REVIEW

Intestinal microbiota: The explosive mixture at the origin of inflammatory bowel disease?

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Author contributions: Ierardi E, Di Leo A and Principi M designed the study, revised the manuscript and approved the final version; Bringiotti R, Lovero R and Losurdo G collected the data and revised the final version before approval.

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Abstract

Inflammatory bowel diseases (IBDs), namely Crohn's disease and ulcerative colitis, are lifelong chronic disorders arising from interactions among genetic, immunological and environmental factors. Although the origin of IBDs is closely linked to immune response alterations, which governs most medical decision-making, recent findings suggest that gut microbiota may be involved in IBD pathogenesis. Epidemiologic evidence and several studies have shown that a dysregulation of gut microbiota (*i.e.*, dysbiosis) may trigger the onset of intestinal disorders such as IBDs. Animal and human investigations focusing on the microbiota-IBD relationship have suggested an altered balance of the intestinal microbial population in the active phase of IBD. Rigorous microbiota typing could, therefore, soon become part of a complete phenotypic analysis of IBD patients. Moreover, individual susceptibility and environmental triggers such as nutrition, medications, age or smoking could modify bacterial strains in the bowel habitat. Pharmacological manipulation of bowel microbiota is somewhat controversial. The employment of antibiotics, probiotics, prebiotics and synbiotics has been widely addressed in the literature worldwide, with the aim of obtaining positive results in a number of IBD patient settings, and determining the appropriate timing and modality of this intervention. Recently, novel treatments for IBDs, such as fecal microbiota transplantation, when accepted by patients, have shown promising results. Controlled studies are being designed. In the near future, new therapeutic strategies can be expected, with non-pathogenic or modified food organisms that can be genetically modified to exert anti-inflammatory properties.

Core tip: This paper focuses on the scientific scenario regarding the potential function of gut microbiota in inflammatory bowel diseases (IBDs). Epidemiologic findings suggest that the heterogeneity and disruption of gut microbiota can be significant in modulating and addressing the immune reactions underlying IBD pathogenesis. Traditional or innovative manipulation strategies of gut microbiota may be possible future treatment options for the management of these disorders.

Bringiotti R, Ierardi E, Lovero R, Losurdo G, Di Leo A, Principi M. Intestinal microbiota: The explosive mixture at the origin of inflammatory bowel disease? *World J Gastrointest Pathophysiol* 2014; 5(4): 550-559 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i4/550.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i4.550

INTRODUCTION

Inflammatory bowel diseases (IBDs) are lifelong chronic disorders arising from interactions among genetic, immunological and environmental factors[1].

Technological advances have allowed novel predictive factors to be assessed, that can identify the disease at an early stage and provide an accurate diagnosis long before the onset of clinical manifestations[2]. Recent findings suggest that, in addition to genetic and environmental factors, interactions with the gut microbiota may play a relevant role in a "perfect storm" driving the pathogenesis of IBDs[3].

MICROBIOTA AND IBD

The human intestinal tract includes several multifaceted microbial populations with an essential function in general health. The human gut contains, in the assortment of 1000 bacterial species, 100-fold more genes than the human genome. The new high throughput sequencing technologies, the presence of 16S rRNA genes in the gut bacterial composition, as well as recent non genomic techniques, have well defined the function of gut microbiota in some human diseases[4,5].

Although the microbiota of the colon is apparently similar in different people, there are marked variations between individuals in bacterial populations of a single species. It has been demonstrated that an increase in biodiversity requires a different metabolic homeostasis and structural stability, while a reduction, due to age, illness or antibiotics, reduce the capacity of the intestinal environment to fight infecting pathogens[6,7]. In fact, epidemiological evidence and experimental studies have suggested that alterations in the gut microbiota (*i.e.*, dysbiosis) can be relevant in intestinal conditions such as chronic IBD[8].

Clinical evidence confirms the role of microbiota in IBD, and an abnormal microbial composition in IBD has been amply demonstrated. The most common site of IBD is the colon, where the highest intestinal bacterial concentrations are found. Additionally, fecal stream diversion can prevent and treat Crohn's disease (CD) and pouchitis. Finally, many studies have shown that antibiotics and probiotics improve the histological, endoscopic and clinical picture[9]. Despite this evidence-based findings, there are still some major unexplained points such as the IBD response to immunosuppressive therapy or the protective role of poor hygienic conditions, which do not appear likely to be related to the microbial state[10].

