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Probiotics and prebiotics in inflammatory bowel disease: microflora 'on the scope'

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The intestinal microflora is a large bacterial community that colonizes the gut, with a metabolic activity equal to an organ and various functions that affect the physiology and pathology of the host's mucosal immune system. Intestinal bacteria are useful in promotion of human health, but certain components of microflora, in genetically susceptible individuals, contribute to various pathological disorders, including inflammatory bowel disease. Clinical and experimental observations indicate an imbalance in protective and harmful microflora components in these disorders. Manipulation of gut flora to enhance its protective and beneficial role represents a promising field of new therapeutic strategies of inflammatory bowel disease. In this review, we discuss the implication of gut flora in the intestinal inflammation that justifies the role of probiotics and prebiotics in the prevention and treatment of inflammatory bowel disease and we address the evidence for therapeutic benefits from their use in experimental models of colitis and clinical trials.

Introduction

Inflammatory bowel disease (IBD) consists of two distinct clinical forms, ulcerative colitis (UC) and Crohn's disease (CD), with unknown aetiology, which nevertheless are considered to share almost identical pathophysiological backgrounds. Current reviews summarize the factors initiating and perpetuating IBD, from four basic viewpoints: genetics, immune dysregulation, barrier dysfunction and role of the microbial flora [1]. The interplay of the intestinal microbes with the mucosal environment, in susceptible individuals, triggers a cascade of reactions that start with the interaction of microbes and their components with the intestinal epithelial cells and dendritic cells via receptors, followed by an interaction of antigen-presenting cells and mucosal lymphocytes, lymphocytes and vascular endothelial cells, and lymphocytes and granulocytes, producing inflammatory mediators and leading to mucosal damage [2–5]. With this in mind, research is underway in these fields, with the ultimate purpose of generating new therapies.

No specific microbe has been proved to cause IBD, despite the fact that several microorganisms have been investigated as implicating factors in the aetiopathogenesis of IBD. These include *Mycobacterium paratuberculosis* [6], *Listeria monocytogenes*, *Chlamydia pneumoniae* [7], *Escherichia coli* and other bacteria [8], and cytomegalovirus [9], but none of the aforementioned has been linked directly with the process. Today more attention is paid to the dynamic balance between intestinal bacteria, particularly commensal flora and host defence mechanisms at the intestinal mucosa, and to their role in the initiation and maintenance of intestinal inflammation [10]. There is strong evidence that changes in the bowel bacterial flora due to environmental or diet factors are of paramount importance in the pathogenesis of IBD [11]. In addition, the role of microflora in intestinal disorders is supported by findings that probiotics can ameliorate IBD or use of antibiotics could benefit certain subsets of IBD patients [12, 13]. This knowledge has led to new therapeutic strategies that target the microflora of patients with IBD using agents such as probiotics, prebiotics and synbiotics, ranging from simple carbohydrates to genetically engineered bacteria with the role of secreting immunoregulatory cytokines.

The microflora: who are 'they'?

The fetal gut is sterile, and colonization with bacteria is sustained by contact between the child and its environment, depending on the mode of delivery [14, 15], hygiene levels [16], medication [17] and type of feeding, as differences in gut microflora composition occur between breast- and formula-fed infants [18, 19]. The bacterial load of the bowel consists of 'native' species that permanently colonize the intestine (fairly stabilized until the fourth year of life) [20] and transient bacteria that are continuously ingested from the external environment. Bacteria play a tremendously important role in the maturation of the gut immune system, as it has been demonstrated in animals

bred in a germ-free environment [21–23], which exhibit crypt hyperplasia, lack of lymphoid follicle development and other structural changes.

Bacteriological cultures at first, and then nucleic acid-based methods [polymerase chain reaction, 16S rRNA probing, fluorescence *in situ* hybridization (FISH)] were used to elucidate the microbial content of the intestinal lumen. Gastric acid, bile and pancreatic secretions result in decreased colonization of the proximal small intestine by most bacteria, but bacterial density increases in the distal small intestine and large bowel. The contents and faeces of the large bowel contain about 10^{11}–10^{12} bacterial cells per gram of wet weight, whereas bacteria form about 50–60% of the faecal mass. This luminal bacterial population composes the faecal microbiota. In healthy humans, four phyla (Bacteroeidetes, Firmicutes, Actinobacteria and Proteobacteria) and three groups (*Clostridium coccoides* group, *C. leptum* subgroup and Bacteroides-Prevotella group, all containing many genera and species) are present in faeces [24, 25]. There are also Fungi and Archaea, but these comprise a very small percentage of the total amount (0.05% and 1%, respectively) [26, 27].

