The Role of Microbiota in Inflammatory Bowel Disease

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Key Words
Crohn’s disease · Inflammatory bowel disease · Intestine · Microbiota · Prebiotics · Ulcerative colitis

Introduction

Inflammatory Bowel Disease

Inflammatory bowel diseases (IBDs) are inflammatory diseases of the gastrointestinal tract (GIT). This group of diseases includes Crohn’s disease (CD), ulcerative colitis (UC), indeterminate colitis and microscopic colitis. Most prevalent are CD and UC, two disease entities that are diagnosed based on clinical, radiological and endoscopic findings, and are a cluster of many possible presentations and disease behaviors. CD may involve any part of the GIT from mouth to anus in a non-continuous fashion with inflammatory changes involving all layers of the intestinal wall. The most common area of involvement is the terminal ileum, though in children involvement of the colon is most common. UC involves the rectum and may extend proximally in various lengths up to involvement of the whole colon causing pancolitis. On histology, the inflammation is usually limited to the mucosa and on endoscopy, macroscopic findings include erythema and loss of vasculature in mild cases and extensive ulcerations in severe cases. Pathology examination shows mainly chronic inflammation in the involved areas. The definition and course of the various clinical types of CD (inflammatory, stricturizing, fistulizing) is beyond the scope of this review, but it is essential to note that in IBD, disease manifestations are not limited to the

Abstract

Crohn’s disease and ulcerative colitis are inflammatory diseases of the gastrointestinal tract that together comprise a spectrum of diseases entitled inflammatory bowel disease (IBD). The human intestine is colonized with commensal microbiota in concentrations that exceed the number of cells in the human body. Under normal physiologic conditions there is an interplay between these bacteria and the human host in a process that maintains the integrity of the immune system in health and disease. Gut microbiota play an important role in IBD. Commensal and pathologic bacteria comprise part of the etiology of IBD, and the gut microbiota in patients with IBD is different from that in healthy controls. Their influence is probably brought about in initiation of disease as well as in flare-ups, and it is logical to assume that manipulation of the gut microbiota could be a preventive measure in IBD evolution and may play a therapeutic role in disease exacerbation. This review will briefly discuss issues related to studying gut microbiota, the interplay of these microbiota with the immunologic system in IBD, and focus on the role of microbiota in the etiology, flare-ups and treatment of IBD.

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0517–8606/09/0671–0027$26.00/0
GIT. Extra-intestinal manifestations are common with arthritis being the most common. Both CD and UC are characterized by periods of remission and periods of relapse with inflammation. In CD, relapses are usually characterized by abdominal pain, diarrhea, fever and weight loss, while in UC, rectal bleeding, diarrhea and abdominal pain are the main features. Laboratory results are compatible with inflammation including leukocytosis, elevated erythrocyte sedimentation rate and C-reactive protein, hypoalbuminemia, and anemia (which could be normocytic due to chronic disease, microcytic due to iron deficiency, or most commonly combined). Imaging studies can help delineate the inflamed sections. The GIT manifestations may or may not be accompanied by extra-intestinal manifestations [1, 2].

**Microbiota of the GIT**

The human intestine is colonized by commensal microbiota in concentrations that exceed the number of cells in the human body. Under normal physiologic conditions, there is an interplay between these bacteria and the human host where the host contributes essential nutrients necessary for the survival of the microbiota and the microbiota assist in maintaining the integrity of the immune system by activating specific receptors, pathways and cytokines [for an elaborate discussion see Fasano, pp 9–18, in this issue]. Historically the GIT microbiota was defined based on the ability to culture specific species, recognizing that a much larger group of ‘non-culturable’ bacteria exists. The definition of ‘non-culturable’ bacteria was not absolute, as these microbacteria required special unavailable conditions and their exact definition had to be postponed until more modern molecular laboratory techniques were developed.

Previously, about 300 culturable colonic species have been identified by classic culture techniques. Modern methods using molecular analysis such as polymerase chain reaction (PCR) and fluorescent in situ hybridization enabled researchers to define many previously non-culturable bacteria. Metagenomics refers to molecular techniques of genetic analysis of bacterial populations performed in parallel, enabling the identification of the different commensal bacteria [3]. Molecular analysis of the microbial composition of fecal and mucosal samples using 16s ribosomal DNA and RNA have enabled definition of 1,800 genera and between 15,000 and 36,000 individual species with the total microbial load in the intestine of $10^{13}$–$10^{14}$ microorganisms with increasing concentrations from the proximal part of the GIT reaching $10^7$–$10^8$ in the distal ileum and $10^{11}$–$10^{12}$ organisms/g luminal contents in the colon [4].