Animal studies

It is known that the non-pathogenic microbiota controls bowel immunity, but interactions in the gut with host microbes can be bidirectional. The mucosal immune system can be affected by the pro-inflammatory potential of abnormal growth of microbiota elements, which ultimately determine or influence an inflammatory reaction and induce the possibility of development of illness. Several animal studies have shown that this interaction is possible and can induce colitis.

Studies in germ-free interleukin 10-deficient (IL-10$^{-/-}$) mice, that fail to acquire spontaneous colitis and immune activation, support this hypothesis[11]. Indeed, some studies show that, regardless of the background strain of these animals, the onset and degree of spontaneous colitis depends on the composition of the enteric gut microbiota[11,12]. Penetrance of colitis increases to nearly 100% when the immune system response is characterized by a T-helper 1 (Th1) interferon (IFN)-γ reaction[12].

Therefore, in this model of colitis, it has been demonstrated that the disease may show different characteristics and distribution based on the intestinal bacteria present. Furthermore, in IL-10-/- germ-free mice, bacterial colonization of non-pathogenic bacteria such as *Escherichia coli* (*E. coli*) or *Bilophila wadsworthia* provokes different types of colitis[13]. In particular, *Bilophila wadsworthia* produces a low grade colitis involving the distal colon, associated with an exclusively Th1-mediated immune response. In contrast, *E. coli* leads to an early (3-wk) development of mild-to-moderate inflammation that is more severe in the cecum. In the same study, *Bacteroides vulgatus*, but not *E. coli*, provokes mucosal inflammation of the colon in HLA-B27 transgenic mice without bone marrow involvement as in transplanted CD3 transgenic mice[13].

Finally, novel experimental evidence demonstrated that *Klebsiella* may provoke moderate pancolitis while *Bifidobacterium animali*s could cause a mild degree of inflammation in the distal colon and duodenum[14,15].

Human studies

A few studies in humans have suggested that IBD patients have an altered balance of intestinal microbiota in the active phase. Bacterial 16S rRNA gene examination did not show relevant differences in bacterial constitution in the intestinal mucosa of CD and ulcerative colitis (UC) patients. Moreover, in UC patients, a decrease in bacterial load was observed even if it was not significant when compared to that of CD patients[16-18].

Another interesting finding, a thinner and less sulphated mucus in patients affected by UC, has been demonstrated and may account for an increased number of bacteria colonizing the mucosa[19,20]. Indeed, a poor mucus layer with a microbiota overgrowth could enhance the presentation of bacterial antigens to the immune system of the gut mucosa. In UC patients, the colonic surface and inflamed areas are colonized by a broad variety of bacteria. For example, in UC specimens *Clostridium histolyticum* and *lituseburense* accounted for 21% of the microbiota. *Enterobacteriaceae* such as *Escherichia* and *Klebsiella* have also been considered to be implicated in the pathogenetic mechanism of UC. Indeed, their aptitude to adhere to enterocytes, allowing them to penetrate the mucosal layer and deliver enterotoxins, might account for this hypothesis[21,22].

Genetics in IBD pathogenesis

The interaction between genetic factors, and a dysregulated response of the immune system to bacterial antigens are still strongly supported hypotheses in the pathogenesis of IBD. Indeed, genome-wide association studies (GWAS) showed that several genes were associated with

IBD susceptibility[23]. These genes, risk factors for CD and UC, encode for proteins that may regulate the microbiota (NOD2/CARD15) or may control host responses (IL-12-IL23R pathway or autophagy)[24,25], and constitute a barrier function notably for UC[26].

One of these proteins, NOD2, may be crucial for distinguishing between non-pathogenic and pathogenic organisms; indeed, it initiates signal transduction thus promoting NFκB translocation into the nucleus, where the expression of specific genes determinates the response of primary and adaptive immune mechanisms[27-29].

The multifunctional genetic linkage of NOD2/CARD15 is demonstrated by the protein's ability to identify bacterial muramyl-dipeptide and by its impact on the homeostasis of non-pathogenic bacteria, regulatory T cells (Tregs), and viral identification by immune system[24]. Although NOD2 homozygosity may carry a 20-fold increased risk for CD, notably in the ileal location, less than 20% of patients affected by CD are homozygous for NOD2[30,31]. So, while these studies and GWAS have provided important details about IBD pathogenesis, investigations on the distribution of genetic variants in different populations poorly explain the large discrepancies in IBD prevalence between different geographic areas as well as the increasing incidence of IBDs in Western countries over the past 5 decades[2].

