World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i4.550

World J Gastrointest Pathophysiol 2014 November 15; 5(4): 550-559
ISSN 2150-5330 (online)

REVIEW

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

Roberto Bringiotti, Enzo Ierardi, Rosa Lovero, Giuseppe Losurdo, Alfredo Di Leo, Mariabeatrice Principi

Roberto Bringiotti, Enzo Ierardi, Rosa Lovero, Giuseppe Losurdo, Alfredo Di Leo, Mariabeatrice Principi, Gastroenterology, Department of Emergency and Organ Transplantation, University of Bari, 70124 Bari, Italy

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.

Correspondence to: Enzo Ierardi, Professor, Gastroenterology, Department of Emergency and Organ Transplantation, University of Bari, Policlinico, Piazza Giulio Cesare, 70124 Bari, Italy. ierardi.enzo@gmail.com

Telephone: +39-08-05592577 Fax: +39-08-05593088
Received: January 14, 2014 Revised: May 2, 2014
Accepted: July 27, 2014
Published online: November 15, 2014

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.

Key words: Intestinal microbiota; Inflammatory bowel diseases; Probiotics; Prebiotics; Symbiotics

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 animalis* 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 fstulae of CD, superinfections, and post-surgical wound infections. Nonetheless, treatment with antibiotics for active luminal CD and UC is not widely accepted as a frst-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 suffciently support the effcacy 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 confned to the ileum. They are recommended for pouchitis in the European Crohn's and Colitis Organisation statements, which also indicate that ciprofoxacin 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 benefcial 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 299*V*	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 fragilis* 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.

REFERENCES

1 **Loftus EV**. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology* 2004; **126**: 1504-1517 [PMID: 15168363 DOI: 10.1053/j.gastro.2004.01.063]

2 **Molodecky NA**, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012; **142**: 46-54.e42; quiz e30 [PMID: 22001864 DOI: 10.1053/j.gastro.2011.10.001]

3 **Leone V**, Chang EB, Devkota S. Diet, microbes, and host genetics: the perfect storm in inflammatory bowel diseases. *J Gastroenterol* 2013; **48**: 315-321 [PMID: 23475322 DOI: 10.1007/s00535-013-0777-2]

4 **Ley RE**, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 2006; **124**: 837-848 [PMID: 16497592 DOI: 10.1016/j.cell.2006.02.017]

5 **Qin J**, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Bork P, Ehrlich SD, Wang J. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; **464**: 59-65 [PMID: 20203603 DOI: 10.1038/nature08821]

6 **Macfarlane S**, Macfarlane GT. Bacterial diversity in the human gut. *Adv Appl Microbiol* 2004; **54**: 261-289 [PMID: 15251284 DOI: 10.1016/S0065-2164(04)54010-8]

7 **Bartosch S**, Fite A, Macfarlane GT, McMurdo ME. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. *Appl Environ Microbiol* 2004; **70**: 3575-3581 [PMID: 15184159 DOI: 10.1128/AEM.70.6.3575-3581.2004]

8 **Frank DN**, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA* 2007; **104**: 13780-13785 [PMID: 17699621 DOI: 10.1073/pnas.0706625104]

9 **Sartor RB**. Microbial influences in inflammatory bowel diseases. *Gastroenterology* 2008; **134**: 577-594 [PMID: 18242222 DOI: 10.1053/j.gastro.2007.11.059]

10 **Round JL**, Lee SM, Li J, Tran G, Jabri B, Chatila TA, Mazmanian SK. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* 2011; **332**: 974-977 [PMID: 21512004 DOI: 10.1126/science.1206095]

11 **Sellon RK**, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, Rennick DM, Sartor RB. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect Immun* 1998; **66**: 5224-5231 [PMID: 9784526]

12 **Kullberg MC**, Ward JM, Gorelick PL, Caspar P, Hieny S, Cheever A, Jankovic D, Sher A. Helicobacter hepaticus triggers colitis in specific-pathogen-free interleukin-10 (IL-10)-deficient mice through an IL-12- and gamma interferon-dependent mechanism. *Infect Immun* 1998; **66**: 5157-5166 [PMID: 9784517]

13 **Kim SC**, Tonkonogy SL, Albright CA, Tsang J, Balish EJ, Braun J, Huycke MM, Sartor RB. Variable phenotypes of enterocolitis in interleukin 10-deficient mice monoassociated with two different commensal bacteria. *Gastroenterology* 2005; **128**: 891-906 [PMID: 15825073 DOI: 10.1053/j.gastro.2005.02.009]