Four divisions comprise the majority of the GIT microflora: Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria [4]. During the evolution of the GIT, a specific pattern recognition of bacterial components by eukaryotic cells has developed including lipopolysaccharides, peptidoglycans, ssRNA, muramyl dipeptide, and flagellins. The specific pattern recognition signals are mediated by toll-like receptors (TLRs) and nucleotide oligomerization domains (NODs), components of the immune system that play a crucial role in establishing an adequate or pathological immune response [5]. The microbiota plays a key role in the crosstalk with the host immune system, evoking a myriad of responses that are regulated by numerous pro- and anti-inflammatory cytokines. Studying the crosstalk pathways is tackled with methodological obstacles. The enormous number of species turns the task of defining the specific role of each bacterium into an almost impossible one. Furthermore, when elucidating the role of the microbiota in IBD, the distinction between bacteria found in the lumen and bacteria adherent to the mucosa is difficult to solve. To this extent, some of the studies analyze fecal bacteria, whereas bacteria adherent to the mucosa itself were studied in some surgical or biopsy specimens [4]. This may explain the observation that different bacteria were identified in these two types of studies. With all these limitations, this review will summarize the available data on the interplay between the host and bacteria in IBD.

**Microbiota and Etiology of IBD**

There are at least four theories relating to microbiota and the evolution of IBD which will be discussed. These include: change in the population of commensal bacteria resulting in ‘less friendly’ microbiota causing inflammation; pathogenic bacteria causing IBD; a change in commensal bacteria turning it to pathogenic bacteria, and abnormal reaction to normal commensal bacteria.

**Change in the Population of Commensal Bacteria**

The population of commensal bacteria of healthy individuals is different from that of patients with IBD. This is true for pediatric as well as adult patients, and for bacteria isolated from the intestinal lumen as well as bacteria adherent to the mucosa [6–11]. The results are inconsistent regarding the differences in active and inactive disease, CD vs. UC, and differences in luminal vs. adherent
bacteria. Most of the studies demonstrate that the level of Firmicutes, especially Clostridium species, is decreased and the level of Enterobacteriaceae, including Escherichia coli, is increased in IBD. Furthermore, E. coli is abundant in ulcers and fistulae. Nevertheless, it is not known whether these changes represent a primary phenomenon where these bacteria play a role in the evolution of IBD, or whether they represent a secondary phenomenon where immune dysregulation or inflammation result in changes in the isolated bacteria. Whether primary or secondary, as the difference in the commensal bacterial population is consistent, it is logical to assume that it plays a role in the pathogenesis of IBD.

**Pathogenic Bacteria**

More than one bacterium has been postulated to cause CD, of which Mycobacterium avium (MAC) is the one most intensively studied. There are many clinical similarities between human CD and cattle MAC. In addition, MAC was cultured and detected by PCR and ELISA in the feces and mucosa of patients with CD. A meta-analysis performed by Feller et al. [13], examining PCR in tissue samples from IBD patients vs. controls, revealed a pooled odds ratio (OR) of 7.01 (95% CI 3.95–12.4) for CD and 4.13 (95% CI 1.57–10.9) for UC. Moreover, 6-mercaptopurine, a purine analog immunosuppressive drug used in the treatment of CD, was paradoxically found to inhibit MAC growth in culture [14]. It is compelling to think of a causative effect between CD and MAC, but data from a study by Selby et al. [15] shed new light on the subject. A prospective 2-year randomized control study showed no efficacy of anti-MAC triple therapy (clarithromycin, rifabutin, and clofazimine) for CD. Some researchers concluded that this evidence is the final proof that MAC is of no importance in the pathogenesis of CD, but the debate is far from being settled [13–15] give reference.

