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Intestinal microbiota, probiotics and prebiotics in inflammatory bowel disease

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Abstract

It has been presumed that aberrant immune response to intestinal microorganisms in genetically predisposed individuals may play a major role in the pathogenesis of the inflammatory bowel disease, and there is a good deal of evidence supporting this hypothesis. Commensal enteric bacteria probably play a central role in pathogenesis, providing continuous antigenic stimulation that causes chronic intestinal injury. A strong biologic rationale supports the use of probiotics and prebiotics for inflammatory bowel disease therapy. Many probiotic strains exhibit anti-inflammatory properties through their effects on different immune cells, pro-inflammatory cytokine secretion depression, and the induction of anti-inflammatory cytokines. There is very strong evidence supporting the use of multispecies probiotic VSL#3 for the prevention or recurrence of post-operative pouchitis in patients. For treatment of active ulcerative colitis, as well as for maintenance therapy, the clinical evidence of efficacy is strongest for VSL#3 and *Escherichia coli* Nissle 1917. Moreover, some prebiotics, such as germinated barley foodstuff, *Psyllium* or oligofructose-enriched inulin, might provide some benefit in patients with active ulcerative colitis or ulcerative colitis in remission. The results of clinical trials in the treatment of active Crohn's disease or the maintenance of its remission with probiotics and prebiotics are disappointing and do not support their use in this disease. The only exception is weak evidence of advantageous use of *Saccharomyces boulardii* concomitantly with medical therapy in maintenance treatment.

Key words: Gut; Microbiota; Inflammatory bowel disease; Probiotic; Prebiotic

Core tip: Intestinal microbiota seems to play an important role in the pathogenesis of inflammatory bowel disease. There is very strong evidence supporting the use of certain probiotics and prebiotics in the therapy of ulcerative colitis and pouchitis, whereas their beneficial role in Crohn's disease has not yet been proven. This article describes the role of gut microbiota in the pathogenesis of inflammatory bowel disease and delineates the possible mechanisms of certain probiotics and prebiotics in disease treatment and maintenance of remission.

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REVIEW OF THE FACTS ON GUT MICROBIOTA IN INFLAMMATORY BOWEL DISEASE

A variety of factors, which may be environmental, genetic, immunological, and microbial in nature, contribute to

the development of inflammatory bowel disease (IBD)[1]. Although the exact etiology of IBD remains unclear, it is believed to be the result of complex aberrant immune responses to as yet undetermined environmental factors (most likely intestinal microorganisms) in the gastrointestinal tract of genetically susceptible hosts[2].

The human gut normally hosts roughly 10^{14} bacterial organisms of up to 1000 different species; this bacterial community can add up to 1-2 kg[1]. In total, the number of intestinal bacteria is approximately ten times the number of cells constituting the human body, with the collective bacterial genome, also referred to as the microbiome, containing 100-fold more genes than the entire human genome[3,4]. More than 99% of the gut microbiota is composed of species within 4 bacterial divisions: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*[5,6]. Greater variations exist below the phylum level, and certain butyrate-producing bacteria, including *Faecalibacterium prausnitzii, Roseburia intestinalis*, and *Bacteroides uniformis*, have been identified as key members of adult gut microbiota[7]. The predominant species in the proximal small intestine are aerobic and Gram-positive. In the distal small bowel, Gram-negative species begin to outnumber Gram-positive bacteria[8]. Distally to the ileocecal valve, bacterial concentrations increase sharply[8], and the most densely populated region of the gastrointestinal tract is the colon, with up to 10^{12} bacteria per gram of intestinal content and a population consisting predominantly of the *Bacteroides, Bifidobacteria, Fusobacteria, Clostridia*, and *Peptostreptococci* groups[1]. The majority of intestinal bacteria belong to the phyla *Bacteroidetes* (64% of attached colonic species) or *Firmicutes* (23% of normal species)[1,5]. *Enterobacteriaceae* such as *Escherichia coli* are relatively minor components of the *Proteobacteria* division (8% of all bacteria)[5].