Apart from the faecal microbiota, other studies have been implemented to clarify the identity of bacteria present on the mucosal surface, suggesting that they represent a different population that is in direct interaction with the intestinal cells and the mucosal immune system of the gut. This bacterial population consists of the mucosal microbiota [28]. The above-mentioned concern the bacteria, and other studies were made to clarify the bacteria present on the mucosal surface. Swidsinski *et al.* used FISH with a wide selection of probes in biofilms from biopsies from the ileum, ascending and sigmoid colon, showing the presence of *Eusobacterium rectale*, *C. coccoides*, Bacteroides-Prevotella, *Bacteroides fragilis* and *Fusobacterium prausnitzii* groups only, in the mucosa of controls [29]. Mylonaki *et al.* identified Bifidobacterium as the predominant genus, followed by Lactobacilli and Bacteroides in specimens from colorectal biopsies of controls [30].

It has been observed that almost as much as 50% of bacteria traced with molecular methods could not be cultured in the laboratory using standard techniques. This reveals the difficulties encountered in attempting to identify all microorganisms making up the microflora [31]. The presence of mucus, which is of great importance in the nesting of the commensal flora, must be taken into account in the process of identifying bacteria. As Tannock has pointed out, DNA-based techniques 'reveal only who might have been there' [20]. Furthermore, RNA-based techniques detect bacteria that are metabolically active at the time of the experiment. Therefore, the use of function-revealing techniques such as transcriptomics and proteomics is necessary in order to reveal the exact role of the microflora. Moreover, it has been suggested that, besides recognizing the presence of a certain species of bacteria, it is necessary to know its exact position in the infrastructure of the microflora, thus introducing the term 'spatial organization of the mucosal flora' [29].

Interactions between microflora and gut mucosa

Certain cell populations in the intestinal mucosa continuously monitor the gut flora, recognize pathogens and transfer signals to other immune cells that trigger inflammation or help to avoid inadequate stimulation, by two major host pattern recognition receptor (PRP) systems, the Toll-like receptors (TLRs) and the nucleotide oligomerization domain (NOD). Intestinal epithelial cells recognize microbes and their products via TLRs and subsequently activate the host immune system and protective mechanisms that allow differentiation between commensal or pathogenic microorganisms [5]. Dendritic cells have been found to project long processes through the intestinal epithelial cells to sample luminal microbial products, which leads to direct dendritic cell–microbe contact via TLR receptors [32]. Recognition of TLR ligands activates immature $CD11c^+$ $CD11b^+$ dendritic cells to secrete interleukin (IL)-23, a dominant driver of inflammation in murine models of colitis [33]. TLRs are expressed in myeloid cells and the Golgi apparatus of intestinal epithelial cells, playing a key role in the recognition of bacterial lipopolysaccharides (LPS) and inducing the secretion of inflammatory cytokines and activation of nuclear factor (NF)-κB [34]. In IBD, a different expression pattern of TLRs on intestinal epithelial cells has been cited, whereas TLR-4 expression is upregulated in CD [35]. TLR-9, participating in the recognition of bacterial DNA, has been implicated by Rachmilewitz *et al.* as the site through which the anti-inflammatory effect of probiotics is expressed, in experimental colitis [36].

Another fact that contributes to our understanding of the intriguing role of the microflora in the initiation and perpetuation of IBD is the knowledge that mutations in bacteria-recognizing proteins of intestinal (*inter alia*) cells are associated with IBD. In particular, Hugot *et al.*, Ogura *et al.* and Hampe *et al.* have independently recognized the *NOD2* gene as strongly linked with CD [37–39]. NOD2, otherwise known as caspase activating recruitment domain 15 (CARD15), is an intracellular protein found in monocytes, dendritic cells, Paneth cells and intestinal epithelial cells that belongs to a superfamily of apoptotic protease activating factor 1 (Apaf-1)-related proteins, which are correlated to signalling of apoptosis and activation of the NF-κB pathway [40–42]. CARD15 is important in the recognition of bacterial peptidoglycans mainly found in Gram-negative bacteria through the binding of muramyl dipeptide, leading to NF-κB activation [43], and in the modulation of the secretion of defensins by Paneth cells [44, 45]. Three main variants of NOD2/CARD15 have been investigated and associated mainly with CD, whereas there

is poor linkage to UC [45]. Recently, *CARD15* mutations have been identified as an independent risk factor for IBD [46]. Many studies have focused on elucidating the mechanisms that facilitate differentiation between harmless commensal flora and pathogenic bacteria that help to avoid inadequate stimulation of intestinal inflammation, but an analytic presentation of these is beyond the scope of this review.

