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Review

Prebiotics, synbiotics and inflammatory bowel disease

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The normal colonic microflora is intimately involved in the aetiology of inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn's disease (CD). These conditions are often refractile to conventional treatments involving the employment of anti-inflammatory and immunosuppressant drugs, and this has led to a search for alternative therapies based on the use of probiotics, prebiotics and synbiotics. The majority of investigations in this area have been done with probiotics, and while there is increasing interest in the abilities of prebiotics and synbiotics to control the symptoms of IBD, very few randomised controlled trials have been reported. Although the results have been variable, human and animal studies have demonstrated that in many circumstances, these functional foods can alter the composition of the colonic microbiota, reduce inflammatory processes in the gut mucosa, and have the potential to induce disease remission. More work is needed to understand the effects of prebiotics and synbiotics on microbial communities in the gut, and their interactions with the host's immune system.

Keywords: Bacteria / Inflammatory bowel disease / Large gut / Prebiotics / Synbiotics

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1 Introduction

The human large intestine contains a diverse microbiota composed of several hundred different species and strains of bacteria [1], which plays an important role in host physiology and metabolism [2]. Although the colonic microbiota is superficially similar in different people, marked interindividual variations exist in various bacterial populations at species level [3]. The ecological principles that govern the large intestinal ecosystem are similar to those in other complex microbial environments, such that increasing species diversity is known to enforce metabolic homeostasis and structural stability [4], while degenerative changes in species composition, through ageing, disease or antibiotic treatment, reduce the ability of the ecosystem to resist invading pathogens [5]. The composition and metabolic activities of the microflora are controlled by many factors, particularly diet and environment [6] although host physiology and gut anatomy are also important determinants of microbiota structure and function [7]. Until the advent and widespread use of prebiotics over the last decade, the general consensus was that the composition of the microbiota was relatively unaffected by diet, however, it is now quite clear that even relatively small amounts of nondigestible oligosaccharides can have significant effects on bacterial community structure in the gut [6].

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Abbreviations: CD, Crohn's disease; **DSS,** dextran sodium sulphate; **FOS,** fructo-oligosaccharide; **GOS,** galacto-oligosaccharides, **IBD,** inflammatory bowel disease; **RS,** resistant starch; **TLR,** toll-like receptor; **TNBS,** trinitrobenzene sulphonic acid; **TNF-α,** tumour necrosis factor alpha; **UC,** ulcerative colitis

2 Prebiotics and synbiotics

Prebiotics are food ingredients that selectively stimulate the growth, and or, activity of intestinal bacteria that have health promoting properties for the host. Currently, the vast majority of prebiotics are nondigestible oligosaccharides, of which galacto-oligosaccharides (GOS), lactulose, inulins and their fructo-oligosaccharide (FOS) derivatives have been the most widely studied [8, 9]. Like probiotics [10], prebiotics can be used to alter the species composition and metabolic properties of the colonic microbiota, however, prebiotics can only affect the growth of organisms that are already in the gut, whereas probiotics are allochthonous to the environment into which they are being introduced. In ecological terms, probiotics therefore have the difficult task of competing with indigenous bacteria in the gut that

already occupy most of the available spatial and metabolic niches. This has led to the development of synbiotics, which are combinations of probiotics and prebiotics [8]. The underlying rationale for their use is that if the correct combinations are used, the prebiotic will assist the probiotic to establish in the bowel.

The effects of relatively small amounts (5 – 20 g/day) of lactulose, inulin, FOS and GOS on the composition of the colonic microbiota has been documented extensively in human and animal studies [11 – 13]. Although a number of intestinal bacteria can utilise prebiotics directly [14], or indirectly by crossfeeding on their hydrolysis products and intermediate products of their metabolism such as lactate [15], specific microbiological effects of these substances on the microbiota include increasing numbers of lactobacilli and bifidobacteria in the gut lumen [16] and on the mucosal surface [17].