There is plenty of evidence supporting the hypothesis of the involvement of intestinal microbiota in IBD pathogenesis. Crohn's disease (CD) and ulcerative colitis (UC) tend to occur in the colon and distal ileum, which contain the highest intestinal bacterial concentrations[5]. A pathogenic role of luminal constituents is suggested by the prevention and treatment of Crohn's disease by the diversion of fecal stream and reactivation of inflammation within one week following reinfusion of ileostomy contents[9]. In patients with CD, division of the fecal stream proximally to the inflamed mucosa results in reduction of inflammation and induction of healing in the excluded parts of the gut, while relapse occurs with restoration of fecal stream and re-exposure to luminal contents[9,10]. Similarly, ulcerative colitis patients who undergo ileal pouch-anastomosis surgery develop mucosal inflammation after bacterial colonization of the pouch[11]. A recent meta-analysis by Khan *et al*[12] has shown the significant beneficial effects of antibiotics over placebo for induction of remission in both CD and UC. Antibiotic treatment also appears to provide clinical benefits in patients with CD and inflammation of the ileal pouch[13]. Furthermore, there are many studies on animal models supporting the role of gut microbiota in the development of IBD. In experimental animal models of IBD, genetically-engineered animals developed spontaneous colitis under standard laboratory conditions, but remained colitis-free when they were raised in a sterile, germ-free environment, thus indicating that bacterial exposure and colonization are essential for the development of colitis[13-16]. Additionally, it has been shown that animal models with chemically induced colitis do not develop intestinal inflammation if they are pretreated with antibiotics[17].

The majority of genes found to be associated with an increased risk for the development of IBD are those encoding proteins that act to preserve the mucosal barrier and/or regulate the host immune system. A major breakthrough in understanding the linkage between genetic predisposition and IBD development was the discovery of the NOD2/CARD15 gene, which encodes a protein belonging to the family of pattern-recognition receptors responsible for microbial recognition, induction of antimicrobial genes, and control of the host adaptive immune response[18]. The genetic defects found in IBD CD patients might make these individuals particularly susceptible to infection by intracellular bacteria such as *Mycobacterium avium paratuberculosis*, *Listeria monocytogenes*, and adherent-invasive *Escherichia coli*[19]. Mutations in genes for toll-like receptors, as well as for the CARD4/NOD1 receptor, may also be associated with increased susceptibility for IBD[20-23].

Patients with CD have increased intestinal permeability, which could reflect mucosal barrier defects that promote bacterial translocation through the intestinal mucosa[24]. The intestinal mucus barrier is significantly altered in UC patients, particularly in terms of mucus composition and phospholipid concentration[25]. Altered function of defensins, antimicrobial peptides with bactericidal activities, might also be involved in IBD[1,24].

Despite much evidence that intestinal microorganisms are required for the triggering and perpetuation of inflammation in IBD, it still remains enigmatic whether a single specific microorganism or a group of microbial agents sharing distinctive characteristics could be responsible, or if it is actually the aberrant immune response to the dysbiosis of the commensal intestinal microbiota that plays the most major role.

Mycobacterium avium paratuberculosis used to be a particularly strong candidate as the single etiologic agent in CD in the past, since it has been shown to cause granulomatous enterocolitis in cattle that closely resembles CD in humans[26]. However, a two-year trial of combined antibiotic therapy with clarithromycin, rifabutin, and clofazimine (drugs efficient against *Mycobacteria*) did not reveal any difference in disease activity in CD patients with or without antibiotic treatment[27]. Increased numbers of invasive mucosa-associated or even intramucosal *Escherichia coli* (*E. coli*) have been reported in patients with CD and UC; a new potentially pathogenic group called adherent-invasive *E. coli* (AIEC)[20,22,28-30]. AIEC are able to adhere to and invade intestinal epithelial cells with a macropinocytosis-like process. They are capable of surviving and

replicating within macrophages, and are known to induce the release of large amounts of pro-inflammatory cytokines, such as TNF-α, by the infected host cell[23].

Although microbial pathogens have been postulated to cause Crohn's disease and ulcerative colitis since their original descriptions, it is now generally accepted that commensal enteric bacteria, either incidentally or specifically, play an important or even central role in the pathogenesis of inflammatory bowel disease, and provide the constant antigenic stimulation that continuously activates pathogenic T cells to cause chronic intestinal injury[1,5]. Four broad mechanisms have been proposed to drive pathogenic immunologic responses to luminal microbial antigens: microbial pathogens inducing intestinal inflammation, dysbiosis of commensal microbiota with a decreased ratio of protective/aggressive commensal bacterial species, host genetic defects in containing commensal microbiota, and defective host immunoregulation. These mechanisms increase exposure of bacterial antigens to mucosal T cells or alter host immune responses to commensal bacteria[5].

