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Modulating the microbiota in inflammatory bowel diseases: prebiotics, probiotics or faecal transplantation?

Kristin A. Verbeke[1,2]*, Leen Boesmans[1,2] and Eef Boets[1,2]

[1]*KU Leuven, Translational Research in Gastrointestinal Disorders, Herestraat 49, O&N1, box 701 B – 3000 Leuven, Belgium*

[2]*Leuven Food Science and Nutrition Research Center, Leuven, Belgium*

Crohn's disease (CD) and ulcerative colitis (UC) are the two major phenotypes of inflammatory bowel diseases (IBD) which constitute a spectrum of chronic, debilitating diseases characterised by a relapsing inflammation of the intestinal mucosal lining. Evidence from a variety of disciplines implicates the intestinal microbiota in the pathogenesis of idiopathic IBD and their complications, including pouchitis. Many studies have reported a dysbiosis in IBD, characterised by a decrease in diversity, a decreased abundance of some dominant commensal members (such as *Clostridium* IV and XIVa) and an increase in detrimental bacteria (such as sulphate reducing bacteria and *Escherichia coli*). Therapies such as prebiotics and probiotics aim to selectively manipulate the intestinal microbiota and have been evaluated as an attractive therapeutic option with few side effects. The multispecies product VSL#3 was found effective in preventing and maintaining remission in pouchitis, whereas both VSL#3 and *E. coli* Nissle were effective in maintaining remission in UC. A more drastic approach to restore the composition of the microbiota and correct the underlying imbalance is a faecal microbiota transplantation (FMT). FMT has been successfully applied to treat patients with even recalcitrant *Clostridium difficile* infection. Particularly in UC, the majority of studies suggest that FMT may be an effective treatment option although the evidence is still limited. It is anticipated that our increasing knowledge on the composition and function of the intestinal microbiota components will allow in the future for a better selection of highly performing bacteria with specific functions required for specific benefits.

Prebiotics: Probiotics: Faecal microbiota transplantation: Inflammatory bowel diseases

Inflammatory bowel diseases (IBD) of which Crohn's disease (CD) and ulcerative colitis (UC) are the major phenotypes and are characterised by chronic relapsing and remitting inflammation of the intestinal mucosa. CD and UC typically manifest in young adults with no difference in prevalence between males and females[(1)]. IBD patients present with symptoms, including abdominal pain, diarrhoea, rectal bleeding and weight loss.

The aetiology of IBD is not completely understood but is generally considered to be complex and multifactorial. Abnormal communication between gut microbial communities and the mucosal immune system has been identified as the core defect that leads to chronic intestinal inflammation[(2)]. In one view, the defect lies in the mucosal immune system and results in excessive immunological responses to the microbiota that is qualitatively and quantitatively normal. In another view, changes in the composition of the gut microbiota and/or a deranged epithelial barrier function elicit pathological responses from a normal mucosal immune system[(3)]. Animal models provide evidence for both possibilities. The hypothesis that an altered composition of the gut microbiota plays

Abbreviations: CD, Crohn's disease; CDI, *Clostridium difficile* infection; GPR, G-protein receptor; FMT, faecal microbiota transplantation; IBD, Inflammatory bowel diseases; UC, ulcerative colitis.

***Corresponding author:** K. A. Verbeke, fax +32 16 330723, email kristin.verbeke@med.kuleuven.be

a key role in the pathogenesis of IBD is currently the focus of intensive research[4].