A variety of host and microbial interactions in the digestive tract have been associated with the use of prebiotics. For example, in the mouth and upper digestive tract they are believed to be protective against dental caries [18], improve calcium and magnesium absorption in the large intestine [19, 20], mimic cellular binding sites for pathogenic bacteria [21, 22], and exert osmotic effects that can sometimes result in diarrhoea [23]. In the large bowel, prebiotic fermentation results in SCFA, lactate, CO_2 and H_2 formation and increased production of microbial cell mass [24], resulting in improved laxation [8, 25]. Moreover, a number of studies in mice and rats challenged with carcinogens and mutagens have shown that prebiotics alone, or in synbiotic combinations, can have strong anticancer effects by reducing damage to DNA in colonic epithelial cells [26], reducing numbers of aberrant crypt foci on the mucosal surface [27] and preventing tumour formation [28].

Prebiotics or synbiotics may be more efficient than probiotics alone since they are able to selectively stimulate bacteria already resident in the gut, along with any probiotics that may be administered, whereas probiotics need to compete with the indigenous microbiota and large amounts of these organisms need to be given to ensure transit and survival through the upper digestive tract.

3 Inflammatory bowel disease (IBD)

IBD is the collective term applied to a group of idiopathic gut disorders that while having distinct presentations, treatments and outcomes, are all characterised by inflammation of the intestinal mucosa that cannot be attributed to infectious agents. The two main conditions are Crohn's disease (CD) and ulcerative colitis (UC), while 10 – 15% of cases are difficult to distinguish and are given the term indeterminate colitis [29, 30].

A variety of aetiologic factors have been linked to CD and UC, including socioeconomic determinants such as smoking, and use of the oral contraceptive pill, but the actual pathogenesis is still not completely understood [31 – 35]. Several hypotheses remain under consideration, and revolve around genetic predisposition, and an uncontrolled immune response towards bacterial stimuli [36 – 39]. Indeed, studies with animal models have shown that bacteria growing in the gut are essential for IBD to occur [40]. A significant number (4.5 – 16.6%) of Crohn's patients have a family history of the disease, suggesting a polygenic inheritance [41, 42]. If a patient has CD, there is a ten-fold risk that a first degree relative will develop the disease, and if a patient has UC, there is a two-fold risk that a first degree relative will develop CD [43]. The NOD 2/CARD 15 (IBD-1), a relatively recently identified mutation in chromosome 16, can be present in up to 30% of CD patients [44], however, it is also found in non-Crohn's patients, and in Japan, this mutation is quite common in the normal population. However, in Caucasians, the presence of the IBD-1 mutation can predict terminal ileum stenotic lesions [45]. Although not routinely measured, there are a variety of different markers that suggest underlying immune dysregulation, *e.g.* antisaccharomyces antibody (ASCA), antiepithelial antibodies (AEA), pancreatic autoantibody (PAB), antiendothelial cell antibody (AECA) and peripheral antineutrophil cytoplasmic antibody (p-ANCA), and the mainstay treatments for both diseases involve immunosuppression using prednisolone. Simplistically, the immunological responses in these diseases can be linked to Th1 and Th2 inflammatory response pathways. Although there is some overlap, these involve different cytokine networks that are involved in triggering inflammatory reactions seen in the gut mucosa [46 – 49]. The principal cytokines involved include tumour necrosis factor alpha (TNF-α), IFN-γ, IL-12, IL-6 and IL-18, amongst others [50]. In UC, but not CD, human beta defensins (HBD) are antimicrobial peptides secreted by epithelial cells in inflamed gut tissue, but unlike the pro-inflammatory cytokines, these molecules are not produced by infiltrating inflammatory cells in the underlying mucosal tissue, they therefore constitute useful markers of resolution of inflammation on the epithelial surface [51, 52].