14 **Sartor RB**. Microbial influences in inflammatory bowel disease: role in pathogenesis and clinical implications. In: Sartor RB, Sandborn WJ, editors. Kirsner's inflammatory bowel diseases. Philadelphia: Elsevier, 2004: 138-162

15 **Moran JP**, Walter J, Tannock GW, Tonkonogy SL, Sartor RB. Bifidobacterium animalis causes extensive duodenitis and mild colonic inflammation in monoassociated interleukin-10-deficient mice. *Inflamm Bowel Dis* 2009; **15**: 1022-1031 [PMID: 19235917 DOI: 10.1002/ibd.20900]

16 **Kornbluth A**, Sachar DB. Ulcerative colitis practice guidelines in adults (update): American College of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol* 2004; **99**: 1371-1385 [PMID: 15233681 DOI: 10.1111/j.1572-0241.2004.40036.x]

17 **Ott SJ**, Musfeldt M, Wenderoth DF, Hampe J, Brant O, Fölsch UR, Timmis KN, Schreiber S. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut* 2004; **53**: 685-693 [PMID: 15082587 DOI: 10.1136/gut.2003.025403]

18 **Swidsinski A**, Ladhoff A, Pernthaler A, Swidsinski S, Loening-Baucke V, Ortner M, Weber J, Hoffmann U, Schreiber S, Dietel M, Lochs H. Mucosal flora in inflammatory bowel disease. *Gastroenterology* 2002; **122**: 44-54 [PMID: 11781279 DOI: 10.1053/gast.2002.30294]

19 **Pullan RD**, Thomas GA, Rhodes M, Newcombe RG, Williams GT, Allen A, Rhodes J. Thickness of adherent mucus gel on colonic mucosa in humans and its relevance to colitis. *Gut* 1994; **35**: 353-359 [PMID: 8150346 DOI: 10.1136/gut.35.3.353]

20 **Corfield AP**, Myerscough N, Bradfield N, Corfield Cdo A, Gough M, Clamp JR, Durdey P, Warren BF, Bartolo DC, King KR, Williams JM. Colonic mucins in ulcerative colitis: evidence for loss of sulfation. *Glycoconj J* 1996; **13**: 809-822 [PMID: 8910008 DOI: 10.1007/BF00702345]

21 **Kleessen B**, Kroesen AJ, Buhr HJ, Blaut M. Mucosal and invading bacteria in patients with inflammatory bowel disease compared with controls. *Scand J Gastroenterol* 2002; **37**: 1034-1041 [PMID: 12374228 DOI: 10.1080/00365520232037822 0]

22 **Kotlowski R**, Bernstein CN, Sepehri S, Krause DO. High prevalence of Escherichia coli belonging to the B2+D phylogenetic group in inflammatory bowel disease. *Gut* 2007; **56**: 669-675 [PMID: 17028128 DOI: 10.1136/gut.2006.099796]

23 **Montgomery SM**, Ekbom A. Epidemiology of inflammatory bowel disease. *Curr Opin Gastroenterol* 2002; **18**: 416-420 [PMID: 17033315 DOI: 10.1097/00001574-200207000-00004]

24 **Henderson P**, Satsangi J. Genes in inflammatory bowel disease: lessons from complex diseases. *Clin Med* 2011; **11**: 8-10 [PMID: 21404774 DOI: 10.7861/clinmedicine.11-1-8]

25 **Stappenbeck TS**, Rioux JD, Mizoguchi A, Saitoh T, Huett A, Darfeuille-Michaud A, Wileman T, Mizushima N, Carding S, Akira S, Parkes M, Xavier RJ. Crohn disease: a current perspective on genetics, autophagy and immunity. *Autophagy* 2011; **7**: 355-374 [PMID: 20729636 DOI: 10.4161/auto.7.2.13074]