The evidence strongly supports that IBDs are polygenetic disorders and their heterogeneity relates to the complexity of their genetic background as well as to different lifestyle and environmental factors, including variations in microbiota composition.

Environmental triggers

It is known that nutrition, medications (NSAIDs) and smoking affect the composition of the gut microbiota and it is known that changes in this multifaceted structure are contributing factors in the origin of some disorders, including IBDs.

Smoking is a relevant risk factor in CD pathogenesis[32-36]. Indeed, it may alter the intestinal microbiota and its cessation may further modify intestinal microbial composition. Indeed, simultaneous increased *Firmicutes* and *Actinobacteria* and decreased *Proteobacteria* and *Bacteroidetes* characterize smoking cessation; in contrast, the composition of the flora in continuous smokers and non-smokers remains stable[37].

Many studies have reported a modification of the gut microbiota composition in populations migrating from developing to developed countries[38]. In these subjects, diet, family size, antibiotic consumption, urbanization, reduced parasitism, and a reduction in exposure to childhood infections, such as hepatitis A and *Helicobacter pylori,* are associated with changes in the microbiota.

Neonates show a sterile or, at least, a very low microbial load in the intestine[39]. Bacterial strains colonize the infant bowel after delivery according to various factors, such as method of delivery, breast- or bottle-feeding, and antibiotic administration[40]. There is early colonization of

Lactobacillus and *Prevotella* after vaginal delivery and greater colonization of *Firmicutes* in neonates delivered by cesarean section, that predisposes to a greater susceptibility to some pathogens and a higher risk of atopic disease[41,42]. Therefore, growth from newborn to early childhood and finally adulthood is associated with changes in the gut microbiota, featuring a reduction in *Lactobacillus* and *Bifidobacteria* and an increase in *Firmicutes, Clostridia* and *Bacteroides* species, that may lead to a high risk of allergic and immunological diseases[43]. This raises the hypothesis that a decreased biodiversity within non-pathogenic microbiota, with an altered immunity maturation, could negatively influence the immune recognition and activation, and thereby confer a risk for developing IBD in adulthood[38].

Regarding the impact of a high-fat dietary intake on the non-pathogenic microbiota, it has been demonstrated that it can radically remodel the intestinal microbiota[44,45]. Moreover, there is evidence that non-absorbed carbohydrates (inulin and fructooligosaccharides) promote the growth of beneficial species, supplying a substrate for the production of short-chain fatty acids (SCFAs)[46].

Recently, novel studies have focused on the role of NSAIDs in inducing and maintaining mucosal damage, thus contributing to the genesis of IBD. In particular, several studies demonstrated that NSAIDs were able to cause injury by means of microbiota modulation[47]. NSAIDs, indeed, can promote the overexpression of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and IFN-γ through changes in the microbiota[48], and further allow bacterial translocation through the intestinal barrier. This hypothesis is confirmed by evidence that the levels of such proinflammatory cytokines are significantly increased in IBD patients.

Microbiota and IBDs: Comments on the literature

There can be no doubt, in view of all the experimental data, that the microbiota can be considered a key factor in the origin of IBDs and not a bystander. Studies performed on animal models provide strong evidence for a primary role played by microbiota in IBDs but human studies do not fully support this pathogenic hypothesis owing to the lack of sufficient scientific proof. For instance, it is well-known that, in CD, the entire alimentary tract from the oral cavity to the anus may be involved, but no data from human studies are available on this topic. Conversely, animal studies have demonstrated that the microbiota composition may influence the onset of IBDs in a selected part of the digestive system. El Aidy *et al*[49] investigated the responses of the jejunal mucosa to bacterial colonization in germ-free mice, showing a consequent shift to anaerobic metabolism, a condition that may strongly influence mucosal oxygenation in IBD. Moreover, in an experimental model of small bowel CD, a single strain of *E. coli* (LF82) has been demonstrated to stimulate the production of proinflammatory cytokines, an effect that was counteracted by lactoferrin, another microbial product[50].