**Change in Commensal Bacteria**

Ileal lesions in CD patients are colonized by pathogenic adherent-invasive E. coli (AIEC) that harbor various pathogenic and virulence factors. AIEC is prevalent in and adherent to the mucosa of CD patients: ~36% of mucosa specimens from CD patients vs. ~6% of controls [16]. In a mouse model, a pathogenic flagellated AIEC aggregated severe colitis in a model of chemical colitis (dextran sulfate sodium, DSS) [17]. A rise in the mRNA of membrane and cytosolic receptors (TLR5 and IPAF, respectively) for flagellin was observed, and thereupon an increase in the mRNA of proinflammatory cytokines (IL-1β and IL-6). This shows that AIEC can evoke an inflammatory response similar to IBD. CD patients have increased expression of CEACAM6 which acts as a receptor for AIEC and supports the adherence and mucosal colonization of the bacteria in these patients [18]. AIEC causes amplification of CEACAM6 expression, thereby causing further amplification of the inflammatory response [18]. On the whole (fig. 1, 2), AIEC have been shown to effectively colonize, adhere, invade, survive, replicate and induce proinflammatory cytokine production, and form granulomas within the mucosa of CD patients [19]. Although AIEC may serve as a model for the pathogenesis of IBD, the exact role of AIEC is yet to be determined. Another interesting relationship between IBD and gut microflora is exhibited by the relationship of IBD and antibodies against Saccharomyces cerevisiae (ASCA) [20]. An increased concentration of ASCA is found in 60–70% of CD patients and 10–15% UC patients. These antibodies seem not to be autoantibodies, but rather antibodies against bacterial or fungal species. S. cerevisiae, a yeast commonly used in the food industry for baking and brewing, has not been directly implicated in the pathogenesis of CD. Interestingly, in a study of patients with CD who had frozen blood samples taken as part of a national survey at the age of 18, ASCA were present in 10/32 (31.3%) CD patients before clinical diagnosis compared with 0/95 in controls (p < 0.001) [21]. In that study, none of the 8 patients with serum samples available before diagnosis of UC were ASCA-positive, and the mean interval between ASCA detection and diagnosis was 38 months. Similarly, several studies reported an increased prevalence of ASCA in healthy relatives of patients with CD. Furthermore, ASCA is more prevalent in familial cases of CD than in sporadic cases [for review see, 20]. Thus, the increased occurrence of ASCA in first-degree relatives of patients with CD and the early detection of ASCA in future CD cases are compatible with the role these antibodies play as markers of genetic disease susceptibility.

**Abnormal Reaction to Commensal Bacteria**

An abnormal response to normal commensal bacteria can cause overt disease in humans prone to develop CD. The abnormal response can occur at various levels including factors affecting the mucosal barrier, bacterial killing or affecting a dysregulated immune response.

**Enhanced Mucosal Permeability (Mucosal Barrier)**

The first line of defense against invasion of noxious agents and bacteria is the gut mucosal barrier, comprised
of a single layer of epithelial cells that are fused together by tight junctions. These tight junctions prevent the paracellular passage of macromolecules and establish the impermeability of the gut to such molecules. In addition, a secreted mucous layer provides defense to the enterocyte from noxious agents and bacteria. It is obvious that a defect in barrier integrity will result in increased permeability. Therefore, erosions or ulcers at the level of the tight junctions or inflammations that impair tight junction function will result in increased permeability. Indeed, increased intestinal permeability is a well-known and established phenomenon in IBD patients [22], and in these patients, mucosal permeability correlates with inflammation. Furthermore, increased intestinal permeability was found in the healthy relatives of patients with CD, especially after exposure to non-steroidal anti-inflammatory drugs [23–26], suggesting a primary defect in the mucosal barrier as a predisposing factor for the development of IBD. Structural proteins such as claudin are important elements in establishing gut integrity; while claudin 2 is pore forming, claudins 5 and 6 are tight junction components [25]. Patients with CD have upregulation of
claudin 2 and downregulation of claudins 5 and 6 [27, 28], thereby causing enhanced permeability. In that context, IL-13 upregulates claudin 2 and is elevated in some patients with CD, and tumor necrosis factor (TNF)-α is known to enhance mucosal permeability as well.