In normal hosts, commensal bacteria activate a sequential program of homeostatic responses by epithelial cells, macrophages, dendritic cells, and T and B lymphocytes that permit coexistence with microbes and their products[5,31,32]. In IBD, genetically predisposed individuals appear to lose the normal tolerance to commensal bacteria, leading to a chronically active inflammation process in which the microbiota provide constant stimulus for the host immune system, causing perpetuation of the disease[17]. Tissue damage might result from an immunologic misperception of indigenous flora as dangerous organisms or from the failure of normal regulatory constraints on mucosal immune responsiveness to intestinal bacteria[33]. There is growing evidence that the interplay between intestinal microbes and the mucosa of susceptible individuals triggers a cascade of reactions that starts with the interaction of microbes with specific receptors on intestinal epithelial cells, dendritic cells, and other antigen-presenting cells, followed by the interaction of these activated cells with lymphocytes, resulting in their differentiation into different subsets, driving either Th1 or Th2 inflammatory responses with the production of a wide range of inflammatory mediators, and consequently leading to mucosal damage[34]. CD is regarded to be a Th1 immune reaction driven state, whereas UC is a Th2 immune state. Bacterial recognition is dependent on transmembrane pattern recognition receptors of intestinal epithelial cells, including toll-like receptors (TLR) and the intracellular NOD-like receptor family[5,31,35,36]. Ligation of these bacterial receptors stimulates central signaling cascades that include the nuclear factor-kappaB (NFκB) pathway, one of the key pathways in mucosal homeostasis that is shown to be elevated in the chronic inflammation tissue of the IBD[5,37].

Composition of gut microbiota in patients with IBD has been extensively studied over the last decade. Although methodologies and results may differ, some generalizations are possible[38]. Numerous studies revealed that fecal microbiota has a different composition in IBD patients compared to healthy controls, and some differences between microbial populations in CD and UC were found. Similar findings were described for mucosa-associated microbiota, a bacterial population present on the mucosal surface that is in direct interaction with intestinal epithelial and immune system cells[39-42]. Moreover, differences were observed between active and non-active stages of the disease as well as between inflamed and non-inflamed regions of the intestine[41,43,44]. When studying intestinal flora in IBD, it is important to keep in mind certain facts. Firstly, only up to 30% of the total microflora can be identified using conventional bacteriological techniques[38], however using molecular techniques has greatly improved the detection rate, though significant numbers of bacteria can still be left undetected[38,45]. Secondly, many strains found in IBD do not belong to major phylogenetic groups represented in healthy individuals[38,46]. Furthermore, a distinction should be made between mucosal flora and fecal flora. The composition of these two domains is unique, which seems to be important in IBD[38,47].

Concentrations of mucosal bacteria are high in patients with bowel inflammation, especially those with CD, whereas they are low in healthy controls. Bacterial invasion of mucosa was evident in up to 83% of biopsies from IBD patients, while no bacteria were detected in tissue samples from controls[45,48]. Functional alterations are most evident in adherent, invasive *Escherichia coli* that colonize the ileum of Crohn's disease patients[49]. Fluorescent in situ hybridization studies demonstrate dramatically increased mucosa-associated bacteria in active Crohn's disease, and to a lesser extent in ulcerative colitis[48]. The fecal microbiota differs from the mucosa-associated microbiota[6], with the latter probably being more relevant for intestinal immunomodulation[48].

Reduced microbial diversity in inflammatory bowel disease has been previously reported[50-52]. Ott *et al*[50] demonstrated that mucosal inflammation in IBD was associated with a loss of normal anaerobic bacteria; the reduction in diversity in IBD was due to a significant loss of *Bacteroides*, *Eubacterium*, and *Lactobacillus* species. The reduction in mucosa-associated *Bifidobacteria* and increase in *E. coli* and *Clostridia* in patients with IBD supports the hypothesis that an imbalance between potentially beneficial and pathogenic bacteria may contribute to its pathogenesis[50,53-55]. Manichanh *et al*[52] used a metagenomic approach to demonstrate the reduced complexity of the bacterial phylum *Firmicutes*, in particular *Clostridium leptum*, in CD patients compared to healthy controls. In general, fewer *Bacteroidetes* and *Firmicutes* were found[56,57], including *Faecalibacterium prausnitzii* and bacterial species with a large butyrate-generating and anti-inflammatory capacity[39,42,57,58], as well as a reduced diversity within this phylum[59]. The counts of other short chain fatty acid (SCFA) producing bacteria such as *Bifidobacteria* are also reduced and consequently concentrations of SCFA in the intes-

tine decrease[60-62]. Other studies have shown, however, that the number of mucosa-associated bacteria increased with the increase of *Enterobacteriaceae*, including adherent-invasive *E. coli*[43,62-64].