26 **Barrett JC**, Lee JC, Lees CW, Prescott NJ, Anderson CA, Phillips A, Wesley E, Parnell K, Zhang H, Drummond H, Nimmo ER, Massey D, Blaszczyk K, Elliott T, Cotterill L, Dallal H, Lobo AJ, Mowat C, Sanderson JD, Jewell DP, Newman WG, Edwards C, Ahmad T, Mansfield JC, Satsangi J, Parkes M, Mathew CG, Donnelly P, Peltonen L, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin A, Craddock N, Deloukas P, Duncanson A, Jankowski J, Markus HS, Mathew CG, McCarthy MI, Palmer CN, Plomin R, Rautanen A, Sawcer SJ, Samani N, Trembath RC, Viswanathan AC, Wood N, Spencer CC, Barrett JC, Bellenguez C, Davison D, Freeman C, Strange A, Donnelly P, Langford C, Hunt SE, Edkins S, Gwilliam R, Blackburn H, Bumpstead SJ, Dronov S, Gillman M, Gray E, Hammond N, Jayakumar A, McCann OT, Liddle J, Perez ML, Potter SC, Ravindrarajah R, Ricketts M, Waller M, Weston P, Widaa S, Whittaker P, Deloukas P, Peltonen L, Mathew CG, Blackwell JM, Brown MA, Corvin A, McCarthy MI, Spencer CC, Attwood AP, Stephens J, Sambrook J, Ouwehand WH, McArdle WL, Ring SM, Strachan DP. Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region. *Nat Genet* 2009; **41**: 1330-1334 [PMID: 19915572 DOI: 10.1038/ng.483]

27 **Maeda S**, Hsu LC, Liu H, Bankston LA, Iimura M, Kagnoff MF, Eckmann L, Karin M. Nod2 mutation in Crohn's disease potentiates NF-kappaB activity and IL-1beta processing. *Science* 2005; **307**: 734-738 [PMID: 15692052 DOI: 10.1126/science.1103685]

28 **Cooney R**, Baker J, Brain O, Danis B, Pichulik T, Allan P, Ferguson DJ, Campbell BJ, Jewell D, Simmons A. NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. *Nat Med* 2010; **16**: 90-97 [PMID: 19966812 DOI: 10.1038/nm.2069]

29 **Travassos LH**, Carneiro LA, Ramjeet M, Hussey S, Kim YG, Magalhães JG, Yuan L, Soares F, Chea E, Le Bourhis L, Boneca IG, Allaoui A, Jones NL, Nuñez G, Girardin SE, Philpott DJ. Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nat Immunol* 2010; **11**: 55-62 [PMID: 19898471 DOI: 10.1038/ni.1823]

30 **Gearry RB**, Roberts RL, Burt MJ, Frampton CM, Chapman BA, Collett JA, Shirley P, Allington MD, Kennedy MA, Barclay ML. Effect of inflammatory bowel disease classification changes on NOD2 genotype-phenotype associations in a population-based cohort. *Inflamm Bowel Dis* 2007; **13**: 1220-1227 [PMID: 17538984 DOI: 10.1002/ibd.20190]

31 **Lesage S**, Zouali H, Cézard JP, Colombel JF, Belaiche J, Almer S, Tysk C, O'Morain C, Gassull M, Binder V, Finkel Y, Modigliani R, Gower-Rousseau C, Macry J, Merlin F, Chamaillard M, Jannot AS, Thomas G, Hugot JP. CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *Am J Hum Genet* 2002; **70**: 845-857 [PMID: 11875755 DOI: 10.1086/339432]

32 **Nos P**, Domènech E. Management of Crohn's disease in smokers: is an alternative approach necessary? *World J Gastroenterol* 2011; **17**: 3567-3574 [PMID: 21987601 DOI: 10.3748/wjg.v17.i31.3567]

33 **Cosnes J**, Carbonnel F, Beaugerie L, Le Quintrec Y, Gendre JP. Effects of cigarette smoking on the long-term course of Crohn's disease. *Gastroenterology* 1996; **110**: 424-431 [PMID: 8566589 DOI: 10.1053/gast.1996.v110.pm8566589]

34 **Lindberg E**, Järnerot G, Huitfeldt B. Smoking in Crohn's disease: effect on localisation and clinical course. *Gut* 1992; **33**: 779-782 [PMID: 1624159 DOI: 10.1136/gut.33.6.779]

35 **Mahid SS**, Minor KS, Soto RE, Hornung CA, Galandiuk S. Smoking and inflammatory bowel disease: a meta-analysis. *Mayo Clin Proc* 2006; **81**: 1462-1471 [PMID: 17120402 DOI: 10.4065/81.11.1462]

36 **Beaugerie L**, Massot N, Carbonnel F, Cattan S, Gendre JP, Cosnes J. Impact of cessation of smoking on the course of ulcerative colitis. *Am J Gastroenterol* 2001; **96**: 2113-2116 [PMID: 11467641 DOI: 10.1111/j.1572-0241.2001.03944.x]

37 **Biedermann L**, Zeitz J, Mwinyi J, Sutter-Minder E, Rehman A, Ott SJ, Steurer-Stey C, Frei A, Frei P, Scharl M, Loessner MJ, Vavricka SR, Fried M, Schreiber S, Schuppler M, Rogler G. Smoking cessation induces profound changes in the composition of the intestinal microbiota in humans. *PLoS One* 2013; **8**: e59260 [PMID: 23516617 DOI: 10.1371/journal.pone.0059260]