When the gut barrier is compromised and permeability increases, commensal and pathogenic bacteria can enter the epithelial cells and cause local inflammation or transverse the mucosa altogether and migrate to local lymph nodes or even further. Pathologic T cells can be activated as demonstrated in mice with a specific mucosal barrier defect [29]. Whether increased permeability is a primary or a secondary phenomenon has not been resolved, but either way it is apt to increase inflammation.

Defective Bacterial Killing

Effective defense of the gut mucosa necessitates selective permeability, luminal sampling of antigens (i.e. dendritic cells), secretion of antimicrobial and defense proteins (i.e. immunoglobulin A, defensins, trefoil factor) and, when invasion occurs, effective bacterial killing. Once bacteria have invaded the epithelial cells, CD patients show low killing capability. Recent findings shed some light on the etiology of this phenomenon though the exact cause is yet to be elucidated. NOD2 (now termed CARD15) is an intra-cytoplasmatic receptor. Its C-terminal has a leucine-rich repeat domain that is important in the binding of bacteria, specifically a muramyl dipeptide. Activation of NOD2 via the leucine-rich repeat domain causes induction of NF-κB, a regulatory proinflammatory cytokine. There are three mutations in NOD2 that are common in patients with CD. All three mutations cause failure of NF-κB activation [30, 31], decreased production of α-defensin [32] and defective intracellular killing [33]. Nuding et al. [34] studied the endpoint of killing capability using a flow cytometric assay of colonic extracts of CD and UC patients and healthy controls. Extracts from CD patients had much lower killing activity than those of either healthy controls or UC patients. Furthermore, decreased bacterial killing in patients with CD is augmented by defective clearance of intracellular pathogens by colonocytes [33]. Although beyond the scope of this review, genome-wide association studies have identified variants in the autophagy-related 16-like 1 (S. cerevisiae) gene (ATG16L1), a protein complex essential for autophagy, as associated with the risk of developing CD [35]. Autophagy is a basic biological process in which cytoplasmic portions are sequestered by a membrane for delivery to lysosomes. Autophagy plays an important role in the clearance of pathogens, and thus changes in autophagy genes will either protect or enhance the risk of developing CD [36].

Defective Immunoregulation

Immunoregulation is a delicate and precisely orchestrated process. Stimulatory and inhibitory mechanisms act simultaneously in coordination in order to combat pathogens whilst maintaining gut integrity and health. Bacterial recognition by transmembrane TLR and intracellular NOD receptors causes a cascade of inflammatory reactions through activation of NF-κB, mitogen-activated protein kinase and AKT/phosphatidylinositol-3’-kinase. TNF-α, IL-6 and interferon (IFN)-γ are all elevated and contribute to chronic inflammation and tissue damage [37]. This is counteracted by anti-inflammatory mechanisms such as IL-10, peroxisomal proliferator-activated receptor (PPAR)-γ, TGF-β, IFN-α and IFN-β and eicosanoids. This complicated process is dependent upon communication between epithelial and lamina propria immune cells [4]. In health, all pathways result in maintaining gut integrity with well-contained local inflammatory responses. In some IBD patients it seems that there is a disruption in this delicate homeostasis. As briefly mentioned earlier, CD has multiple phenotypes: inflammatory, stricturing, fistulizing, so it is conceivable that different dysregulated pathways are responsible for different disease phenotypes. Regardless of the specific dysregulated pathway, it seems that immunologic dysregulation combines with commensal or pathogenic bacteria in the inflammatory process. Interestingly, immunologic process abnormalities might differ at the beginning of disease vs. later on. Kugathasan et al. [38] found that IL-12 induced T cells from early CD to acquire a T-helper (Th) type 1 response characterized by high IFN-γ production, similar to acute infectious colitis, whereas this phenomenon was of a much lower magnitude in patients with longstanding disease.

In the context of T cell function, it is important to remember that autophagy genes, involved in the process of pathogen clearance, are essential for T-cell development, survival and proliferation [39], and it appears that autophagy plays a part both in host immune defense mechanisms and is a tool for bacteria to enhance their pathogenicity [36].