A comprehensive study of 190 resected tissue samples by Frank *et al* showed decreased numbers of the phyla *Firmicutes* and *Bacteroidetes* with concomitant increases in *Proteobacteria* and *Actinobacteria*[59]. In a study of adult patients, Gophna *et al* compared the tissue-associated intestinal microbiota in biopsy samples from patients with CD and UC, as well as from healthy controls. Their findings showed a significant increase of *Proteobacteria* and *Bacteroidetes* in CD patients and a decrease in *Clostridia* in this group. Comparison between the ulcerative colitis and healthy control groups displayed no significant differences. Based on the finding that the microbiota was of similar composition in samples from inflamed and non-inflamed tissues within the same individual, they concluded that imbalance in microbiota in CD is probably not sufficient to cause inflammation[64].

Nwosu *et al*[65] investigated correlation of age dependency and IBD. Their findings demonstrated an apparent opposite age-related trend for *Bacteroides* and *Escherichia* between UC and CD, suggesting an immunological effect of *Bacteroides* on promoting CD at early age while later having a protective role, suggesting that these differences reflect underlying immunological disorders for CD and UC.

Up to 95% of patients with active colitis may harbor sulfate reducing bacteria (SRB)[55,66,67]. Fecal samples of patients with UC have been shown to have greater than normal levels of SRB and it has been suggested that SRB may play an important role in UC pathogenesis. Theoretically, the impairment of butyrate metabolism within colonocytes may lead to increased villous atrophy, which is one of the features of active inflammation of colonic mucosa[54].

Pediatric populations are useful for research into gut microbiota in IBD, as most pediatric patients are treatment-naïve or newly diagnosed. Although most research has been performed on adults, microbiota of pediatric IBD has been increasingly investigated over the last few years. The first larger pediatric microbiota investigation in IBD patients by Conte *et al*[29] showed a higher number of mucosa-associated facultative-anaerobic and aerobic bacteria in the ileum, cecum, and rectum of children with IBD than in controls, with the highest numbers found in patients with indeterminate colitis and Crohn's disease. They also found a good deal of individual variability in the concentrations of mucosa-associated bacteria within the different groups of patients examined, although the highest heterogeneity of species was found in the ileal mucosa of patients with Crohn's disease.

Microbial dysbiosis was also demonstrated using fecal samples in 19 children with newly diagnosed Crohn's disease. This study showed significantly lower concentrations of *Firmicutes*, mainly due to changes in detection within the *Clostridia* class, and higher concentrations of *Proteobacteria* and *Bacteroidetes*, whereas the concentration of *Actinobacteria* was similar in CD patients and controls. Furthermore, Kaakoush *et al*[68] concluded that the ratio of *Bacteroidetes* to *Firmicutes* increased with the PCDAI activity index of the patients.

Lionetti *et al*[69] suggested that a possible mechanism of action of enteral nutrition in inducing disease remission in pediatric patients with Crohn's disease is the modification capacity of the gut microbiota. This was supported by the findings that in 8 out of 9 pediatric Crohn's patients, enteral nutrition alone induced disease remission. In all children with CD, analysis of gel band distribution revealed profound modification of the fecal microflora after exclusive enteral nutrition therapy, whereas in healthy controls no modification of microflora was detected and a bacterial profile analysis remained stable during the 3-mo observation period.

Horizontal distribution of the fecal microbiota in adolescents with IBD was investigated by Gosiewski *et al*[70], who demonstrated that distribution of the microbiota in the colon is layered. Their results demonstrated that the quantitative composition of the bacterial microbiota changed in the consecutive fecal fractions and tissue samples of patients with CD and UC, whereas in the control group there were no differences in microbiota composition in consecutive fecal and tissue samples. The largest differences in the total proportion of bacteria were visible in the *Bifidobacterium* genus, whose number declined with consecutive fractions, whereas in controls it remained high in all fractions. Also, in patients with CD, the percentage of bacteria from the *Streptococcus* genus and *Enterobacteriaceae* in subsequent fractions increased in comparison to the control group, and in patients with UC similar findings were described for *Lactobacilli*. Investigation of the *Bacteroides spp.* showed that their percentage dropped in the consecutive fecal fractions in CD, similarly to the control group, whereas in patients with UC it increased. Only in the UC group was the bacterial flora attached to the mucous layer found to exert degrading action on the protective mucin[70]. Mucus layer thickness in adolescents with IBD was studied in a group by Fyderek *et al*[71]. They demonstrated that the mucus layer in the inflamed sites was significantly thinner as compared to controls and to non-inflamed sites in IBD patients. Furthermore, they reported that *Streptococcus spp.* were predominant in the inflamed mucosa in CD patients, and *Lactobacilli spp.* were predominant in UC patients.