The role of the microbiota and the epithelial barrier in the pathogenesis of inflammatory bowel diseases

The human intestine contains more than 10^{14} bacteria that comprise, according to a recently developed quantitative low-error amplicon sequencing technique, slightly more than 100 different bacterial species[5]. Both human and animal studies have indicated a role for the intestinal microbiota in the onset and perpetuation of IBD. In most models of IBD, the animals remain healthy when raised in germ-free conditions and only develop the disease after colonisation with a commensal pathogen-free microbiota[6]. In CD, it has been convincingly shown that the restoration of the faecal stream induced a recurrence of CD in the excluded colon and ileum[7,8]. In addition, antibiotic treatment has been shown beneficial in at least a subset of IBD patients[9]. This combined evidence has resulted in intensive efforts to discover a specific microbial agent in the cause of IBD. However, there is little support for a single pathogen in the aetiology of IBD, as no consistent evidence has been found that IBD in human subjects is caused by a persistent pathogenic microorganism[10]. In contrast, a common feature in both the intestinal microbiota of UC and CD patients is a reduced diversity of bacterial species and a lower temporal stability of the microbiota composition[11–13]. On the phylum level, the microbiota in IBD patients is characterised by lower numbers of Bacteroidetes and Firmicutes (including *Clostridium* IV and XIVa groups) and a concomitant increase in Proteobacteria and Actinobacteria[4,14,15]. Furthermore, an increase in the family of *Enterobacteriaceae* has been reported in patients with UC[4]. Lower levels of *Faecalibacterium prausnitzii* in the mucosa-associated microbiota were shown in patients with CD[16] and UC[17].

In healthy subjects, the intestinal immune system provides protection mechanisms at multiple levels to maintain homeostasis and to prevent access of this enormous number of resident microbes to the systemic circulation. First, the secretion of mucus, several antimicrobial molecules and IgA by different epithelial cells minimise the chances for the direct contact of bacteria with the epithelial cells. Secondly, commensal microorganisms that have been able to penetrate the epithelial barrier will be rapidly phagocytosed and destroyed by intestinal macrophages. Finally, compartmentalisation is accomplished by unique anatomic adaptations that limit commensal bacterial exposure to the immune system. Some microbes are sampled by intestinal dendritic cells. The loaded dendritic cells traffic to the mesenteric lymph nodes through the intestinal lymphatic vessels but do not penetrate further into the body[18].

A disruption of this dynamic balance between microbes and host response will result in chronic intestinal inflammation and tissue injury and might play a role in the pathogenesis of IBD.

Manipulation of the microbiota with probiotics and prebiotics in inflammatory bowel diseases

Conventional drug therapy in IBD primarily aims to suppress the enhanced immune response to induce or to maintain remission. Commonly-used drugs include corticosteroids, aminosalicylates and immune suppressants such as methotrexate and azathioprine. The development of biological agents such as monoclonal antibodies against TNF-α that target the adaptive immune system, has significantly improved the quality of life of many patients with IBD. Nevertheless, only about one third of the patients will achieve remission and many of the primary responders will eventually lose their response over time. Therefore, development of new therapies remains essential. An overview of emerging and new therapies currently in clinical trials is provided elsewhere[19].

Treatments that manipulate the intestinal microbiota composition and/or activity such as interventions with probiotics and prebiotics might constitute an attractive alternative therapeutic option. Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host[20]. In contrast, the concept of prebiotics aims to stimulate the proliferation of advantageous indigenous bacteria already present in the gut by manipulation of the substrates available to the microbiota. Prebiotics have been defined as selectively fermentable ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health[21].

Probiotics in inflammatory bowel diseases

As early as 1877, Pasteur and Joubert observed an antagonistic interaction between some bacterial strains. In the early 1900s, the Russian scientist Metchnikoff attributed the longevity of rural Bulgarians to the consumption of fermented milk product. However, during the second half of the twentieth century, the focus was more on antibiotics to interfere with intestinal microbiota composition. The growing awareness of the side effects associated with antibiotic use, an increasing fear of antibiotic resistant microbial strains and the fear that industry would not manage to develop new antibiotics at a sufficient rate resulted in a renewed and more general interest in the concept of probiotics and prebiotics[22]. The safety and low burden of side effects of probiotic and prebiotic interventions compared with corticosteroids, immunosuppressants and antibiotics, are particularly appealing. The first study, describing probiotic administration with *Escherichia coli* Nissle 1917 to patients with inactive ulcerative colitis was published in 1997[23]. The probiotic strain was found as effective as the standard therapy mesalazine to keep patients in remission. Since then, probiotics have been mentioned in many review papers as promising new therapy for IBD[24–26], whereas others were much more sceptical[27]. Nevertheless the number of clinical trials that investigated the efficacy of probiotics in IBD remains relatively limited. The promising results obtained with *E. coli*