38 **Bernstein CN**, Shanahan F. Disorders of a modern lifestyle: reconciling the epidemiology of inflammatory bowel diseases. *Gut* 2008; **57**: 1185-1191 [PMID: 18515412 DOI: 10.1136/gut.2007.122143]

39 **Jiménez E**, Marín ML, Martín R, Odriozola JM, Olivares M, Xaus J, Fernández L, Rodríguez JM. Is meconium from healthy newborns actually sterile? *Res Microbiol* 2008; **159**: 187-193 [PMID: 18281199 DOI: 10.1016/j.resmic.2007.12.007]

40 **Fouhy F**, Ross RP, Fitzgerald GF, Stanton C, Cotter PD. Composition of the early intestinal microbiota: knowledge, knowledge gaps and the use of high-throughput sequencing to address these gaps. *Gut Microbes* 2012; **3**: 203-220 [PMID: 22572829 DOI: 10.4161/gmic.20169]

41 **Adlerberth I**, Wold AE. Establishment of the gut microbiota in Western infants. *Acta Paediatr* 2009; **98**: 229-238 [PMID: 19143664 DOI: 10.1111/j.1651-2227.2008.01060.x]

42 **Palmer C**, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. *PLoS Biol* 2007; **5**: e177 [PMID: 17594176 DOI: 10.1371/journal.pbio.0050177]

43 **Blaser MJ**, Falkow S. What are the consequences of the disappearing human microbiota? *Nat Rev Microbiol* 2009; **7**: 887-894 [PMID: 19898491 DOI: 10.1038/nrmicro2245]

44 **Hildebrandt MA**, Hoffmann C, Sherrill-Mix SA, Keilbaugh SA, Hamady M, Chen YY, Knight R, Ahima RS, Bushman F, Wu GD. High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology* 2009; **137**: 1716-1724.e1-2 [PMID: 19706296 DOI: 10.1053/j.gastro.2009.08.042]

45 **Murphy EF**, Cotter PD, Healy S, Marques TM, O'Sullivan O, Fouhy F, Clarke SF, O'Toole PW, Quigley EM, Stanton C, Ross PR, O'Doherty RM, Shanahan F. Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models. *Gut* 2010; **59**: 1635-1642 [PMID: 20926643 DOI: 10.1136/gut.2010.215665]

46 **Devkota S**, Wang Y, Musch MW, Leone V, Fehlner-Peach H, Nadimpalli A, Antonopoulos DA, Jabri B, Chang EB. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. *Nature* 2012; **487**: 104-108 [PMID: 22722865 DOI: 10.1038/nature11225]

47 **Montenegro L**, Losurdo G, Licinio R, Zamparella M, Giorgio F, Ierardi E, Leo AD, Principi M. Non steroidal anti-inflammatory drug induced damage on lower gastro-intestinal tract: is there an involvement of microbiota? *Curr Drug Saf* 2014; **9**: 196-204 [PMID: 24809527]

48 **Hayashi Y**, Aoyagi K, Morita I, Yamamoto C, Sakisaka S. Oral administration of mesalazine protects against mucosal injury and permeation in dextran sulfate sodium-induced colitis in rats. *Scand J Gastroenterol* 2009; **44**: 1323-1331 [PMID: 19891583 DOI: 10.3109/00365520903262414]

49 **El Aidy S**, Merrifield CA, Derrien M, van Baarlen P, Hoo-

iveld G, Levenez F, Doré J, Dekker J, Holmes E, Claus SP, Reijngoud DJ, Kleerebezem M. The gut microbiota elicits a profound metabolic reorientation in the mouse jejunal mucosa during conventionalisation. *Gut* 2013; **62**: 1306-1314 [PMID: 22722618 DOI: 10.1136/gutjnl-2011-301955]

50 **Bertuccini L**, Costanzo M, Iosi F, Tinari A, Terruzzi F, Stronati L, Aloi M, Cucchiara S, Superti F. Lactoferrin prevents invasion and inflammatory response following E. coli strain LF82 infection in experimental model of Crohn's disease. *Dig Liver Dis* 2014; **46**: 496-504 [PMID: 24631031 DOI: 10.1016/j.dld.2014.02.009]