In a study of 15 treatment-naïve pediatric patients with CD and 26 healthy controls, Kellermayer *et al*[72] investigated mucosal microbiota with high-throughput methodologies. Using distance-based redundancy analysis, they showed that there was significant separation between the CD-associated colonic mucosal microbiota and the microbiota of controls. They also showed that patients with granulomatous CD had a higher number of genera and species, significantly differentiating the colonic mucosal microbiota from controls and patients without granulomas. The most prominent genera distinguishing granulomatous CD from non-granulomatous were *Rumi-*

nococcus, *Roseburia*, *Eggerthella* (all three decreased), and *Porphyromonas* (increased). There was a trend for the genera *Faecalibacterium* to be decreased in the transverse colonic mucosa of granulomatous patients with CD compared with non-granulomatous disease[72].

A Scottish group by Hansen *et al* has been intensively investigating pediatric gut microbiota in IBD patients over the last few years. They have reported differences in colonic mucosal bacteria between pediatric UC patients and controls. Contrary to findings from previous studies, they reported a reduction in *Bacteroidetes* and an increase in *Firmicutes*[73]. They also described a reduction in bacterial diversity and an increased concentration of *Faecalibacterium prausnitzii* in de-novo pediatric CD patients, a finding contradicting the current protective role model of *F. prausnitzii* in CD[74]. In the latest study by this group, microaerophilic microbiota of pediatric IBD onset has been researched. *Campylobacter* appears to be commonly isolated from pediatric colonic biopsies, but does not seem to be strongly associated with IBD. As a common commensal in pediatric gut microbiota, *Sutterella wadsworthensis* has also been reported[75]

Despite many discoveries in the last two decades, it remains unknown whether the intestinal microbiota triggers and maintains the chronicity of inflammatory response in IBD, or is altered as a secondary response to intestinal inflammation[76].

PROBIOTICS AND PREBIOTICS

Probiotics are specific live microorganisms which, when ingested in sufficient amounts, can promote health in the host[77]. In order to qualify as probiotic, microorganisms must fulfill a number of criteria[78]. They should be strictly specified at the genus, species, and strain levels, and specific strains should be registered and disposed in an international culture collection. Thus, generalizations concerning the efficacy of a whole species or even genus might be misleading. Probiotics should be extremely safe; their safety is supported by the fact that many strains are of human origin and have a long history of safe use. Many probiotics and their applications have been granted GRAS (generally regarded as safe) status. Although this classification should not be generalized, it does not warrant permanent surveillance for potential risks, such as invasiveness and potential for transfer of antibiotic resistance to other microorganisms[79,80]. Because the effects of probiotic microorganisms are generally dependent on their viability, their stability during processing and storage, as well as their ability to survive intestinal transit through the stomach and proximal small bowel to finally adhere to mucosa and colonize the intestine, should be demonstrated[78]. The final, but perhaps one of the most important, criteria for specific microorganism to be qualified as probiotic is a scientifically proven effect on the promotion of health or prevention and treatment of a specific disease[78].

Prebiotics are non-digestible food ingredients that selectively stimulate favorable bacterial growth and/or promote activity of a limited number of health-promoting bacteria, hence benefiting the host[81,82]. However, prebiotics can also be applied to enhance the survival and action of ingested probiotic bacteria. When probiotics and prebiotics are combined in one product to achieve synergistic effects they are usually called synbiotics. The vast majority of prebiotic substances are carbohydrates that are indigestible for human digestive enzymes but can be fermented by beneficial bacterial genera in the colon and serve as a substrate for their metabolism. Some of them can be found in natural foods, such as human milk oligosaccharides in mother's milk, while others are added to food. Good examples of prebiotics are fructo-oligosaccharides (FOS), inulin, galacto-oligosaccharides (GOS), soybean oligosaccharides, and complex polysaccharides that constitute dietary fiber[81].