Immunologic dysregulation is part of IBD etiology, and this goes hand in hand with the presence of bacteria, which is essential for the development of IBD. Animal models for IBD as well as the behavior of the disease with diverting ileostomy provided conclusive evidence that bacteria are an obligatory component of inflammation.
For example, IL-10-deficient mice develop spontaneous colitis, but no colitis is observed when these mice are living in a sterile environment. Thus it appears that in the presence of a primary immunologic defect or defects the presence of commensal bacteria is crucial [40]. While animal models are a good example of the essential role of gut microbiota for the development of IBD, diverted ileostomy provides evidence in humans of the importance of the gut microflora in maintaining inflammation. When fecal flow, rich in bacteria, is diverted, as in ileostomy, the excluded part goes into remission (free of inflammation), whereas when continuity is reestablished and the bacteria-rich content is reintroduced, inflammation returns [41–43].

Infections as a Predisposing Factor for the Onset of IBD and Flare-Ups

There are data suggesting that infection predisposes for the onset of IBD and contributes to exacerbations thereafter. Various epidemiologic studies demonstrate a greater incidence of IBD after infectious gastroenteritis. Powell and Wilmot [44] were the first to describe UC after amebic dysentery [45]. Results of a large cohort of adults with an episode of acute gastroenteritis and matched controls demonstrated doubling of the risk of developing IBD after infectious gastroenteritis [46]. A recent epidemiologic study in England, using the computerized data available for all hospitalized patients, found the same seasonal variation in hospitalization for CD, UC and bacterial gastroenteritis without any correlation with viral gastroenteritis [47]. Thus, currently available data suggest a causative relationship between infectious gastroenteritis and the onset of IBD.

Flare-ups of IBD are known to be associated with various enteric pathogens such as Clostridium difficile, enteropathogenic E. coli and species of Salmonella, Shigella and Campylobacter. In a study by Meyer et al. [48] 10.5% of stool cultures were positive for enteric pathogens. Of 213 patients, C. difficile was responsible for 5.5% of the cases (11 patients), while Campylobacter spp. (5 patients), Entamoeba histolytica (3 patients), Salmonella spp., Plasmodonias shigelloides, Strongyloides stercoralis and Blastocystis hominis (1 patient each) comprised the other pathogens [49]. Performing stool cultures to exclude C. difficile toxins and the presence of parasites, is recommended for IBD patients with a flare-up. If a pathogen is diagnosed, antibiotic treatment should be initiated in sharp contrast to recommendations for healthy individuals where anti-biotic treatment is seldom indicated. C. difficile is a pathogen with emerging importance in hospitalized patients in general and especially in patients with IBD. In recent years, C. difficile has become more virulent causing more severe disease associated with greater morbidity and mortality. Furthermore, these strains are more resistant to various antibiotics [50, 51]. C. difficile colitis in patients hospitalized with IBD has risen about 2- to 3-fold from 1998 to 2004, with the incidence reaching 5% of admissions [52, 53]. Interestingly, C. difficile prevalence has been reported to be higher in IBD patients than in non-IBD patients [52], and in another cohort higher in UC compared to CD [53]. C. difficile colitis in a patient with IBD is associated with a much worse prognosis including greater mortality, higher rates of colectomy, longer length of hospital stay, and a more expensive hospitalization.

The scope of this review is fecal bacteria and IBD, but when discussing infections and IBD one should bear in mind other pathogens, of which the most clinically relevant are viral infections with cytomegalovirus (CMV). CMV infection is of special importance in patients suffering from colitis. In a setup of non-remitting colitis, CMV should be searched for and treated properly when diagnosed.

In summary, gastrointestinal infections may play a part in the pathogenesis of IBD and play an important role in disease flare-up, creating a delicate interaction between host, commensal bacteria and pathogenic bacteria in the initiation, maintenance and flare-ups of inflammation. When evaluating IBD patients in relapse, physicians should routinely search for infections and treat them appropriately.