51 **Couturier-Maillard A**, Secher T, Rehman A, Normand S, De Arcangelis A, Haesler R, Huot L, Grandjean T, Bressenot A, Delanoye-Crespin A, Gaillot O, Schreiber S, Lemoine Y, Ryffel B, Hot D, Nùñez G, Chen G, Rosenstiel P, Chamaillard M. NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. *J Clin Invest* 2013; **123**: 700-711 [PMID: 23281400 DOI: 10.1172/JCI62236]

52 **Sartor RB**. Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. *Gastroenterology* 2004; **126**: 1620-1633 [PMID: 15168372 DOI: 10.1053/j.gastro.2004.03.024]

53 **Ursing B**, Alm T, Bárány F, Bergelin I, Ganrot-Norlin K, Hoevels J, Huitfeldt B, Järnerot G, Krause U, Krook A, Lindström B, Nordle O, Rosén A. A comparative study of metronidazole and sulfasalazine for active Crohn's disease: the cooperative Crohn's disease study in Sweden. II. Result. *Gastroenterology* 1982; **83**: 550-562 [PMID: 6124474]

54 **Sutherland L**, Singleton J, Sessions J, Hanauer S, Krawitt E, Rankin G, Summers R, Mekhjian H, Greenberger N, Kelly M. Double blind, placebo controlled trial of metronidazole in Crohn's disease. *Gut* 1991; **32**: 1071-1075 [PMID: 1916494]

55 **Colombel JF**, Lémann M, Cassagnou M, Bouhnik Y, Duclos B, Dupas JL, Notteghem B, Mary JY. A controlled trial comparing ciprofloxacin with mesalazine for the treatment of active Crohn's disease. Groupe d'Etudes Thérapeutiques des Affections Inflammatoires Digestives (GETAID). *Am J Gastroenterol* 1999; **94**: 674-678 [PMID: 10086650]

56 **Arnold GL**, Beaves MR, Pryjdun VO, Mook WJ. Preliminary study of ciprofloxacin in active Crohn's disease. *Inflamm Bowel Dis* 2002; **8**: 10-15 [PMID: 11837933]

57 **Prantera C**, Zannoni F, Scribano ML, Berto E, Andreoli A, Kohn A, Luzi C. An antibiotic regimen for the treatment of active Crohn's disease: a randomized, controlled clinical trial of metronidazole plus ciprofloxacin. *Am J Gastroenterol* 1996; **91**: 328-332 [PMID: 8607501]

58 **Greenbloom SL**, Steinhart AH, Greenberg GR. Combination ciprofloxacin and metronidazole for active Crohn's disease. *Can J Gastroenterol* 1998; **12**: 53-56 [PMID: 9544412]

59 **Leiper K**, Morris AI, Rhodes JM. Open label trial of oral clarithromycin in active Crohn's disease. *Aliment Pharmacol Ther* 2000; **14**: 801-806 [PMID: 10848665]

60 **Steinhart AH**, Feagan BG, Wong CJ, Vandervoort M, Mikolainis S, Croitoru K, Seidman E, Leddin DJ, Bitton A, Drouin E, Cohen A, Greenberg GR. Combined budesonide and antibiotic therapy for active Crohn's disease: a randomized controlled trial. *Gastroenterology* 2002; **123**: 33-40 [PMID: 12105831]

61 **Rutgeerts P**, Hiele M, Geboes K, Peeters M, Penninckx F, Aerts R, Kerremans R. Controlled trial of metronidazole treatment for prevention of Crohn's recurrence after ileal resection. *Gastroenterology* 1995; **108**: 1617-1621 [PMID: 7768364]

62 **Rutgeerts P**, Van Assche G, Vermeire S, D'Haens G, Baert F, Noman M, Aerden I, De Hertogh G, Geboes K, Hiele M, D'Hoore A, Penninckx F. Ornidazole for prophylaxis of postoperative Crohn's disease recurrence: a randomized, double-blind, placebo-controlled trial. *Gastroenterology* 2005; **128**: 856-861 [PMID: 15825069]

63 **Turunen U**, Färkkilä V. Long-term treatment of ulcerative colitis with ciprofloxacin. *Gastroenterology* 1999; **117**: 282-283 [PMID: 10428613]

64 **Mantzaris GJ**, Archavlis E, Christoforidis P, Kourtessas D, Amberiadis P, Florakis N, Petraki K, Spiliadi C, Triantafyllou G. A prospective randomized controlled trial of oral ciprofloxacin in acute ulcerative colitis. *Am J Gastroenterol* 1997; **92**: 454-456 [PMID: 9068468]