Probiotics or prebiotics may achieve their therapeutic effect in IBD through many different mechanisms. They influence the composition of intestinal microbiota and alter the metabolic properties of the microbiome[76]. By increasing the production of short-chain fatty acids, they may lower the pH of the colonic environment and thus inhibit the growth of potentially pathogenic microorganisms. Butyrate plays a trophic role as a nutrient for colonocytes and enhances repair of injured gut epithelium in IBD. Moreover, evidence shows that butyrate acts directly as an anti-inflammatory agent by inactivating the intracellular transcriptional factor NFκB pathway, consequently attenuating synthesis of inflammatory cytokines[8]. A large number of probiotic strains are able to produce antibacterial substances, such as hydrogen peroxide, hydrogen sulfide, lactic acid, and specific bacteriocins[83], as well as displace deleterious microbes from the luminal-mucosal interface by competing for binding sites on the epithelial cell surface or mucus layer[84,85].

Probiotics communicate with epithelial cells and different sets of cells implicated in both innate and acquired immune response via pattern-recognition receptors[3]. They can enhance gut barrier function and reduce intestinal permeability for intestinal microorganisms and other antigens[86]. For example, several strains of *Lactobacilli* can up-regulate MUC3 gene expression, resulting in increased mucus production by intestinal goblet cells[87,88]. Several probiotic strains can induce the production and secretion of different anti-microbial peptides by epithelial cells, such as defensins, lysozyme, lactoferrin, or phospholipase, and directly decrease permeability of the epithelial layer by enhancing tight junctions and reducing epithelial cell apoptosis[85,89,90].

Each probiotic strain may have distinct immunoregulatory properties, thus probiotics can indirectly or directly modulate intestinal immune response. In very simplified terms, probiotics can be classified into two groups with regards to their influence on the immune system: one exhibiting immunostimulating activities and the other anti-inflammatory properties[91]. Numerous studies have revealed the mechanisms by which probiotics down-reg-

ulate the inflammatory immune response, including those with proven clinical efficacy in the therapy of IBD. Some probiotic strains may induce maturation of intestinal dendritic cells, an important part of antigen presenting and immune regulation, and extend their survival[92]. Several probiotics act through strengthening the regulatory T cell (Treg) response. Tregs are antigen-specific T cells which prevent autoimmunity and preserve tolerance towards harmless antigens, including intestinal commensal microbiota[84]. They can control excessive NFκB pathway activation, decrease production of pro-inflammatory cytokines (*e.g.*, TNFα, INFγ, and IL-8), and induce the production and secretion of anti-inflammatory cytokines such as IL-10 and TGFβ[3,91,93,94].

It is possible that there are further mechanisms of probiotic action that have not yet been demonstrated. Regarding the fact that pathogenesis of each type of IBD differs and that mechanisms of action of probiotics are strain-specific and very different, we might expect that different probiotics would be effective for each type and phase of the disease.

Over the last two decades, several interventional clinical studies comparing the efficacy of probiotic therapy against placebo or standard therapy with drugs have been published. The use of different study designs (*e.g.*, concomitant use of other forms of therapy) and various probiotic strains and doses, with only a few studies resembling one another in such a manner to be able to uniformly compare the results, makes it very difficult to derive any firm conclusions.

TREATMENT OF ACTIVE ULCERATIVE COLITIS

Clinical studies on the efficacy of probiotics for the induction of remission in ulcerative colitis gave encouraging, albeit conflicting, results. Bennet and Brinkman first reported a successful induction of long-lasting remission by a single enema of the fecal microbiota of a healthy donor in a patient with active UC[95]. Borody *et al*[96] published six cases of patients with UC resistant to medical therapy with steroids and immunomodulators who underwent transplantation of fecal microbiota from healthy donors by repeated enemas after 7-10 d of pre-therapy with vancomycin, metronidazole, rifampicin, and bowel lavage with polyethylene glycol. Complete reversal of UC was achieved in all patients, and they were all able to stop anti-inflammatory therapy after 6 wk. After 1 to 13 years of follow-up, all patients remained in complete clinical, endoscopic, and histologic remission without any adjunctive therapy.