There has been much discussion as to whether infec-

Table 1 Antibiotic therapy in inflammatory bowel diseases

Ref.	Year	Antibiotics	Duration	Result
Crohn's disease-primary therapy				
Ursing *et al*[53]	1982	Metronidazole 800 mg/d	16 wk	No difference from sulfasalazine
Sutherland *et al*[54]	1991	Metronidazole 10 or 20 mg/kg	16 wk	Superior to placebo (↓ CDAI), no difference in remission
Colombel *et al*[55]	1999	Ciprofloxacin 500 mg 2 × d	6 wk	No difference from mesalamine
Arnold *et al*[56]	2002	Ciprofloxacin 500 mg 2 × d	6 mo	Superior to placebo (CDAI)
Prantera *et al*[57]	1996	Ciprofloxacin 500 mg 2 × d + metronidazole 250 mg 4 × d	12 wk	No difference from prednisolone
Greenbloom *et al*[58]	1998	Ciprofloxacin 500 mg 2 × d + metronidazole 250 mg 3 × d	10 wk	Uncontrolled, 68% remission
Leiper *et al*[59]	2000	Clarithromycin 250 mg 2 × d	4 wk	Uncontrolled, 64% response, 48% remission
Steinhart *et al*[60]	2002	Ciprofloxacin 500 mg 2 × d + metronidazole 250 mg 3 × d	8 wk	No improvement over budesonide alone (33% *vs* 38% remission)
Crohn's disease-prevention of postsurgical relapse				
Rutgeerts *et al*[61]	1995	Metronidazole 20 mg/kg	12 wk	↓ clinical relapse 1 yr *vs* placebo
Rutgeerts *et al*[62]	2005	Ornidazole 1 g/d	52 wk	↓ severe endoscopic relapse *vs* placebo
Ulcerative colitis-primary therapy				
Turunen *et al*[63]	1999	Cipro 500 mg 2 × d	6 mo	Superior to placebo
Mantzaris *et al*[64]	1997	Cipro 500 mg 2 × d	6 mo	No benefit *vs* placebo
Casellas *et al*[65]	1998	Amoxicillin 1 g/ Clavulanic acid 250 mg	5 d	↓ mucosal IL-8 and eicosanoids *vs* placebo
Turner *et al*[66]	2014	metronidazole, amoxicillin, doxycycline (Paediatrics)		Remission (46.6%)
Pouchitis				
Shen *et al*[67]	2001	Metronidazole 20 mg/kg or Cipro 500 mg 2 × d	6 mo	Both effective, Cipro > metronidazole
Gionchetti *et al*[68]	2000	Cipro 500 mg 2 × d and Rifaximin 1 g 2 × d	5 d	89% response, 33% remission, uncontrolled

tious factors could be a trigger for IBD. No evidence is available from human studies, but animal models offer interesting insights. Couturier-Maillard *et al*[51] demonstrated that microbiota transplantation from healthy wild-type mice may reduce the IBD risk in Nod2-deficient mice and lead to long-term alterations in the gut microbiota. On the other hand, disease risk was promoted in wild-type mice that were recolonized with dysbiotic fecal microbiota from NOD2-deficient mice. In conclusion, animal models must be seen only as a starting point for microbiota investigation in humans, and the main lesson that we can deduce is that an imbalance of bacterial species is one of the main reasons that can explain the different types of colitis induced by the effect of different bacteria.

PHARMACOLOGICAL MANIPULATION OF MICROBIOTA IN IBDs

Antibiotics

Antibiotics are known to have an important role in the management of septic complications of IBD, *e.g.*, intra-abdominal and perianal abscesses and fistulae of CD, superinfections, and post-surgical wound infections. Nonetheless, treatment with antibiotics for active luminal CD and UC is not widely accepted as a first-line choice. Although the use of antibiotics against pathogenic bacteria is proven and based on reliable evidence of experimental enterocolitis and IBD, there are some clinical trials that do not sufficiently support the efficacy of these drugs in patients affected by IBD[52].

The most representative published studies are summarized in Table 1[53-68]. Metronidazole, ciprofloxacin, or the contemporary use of these agents are useful in Crohn's colitis, ileocolitis and pouchitis, but not in disease confined to the ileum. They are recommended for pouchitis in the European Crohn's and Colitis Organisation statements, which also indicate that ciprofloxacin appears to have fewer adverse effects (statements 8C, 8D)[69].