The role of microflora in IBD

Clinical and experimental facts have led to the assumption that bacteria in general and commensal microflora more specifically play a key role in the onset and perpetuation of IBD. Harper *et al.* in 1985 showed that reintroducing the small bowel effluent of patients with CD treated with split colostomy, rather than its sterile ultrafiltrate (which contained no bacterial cells or other great particles), into the 'hibernating' colon induced inflammation of the area. Furthermore, diversion of the faecal stream in CD reduces inflammation of the gut and induces healing in the excluded part of the bowel, whereas pouchitis does not occur before ileostomy takedown [47]. Another observation which strongly supports this theory is the predominance of IBD lesions in areas of the highest bacterial exposure, i.e. terminal ileum, colon and ileal pouches. Antibiotics have been proven of some value in treating IBD [13, 48, 49], even though there is evidence of actions other than purely antimicrobial, as demonstrated by experimental models of inflammation and ciprofloxacin [50].

The role of bacteria in IBD is strongly corroborated by germ-free animal experiments. Genetically engineered (transgenic and gene knock-out) rats exhibit chronic intestinal inflammation under standard laboratory conditions, but fail to do so when raised in a germ-free environment [51–53], and chemically induced colitis with trinitrobenzene sulphonic acid (TNBS) occurs in normal rats, but not in rats previously treated with antibiotics [54]. Moreover, adding different species of bacteria or different LPS parts from the same strain in transgenic animals invokes different lymphocyte subpopulation activation [55]. These findings suggest that not all bacteria trigger the same antigenic stimulation, which implies that treatment with probiotic strains depends primarily on the strain chosen.

The faecal, as well as the mucosal microbiota has been found to differ between healthy subjects and IBD patients [20]. Although results are conflicting about the dominant species in each case, one may say that there are distinct characteristics that differentiate the microbiota colonizing the tract of patients with IBD in comparison with that of healthy people. There is higher biodiversity of species in healthy subjects [56], and dominant species comprise about 90% of the total bacterial population, whereas in IBD patients biodiversity is lower and there is a high percentage (almost 30%) of 'unusual' species [57]. It has also been shown that healthy subjects are characterized by a higher percentage of Firmicutes [58]. Conte *et al.* have recently published their data comparing paediatric patients, which support the accumulating evidence of microflora alterations in IBD [59]. In healthy people, equilibrium exists inside the gastrointestinal tract between protective and harmful bacteria. The term dysbiosis has been introduced to suggest that this equilibrium is broken in IBD, resulting in chronic intestinal inflammation [60].

Human milk is the prototype and best synbiotic known. It contains many biologically active components, amongst which are proteins used to inhibit growth of pathogenic bacteria and viruses such as lactoferrin and IgA [61], lactic acid bacteria shown to be of endogenous origin and not contaminants from the breast skin [62], oligosaccharides with a clear prebiotic effect [63], antioxidants, epithelial growth factors, cellular protective agents and enzymes that degrade mediators of inflammation [64]. The effect of human milk feeding in premature infants is clearly beneficial, with many studies demonstrating its excellence, in comparison with artificial formulas, in attenuating late-onset sepsis, necrotizing enterocolitis, diarrhoea and urinary tract infections [65–67].

Statistics tell us that IBD is increasing in Western countries, whereas in developing countries, where sanitation levels are low, it is much less common. This phenomenon includes not only IBD but also atopic diseases, as supported by studies that have detected differences in the strains of the faeces from children with low prevalence of atopic diseases from developing countries compared with children with high prevalence of atopic diseases from Western countries [68, 69]. The Hygiene Hypothesis, based on the fact that lifestyle has changed from rural or semirural to purely industrial in Western countries, where interaction with environmental bacteria is not favourable and sometimes not desirable, poses the question: are infections in early childhood the key to the formation of the intestinal microflora and thus the modulation of the immune system as an entity?

Pro-, pre- and synbiotics in the treatment of IBD

The accumulating knowledge that microbiota modulates gut physiology and immunological function in IBD, described above, has led scientists to investigate the efficacy of probiotics, prebiotics and synbiotics in the treatment of IBD. This therapeutic strategy aims to restore the balance of the gastrointestinal microflora in order to reduce or prevent intestinal inflammation. Several microbial strains, carbohydrate mixtures and their combinations have been tested in experimental models and clinical trials, and their results in the therapeutic manipulation of bowel microbiota will be summarized in the following paragraphs.