Several studies investigated the efficacy of multispecies probiotic VSL#3 containing four strains of *Lactobacilli* (*L. casei*, *L. plantarum*, *L. acidophilus*, and *L. delbrueckii* subsp. *bulgaricus*), three strains of *Bifidobacteria* (*B. longum*, *B. breve*, and *B. infantis*) and one strain of *Streptococcus* (*S. salivarius* subsp. *thermophilus*). Tursi *et al*[97] compared the efficacy and safety of low-dose balsalazide (2.25 g/d) plus 3 g/d VSL#3 (group A, $n = 30$), with medium-dose balsalazide alone (group B, $n = 30$), and with mesalazine (group C, $n = 30$) in the 8-wk treatment of mild to moderate active ulcerative colitis. Efficacy was assessed by assessment of symptoms, endoscopic appearance, and histological evaluation. Balsalazide/VSL#3 was significantly superior to balsalazide alone and to mesalazine in obtaining remission (85.71% *vs* 80.77% *vs* 72.73%, respectively; $P < 0.02$). The balsalazide/VSL#3 combination was faster in obtaining remission than balsalazide alone or mesalazine (4, 7.5, and 13 d, respectively), and was also better in improving all parameters evaluated. Moreover, balsalazide with or without VSL#3 was better tolerated than mesalazine. The authors concluded that balsalazide/VSL#3 might be a very good choice in the treatment of active mild-to-moderate active ulcerative colitis. Bibiloni *et al*[98] studied the efficacy and safety of VSL#3 for induction of remission in an open-label study in 34 ambulatory patients with mild to moderate active UC. Among 32 patients who completed 6-wk treatment with VSL#3 3.6×10^9 CFU/d, remission (defined as UCDAI < or = 2) was achieved in 53% and response (decrease in UCDAI > or = 3, but final score > or =3) in 24%. In 9% of patients there was no response, in another 9% worsening of the condition was observed, and in 5% there was no final endoscopic assessment. The investigators reported no biochemical or clinical adverse events related to VSL#3. In addition, they confirmed the presence of VSL#3 species by DNA sequencing of 16S rRNA in biopsies collected from patients in remission. A small open-label pilot study on 18 pediatric patients between the ages of 3-17 years with mild to moderate acute UC using VSL#3 for 8 wk was performed by Huynh *et al*[99]. The simple clinical colitis activity index (SCCAI) was used to assess disease activity. Remission (defined as SCCAI $\leqslant 3$) was achieved in 56% and response (decrease in SCCAI $\geqslant 2$, but final score $\leqslant 5$) in 6%, with no change or worsening reported in 39% of patients. Five patients were withdrawn due to lack of improvement and only 13 patients completed 8 wk of VSL#3 treatment. VSL#3 was well tolerated, and no biochemical or clinical adverse effects attributed to VSL#3 were identified.

Tursi *et al*[100] compared the efficacy of VSL#3 in a dosage of 3.6×10^9 CFU ($n = 65$) with placebo ($n = 66$) in achieving remission in UC patients on concomitant therapy with aminosalicylates and/or immunosuppressants. After 8 wk of treatment, the decrease in UCDAI of 50% or more was significantly higher in the VSL#3 group (63.1%) than in the placebo group (40.8%) ($P = 0.010$). A decrease of three points or more in the UCDAI score was achieved in 60.5% in the VSL#3 group *vs* 41.4% in the placebo group ($P = 0.017$). They also found a significant difference in rectal bleeding ($P = 0.014$) but not in stool frequency, physician's rate of disease activity, or endoscopic score. Remission was slightly higher in the VSL#3 group than in the placebo group (47.7% *vs* 32.4%; $P = 0.069$).

In a randomized, multicenter, double-blind, controlled

trial, Sood *et al*[101] compared the efficacy of VSL#3 applied twice daily in a dosage of 3.6×10^9 CFU ($n = 77$) to placebo ($n = 70$) for induction of remission of mild to moderate UC. The primary endpoint was a 50% decrease in the ulcerative colitis disease activity index (UCDAI) at 6 wk. The secondary endpoints included remission by 12 wk and reduction in total individual UCDAI parameters from baseline at 12 wk. At week 6, the percentage of patients with an improvement in UCDAI score that was greater than 50% was significantly higher in the group given VSL#3 (32.5%) than the group given placebo (10%) ($P = 0.001$). At week 12, 42.9% patients given VSL#3 achieved remission, compared with only 15.7% patients given placebo ($P < 0.001$). Furthermore, significantly more patients given VSL#3 (51.9%) achieved a decrease in their UCDAI that was greater than 3 points, compared with those given placebo (18.6%) ($P < 0.001$). The VSL#3 group had significantly greater decreases in UCDAI scores and individual symptoms at weeks 6 and 12 compared with the placebo group.