CD is a complex inflammatory disorder. Patients with the condition can be asymptomatic or have a life-threatening presentation. The disease is distinguished by patchy, transmural inflammation that may affect any part of the gastrointestinal tract, from the oral cavity to the rectum. Patients usually present with a range of symptoms including malaise, weight loss, abdominal pain, diarrhoea, anorexia, fever and a variety of extraintestinal manifestations [53, 54]. The faecal microflora of IBD patients has been a focus of attention for over 50 years. During this time, many reports have been made of individual bacterial species thought to provoke or enhance the clinical manifestations of colitis, but there is no consistent evidence for the involvement of specific microbial agents. Some bacteria may be

detrimental when dysfunction of the mucosal barrier is present. Injection of *Bacteroides* or clostridia into the rat intestinal wall was shown to induce an inflammatory reaction, which did not occur with bifidobacteria or lactobacilli [55, 56]. High serum antibody titres to the outer of membrane protein of *Bacteroides vulgatus* have been measured in patients with UC [57]. Higher numbers of *E. coli* have been found in the mucus layer and not adherent to the surface layer of the epithelium in patients with UC compared to controls [58], but the presence of these organisms could only be detected in small number of tissue samples [59], and other *E. coli* subtypes have shown no differences in adherence properties [60]. Thus investigations into individual causative agents in IBD is incomplete and the relevance of *E. coli* continues to remain controversial. Changes can be seen in microbial community structure in the gut, but it is unclear whether this is linked to disease aetiology, or if they are a result of changes in the gut environment caused by the disease itself. Analysis of faecal bacterial populations in Crohn's patients has shown that high species diversity is maintained in these individuals and that enterobacterial numbers are increased. It was also reported that the *Bacteroides* and *Clostridium coccoides* groups declined in importance [61], but this was not confirmed in subsequent studies in which a metagenomic approach to studying faecal communities was used [62]. The latter investigation indicated that there was a marked reduction in the proportion of organisms belonging the phylum Firmicutes in CD patients, especially the *Clostridium leptum* group, compared to normal, while there was no difference in the *Bacteroides* and *C. coccoides* groups.

UC is distinct from CD in several ways, endoscopically, histologically and in location of the disease. UC presents as a chronic, episodic inflammation of the large bowel, beginning distally in the rectum and progressing towards the proximal colon, and is characterised by bloody diarrhoea in the absence of a positive stool culture for pathogenic bacteria, ova or parasites [54, 63]. As with CD, evidence for a specific transmissible agent in UC aetiology is weak, and it is possible that a number of members of the commensal microbiota may be linked to the disease [36]. However, because of their abilities to produce large amounts of sulphide, which inhibits butyrate metabolism and is highly toxic to the colonic epithelium [64], there may be a role for dissimilatory sulphate-reducing bacteria in either the causation or maintenance of UC [65, 66].

4 Mucosal populations

In numerical terms, the vast majority of intestinal bacteria occur in the gut lumen, however, it is becoming increasingly evident that distinct microbial communities establish in biofilms on mucosal surfaces in the large bowel [67, 68]. In quantitative terms, there is considerably more microbial cell mass in gut lumen, however, mucosal communities exist in close juxtaposition to host tissues and it is likely that they interact to greater degree with the gut immune system than luminal organisms. Many cellular components of intestinal microorganisms have strong immunomodulatory properties, especially peptidoglycans, lipoteichoic acids and endotoxic lipopolysaccharides [69], which affect the activities of intestinal epithelial cells, blood leukocytes, B and T lymphocytes and other accessory cells of the mucosal immune system [70].

Relatively few studies have been made on mucosal communities in humans, however, evidence from human studies has also suggested that mucosal bacterial populations in UC may be altered towards a more proinflammatory phenotype [71, 72]. Microbiological sampling of rectal biopsies taken during endoscopy from nine patients with active UC, and 10 noninflammatory controls, resulted in the isolation of 72 different bacterial species, belonging to 18 genera [68]. Comparison of the UC patients and noninflammatory controls indicated that only 20 species were common to both groups. However, detailed population analysis showed that only the differences in mucosal bifidobacterial numbers, which were 30-fold lower in UC, were statistically significant ($p = 0.005$). Mucosal peptostreptococci were only detected in UC patients, who also had proportionally more facultative anaerobes than the controls. It was concluded that interindividual variations in mucosal biofilm composition made it difficult to assign a role for specific bacteria or groups of organisms in UC aetiology, although reduced numbers of bifidobacteria and increased Gram positive cocci may implicate involvement of these organisms at some stage of the disease.