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Nissle 1917 were later confirmed in two large, randomised controlled trials[28,29]. In contrast, a dose-finding study with *E. coli* Nissle 1997 in ninety patients with mild-to-moderate active UC, only found a higher response rate in the treatment group compared with placebo in the per-protocol analysis but not in the intention-to-treat analysis[30]. The fact that a considerable number of patients was excluded from per-protocol analysis because of major protocol violation or discontinued the study because of lack of efficacy might explain these observations. The most extensively tested probiotic preparation is VSL#3, a highly concentrated mixture of four strains of *Lactobacillus* (*L. casei*, *L. plantarum*, *L. acidophilus* and *L. delbrueckii* subsp. *bulgaricus*), three strains of *Bifidobacterium* (*B. longum*, *B. breve* and *B. infantis*) and one strain of *Streptococcus* (*S. salivarius* subsp. *thermophilus*). In several trials, the potential of VSL#3 was evaluated to induce remission in patients with active UC[31–37], to maintain remission in patients with UC[34,38], to maintain remission or prevent postoperative relapse in patients with CD[39,40] or to prevent or treat pouchitis[41–44]. As the functional properties and benefits of probiotic strains are strain-dependent and cannot be extrapolated to other strains[45], not even strains of the same genus, meta-analysis of trials using different probiotic products should be interpreted very cautiously. However, two recent meta-analyses that performed subgroup analyses per probiotic, indicated a significant benefit of VSL#3 over control in inducing remission in UC (relative risk 1·69 (95 % CI 1·17, 2·43)[46] and relative risk 1·74 (95 % CI 1·19, 2·55)[47]). The trial conducted by Miele[34] also suggested a benefit for VSL#3 in maintaining remission in UC in children, whereas meta-analysis of three trials[41–43] suggested that VSL#3 significantly prevented relapse in patients with pouchitis (relative risk 0·18 (95 % CI 0·10, 0·34)[47]).

Lactobacillus rhamnosus GG was evaluated in a large randomised controlled trial including 187 patients with inactive UC. After 6 and 12 months treatment, the number of subjects remaining in remission was not different in the group receiving *L. rhamnosus* GG compared with a control group that received mesalazine (2400 mg/d) or the group that received the combination treatment[48]. In contrast, the same probiotic did not show clinical benefit over control in the treatment of patients with CD[49–51] and was ineffective as primary therapy in patients with ileal pouch inflammation[52]. Similarly, *Lactobacillus johnsonii* was ineffective in preventing relapse in patients with inactive CD[53,54].

Although Guslandi *et al.*[55] found a significantly lower relapse rate in thirty-two patients with inactive CD after treatment with *Saccharomyces boulardii* with mesalazine *v.* mesalazine alone, a recent randomised controlled trial in 165 patients did not find beneficial effects of *S. boulardii* over placebo[56].

The use of VSL#3 received an 'A' recommendation by Floch *et al.*[57], meaning 'strong, positive studies in the literature' for preventing and maintaining remission in pouchitis as well as for maintaining remission in UC. For the latter indication, also *E. coli* Nissle received an 'A' recommendation.

This differential effect of probiotics in UC *v.* CD may highlight the fact that IBD is a multifactorial disease with a high variety in phenotypes and severity[3]. Indeed, the notion that IBD is actually a syndrome comprising several disease subtypes, is gaining more and more acceptance[58].

