLA-B27-beta2-microglobulin gene spontaneously develop immune-mediated colitis of variable severity at 2-4 months of age. The disease is characterized by non-bloody diarrhoea and marked inflammatory infiltration of the caecal and colonic mucosa. Hoentjen and coworkers<sup>32</sup> tested a mixture of oligofructose and inulin in this model of spontaneous colitis, and observed a significant anti-inflammatory effect in rats fed with the prebiotic mixture. Prebiotic treatment reduced gross morphological scores and histological grading of the lesions. In addition, prebiotic treatment reduced the expression of pro-inflammatory cytokines such as IL-1 $\beta$ , but enhanced the expression of regulatory type cytokines (TGF- $\beta$ ).

The effects of the prebiotic lactulose have also been tested in some animal models of intestinal inflammation. Mice deficient of the IL-10 gene spontaneously develop colitis. In the neonatal period, these knockout mice have a decreased level of Lactobacillus species in the colon and an increase in adherent and translocated bacteria<sup>33</sup>. Oral administration of lactulose was shown to normalize counts of lactobacilli in faeces and prevented the development of colitis. Likewise, protective effects of lactulose have been demonstrated in the DSS and TNBS models<sup>34,35</sup>. Taken together, all these experimental data give a strong indication of the anti-inflammatory effects of prebiotics in a wide range of animal models of IBD.

### Clinical studies

A randomized, placebo-controlled, double-blind, crossover clinical trial tested the effect of inulin in patients with chronic

pouchitis<sup>36</sup>. This clinical condition is characterized by chronic mucosal inflammation of the ileal pouch-anal anastomosis in patients that have had a total colectomy. The ileal pouch is surgically constructed in order to function as a faecal reservoir. The inflammatory disorder impairs the function of the reservoir and results in persistent diarrhoea with mucus and blood. Twenty patients with mild disease activity entered the trial and were randomized to begin with either placebo or inulin (24 g per day) for three weeks, using a double-blinded crossover design with a washout period of four weeks. Compared with placebo, dietary supplementation with inulin significantly reduced endoscopic and histological parameters of inflammation of the mucosa of the ileal reservoir (Table 1). The effect was associated with an increase in faecal butyrate and a decrease in the counts of *Bacteroides* in faeces.

Furrie et al.<sup>27</sup> reported a randomized, placebo-controlled, double-blind clinical trial in two parallel groups of patients with ulcerative colitis. Eligible patients had mild disease activity and were on stable medication. Eighteen patients were randomized to receive for a period of 1 month either a synbiotic preparation (oligofructose-enriched inulin at 12 g per day, and *Bifidobacterium longum* at 200 billion colony forming units per day) or placebo (maltodextrin). Synbiotic treatment induced significant reduction of mucosal expression of proinflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ ) and inducible beta-defensins. Histological examination of biopsies showed marked decrease in inflammatory cell infiltrate and crypt abscesses in patients receiving the synbiotic, together with improved sigmoidoscopy scores and clinical activity indices, but differences were not significant due to the reduced number of patients enrolled.

The effect of oligofructose-enriched inulin in patients with active ulcerative colitis was recently tested in a randomized, placebo-controlled, double-blind pilot trial with two parallel groups<sup>37</sup>. Eligible patients had been previously in remission

with mesalazine as maintenance therapy or no drug, and presented to the hospital for relapse of mild-moderate activity. They were treated with mesalazine (3 g/day) and randomly allocated to receive either oligofructose-enriched inulin (12 g/day) or placebo (12 g/day of maltodextrin) for two weeks. The primary endpoint was the anti-inflammatory effect of the prebiotic as assessed by objective, non-invasive markers of intestinal inflammation, i.e. faecal concentration of calprotectin. Calprotectin is a protein found in granulocytes that resists metabolic degradation and can be measured in faeces. Interestingly, at day 7, an early significant reduction of calprotectin was observed in the group receiving oligofructose-enriched inulin but not in the placebo group. At the end of the study period, disease activity scores were significantly reduced in the two groups. Use of this prebiotic may improve response to medical therapy with mesalazine, but this point needs further investigation in a trial with adequate number of patients.

Prebiotics have also been tested in Crohn's disease. In a small open-label trial, 10 patients with active ileo-colonic Crohn's disease were given 15 g of oligofructose per day for 3 weeks<sup>38</sup>. All but two patients exhibited a decline in the Harvey Bradshaw index of disease activity after three weeks on oral oligofructose, and the group as a whole showed a significant fall in disease activity as compared to baseline. There was a significant increase in bifidobacteria numbers in faeces but not in rectal biopsies. However, this study did not include a placebo-control group. A controlled study in Crohn's disease patients with appropriate sample size is now being performed by the same group of investigators.

Taken together, experimental and clinical data so far support the hypothesis that prebiotics such as inulin and oligofructose can offer an opportunity to prevent or mitigate intestinal inflammatory lesions in human Crohn's disease, ulcerative colitis, and pouchitis. Controlled clinical trials of appropriated sample size are still needed to confirm this hypothesis.

**Table 1.** Effect of Dietary Inulin Supplementation on Pouchitis Disease Activity Index (PDAI)

	Placebo	Inulin	P
<b>Clinical score</b>			
Stool frequency	0.53 (0.19)	0.47 (0.15)	0.65 (NS)
Rectal bleeding	0.05 (0.06)	0.05 (0.06)	0.10 (NS)
Fecal urgency/abdominal cramps	0.68 (0.18)	0.47 (0.19)	0.16 (NS)
Fever	0 (0)	0 (0)	0.10 (NS)
Total clinical score	1.26 (0.29)	1.00 (0.27)	0.17 (NS)
<b>Endoscopic score</b>			
Edema	0.16 (0.09)	0 (0)	0.08 (NS)
Granularity	0.05 (0.06)	0.05 (0.06)	1.00 (NS)
Friability	0.32 (0.12)	0.16 (0.09)	0.18 (NS)
Loss of vascularity	0.05 (0.06)	0.11 (0.08)	0.56 (NS)
Mucous exudates	0.32 (0.12)	0.05 (0.06)	0.03
Ulceration	0.58 (0.12)	0.58 (0.12)	1.00 (NS)
Total endoscopic score	1.47 (0.32)	0.95 (0.22)	0.04
<b>Histologic score</b>			
Polymorph infiltration	1.44 (0.15)	1.11 (0.14)	0.05 (NS)
Ulceration per low-power field	1.17 (0.13)	1 (0)	0.18 (NS)
Total histologic score	2.61 (0.26)	2.11 (0.14)	0.04
<b>Total PDAI score</b>	<b>5.39 (0.62)</b>	<b>4.05 (0.44)</b>	<b>0.01</b>

Data are means and standard error of the mean, in brackets, and were published by Welters and coworkers (ref. 36). NS = not significant.

#### Conflict of interest statement

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