Bacteria and Treatment of IBD

IBD is characterized by periods of remission and relapses. Treatment is aimed at maintaining remission and treating flare-ups. The therapeutic arsenal for IBD includes 5-aminosalicylic acid drugs which are anti-inflammatory drugs; immunomodulating agents (steroids, 6-mercaptopurine and its pro-drug azathioprine, methotrexate), and biologic agents such as anti-TNF antibodies. Due to the established role of the gut microbiota and enteric pathogens in IBD, manipulation of the microflora, mainly by probiotic supplementation, and treatment with antibiotics have been studied extensively both in UC and CD, and these studies in animals and humans will be discussed here briefly.
Probiotics and IBD

Probiotics is a general term encompassing a large group of organisms. Although each probiotic bacterium has its own characteristics, in general probiotics have proven anti-inflammatory activity both in vivo and in vitro. Based on the known interaction between bacteria and the intestinal immune system, it is logical to assume that specific strains will have the ability to overcome the predetermined inflammatory dysregulation present in IBD. Currently, probiotics have proven efficacious in pouchitis and in the maintenance of remission and exacerbation in UC. At present, despite a theoretical rationale that supports the use of probiotics in CD, it has not proved efficacious either in the maintenance of remission and acute exacerbations or as postoperative treatment [54–58].

Theoretical Rationale and Animal Studies

Lilly et al. [59] defined probiotics in 1965 as selective nonpathogenic living microorganisms including some commensal bacterial flora, which have beneficial effects on host health and disease prevention and/or treatment. The Food and Agriculture Organization of the United Nations World Health Organization has defined probiotics as ‘live microorganisms which, when consumed in adequate amounts as part of food, confer a health benefit on the host’. Since animal studies suggest that some probiotic effects can be achieved by nonviable bacteria and even by bacterial DNA, probiotics have more recently been defined as ‘microbial cell preparations or components of microbial cells with a beneficial effect on the health and wellbeing of the host’ [60, 61]. Common probiotics include different Lactobacilli bacteria and the yeast Saccharomyces. Different probiotics have different effects, and it might even be that some bacteria are presumed to be probiotics, while actually consuming them provides no benefit at all. It seems that different probiotics might confer benefits in different disease states and for different patients. In addition, the dosage of probiotics is important. A combination of probiotics might be more effective than one species of probiotic, as is the case for VSL#3.

Vanderpool et al. [62] summarized the recent findings regarding probiotic activity. The following mechanisms of action were identified.

(1) Blockage of pathogenic bacteria via bactericidal substance: Lactobacilli as well as Bifidobacteria were found to produce unique antibacterial substances each with a different mode of action, so that probiotics from different groups might exert a positive effect synergistically. Lactobacilli produce lactic acid, thus lowering luminal pH and thereby inhibiting Gram-negative bacterial growth.

(2) Competing with pathogens and toxins for adherence to the intestinal epithelium: These phenomena were noticed even when probiotic bacteria were given after pathogenic bacteria adhesion. Probiotics enhance production of β-defensin and other protective substances to prevent pathogen and toxin adherence.

(3) Regulating immune responses by enhancing the innate immunity and modulating pathogen-induced inflammation via membrane receptors: TLRs are recognized by commensals as well as probiotics. Probiotics in general enhance production of anti-inflammatory cytokines such as IL-10 but not of proinflammatory cytokines such as TNF-α and IL-12 and INF-γ. On the other hand, probiotic bacteria enhance the anti-pathogenic bacterial immune response. There are 11 TLRs, with 9 of the mammalian TLRs having specified ligands [63]. While the probiotic combination of many probiotic species, VSL#3, reduces chemical colitis by DSS, this does not occur in TLR9-deficient mice or when heat-treated bacteria are given. However, in TLR2- and TLR4-deficient mice this protective phenomenon is maintained whether live bacteria or irradiated bacteria are used. It is therefore deduced that TLR9 plays a role in probiotic-mediated defense and non-denatured bacterial proteins are the ligands.

(4) Regulating intestinal epithelial homeostasis: The epithelial barrier is an important defense against pathogenic bacteria and toxins. L. casei, VSL#3, E. coli Nissle 1917, L. acidophilus and B. thetaiotaomicron have all been shown to promote barrier integrity. Mechanisms of action for different bacteria include promotion of tight junction function and prevention of increased paracellular permeability after enteropathogenic exposure. In this regard the use of multiple probiotic strains might prove beneficial.

A graphic summary of ways by which probiotics exert their influence on enterocytes is provided in figure 3.