Probiotics

Probiotics are living microorganisms, able to survive stomach acid and bile, maintain viability throughout extended periods of storage, and safe for human consumption, inducing beneficial results in the host [70]. Several mechanisms of action of probiotics relative to prevention and treatment of IBD have been reported (Table 1), such as antimicrobial activity and suppression of bacterial growth, immunomodulation and initiation of an immune response, enhancement of barrier activity, and suppression of human T-cell proliferation [71–75]. Probiotics have also been found to induce their effect by means of their DNA, as shown by experiments using probiotic DNA [36, 76, 77] and subcutaneous administration of probiotic DNA [78]. Derived originally from cultured food, especially dairy products, this group includes Lactobacillus species, Bifidobacterium species, *E. coli* Nissle 1917 (a nonpathogenic *E. coli* strain), *Saccharomyces boulardii*, *C. butyricum*, VSL#3 and *Lactococcus lactis* genetically engineered to secrete IL-10. An interesting new approach to what we could call a probiotic is helminths.

Table 1

The effects of the probiotics on the mechanisms of intestinal pathophysiology

Probiotics	Effects on intestinal pathophysiology
Lactobacillus	Inhibition of NF-κB nuclear translocation, blockage of IκB degradation (*L. reuteri*) Inhibition of production of IL-6 (*L. casei*) Upregulation of intestinal MUC3 and MUC3 mRNA expression Inhibition of apoptosis of intestinal epithelial cells (L. GG) Decreased translocation of commensal bacteria via the mesenteric lymph nodes and liver (*L. plantarum*, L. GG) Induction of COX-2 expression (*L. rhamnosus*)
Bifidobacterium	Suppression of the growth of *Bacteroeides vulgatus* (*B. infantis*) Increase in IL-10 secreted by mesenteric lymph nodes (Bifidobacterium-fermented milk) Reduction of MPO activity, tissue contents of immunoglobulin, TNF-α (Bifidobacterium-fermented milk) Alteration of bacterial translocation and SCFA production (*B. infantis*) Inhibition of disorderd T-cell activation
***Escherichia coli* Nissle 1917**	Downregulation of the expansion of newly recruited T cells into the mucosa Intestinal inflammation regulation via TLR-2 and TLR-4 Restoration of disrupted epithelial barrier in the colonic epithelial cell line T84
Saccharomyces boulardii	Limitation of infiltration of T-helper 1 cells into the mucosa NF-κB blocking and IL-8 downregulation
Clostridium butiricum	Production of high levels of short chain fatty acids
VSL#3	Reduction of secretion of TNF-α and interferon-γ Improvement of the colonic barrier function Inhibition of Salmonella Dublin invasion into T-84 cells Convertion of linoleic acid into conjugated linoleic acid Inhibition of TNF-α-induced IL-8 secretion, mitogen-activated protein kinase activation and NF-κB activation in HT-29 cells (CpG DNA) Upregulation of mucin expression
Helminthes	Skewing of the immune response towards Th2

Lactobacillus

Lactobacilli have been used in several experimental models and clinical trials to examine their effect on gut pathophysiology. In experimental models, various Lactobacillus strains evoke differential regulation of a number of genes involved in essential physiological functions such as immune responses [71, 79] and attenuate damage to the colon by acetic acid and methotrexate [80]. *Lactobacillus casei* inhibits production of IL-6 in LPS-stimulated large intestinal lamina propria mononuclear cells and downregulates nuclear translocation of NF-κB in SAMP1/Yit mice [81]. Several Lactobacillus strains upregulate intestinal MUC3 and MUC3 mRNA expression [82]. *Lactobacillus rhamnosus* was found to induce cyclooxygenase-2 expression in human T84 colon epithelial cells [83] and *Lactobacillus paracasei* ssp. *paracasei* B21060 suppresses human T-cell proliferation [75]. Lactobacillus GG improves intestinal barrier function by inhibition of apoptosis of intestinal epithelial cells [84], prevents recurrence of colitis in HLA-B27 transgenic mice after antibiotic treatment [85] and attenuates TNBS-induced colitis [86]. Benefit has been shown with oral administration of *Lactobacillus salivarius* spp. *salivarius* CECT5713 in TNBS-induced colitis [87, 88]. Dextran sulphate sodium (DSS)-induced colitis was attenuated by daily administration of *Lactobacillus crispatus*, *Lactobacillus plantarum* and Lactobacillus GG in various animal models [89–91]. In IL-10 knock-out mice, *Lactobacillus plantarum* 299v [92] and *Lactobacillus salivarius* ssp. *salivarius* UCC118 [93] have been shown to reduce intestinal inflammation.

Despite its favourable results in experimental models of IBD, Lactobacilli have not been proven to induce remission in either UC or CD patients in various clinical settings [94–98]. The only exception is an open trial in children with CD [99]. In terms of maintenance of remission only one study has shown prolongation of the relapse-free time in UC patients by administration of Lactobacillus GG [94]. On the other hand, no benefit has been shown in maintenance of either medically or surgically induced remission in CD [95, 96].