65 **Casellas F**, Borruel N, Papo M, Guarner F, Antolín M, Videla S, Malagelada JR. Antiinflammatory effects of enterically coated amoxicillin-clavulanic acid in active ulcerative colitis. *Inflamm Bowel Dis* 1998; **4**: 1-5 [PMID: 9552221]

66 **Turner D**, Levine A, Kolho KL, Shaoul R, Ledder O. Combination of oral antibiotics may be effective in severe pediatric ulcerative colitis: A preliminary report. *J Crohns Colitis* 2014; Epub ahead of print [PMID: 24958064 DOI: 10.1016/j.crohns.2014.05.010]

67 **Shen B**, Achkar JP, Lashner BA, Ormsby AH, Remzi FH, Brzezinski A, Bevins CL, Bambrick ML, Seidner DL, Fazio VW. A randomized clinical trial of ciprofloxacin and metronidazole to treat acute pouchitis. *Inflamm Bowel Dis* 2001; **7**: 301-305 [PMID: 11720319]

68 **Gionchetti P**, Rizzello F, Venturi A, Brigidi P, Matteuzzi D, Bazzocchi G, Poggioli G, Miglioli M, Campieri M. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. *Gastroenterology* 2000; **119**: 305-309 [PMID: 10930365 DOI: 10.1053/gast.2000.9370]

69 **Dignass A**, Lindsay JO, Sturm A, Windsor A, Colombel JF, Allez M, D'Haens G, D'Hoore A, Mantzaris G, Novacek G, Oresland T, Reinisch W, Sans M, Stange E, Vermeire S, Travis S, Van Assche G. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 2: current management. *J Crohns Colitis* 2012; **6**: 991-1030 [PMID: 23040451 DOI: 10.1016/j.crohns.2012.09.002]

70 **Matthes H**, Krummenerl T, Giensch M, Wolff C, Schulze J. Clinical trial: probiotic treatment of acute distal ulcerative colitis with rectally administered Escherichia coli Nissle 1917 (EcN). *BMC Complement Altern Med* 2010; **10**: 13 [PMID: 20398311 DOI: 10.1186/1472-6882-10-13]

71 **Sood A**, Midha V, Makharia GK, Ahuja V, Singal D, Goswami P, Tandon RK. The probiotic preparation, VSL#3 induces remission in patients with mild-to-moderately active ulcerative colitis. *Clin Gastroenterol Hepatol* 2009; **7**: 1202-1209, 1209.e1 [PMID: 19631292 DOI: 10.1016/j.cgh.2009.07.016]

72 **Pronio A**, Montesani C, Butteroni C, Vecchione S, Mumolo G, Vestri A, Vitolo D, Boirivant M. Probiotic administration in patients with ileal pouch-anal anastomosis for ulcerative colitis is associated with expansion of mucosal regulatory cells. *Inflamm Bowel Dis* 2008; **14**: 662-668 [PMID: 18240282 DOI: 10.1002/ibd.20369]

73 **Bibiloni R**, Fedorak RN, Tannock GW, Madsen KL, Gionchetti P, Campieri M, De Simone C, Sartor RB. VSL#3 probiotic-mixture induces remission in patients with active ulcerative colitis. *Am J Gastroenterol* 2005; **100**: 1539-1546 [PMID: 15984978 DOI: 10.1111/j.1572-0241.2005.41794.x]

74 **Tsuda Y**, Yoshimatsu Y, Aoki H, Nakamura K, Irie M, Fukuda K, Hosoe N, Takada N, Shirai K, Suzuki Y. Clinical effectiveness of probiotics therapy (BIO-THREE) in patients with ulcerative colitis refractory to conventional therapy. *Scand J Gastroenterol* 2007; **42**: 1306-1311 [PMID: 17852859 DOI: 10.1080/00365520701396091]

75 **Lorea Baroja M**, Kirjavainen PV, Hekmat S, Reid G. Anti-inflammatory effects of probiotic yogurt in inflammatory bowel disease patients. *Clin Exp Immunol* 2007; **149**: 470-479 [PMID: 17590176 DOI: 10.1111/j.1365-2249.2007.03434.x]

76 **Zocco MA**, dal Verme LZ, Cremonini F, Piscaglia AC, Nista EC, Candelli M, Novi M, Rigante D, Cazzato IA, Ojetti V, Armuzzi A, Gasbarrini G, Gasbarrini A. Efficacy of Lactobacillus GG in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther* 2006; **23**: 1567-1574 [PMID: 16696804 DOI: 10.1111/j.1365-2036.2006.02927.x]