Numerous animal model studies of IBD have substantiated the rationale of probiotics for the prevention and treatment of IBD. The Lactobacillus strain, as well as a bifidobacterium, caused attenuation of colitis in IL-10-deficient mice. This was explained by the observed reduction of Th1 cytokines and the induction of TGF-β [64]. L. plantarum caused elevation in the anti-inflammatory cytokine IL-10 in inflamed colonic mucosa [63], while VSL#3 improved DSS-induced colitis in weaning rats [65]. S. bouardii, a nonpathogenic yeast, inhibited inflammation in a mouse model through action on the
migration of T lymphocytes [66]. The *Lactobacillus casei* strain induced improvement in murine chronic IBD, while causing downregulation of proinflammatory cytokines IL-6 and IFN-γ [67].

Not all probiotics are effective. Geier et al. [68] studied the effect of four candidate probiotics on DSS-induced colitis in rats. *Lactobacillus fermentum* BR11 was found effective, while two others *Streptococcus thermophilus* TH-4 and *Bifidobacterium lactis* BB12 were not effective. *Lactobacillus rhamnosus* GG actually caused some exacerbation of inflammation [68]. In a conceptually breakthrough experiment, genetically modified *Lactococcus lactis* were engineered to secrete IL-10. Treatment with these IL-10-secreting bacteria caused attenuation of colitis in a mouse model [69].

On the whole, there is a vast theoretical rationale for treatment of IBD with probiotics. Reluctantly, clinical trials thus far have only proved efficacy in a somewhat small and specific group of patients. There are multiple explanations for the failure to observe a significant effect. It is quite common that animal studies and human studies do not agree with each other and even arrive at contradictory results. However, based on in vitro data and findings in animals, it might just be that we have not yet found the optimal probiotics or combinations of probiotics. Probiotics are a large group of microbes with different mechanisms of action, and some may act synergistically whereas others may actually interfere with positive actions of other bacteria. Deducing from findings in acute gastroenteritis [70], it could well be that studies in IBD may have not used the correct dosage.

**Prebiotics and Synbiotics and IBD**

Despite evidence for the effect of non-living bacteria on gut inflammation [71], it could well be that optimal conditions provided for living bacteria are to be addressed.

Prebiotics were defined by Gibson and Roberfroid as ‘nondigestible food components that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thereby improving host health’ [72]. Experimental evidence supports the hypothesis that prebiotics such as inulin and oligofructose play a role in modifying and even preventing inflammation in CD, UC, and pouchitis [for review see 73]. In a preliminary non-controlled study in 10 adult subjects with CD, fructooligosaccharides induced significant clinical improvement and an increase in fecal bifidobacteria concentration, as well as the percentage of IL-10-positive dendritic cells and the percentage of dendritic cells expressing TLR2 and TLR4 [74].

The term synbiotic is used when both probiotics and prebiotics are used [72]. While, again in a small cohort of adult CD patients, synbiotics provided a mild clinical effect [75], in another setting (post-surgical recurrence) with different species, they provided no measurable effect [57].

**Pouchitis**

Pouchitis is a term used to describe inflammatory involvement of a neo-rectum performed with various surgical techniques in patients after total proctocolectomy.
This procedure is usually preformed in patients with UC and familial syndromes necessitating total proctocolectomy. The procedure is also performed in CD colitis but involves high complication and failure rates. Probiotics are efficient in the primary prevention of a first episode of pouchitis [76], treatment of pouchitis, and secondary prevention or recurrent attacks. A recently published meta-analysis of 5 randomized controlled studies representing 258 patients summarized the data regarding treatment of pouchitis with probiotics. About two thirds of the patients were treated with VSL#3 and the remaining third received L. rhamnosus GG [77]. VSL#3 is a highly concentrated combination of 450 billion live bacteria per sachet. It is a mixture of 8 probiotic lactobacilli bacteria: Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus bulgaricus, and Streptococcus thermophilus. The OR for relapse of disease outcomes among probiotics treatment (combined) in the 5 trials was \( \sim 0.04 \) (\( p < 0.0001 \)). A statistically significant OR of \( \sim 0.02 \) for relapse rate was calculated with data from 4 studies in which the treatment given was VSL#3. Studies were of great heterogeneity with VSL#3 having the greatest effectiveness.

Thus, for pouchitis, there is indisputable evidence that probiotics are effective for the treatment as well as for primary and secondary prevention of pouchitis.