Bifidobacterium

Bifidobacterium infantis has been shown, *in vitro* and *in vivo*, to suppress the growth of *Ba. vulgatus* [100], and to attenuate intestinal inflammation in IL-10 knock-out mice [93]. Various Bifidobacterium strains (*breve*, *catenulatum*, *longum* and *infantis*) resulted in amelioration of intestinal inflammation in DSS-induced colitis in mice [101, 102]. Bifidobacterium-fermented milk (*Bi. breve*, *Bi. bifidum*, *Lactobacillus acidophilus*) administration in SAMP1/Yit mice led to reduction of histological injury scores, ileal tissue weight, myeloperoxidase activity, tissue contents of

immunoglobulin, tumour necrosis factor (TNF)-α, and increases in IL-10 secreted by mesenteric lymph nodes [103]. Another recent study has demonstrated that mice fed with skim milk containing 0.3% (w/w) *Bi. bifidum* did not develop $CD4^{(+)}$ $CD45RB^{(high)}$ T-cell-mediated IBD compared with mice fed with skim milk without the probiotic [104]. In clinical trials, Bifidobacterium-fermented milk was found to reduce the UC activity index and exacerbation of symptoms compared with placebo [105, 106]. On the other hand, Bifidobacterium-fermented milk used by Laake *et al.* in an open trial with 10 patients suffering from active pouchitis demonstrated no benefit [107].

Escherichia coli *Nissle 1917*

The probiotic Nissle 1917 (EcN) is an *E. coli* strain that has been used for decades in Central Europe for the treatment and prevention of intestinal disorders. In experimental conditions, *E. coli* Nissle 1917 downregulates the expansion of newly recruited T cells into the mucosa and limits intestinal inflammation via TLR-2 [108] and TLR-4 [109] pathways. DSS-induced colitis in mice is prevented by administration of soluble bacterial antigens from this nonpathogenic *E. coli* strain [110]. Recently, Zyrek *et al.* have shown *in vitro* that EcN restores the disrupted epithelial barrier in the colonic epithelial cell line T84 [111]. In two clinical trials, *E. coli* Nissle 1917 was found equivalent to mesalamine at attaining [112], as well as maintaining remission of UC for 12 months [113]. In maintenance of remission of CD, no difference was found in remission between *E. coli* Nissle 1917 and placebo [114], but it should be noted that there is strong evidence for the effect of *E. coli* Nissle 1917 in the maintenance of remission of UC [115, 116].

Saccharomyces boulardii

Saccharomyces boulardii is a nonpathogenic yeast used for treatment of diarrhoea. This yeast was recently shown to attenuate the migration of T-helper 1 cells into the mucosa, altering the cascade of cytokines [117] and also to produce a low-molecular-weight factor blocking NF-κB activation and IL-8 expression [118]. Combination of *S. boulardii* with mesalamine was found to induce a significant prolongation of CD remission [119] and to have a successful outcome in patients with active UC [120].

Clostridium butyricum

Clostridium butyricum, an enterobacterium, produces high levels of short chain fatty acids that have been reported to be important in intestinal physiology. Two studies have been reported in rodents, to examine the effect of this microorganism. In the first, a *C. butyricum* derivative was tested in a DSS-colitis model successfully [121], while Okamoto *et al.* studied the M588 strain and demonstrated that it attenuated intestinal inflammation and suggested that oral administration of *C. butyricum* may be useful instead of butyrate enema in the treatment of UC [122].

Vsl#3

VSL#3 is a probiotic preparation consisting of four strains of lactobacilli (*acidophilus*, *bulgaricus*, *casei*, *plantarum*), three strains of bifidobacteria (*breve*, *infantis*, *longum*), and *Streptococcus thermophilus* that are normal components of the human gut microflora. Administration of this mixture to IL-10 knockout mice reduces intestinal inflammation and secretion of TNF-α and interferon (IFN)-γ from the mucosa and improves the colonic barrier function [123]. It has been shown to inhibit Salmonella Dublin invasion into T-84 cells both *in vitro* and *in vivo* [124] and to convert linoleic acid into the anti-inflammatory conjugated linoleic acid [125]. Non-methylated genomic DNA (CpG) extracted from VSL#3 inhibits TNF-α-induced IL-8 secretion, mitogen-activated protein kinase activation and NF-κB activation in HT-29 cells, and attenuates intestinal inflammation in murine models of colitis through the TLR-9 receptor [36, 76]. This mixture has recently demonstrated a role in potentiating mucin expression in experimental models [126]. However, in a mice model of DSS colitis the modification of microflora by supplementation with the VSL#3 did not repair the colonic barrier breakdown and did not heal chronic DSS-induced colitis [127].