The proposed mechanisms of action that might explain the benefits of probiotics in IBD have mainly been studied in *in vitro* and *in vivo* animal experiments. The effects executed by a certain probiotic depend on its metabolic properties, the molecules presented at its surface or on the components secreted[59]. Probiotic bacteria may affect the composition of the intestinal microbiota by competition for adhesion sites and nutrients or by secretion of bacteriocins or acids with antimicrobial activity against Gram-positive and Gram-negative bacteria[60]. In addition, probiotics can enhance the intestinal barrier function by increasing the production of mucus or the production of anti-microbial peptides, such as β-defensins, lysozyme, lactoferrin or phospholipase by the epithelial cells[61]. For example, VSL#3 increased the basal luminal mucin content in rats by 60 % and significantly stimulated colonic mucin secretion and MUC2 gene expression in isolated rat colonic loops[62]. Finally, probiotics may affect the mucosal immune system by increasing the production of intestinal anti-inflammatory cytokines such as IL-10 and transforming growth factor β[63–65], by modulating the NF-κB pathway[66] and by influencing the cross-talk between natural killer cells and dendritic cells[67].

Prebiotics in inflammatory bowel diseases

Clinical studies that evaluated the impact of prebiotics in IBD are scarce. In a small-scale uncontrolled study in ten subjects with active ileocolonic CD, fructo-oligosaccharides were administered in a dose of 15 g/d. After 3 weeks, the Harvey–Bradshaw Index, which is a simplified version of the Crohn's Disease Activity Index score, was significantly reduced and faecal numbers of bifidobacteria were increased. In addition, the numbers of IL-10 positive dendritic cells as well as Toll-like receptors-2 and -4 positive dendritic cells were significantly increased[68]. Based on these promising results, the same group of researchers performed an adequately powered follow-up study in 103 patients with active CD patients[69]. Patients received the same dose of fructo-oligosaccharides (15 g/d; *n* 49) or placebo (*n* 53) for 4 weeks. However, neither in the intention-to-treat nor in the per-protocol analysis, a significant improvement in disease activity was achieved and the levels of faecal bifidobacteria or *F. prausnitzii* were not modified. In addition, the incidence and severity of gastrointestinal symptoms was significantly increased. A similar study was performed independently in sixty-seven patients with inactive and mild-to-moderate active CD[70]. Patients received 2 × 10 g/d oligofructose-enriched inulin (*n* 34) or placebo (*n* 33) for 4 weeks. Similar to Benajmin *et al.* and in contrast to the studies in healthy subjects, oligofructose-enriched inulin did not increase faecal numbers of *F. prausnitzii* or *B. adolescentis*.

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However, a significant increase in *B. longum* and a reduction in *Ruminococcus gnavus* was observed. In the subgroup of patients with active disease (*n* 8), the increase in numbers of *B. longum* was positively correlated to an improvement in disease activity (R 0·894; $P = 0·02$). In addition, metabolomic analysis of the faecal samples confirmed a depletion in butyrate in patients with active CD that was restored to levels comparable with healthy controls after intervention with oligofructose-enriched inulin[71].

Once they reach the colon, prebiotics are rapidly fermented by the resident bacteria. The major metabolites include SCFA (acetate, propionate, butyrate) besides some lactate and gasses such as carbon dioxide, hydrogen and methane. These SCFA, and in particular butyrate, have been considered as crucial mediators that might explain the anti-inflammatory activity of prebiotics. Butyrate activates the nuclear peroxisome-proliferator-activated receptor γ[72] which antagonises several proinflammatory pathways. Activation of peroxisome-proliferator-activated receptor γ has been shown effective in the prophylaxis and to a lesser extent in the treatment of several animal models of acute or chronic colitis[73,74]. Besides, the interaction of butyrate with the G-protein receptor (GPR) 43 also influences the inflammatory response, as GPR43-deficient mice showed exacerbated or unresolving inflammation in models of colitis, arthritis and asthma[75]. GPR43, also known as free fatty acid receptor 2, is a seven-transmembrane receptor largely expressed throughout the gut that is considered a key player in the cross-talk between the gut microbes and the host[76]. In a mouse model, activation of GPR43 by SCFA regulated the size and function of the colonic regulatory T cell pool and protected in this way against colitis[77]. More recently, the niacin receptor GPR109a (also known as the hydroxycarboxylic acid receptor 2) was identified as another receptor on colonic cells important for colonic health. Singh *et al.*[78] showed that GPR109a signalling by butyrate promoted anti-inflammatory properties in colonic macrophages and dendritic cells and enabled them to induce differentiation of regulatory T cells and IL-10-producing T cells.