77 **Kato K**, Mizuno S, Umesaki Y, Ishii Y, Sugitani M, Imaoka A,

Otsuka M, Hasunuma O, Kurihara R, Iwasaki A, Arakawa Y. Randomized placebo-controlled trial assessing the effect of bifidobacteria-fermented milk on active ulcerative colitis. *Aliment Pharmacol Ther* 2004; **20**: 1133-1141 [PMID: 15569116 DOI: 10.1111/j.1365-2036.2004.02268.x]

78 **Braat H**, Rottiers P, Hommes DW, Huyghebaert N, Remaut E, Remon JP, van Deventer SJ, Neirynck S, Peppelenbosch MP, Steidler L. A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. *Clin Gastroenterol Hepatol* 2006; **4**: 754-759 [PMID: 16716759 DOI: 10.1016/j.cgh.2006.03.028]

79 **Bai AP**, Ouyang Q. Probiotics and inflammatory bowel diseases. *Postgrad Med J* 2006; **82**: 376-382 [PMID: 16754706 DOI: 10.1136/pgmj.2005.040899]

80 **Prantera C**, Scribano ML, Falasco G, Andreoli A, Luzi C. Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn's disease: a randomised controlled trial with Lactobacillus GG. *Gut* 2002; **51**: 405-409 [PMID: 12171964 DOI: 10.1136/gut.51.3.405]

81 **Schultz M**, Veltkamp C, Dieleman LA, Grenther WB, Wyrick PB, Tonkonogy SL, Sartor RB. Lactobacillus plantarum 299V in the treatment and prevention of spontaneous colitis in interleukin-10-deficient mice. *Inflamm Bowel Dis* 2002; **8**: 71-80 [PMID: 11854603 DOI: 10.1097/00054725-200203000-00001]

82 **Dieleman LA**, Goerres MS, Arends A, Sprengers D, Torrice C, Hoentjen F, Grenther WB, Sartor RB. Lactobacillus GG prevents recurrence of colitis in HLA-B27 transgenic rats after antibiotic treatment. *Gut* 2003; **52**: 370-376 [PMID: 12584218 DOI: 10.1136/gut.52.3.370]

83 **Fabia R**, Ar'Rajab A, Johansson ML, Willén R, Andersson R, Molin G, Bengmark S. The effect of exogenous administration of Lactobacillus reuteri R2LC and oat fiber on acetic acid-induced colitis in the rat. *Scand J Gastroenterol* 1993; **28**: 155-162 [PMID: 8382837 DOI: 10.3109/00365529309096063]

84 **Gionchetti P**, Rizzello F, Helwig U, Venturi A, Lammers KM, Brigidi P, Vitali B, Poggioli G, Miglioli M, Campieri M. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. *Gastroenterology* 2003; **124**: 1202-1209 [PMID: 12730861 DOI: 10.1016/S0016-5085(03)00171-9]

85 **Cui HH**, Chen CL, Wang JD, Yang YJ, Cun Y, Wu JB, Liu YH, Dan HL, Jian YT, Chen XQ. Effects of probiotic on intestinal mucosa of patients with ulcerative colitis. *World J Gastroenterol* 2004; **10**: 1521-1525 [PMID: 15133865]

86 **Malchow HA**. Crohn's disease and Escherichia coli. A new approach in therapy to maintain remission of colonic Crohn's disease? *J Clin Gastroenterol* 1997; **25**: 653-658 [PMID: 9451682 DOI: 10.1097/00004836-199712000-00021]

87 **Marteau P**, Lémann M, Seksik P, Laharie D, Colombel JF, Bouhnik Y, Cadiot G, Soulé JC, Bourreille A, Metman E, Lerebours E, Carbonnel F, Dupas JL, Veyrac M, Coffin B, Moreau J, Abitbol V, Blum-Sperisen S, Mary JY. Ineffectiveness of Lactobacillus johnsonii LA1 for prophylaxis of postoperative recurrence in Crohn's disease: a randomised, double blind, placebo controlled GETAID trial. *Gut* 2006; **55**: 842-847 [PMID: 16377775 DOI: 10.1136/gut.2005.076604]

88 **Van Gossum A**, Dewit O, Louis E, de Hertogh G, Baert F, Fontaine F, DeVos M, Enslen M, Paintin M, Franchimont D. Multicenter randomized-controlled clinical trial of probiotics (Lactobacillus johnsonii, LA1) on early endoscopic recurrence of Crohn's disease after lleo-caecal resection. *Inflamm Bowel Dis* 2007; **13**: 135-142 [PMID: 17206696 DOI: 10.1002/ibd.20063]