Probiotics

Probiotics are viable microorganisms that have been cultured from foods, in particular milk. Various species and bacterial strains that have been used in IBD clinical trials, are believed to have a potential beneficial role. The most evaluated probiotics are *E. coli Nissle*[70], *VSL#3* mixture (four strains of *lactobacilli*, three strains of *bifidobacteria*, and one strain of *Streptococcus salivarius thermophilus*)[68,71-73], *BIO-THREE* mixture (*S. faecalis, C. butyricum,* and *Bacillus mesentericus*)[74], a mixture of *L. rhamnosus* and *L. reuteri*[75], *L. rhamnosus GG*[76], Yakult strains of *Bifidobacterium brevis, Bifidobacterium bifidum* and *L. acidophilus*[77]. Recently, advanced genetic engineering has produced modified species that are able to produce immunosuppressive molecules such as IL-10[78].

These studies have shown that probiotic supplementation can re-establish bacterial homeostasis in the intestine and downregulate gut inflammation that is characteristic of IBD patients, thus modulating the inflammatory/anti-inflammatory balance. A reduction in the number of microbiome elements was also found. Indeed, the administration of probiotics can normalize

Table 2 Probiotic therapy in inflammatory bowel diseases

Model	Probiotic	Effect
Trinitrobenzene sulphonic acid or dinitrobenzene sulphonic acid	*Bi. infantis*	No effect
	L. acidophilus, L. casei and *Bi. animalis*	Reduced inflammation
	VSL#3	No effect
	Lactobacillus GG	No effect
	L. plantarum 299	No effect
	VSL#3 (DNA, subcutaneously)	Reduced inflammation
Iodoacetamide	VSL#3	Reduced inflammation
	Lactobacillus GG	Reduced inflammation
Acetic acid	*L. rhamnosus GG*	No effect
	L. reuteri R2LC	Reduced inflammation
	L. reuteri R2LC	Reduced inflammation
Dextran sodium sulphate	VSL#3 (irradiated and DNA*)	Reduced inflammation
IL-10 knockout mice	*L. salivarius* 118 (subcutaneously)	Reduced inflammation
	L. salivarius	Reduced inflammation
	Bi. infantis	Reduced inflammation
	L. plantarum 299V	Reduced inflammation
	VSL#3	Reduced inflammation
	L. salivarius	Reduced inflammation
	L. reuteri	Reduced inflammation
	VSL#3 (DNA, subcutaneously)	Reduced inflammation
E. coli-induced colitis in IL-2 knockout mice	*B. vulgatus*	Reduced inflammation
B. vulgatus-induced colitis	*Lactobacillus GG*	Prevented recurrent colitis
	L. plantarum 299V	No prevention of recurrent colitis

altered intestinal microbiota in IBD patients, and increase protective species by reducing the pathogen load, positively affecting intestinal permeability, balancing local immune response, producing beneficial substances, and disintegrating pathogenic antigens in the intestinal lumen[79].

In animal models (Table 2), *Lactobacilli* and *Bifidobacteria* reduced the severity of experimental colitis in IL-10 knockout mice[80,81]. In another study *L. plantarum* prevented colitis onset in HLA B27 transgenic rats. This and other reports confirm the protective effects of several probiotics in selected hosts and special inflammatory conditions. Therefore, in experimental colitis induced in B27 transgenic rats, which had remission with broad-spectrum antibiotics, probiotics prevented recurrence of the colitis. However, probiotic treatment alone was unable to produce remission of the induced disease[82].

The beneficial effect of probiotics was demonstrated in rats with colitis induced by instillation of 4% acetic acid, which causes altered intestinal permeability. In particular, after 4 d of acetic acid treatment the activity of myeloperoxidase (MPO) showed a 3-fold increase, in parallel with a 6-fold increase in mucosal permeability in the colonic samples. The use of *L. reuteri* R2LC, after acetic acid administration, reduced the morphological score,

MPO activity, mucosal permeability, and prevented the onset of colitis[83].

In human studies, 9-mo daily use of a probiotic formula, *i.e.*, *VSL#3*, was effective in preventing the relapse of chronic pouchitis after remission induced by antibiotics[68]. Another investigation replicated the same results, and, in addition, showed a decreased frequency of pouchitis when *VSL#3* was given after pouch closure[84].