The increased understanding of the gut microbiota and its physiological and metabolic importance to the host that has developed in recent years has highlighted the importance of mucosal bacteria in regulating immune responses in the gut. Manipulation of the mucosal microbiota to reduce its inflammatory potential is therefore an attractive therapy for UC. One option is to use antibiotics to remove species involved in inducing the inflammatory response, however, antibiotic therapy has had limited success in UC [73]. This has led to a series of animal and human-based studies using so-called functional foods to change the bacterial species composition of the microbiota in IBD. However, while there are many reports of probiotic use in IBD in the literature [74 – 77], very few studies have been carried out using prebiotics or synbiotics.

5 Prebiotics and IBD

5.1 Animal studies

A series of animal studies have been carried out in recent years to determine the effects of different prebiotics in colitis. In the investigation by Videla *et al.* in 2001 [78], rats

with dextran sodium sulphate (DSS)-induced colitis were fed 400 mg of inulin *per* day, either orally in their drinking water at 1% w/v, or 400 mg/day by oral gavage or rectal enema, mixed in 5 mL of saline. Controls either received no treatment or saline gavage or enemas. Ninety five animals were involved in the investigation, and at the end of the study period, rats that had received the prebiotic orally had significantly less mucosal inflammation ($p < 0.05$), as well as reduced release of the inflammatory mediators prostaglandin E2, thromboxane B2 and leukotriene B4 ($p < 0.05$), together with lower tissue myeloperoxidase activity ($p < 0.05$).

This was followed up the following year by an investigation in which rats were fed either whey-derived or lactose-derived GOS for 10 days, before the induction of colitis with trinitrobenzene sulphonic acid (TNBS). The animals were examined 72 h later. The GOS-fed rats were shown to have increased levels of faecal bifidobacteria, but no reduction in the severity of inflammation [79].

In 2003, there were a further two studies involving the use of prebiotics in colitis. The first investigation involved 72 Sprague–Dawley rats with DSS-induced colitis, that were fed 60 g of either a basal diet, resistant starch (RS) or FOS [80]. Results showed that there was no change in body weight over the study period, however, the FOS and RS-fed rats had significantly reduced inflammatory histological scores in the caecum ($p < 0.05$), and a significantly reduced score in the colon for animals receiving the RS diet only ($p < 0.05$). The second study used 82 rats with TNBS-induced colitis and compared the therapeutic value of prebiotics with probiotics [81]. The animals were given intragastric infusions of either FOS, 10^{11} CFU of a lactic acid bacteria probiotic containing *Lactobacillus acidophilus*, *L. casei* subsp. *rhamnosus* and *Bifidobacterium animalis*, or a control of 9 g/L NaCl for 14 days. Both prebiotic and probiotic diets had similar effects, with significant reductions in the gross score of inflammation ($p < 0.001$), reduced myeloperoxidase activities ($p < 0.001$), reduced gut pH ($p < 0.001$) and increased faecal lactate ($p = 0.02$) and butyrate concentrations ($p < 0.001$). Other studies using Wistar rats with DSS-induced colitis involved giving the animals either lactulose (300–1000 mg/kg/day) or aminosalicylic acid (5-ASA; 150 mg/kg/day) orally for 6 days [82]. The prebiotic was found to ameliorate colitis in a dose-dependent fashion, while 5-ASA reduced the severity of colonic lesions in comparison to nontreated rats.

Studies carried out on the protective effects of lactulose in the TNBS model of rat colitis showed that 14 days consumption of lactulose significantly reduced myeloperoxidase activity, colonic TNF-α and leukotriene B4 production, and increased levels of lactobacilli and bifidobacteria in the gut [83]. Hoentjen *et al*. [84] gave HLA-B27 transgenic rats Synergy I, which is a combination of inulin and oligofructose, prior to clinical detection of colitis, and then assessed the animals after 7 wk. The prebiotic-treated rats had significantly reduced gross caecal ($p < 0.005$) scores and caecum ($p < 0.01$) and colonic ($p < 0.005$) inflammatory histology scores, as well as lowered caecal IL-1β ($p < 0.05$), while transforming growth factor beta (TGF-β) was increased ($p < 0.05$). The prebiotic markedly stimulated growth of lactobacilli and bifidobacteria in the caecum, and decreased IFN-γ ($p < 0.01$) in mesenteric lymph nodes.