Other potential protective mechanisms of prebiotic activity include changes in the intestinal microbiota, improvement of the intestinal barrier and regulation of the mucosal and systemic immune responses[79].

Faecal microbiota transplantation

Faecal microbiota transplantation (FMT) provides a more drastic strategy to modify one's microbiota composition and involves the transfer of a faecal suspension from a healthy person into the gastrointestinal tract of a person with colonic disease[80]. In contrast to probiotic administration where the exogenous bacterial strains only transiently populate the intestine, FMT intends to induce a protracted modification of the microbiota. In a study in ten patients treated with FMT for irritable bowel syndrome, constipation or Crohn's colitis, a change in the patients' bacterial populations of the colon to represent those of the healthy donor's microbiota persisted for at least 24 weeks[81].

The first report on FMT in human subjects was by Eiseman and co-workers in 1958 for the treatment of pseudomembranous colitis[82], presumably caused by *C. difficile* infection (CDI). *C. difficile* is a Gram-positive spore-forming bacteria that can be a minor normal component of a healthy microbiota[83] but can become pathogenic when the normal microbiota has been destroyed, typically after the use of a broad spectrum antibiotic. Since that time, >500 additional patients were treated with FMT with success rates of 95 %[80]. More recently, the efficacy of FMT for treatment of CDI was confirmed in a randomised controlled trial[84].

For indications other than CDI, the number of reports is more limited. In IBD, only a small number of case reports or case studies have been reported. The majority of the patients suffered from refractory UC and only a few patients with CD were treated with FMT. However, randomised controlled trials are currently underway (www.clinicaltrials.gov).

The exact mechanism of action of FMT in the treatment of active IBD or CDI is not well known. As both CDI and IBD are characterised by depletions in the normal intestinal microbiota including lower numbers of Bacteroidetes and Firmicutes, it is assumed that FMT acts by reintroducing a complete and stable community of microorganisms that repair or replace the disrupted microbiota and corrects the underlying imbalance[80]. In addition, FMT may also (re)introduce species that produce bacteriocins which eradicate susceptible pathogens. Typically, bacteriocins inhibit the growth of strains closely related to the producer and provide in this way a competitive advantage to strains in a complex ecosystem. An example is the narrow-spectrum Thuricin CD that is produced by *Bacillus thuringiensis* and has activity against *C. diffile*[85].

A meta-analysis including nine reports describing FMT in twenty-six patients with IBD concluded that, although the evidence is limited and weak, the majority of the studies suggest that FMT may be an effective treatment option in IBD[86]. A retrospective analysis of sixty-two patients with UC treated with FMT, reported response to FMT in 91·9 % (fifty-seven/sixty-two) of the patients, of which 67·7 % (forty-two/sixty-two) achieved complete remission and 24·2 % (fifteen/sixty-two) achieved partial response. Eight per cent of the subjects (five/sixty-two) were treatment failures. From the twenty-one patients that underwent repeated colonoscopy, 57·1 % (twelve/twenty-one) had mucosal healing with absence of histological inflammation[87]. In a paediatric study, ten children and young adults with mild-to-moderate colitis received faecal enemas for five consecutive days. Within 1 week, clinical response was shown in 78 % and clinical remission was achieved in 33 %. After 4 weeks, 67 % of the children maintained clinical response[88]. In contrast, in a recent study not included in the earlier mentioned reviews, none of the six UC patients that received a FMT infusion achieved clinical remission, despite a reversal of some of the reported dysbiotic changes in the intestinal microbiota after