Three double-blind randomized controlled trials evaluating the use of VSL#3 in remission of pouchitis have shown that daily administration of VSL#3 after induction of remission by antibiotics [128, 129] or immediately postsurgically [12] prevented relapse of chronic pouchitis compared with placebo groups. On the other hand, Shen *et al.* found no efficacy of VSL#3 treatment in patients with antibiotic-dependent pouchitis [130]. Data from other clinical trials suggest that VSL#3 is effective in the treatment and maintenance of active UC, without adverse effects [131, 132]. The use of VSL#3 for the treatment of IBD-related arthralgia has shown promising features in experimental models and preliminary clinical studies [133]. Despite contradictory data from a number of studies, there is reasonable evidence for the effect of VSL#3 in preventing the recurrence of pouchitis [115, 116, 134].

Genetically engineered Lactococcus lactis

Lactococcus lactis is a food-grade bacterium, known from cheese production, which can be genetically engineered to constantly secrete satisfactory amounts of bioactive cytokines [135]. Transgenic *Lactococcus lactis*, modified to secrete active IL-10, has been used with signs of inflammation attenuation in two murine models of colitis by Steidler and his group [136]. This paper sets the basis for a new rationale of intervention to the microflora in IBD. To examine the potential of using pathogen-derived immunomodulating molecules *in vivo* as novel therapeutics for IBD, Foligne *et al.* used Yersinia LcrV Protein-secreting *Lactococcus lactis* in two murine models of colitis. Oral administration proved to be very effective in preventing and treating acute colitis in both models [137]. However, the use of transgenic bacteria in clinical trials should be

carefully planned, as containment of the modified microorganism within the host and avoidance of unwanted mutations must be ensured, to prevent the emergence of potentially dangerous novel microorganism.

Helminths

The 'hygiene hypothesis' is supported by the fact that IBD is much less common in countries with poor sanitation and low hygiene levels, where helminth infections are common, in comparison with Western countries [138]. It has thus been assumed that helminths may lead to the prevention of IBD, by some unknown mechanism, but there are data showing that they move the antigenic response from Th1 to Th2 [139, 140]. Helminths can reverse intestinal inflammation in animal models of IBD, and changes in the cytokine profile of the infected mice have been observed [141]. Lamina propria mononuclear cells from mice infected with helminths were found to produce less IL-12(p40) and IFN-γ and more IL-4, IL-13, IL-10 and transforming growth factor-beta compared with naive mice [142]. The use of helminths, such as *Trichuris suis*, that are not human parasites for the treatment of IBD patients in clinical trials may sound odd, but the facts are: colonization with their eggs is self-limiting, they do not replicate in the host, there is no direct transmission and it is convenient to produce eggs [141] – the ideal colonization of the gut without invading the host. Starting from animal studies, *T. suis* was used by Summers *et al.* in two trials in the University of Iowa, one in UC (randomized, double-blind, placebo-controlled) [143] and one in CD (open label trial) [144] patients, showing efficacy in both studies. Evidence from this area looks promising, but further clinical trials with helminth material are necessary to confirm these data.

Prebiotics

Prebiotics are indigestible carbohydrates, which stimulate the growth of particular species of the microflora of the host, resulting in an ameliorated enteric function (Table 2). These nondigestible food constituents act primarily by increasing the population of certain bacteria and thus quantitatively altering the microflora [70]. When reaching the colon, they are fermented by anaerobic bacteria, producing short-chain fatty acids (SCFA) and gas (CO_2 and H_2). As a result, intraluminal pH drops [145], favouring the increase of Bifidobacteria, Lactobacilli and nonpathogenic *E. coli* and decreasing Bacteroidaceae. The fermentation of carbohydrates also leads to the production of acetic, propionic and butyrate acids that are involved in several colon-specific and systemic pathways [146]. Acetate is used as cell fuel and propionic acid is involved in cholesterol synthesis, amongst others. Of these, butyrate is of great importance to the metabolism of the colonocyte [147]. It has also been proven that butyrate exerts anti-inflammatory action, by *in vitro* reducing the expression of TNF-α-related cytokines and upregulating IL-10 in mice [148], possibly by inhibition of the nuclear translocation of NF-κB [149]. Butyrate enemas have been used with success in UC [150–152], but the need for continuous administration limits its use. The most commonly used prebiotics in experimental models and clinical trials are lactulose, lactosucrose, oligofructose and inulin, psyllium, germinated barley foodstuff, fructo- and milk-oligosaccharides.