89 **Butterworth AD**, Thomas AG, Akobeng AK. Probiotics for induction of remission in Crohn's disease. *Cochrane Database Syst Rev* 2008; **(3)**: CD006634 [PMID: 18646162 DOI: 10.1002/14651858]

90 **Cummings JH**. Dietary carbohydrates and the colonic microflora. *Curr Opin Clin Nutr Metab Care* 1998; **1**: 409-414 [PMID: 10565385 DOI: 10.1097/00075197-199809000-00007]

91 **Bird AR**, Brown IL, Topping DL. Starches, resistant starches, the gut microflora and human health. *Curr Issues Intest Microbiol* 2000; **1**: 25-37 [PMID: 11709851]

92 **Jacobasch G**, Schmiedl D, Kruschewski M, Schmehl K. Dietary resistant starch and chronic inflammatory bowel diseases. *Int J Colorectal Dis* 1999; **14**: 201-211 [PMID: 10647628 DOI: 10.1007/s003840050212]

93 **Videla S**, Vilaseca J, Antolín M, García-Lafuente A, Guarner F, Crespo E, Casalots J, Salas A, Malagelada JR. Dietary inulin improves distal colitis induced by dextran sodium sulfate in the rat. *Am J Gastroenterol* 2001; **96**: 1486-1493 [PMID: 11374687 DOI: 10.1111/j.1572-0241.2001.03802.x]

94 **Camuesco D**, Peran L, Comalada M, Nieto A, Di Stasi LC, Rodriguez-Cabezas ME, Concha A, Zarzuelo A, Galvez J. Preventative effects of lactulose in the trinitrobenzenesulphonic acid model of rat colitis. *Inflamm Bowel Dis* 2005; **11**: 265-271 [PMID: 15735433 DOI: 10.1097/01.MIB.0000160808.30988.d9]

95 **Welters CF**, Heineman E, Thunnissen FB, van den Bogaard AE, Soeters PB, Baeten CG. Effect of dietary inulin supplementation on inflammation of pouch mucosa in patients with an ileal pouch-anal anastomosis. *Dis Colon Rectum* 2002; **45**: 621-627 [PMID: 12004211 DOI: 10.1007/s10350-004-6257-2]

96 **Lindsay JO**, Whelan K, Stagg AJ, Gobin P, Al-Hassi HO, Rayment N, Kamm MA, Knight SC, Forbes A. Clinical, microbiological, and immunological effects of fructo-oligosaccharide in patients with Crohn's disease. *Gut* 2006; **55**: 348-355 [PMID: 16162680 DOI: 10.1136/gut.2005.074971]

97 **Schultz M**, Munro K, Tannock GW, Melchner I, Göttl C, Schwietz H, Schölmerich J, Rath HC. Effects of feeding a probiotic preparation (SIM) containing inulin on the severity of colitis and on the composition of the intestinal microflora in HLA-B27 transgenic rats. *Clin Diagn Lab Immunol* 2004; **11**: 581-587 [PMID: 15138186]

98 **Furrie E**, Macfarlane S, Kennedy A, Cummings JH, Walsh SV, O'neil DA, Macfarlane GT. Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut* 2005; **54**: 242-249 [PMID: 15647189 DOI: 10.1136/gut.2004.044834]

99 **Gough E**, Shaikh H, Manges AR. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent Clostridium difficile infection. *Clin Infect Dis* 2011; **53**: 994-1002 [PMID: 22002980 DOI: 10.1093/cid/cir632]

100 **Borody TJ**, Khoruts A. Fecal microbiota transplantation and emerging applications. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 88-96 [PMID: 22183182 DOI: 10.1038/nrgastro.2011.244]

101 **Landy J**, Al-Hassi HO, McLaughlin SD, Walker AW, Ciclitira PJ, Nicholls RJ, Clark SK, Hart AL. Review article: faecal transplantation therapy for gastrointestinal disease. *Aliment Pharmacol Ther* 2011; **34**: 409-415 [PMID: 21682755 DOI: 10.1111/j.1365-2036.2011.04737.x]

102 **Eiseman B**, Silen W, Bascom GS, Kauvar AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery* 1958; **44**: 854-859 [PMID: 13592638]

103 **Anderson JL**, Edney RJ, Whelan K. Systematic review: faecal microbiota transplantation in the management of inflammatory bowel disease. *Aliment Pharmacol Ther* 2012; **36**: 503-516 [PMID: 22827693 DOI: 10.1111/j.1365-2036.2012.05220.x]

P- Reviewer: Rajendran VM, Sipos F **S- Editor**: Ji FF
L- Editor: Cant MR **E- Editor**: Wang CH