Treatment of UC with Probiotics

Zigra et al. [78] published a systematic review on the treatment of UC with probiotics summarizing data from 9 controlled randomized trials. The data refer to 972 patients. Different probiotics were used and different control groups were studied. Balsalazide (5-aminosalicylic preparation) and VSL#3 were compared to Balsalazide alone for treatment of mild to moderate UC in 1 study; a synbiotic preparation was compared with placebo in active disease in another study; Lactobacillus GG was compared to mesalazine and Lactobacillus GG in 1 study, and non-pathogenic E. coli was compared to mesalazine in 3 studies. Bifidobacteria was compared with placebo in active UC in 3 studies. Three studies examined the effect of inducing remission; 1 study found a significant effect for the probiotics [79] and 2 showed a trend for efficacy [80, 81]. The pooled OR for response was 2.27 (\( p < 0.05 \)) with nonsignificant heterogeneity. Six studies included patients for maintenance of remission, and in 2 of them significantly higher remission rates were noticed [82, 83]. Bifidobacterium proved efficient or showed a trend toward efficacy in 3 studies. Significant heterogeneity was found between studies and pulled data failed to show a significant effect. Taking into account the heterogeneity of the trials, questions as to the rationale of statistically combining the data arise.

One can deduce that the significant results in some of the studies provide evidence for the value of specific probiotic strains in specific clinical setups.

Antibiotics and IBD

Antibiotics as Primary Treatment

It is common practice to treat active CD, especially when the colon is involved, with antibiotics. While pathogenic bacteria play a role in the pathogenesis of IBD, and infection may be a part of disease relapse, antibiotics have an unknown immunological effect on gut microbiota. When using antibiotics, the most commonly used is a combination of ciprofloxacin and metronidazole.

On the whole, the number of trials evaluating the efficacy of antibiotics as primary treatment for CD is small. Results are heterogeneous with some studies showing efficacy, some a trend towards efficacy, and others demonstrate no effect [84]. It seems that the case for antibiotic treatment in CD is not resolved and decisions should be personalized taking into account the efficacy on the one hand, and the possible side effects of all treatment options.

Antibiotics for Complications of IBD

IBD can take a complicated course. Some of the complications are infectious and as such require antibiotic treatment. Patients suffering from UC, usually with pancolitis, might develop toxic megacolon. This is a situation in which the colon is distended due to inflammation, and the colonic wall is thin. In this situation perforation is impending with peritonitis and sepsis and, therefore, antibiotic treatment is necessary. Toxic megacolon might require colectomy even with the best conservative care. It is better if surgery is planned and emergency colectomy avoided. If perforation has reluctantly occurred emergency surgical treatment with antibiotic coverage is mandatory.

Infectious complications can complicate CD as well. One of the phenotypes of CD is a fistulizing disease that can lead to infected enterocutaneous fistulas and enterovesicular fistulas, both requiring antibiotic treatment. Fistulizing disease can create sinuses which can, when infected, form an abscess. In these case scenarios prompt drainage and antibiotic treatment are needed.
Perforation, whether microperforation or full-blown perforation with peritonitis, requires antibiotics, too. These measures are combined with additional therapeutic measures according to the specific situation.

**Complication of Treatment for IBD and Bacteria**

Drugs affecting the immune system are used in the treatment of IBD. These drugs cause a propensity for opportunistic infections. Immunosuppressant drugs, steroids and anti-TNF treatment can all cause immunosuppression with various infections resulting. It is beyond the scope of this review to elaborate on this subject, except to remind pediatricians and family doctors to ensure proper vaccination prior to the commencement of immuno-suppressed IBD patients.

It is also noteworthy to state, at this point of time, that the safety of probiotics has yet to be demonstrated in immuno-suppressed IBD patients.

**Conclusion**

Gut microbiota plays an important role in IBD. Commensal and/or pathologic bacteria comprise a part of the etiology of IBD. Their ability to promote inflammation is crucial for disease development. The influence of the gut microbiota is probably brought about in the initiation of disease as well as in flares. This knowledge calls into play treatment modalities aimed at modifying gut bacteria either by probiotic treatment or antibiotic treatment. The use of the adequate bacterium or a combination of bacteria, the dosage needed and the role of probiotics and symbiotics are areas of active research aimed at elucidating the mechanisms involved and finding new preventive measures and treatment options for patients with IBD.

**References**


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