Table 2

The effects of the prebiotics on the mechanisms of intestinal pathophysiology

Prebiotics	Effects on intestinal pathophysiology
Common effects	Reduction of intraluminal pH Favouring of Bifidobacteria and Lactobacilli *vs.* Bacteroidaceae Short-chain fatty acid (SCFA) production Regulate colonic mucosa physiology via the production of SCFA
Lactulose	Reduction of MPO activity Production of TNF-α and leukotriene B
Germinated barley foodstuff	Decrease in serum IL-8 and α_1-acid glycoprotein
Fructo-oligosaccharides	Upregulation of IL-10 expression in dendritic cells
Goat's milk oligosaccharides	Decrease the colonic MPO activity, increase MUC-3

Lactulose

Lactulose attenuates inflammation in IL-10 knockout mice [153]. It also reduces myeloperoxidase activity, TNF-α and leukotriene B production and increases Bifidobacteria and Lactobacilli, when administered for 2 weeks prior to induction of TNBS colitis in mice [154]. It has also demonstrated a dose-dependent beneficial effect in DSS-induced colitis [155]. In a pilot study oral lactulose had no beneficial effects in IBD patients as regards clinical activity, endoscopic score or immunohistochemical parameters, but significantly ($P = 0.04$) improved the quality of life [156]. Nevertheless, its side-effects (mainly diarrhoea) limit its use in clinical trials.

Lactosucrose

Lactosucrose is a water-soluble fibre that is shown to increase the percentage of Bifidobacteria and the total amount of bacteria in healthy subjects, but with no effect on SCFA [157]. In a study by Teramoto *et al.*, administration of lactosucrose for 2 weeks in IBD patients led to an increase in Bifidobacteria and a decrease in Bacteroidaceae [158], but despite these favourable effects, lactosucrose has not been further evaluated.

Oligofructose and inulin

These are both composed of multiple saccharide units and have similar functions in the bowel that stimulate growth and activity of Lactobacilli and Bifidobacteria [159]. Their

combination prevents development of colitis in HLA-B27 transgenic mice [160]. Inulin attenuates inflammation and reduces markers of inflammation in DSS-induced colitis, leading also to the increase of lactic acid bacteria and pH drop [161, 162]. Licht *et al.* [163] have reported that oligofructose and inulin feeding (separately) to rats led to a greater percentage of lactic acid bacteria and higher butyrate levels. Inulin and oligofructose (OF-IN), given together, had similar results in healthy humans [159]. In a clinical trial, patients with a relapse of mild to moderate UC received mesalazine in combination with oligofructose-enriched inulin or placebo. Oligofructose-enriched inulin was well tolerated and associated with an early reduction in faecal calprotectin [156].

Psyllium (Ispaghula husk, Plantago ovata)

This is a water-soluble fibre, formerly investigated for its hypocholesterolaemic effect [164]. Dietary fibres ameliorated colonic damage in HLA-B27 transgenic rats via the increased production of SCFA and the synergistic inhibition of proinflammatory mediator production [165]. Supplementary diets rich in fibres significantly attenuate clinical symptoms compared with placebo in UC patients, as reported by a small number of studies [166–168]. A clinical trial in UC patients, in comparison with sulfasalazine, showed no difference in remission period rates, thus accrediting psyllium as an alternative treatment to sulfasalazine for maintaining remission [169]. Psyllium was also shown, in the previous study, to raise faecal butyrate levels.

Germinated barley foodstuff

Germinated barley foodstuff (GBF) is derived from the aleuronic layer and scutellum fractions of germinated barley, and has two characteristics: large water-holding capacity and richness in glutamine. It is composed of a fibre fraction and a protein-rich (glutamine) fraction [170]. In an experimental model of colitis, GBF preventive treatment showed a beneficial effect on the microflora [171] with raised butyrate levels [172], a decrease in serum IL-8 and α_1-acid glycoprotein [173] and suppression of the infiltration of the mucosa by mast cells [174], leading to attenuation of colitis. When used therapeutically by the same group, it demonstrated an equal anti-inflammatory efficacy to that of sulfasalazine, with better handling of diarrhoea [173]. In a multicentred open label trial, oral administration of GBF along with standard treatment in 21 patients with mild to moderate UC for 24 weeks led to an important decrease in clinical activity index, compared with controls receiving standard treatment alone [175]. The same group used GBF in another study [168], to demonstrate the efficacy of GBF in maintaining remission of UC along with standard treatment, with lower recurrence rates and lower steroid dosage in the GBF group.