In cases of mild-to-moderate active UC treated with probiotics, there was an improvement in clinical severity, a reduction in relapses, and induction of remission. Moreover, these findings were accompanied by high histological scores and increased levels of fecal butyrate and other SCFAs[73-77].

Studies in UC patients found that the prevention of flare-ups by probiotics was associated with inactivation of NF-κB, downregulation of TNF-α and IL-1β, and a simultaneous increase in anti-inflammatory cytokines, such as IL-10[85]. Few data are available about the mechanism by which probiotics could modify the composition of the resident microbiota, even though it has been hypothesized that they might increase the load of *Lactobacilli* and/or *Bifidobacteria*[74,85].

On the other hand, clinical trials with the use of probiotics in CD, are less concordant than in UC. Malchow[86] found that *E. coli Nissle* was more effective than placebo in preventing relapse of CD in the remission phase induced by conventional therapy, but supplementation of probiotics was found to be ineffective in prolonging remission after the administration of *L. johnsonii LA1* following surgical resection[87,88]. Similarly, a study of Prantera *et al*[80] did not demonstrate any benefit by 1 year-long *Lactobacillus GG* consumption in the prevention of post-surgical clinical or endoscopic relapses in the neo-terminal ileum.

As reported above, Butterworth *et al*[89] evaluated 12 potentially relevant studies of the efficacy of probiotics in CD, even though 11 did not fulfill the inclusion criteria. In the only study satisfying the stated criteria, patients with moderately active CD received *L. rhamnosus* GG for 6 mo without obtaining an improvement.

Prebiotics
Prebiotics are dietary supplementations, usually non-digestible glycides, which are energy substrates for protective intestinal organisms. Lactosucrose, fructo-oligosaccharides, inulin, bran, psyllium, and germinated barley extracts promote *Lactobacilli and Bifidobacteria* growth, thus inducing SCFA production, in particular butyrate[90-92]. Therefore, these substances are able to re-establish the optimal beneficial/pathogen bacteria ratio in IBD patients. These physiological dietary supplements increase the protective lactic acid bacilli load, with a consequent inhibition of harmful species by decreasing the luminal pH, reducing epithelial adhesion, and producing bactericidal molecules. Animal studies showed a protective effect in rat colitis models (Table 3)[93,94]. Several small controlled studies but only a few randomized controlled

Table 3 Inflammatory bowel diseases prebiotic therapy		
Model	**Prebiotic**	**Effect**
Trinitrobenzene sulphonic acid	Fructo-oligosaccharide	Reduced inflammation
	Galacto-oligosaccharide	No effect on inflammation
Dextran sodium sulphate	Fructo-oligosaccharide	No effect on inflammation
	Resistant starch	Reduced inflammation
	Germinated barley foodstuff	Reduced inflammation
	Germinated barley foodstuff	Reduced inflammation
	Inulin	Reduced inflammation
	Germinated barley foodstuff	Reduced inflammation
IL-10 knockout mice	Lactulose	Reduced inflammation

trials (RCT) in IBD patients have been performed, fewer than the studies with probiotics.

Interestingly, Welters *et al*[95] carried out a clinical trial in 20 patients with an ileal pouch-anal anastomosis who consumed 24 g of inulin or placebo daily for 3 wk. The pH, short chain fatty acids, microflora, and bile acids were determined in the stools, while the inflammatory status was evaluated by clinical, endoscopic and histological parameters. It was proven that the treatment enhanced butyrate levels, reduced pH, and reduced the number of *Bacteroides fragili*s as well as fecal concentrations of secondary bile acids. These findings were accompanied by a reduction in inflammation in the ileal reservoir mucosa.

In another open-label study, 10 patients with active ileocolonic CD were enrolled to receive a daily 15 g dose of fructo-oligosaccharides (FOS) for 3 wk. The Harvey-Bradshaw index was chosen to assess the disease activity, and fluorescence *in situ* hybridization was used to measure *Bifidobacteria* in stools; flow cytometry of dissociated rectal biopsies evaluated mucosal dendritic cell, IL-10 and TLR expression. The results of this study were promising: the use of FOS resulted in a significant reduction in the Harvey Bradshaw index, and a significant increase in fecal *Bifidobacteria* concentrations. The percentage of IL-10 positive dendritic cells was increased from 30% to 53%. Moreover, an increase in the percentage of dendritic cells expressing TLR2 and TLR4 was found (from 1.7% to 36.8% and from 3.6% to 75.4%, respectively)[96].