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FMT[89]. Importantly, whereas treatment of CDI is most often achieved after a single FMT procedure, treatment of UC generally requires multiple FMT infusions indicating that the FMT response in UC is not as robust as in CDI[90]. Only exceptionally, patients with UC may achieve cure with a single FMT administration. In addition, FMT may provide greater therapeutic benefit in patients whose onset of ulcerative colitis was associated with an alteration in the faecal microbiota due to antibiotic use or concomitant CDI[91]. A potential explanation for the less robust FMT response in the treatment of UC might be that the dysbiosis observed in UC is a downstream result of the disease rather than the cause. In that case, modification of the intestinal microbiota might not be the right mechanism for treatment[92]. Alternatively, it is possible that phylogenetic or functional (in)compatibilities between donor and recipient microbiota might govern the outcome of the FMT[93]. For example, it is not known to date whether (mis)matching of the donor's and recipient's enterotype[94] might be relevant.

FMT is generally well tolerated. Most patients treated with FMT experience diarrhoea on the day of infusion, and a small percentage report belching and/or abdominal cramping or constipation[95]. Some recent studies in IBD reported adverse effects during and after FMT[93,96]. Patients experienced transient fever, increased C-reactive protein levels and diarrhoea on the day after the procedure. These symptoms also disappeared within a few days. A recent case report describes a flare of UC in a patient that had been in remission for more than 20 years, after FMT procedure for treatment of CDI[97]. In a long-term follow-up study in seventy-seven patients (follow-up varying between 3 and 68 months with a mean of 17 months), four subjects developed an autoimmune disease after the FMT although there was no clear relationship between the new disease and the FMT[98]. These reports indicate that we need to remain vigilant to potential complications and possibly also long-term safety effects of FMT.

The optimal protocol for the use of FMT needs to be determined. Several parameters including the selection of the donor, screening of the donor, storage of the donor sample, type of diluents, volume of stool required, way of administration, need for antibiotics and bowel lavage of the recipient have not been investigated in a systematic way. Based on retrospective review of the literature, it seems that in most studies, 50–60 g stool from a related donor is suspended in about 300 ml non-bacteriostatic saline solution and is administered via colonoscopy[99]. Recipients have generally received antibiotics before transplantation although animal studies show that antibiotic pretreatment may not be necessary to improve engraftment of the FMT[100].

In the future, standardisation of the protocol for FMT will likely be increased. It has been shown that stool frozen at −80 °C is equally transplantable to fresh stool[101]. The ability to use frozen material allows us to shift from individual donors selected for each patient to standard volunteer donors and ultimately to banking of frozen processed faecal material that is ready to use when needed[102]. In addition, a pilot study successfully used a synthetic stool mixture to treat two patients with CDI. The stool mixture consisted of thirty-three well-characterised isolates representing commensal species that were generally sensitive to a range of antimicrobials and were relatively straightforward to culture[103]. Such a stool substitute offers the advantage that the exact composition of the administered bacteria is known and that the preparation can be extensively tested and controlled and can be reproduced for future treatment.

Conclusion

Manipulation of the microbiota composition has been considered as a promising and safe alternative to the currently used drug therapies in IBD that aim at suppression of the inflammatory response. However, to date, this strategy has not fully lived up to expectations.

Promising results obtained in animal models could not always be translated into human subjects. A potential explanation might be that modulation of the microbiota has limited potential once the disease has developed and should be done earlier in life[104]. Indeed, in many animal studies, the probiotics are administered before the development of the inflammation[99,100], whereas patients are only treated after the occurrence of symptoms. Interfering with the microbiota might be more efficient to prevent diseases such as IBD when it is performed during the period of bacterial colonisation and mucosal barrier development[104].

Nevertheless, our increasing knowledge on the composition and function of the intestinal microbiota components opens perspectives for a better selection of highly performing bacteria with specific functions required for specific benefits.

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Conflicts of Interest

None.

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Authorship

K. V. wrote the manuscript. K. V., L. B. and E. B. performed the literature search and interpreted the data. K. V., L. B. and E. B. critically revised the manuscript.

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