More recently, 20 rats with DSS-induced colitis had 5% of their diet converted to goats milk oligosaccharides [85]. Animals given the additional oligosaccharides did not lose weight ($p < 0.05$) and were shown to have less severe clinical symptoms and inflammation compared to DSS-treated controls ($p < 0.05$).

5.2 Human studies

There have only been three randomised controlled trials (RCT) involving the use of prebiotics in human IBD, two in CD and one in ileal pouch-anal anastomotic disease, all of which were underpowered, but showed promising results. Pouchitis is a chronic, relapsing inflammatory condition that often occurs in the ileal pouch following proctocolectomy for UC. Pouchitis patients respond in a positive clinical manner to probiotics [86], and one successful study is reported of prebiotics for this condition [87]. In a randomised double-blind crossover study, 24 patients with stable asymptomatic pouchitis were given 24 g of inulin dissolved in a commercially available milk-based beverage (Nutridrink®, Nutricia Nederland B. V.) or placebo daily, for 3 wk each [87]. At the end of the prebiotic period, results showed that there was a reduction in the endoscopic and histological pouch disease activity index (PDAI) score, together with lower faecal pH, reductions in faecal *Bacteroides fragilis* ($p = 0.05$) and secondary bile acids. Butyrate concentrations were increased, while symptom scores were low initially and were essentially unchanged. In 2003, Hussey *et al*. [88] did an open-label human trial involving ten children with CD. In a 6 wk prospective, nonrandomised, open-labelled pilot study, children with active disease were given, as their sole source of nutrition, a whey protein, FOS and inulin-containing formula (Peptamen® with Prebio), *via* nasogastric feeding. The children gained weight significantly ($p < 0.0001$) and had significantly reduced ($p < 0.0001$) Crohn's disease activity indices (CDAI), with nine out of ten children having scores indicating little or no disease activity, together with markedly reduced erythrocyte sedimentation rates ($p < 0.01$), a nonspecific biochemical marker of inflammation.

Lindsay *et al*. [89] conducted an open-label study using ten patients with active CD, all of whom were given 15 g of FOS for 3 wk. The Harvey-Bradshaw index, a simplified version of the CDAI, was reduced markedly ($p < 0.01$), and the patients had increased faecal bifidobacterial numbers ($p < 0.001$). There was also an increase the percentage of IL-10 positive dendritic cells ($p = 0.06$), and the percentage

of dendritic cells expressing toll-like receptor (TLR2) and TLR4 ($p = 0.08$ and $p < 0.001$, respectively). TLRs are receptor cells that are activated by microbial ligands to induce and control immune responsiveness. Bifidobacteria in particular has been linked to TLR-4.

Instead of using a fermentable carbohydrate to stimulate the growth of beneficial bacteria in the gut, a growth stimulating factor (1,4-dihydroxy-2-naphoic acid) produced by *Propionibacterium freudenreichii* was used to increase bifidobacterial numbers in a recent nonrandomised open label trial involving 12 UC patients [90]. The patients received 4.5 g of the prebiotic daily for 4 wk. Clinical activity index scores decreased from 7.4 to 4.7, and endoscopy scores from 4.4 to 2.8. Faecal butyrate excretion was increased significantly, but the authors found no differences in bifdobacterial numbers.