Symbiotics

Probiotic therapy can potentially be improved by simultaneous administering of prebiotics (non-digestible and non-absorbable carbohydrates) that enhance probiotic proliferation in the gut. This mixture is referred as a symbiotic. The main benefit of symbiotic formulation is that a prebiotic constituent could positively modulate the increase in local microbiota, which is further regulated by the probiotic component of the synbiotic formulation. In animal models, Schultz *et al*[97] evaluated the effect of a symbiotic preparation composed of a probiotic combination of *Lactobacilli, Bifidobacteria* and inulin (SIM) in HLA-B27-beta(2)-

microglobulin transgenic rats affected by severe colitis. After 4 mo of SIM treatment, the colonic disease showed histological improvement and, furthermore, there was an alteration in the microflora profile, with an increased variety, and specifically, increased growth of *Bifidobacterium animalis* compared with untreated rats.

A few well conducted studies have supported the usefulness of symbiotic supplementation. Furrie *et al*[98], in a double-blinded RCT, developed a symbiotic called Synergy 1, made up of a combination of a probiotic (*Bifidobacterium longum*) and a prebiotic (inulin-oligofructose), which provided a metabolic substrate for the *Bifidobacterium* strain, and obtained promising results in UC patients.

Fecal transplantation

A novel treatment for IBD is fecal microbiota transplantation (FMT). FMT consists of extracting gastrointestinal microbiota from a healthy donor, which is then instilled *via* an enema through a liquid stool suspension. FMT has recently gained ground as a therapy for refractory and/or recurrent *C. difficile* infection[99-102].

In a recent systematic review conducted by Anderson *et al*[103], following Cochrane and PRISMA recommendations, 5320 articles on FMT in patients with IBD were identified. Seventeen articles were selected, including reports on FMT given in single cases to treat IBD, and in the management of infectious diarrhea in IBD. The 17 trials encluded 41 subjects followed up for 2 wk-13 years. FMT was able to produce a reduction in symptoms in most of the IBD patients, allow an interruption in IBD medication, and result in disease remission. In those patients who experienced a simultaneous *C. difficile* infection, complete eradication of the bacterium was achieved. Even though this procedure may face difficult acceptance by patients, the review describes promising results.

Despite insufficient data on FMT in IBD, this procedure is potentially an effective and safe treatment; it may be suggested for subjects who failed conventional treatments. It is necessary to perform new well-designed and randomized trials to enrich the data about FMT in IBD to: (1) evaluate safety and success rate; and (2) to standardize protocols. Without these considerations, FMT could not become a standard part of clinical therapy[103].

CONCLUSION

Patients affected by IBDs, either UC or CD, suffer from a heterogeneic entity whose pathogenic etiology must be explored in the context of a "multihit" phenomenon that precipitates the disease through a multifactorial platform resulting from interactions among genetic, immunological and environmental triggers. Although the microbiota may well play a crucial role in the origin of IBD, up-to-date therapeutic strategies have a primary purpose of suppressing the host response, and so a significant fraction of patients fail to accomplish sustained remission.

While novel techniques in molecular biology and engineering have enabled further discoveries about the gut microbiota, the relationship between intestinal microbiota

and IBD has not yet been completely clarified. A better understanding of the role that some bacterial species play in IBD pathogenesis is essential in order to develop appropriate management strategies.

The possibility of modulating our gut flora by interventions on microbial composition and the correct timing of this operation have important implications on efforts to improve gastrointestinal health. Nevertheless, microbiology should support, but not replace, the genetics of IBD, and meticulous typing of the intestinal microflora should soon take a decisive place in its complete characterization in order to explore the relationship between genes and the environment in health and disease. Finally, future research in microbial intervention needs to be directed towards two areas: (1) improvements in strain selection with the goal of realizing new screening procedures for a better understanding of the mechanisms of action, and ensuring adequate efficacy; (2) a new therapeutic strategy with non-pathogenic organisms of alimentary origin that can be genetically modified with the aim of producing anti-inflammatory substances.

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