Fructo- and milk-oligosaccharides

Fructo-oligosaccharides have been found to attenuate TNBS-induced colitis in rats, promoting the growth of beneficial lactic acid bacteria and raising butyrate levels [176, 177], but not DSS-colitis in mice [178]. The combination of fructo-oligosaccharides and inulin, combining the beneficial effect of the first in the modulation of microflora [179] and of the second in raising butyrate levels [180, 181], has been reported to attenuate macroscopic and histological inflammation in HLA-B27 transgenic rats [160]. Goat's milk oligosaccharides fed to DSS-colitis mice had a beneficial effect in maintenance of their body weight, with decreased colonic myeloperoxidase activity, milder clinical symptoms and increased MUC-3 compared with control [182] and TNBS-colitis rats [183]. In a recent study Vos *et al.* have shown that dietary supplementation of oligosaccharides enhanced Th1-dependent vaccination responses in mice [184]. In an open label study by Lindsay *et al.* in 15 CD patients, fructo-oligosaccharides were shown to raise levels of IL-10 expression in dendritic cells, with an increase of Bifidobacteria [185].

Synbiotics

Synbiotics are combined products of pro- and prebiotics [70]. The above-mentioned beneficial effects of both probiotics and prebiotics in experimental models and clinical trials have led scientists to the thought of combining them in a novel therapeutic scheme called synbiotic. As shown above, every probiotic and prebiotic has distinct features, acting in its own way in modulating the microflora, and it is thus deduced that there are many combinations to be studied. Moreover, the complex organization and function of the intestinal microflora does not ensure that mixtures that we expect to act synergistically, based on the fact that each component of the mixture has a specific role, will do so. Nevertheless, it is necessary to study these treatment options, as the combination of treatments will hopefully lead to more flexible therapeutic schemes, improving compliance of patients and decreasing costs. Roller *et al.* have demonstrated suppression of colonic carcinogenesis by use of a combination of oligofructose-enriched inulin, Lactobacillus and Bifidobacterium, while the same mixture stimulated secretion of IgA and IL-10 by the caecum [186]. One paper has showed that a combination of *Bi. breve*, *Lactobacillus casei* and galacto-oligosaccharides substantially improved bowel function in a girl with short bowel syndrome [187]. Furrie *et al.*, in a randomized double-blind controlled trial of 18 UC patients, used a combination of *Bi. longum*, inulin and oligofructose and demonstrated that this mixture reduces sigmoidoscopy and rectal biopsy inflammation scores, with a concurrent reduction of TNF-α and IL-1β levels [188]. Synbiotics with their combinations offer a large area for clinical trials and potential treatments in IBD and a new field for studies of their effects on the pathogenic mechanisms in intestinal inflammation.

Conclusions

During recent decades probiotics and prebiotics have been used in a large number of experimental models of colitis and clinical trials. Experimental studies *in vitro* and *in vivo* have provided good evidence that bacterial and bacterial components are implicated in the pathogenic mechanisms of gut pathophysiology and that intestinal microflora play a pivotal role in the pathogenesis of IBD. These studies have helped us to identify the changes in the microbiota in the mucosal and luminal environment of bowel and to understand the interaction of these microorganisms with the underlying mucosal immune system in gut. However, a better understanding of this ecosystem is required in order to determine which bacterial strain or prebiotic would be the ideal treatment for a given bowel disorder.

On the other hand, clinical trials have given conflicting results thus far. It is necessary to cite the more profound clinical effect of these treatments in UC remission and prevention of pouchitis in CD, whereas benefits are limited in CD and results must be gathered as for non-gastrointestinal manifestations of IBD in general. These agents, introduced as 'pharmabiotics' by Shanahan [189], have exhibited lack of toxicity so far, whereas they seem to be a good solution in patients who are reluctant to take medication (a very important factor, especially in conditions such as IBD, where treatment is elongated and difficult to cope with); probiotics and prebiotics are 'nature products', not drugs.

Pro- and prebiotics have demonstrated their beneficial impact in healthy subjects, both experimentally and clinically, to an equal, and in our opinion a greater extent than in IBD patients. Results from clinical studies have demonstrated that these therapies are equivalent to those traditionally used, and, in a few cases, better at maintaining remission induced by traditional therapies [94, 106, 134]. The use of pro- and prebiotics should be studied in healthy populations starting from childhood, in order to evaluate their potential as preventive means, apart from IBD therapy. Large cohort studies could be of use in this case. Of course, as in any long-term treatment, the safety of continuous administration of pro- and prebiotics must be first confirmed in experimental models, to avoid unexpected and thus far undemonstrated detrimental effects from this constant antigenic stimulation.

Pro-, pre- and synbiotics now appear to have a pivotal role in the prevention and management of various gastrointestinal disorders that is totally dependent on the combination of bacteria used and the type and stage of the treated bowel disease. These 'pharmabiotics' constitute a heterogeneous group with different properties and biological effects on gut physiology and pathophysiology. In addition, similar bacteria do not share similar therapeutic activities and *in vitro* their properties do not always predispose their effects on the human intestinal physiology as a part of the microenvironment of microflora. Further work with well-designed randomized control clinical trials is necessary in order to understand the undoubted role of these agents in the management of gut physiology in health and disease.

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