6 Synbiotics and IBD

6.1 Animal studies

The only animal study using a synbiotic (SIM) was reported by Schultz *et al*. [91]. SIM consisted of *L. acidophilus* La-5 and *Bifidobacterium lactis* Bb-12 (10^9 cfu/mL) and linulin, and HLA-B27 transgenic rats were randomised to SIM or water for 4 months. Control rats were given metronidazole with or without SIM. Animals fed SIM were reported to have increased biodiversity in the gut microbiota, analysed by PCR combined with denaturing gradient electrophoresis, with a particular increase in detection of *B. animalis*, although there was no detection of any of the added SIM probiotic. Histologically there was a significant improvement in colonic inflammation in the SIM rats at the end of the study ($p < 0.03$). Since the probiotic was not detected, it is plausible that the prebiotic (inulin) may have been responsible for the outcomes found in the study, which would indicate the need for clinical trials involving the use prebiotics alone, as well as in conjunction with probiotics in IBD.

6.2 Human studies

A 4-wk double-blinded RCT was undertaken by Furrie *et al.* [92] in which 18 patients with active UC were enrolled. The test group comprising nine individuals was provided with a synbiotic consisting of 12 g of oligofructose-enriched inulin (Synergy 1) and capsules containing 2×10^{11} freeze-dried *B. longum per* day. The placebo group were given identical capsules of potato starch and sachets of powdered maltodextrose. Bifidobacterial numbers on the rectal mucosa increased 42-fold in patients given the synbiotic, compared to a 4.6-fold increase in the control group. Synbiotic treatment was accompanied by marked reductions in TNF-α and IL-1α in mucosal tissue, together with inducible HBD 2, 3 and 4. No synbiotic effects were seen with respect to IgA and IgG production, red and white blood cell counts, neutrophil function (respiratory bursts), or IL-10 production, however, sigmoidoscopy scores (scale 0 – 6) were lowered in the synbiotic group (start 4.5 ± 1.4, finish 3.1 ± 2.5) compared to the placebo (start 2.6 ± 2.1, finish 3.2 ± 2.2), while histopathology showed marked reductions in inflammatory cells and crypt abscesses, and regeneration of normal epithelium. The study therefore demonstrated that short-term synbiotic treatment of active UC resulted in improvement in the full clinical appearance of chronic inflammation in these patients.

Very little has been reported on the use of synbiotics to treat the symptoms of CD, however, a randomised, placebo-controlled study was reported recently in which 20 patients were given Synbiotic 2000 postoperatively for 24 months, while 10 subjects served as controls [93]. The synbiotic was taken once daily and comprised four lactic acid bacteria (*Lactobacillus raffinolactis*, *L. paracasei*, *L. plantarum*, *Pediococcus pentosaceus*, each 10^{10}) and four sources of fermentable carbohydrate (RS, b-glucans, pectin, inulin, each 2.5 g). Results showed that the synbiotic had no effect on endoscopic or clinical relapse rates within the two groups. The authors speculated that increasing the numbers of patients and the amount of the synbiotic fed to them might be more effective in preventing recurrence of the disease.

7 Conclusions

IBDs such as CD and UC are associated with high morbidity and incur substantial social, commercial and healthcare costs. Many patients do not respond well to standard therapies, which often have undesirable side-effects, therefore, an inexpensive and effective treatment based on the use of prebiotics or synbiotics could make a significant contribution to relieving the clinical and financial burdens of these diseases. The use of well-designed and tested products to treat UC and CD offers several potential advantages in that they are inexpensive, easy to administer, demonstrably safe, and have no side-effects. The few studies that have been reported on the therapeutic use of prebiotics and synbiotics in IBD to date have shown some promise for the future of this area of research, but more randomised controlled trials with larger patient cohorts need to be undertaken. Considerable care needs to be exercised in the selection and methods of deployment of the therapeutic agents that will be used in these investigations.

With the aetiology of IBD still not fully understood, and the complex nature of mucosal bacterial interactions and the host immune system just beginning to be studied in any detail, the selection of synbiotic combinations needs to be made on the basis of a more rigorous scientific understanding of the predicted immune and bacterial effects. There is no doubt that adequately powered clinical trials are required

to take into account subtypes within the groups, both in terms of disease spectrum and individual microflora differences, because these factors may significantly influence overall results. It seems possible that there will be cohorts within overall patient groups that may see benefits from prebiotics alone or in combination as a synbiotic, but this will not be evident without the establishment of larger clinical trials.

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