Miele *et al*[102] performed a 1-year prospective, placebo-controlled, double-blind pediatric study to assess the efficacy of VSL#3 on the induction and maintenance of remission in children with active UC. A total of 29 consecutive patients (mean age: 9.8 years; range: 1.7-16.1 years) with newly diagnosed UC were randomized to receive either a weight-based dose of VSL#3 ($n = 14$) or placebo ($n = 15$) in conjunction with concomitant steroid induction and mesalamine maintenance treatment. The Lichtiger colitis activity index and a physician's global assessment were used to measure disease activity. At baseline (within 6 mo, 12 mo, or at the time of relapse), all patients were assessed endoscopically and histologically. All 29 patients responded to the induction therapy. Remission was achieved in 92.8% children treated with VSL#3 and standard therapy compared to only 36.4% treated with placebo and standard therapy ($P < 0.001$). Moreover, only 21.4% patients treated with VSL#3 relapsed within 1 year of follow-up compared to 73.3% patients from the placebo group ($P = 0.014$). At 6 mo, 12 mo, or at time of relapse, endoscopic and histological scores were significantly lower in the VSL#3 group than in the placebo group ($P < 0.05$). There were no biochemical or clinical adverse events related to VSL#3. This study demonstrated the efficacy of VSL#3 both in the induction and maintenance of remission in pediatric UC patients.

In a small open-label study by Tsuda *et al*[103], the effectiveness of another multispecies probiotic preparation BIO-THREE (containing *Streptococcus faecalis*, *Clostridium butyricum*, and *Bacillus mesentericus*) was tested for treatment of mild to moderate distal UC refractory to conventional therapies. Twenty patients were treated for 4 wk. Clinical symptoms and endoscopic findings were evaluated, and UCDAI scores calculated before and after treatment. In addition, fecal microbiota was analyzed by the terminal restriction fragment length polymorphism (T-RFLP) method. Remission (UCDAI score $\leqslant 2$) was observed in 45% and response (decrease in UCDAI $\geqslant 3$, but final score $\geqslant 3$) in 10%, however in 40% there was no response and in 5% they found worsening (UCDAI > 3) of the disease. T-RFLP analysis indicated an increase in *Bifidobacteria*.

In a single-center, randomized, double-dummy study, Rembacken *et al*[104] examined whether the addition of a non-pathogenic strain of *E. coli* Nissle 1917 to standard medical therapy increased the chance of remission of active ulcerative colitis and whether this probiotic strain was as effective as mesalazine in preventing relapse. Of a total of 116 patients, 59 were randomized to the mesalazine group and 57 to the *E. coli* group. All patients received concomitant standard medical therapy with tapering steroids together with a 1-wk course of oral gentamicin. After remission, patients were maintained on either mesalazine or *E. coli*, and followed-up for 1 year. The investigators found no significant differences between the mesalazine and *E. coli* groups in percentage of patients that achieved remission, mean time to remission, percentage of patients who relapsed, and mean duration of remission. Although the addition of *E. coli* to standard therapy did not increase the induction rate of remission, the results suggested that treatment with this probiotic might have an equivalent effect to mesalazine in maintaining remission of ulcerative colitis.

Kato *et al*[105] conducted a randomized placebo-controlled trial using *Bifidobacteria*-fermented milk (BFM) (containing *Bifidobacterium breve* strain Yakult, *B. bifidum*, and *Lactobacillus acidophilus*) supplementation as a dietary adjunct in treating active ulcerative colitis. Twenty patients with mild to moderate active UC randomly received 100 mL/d of BFM or placebo for 12 wk with conventional treatment. The clinical activity index was significantly lower in the BFM than in the placebo group, and the endoscopic activity index and histological score were significantly reduced in the BFM, but not the placebo group, after treatment. They also observed an increase in fecal butyrate, propionate, and short-chain fatty acid concentrations in the BFM, but not the placebo group. Therefore, the authors concluded that supplementation with this *Bifidobacteria*-fermented milk product is safer and more effective than conventional treatment of active UC alone.

Ishikawa *et al*[106] compared a group of patients with BFM supplementation 100 mL/d ($n = 11$) and a control group ($n = 10$), both receiving standard medical treatment of ulcerative colitis. Colonoscopies, general blood markers, and examinations of intestinal flora, including the analysis of fecal organic acids, were performed at the initiation of the study and after one year. Exacerbation of symptoms was observed in 3 out of 11 subjects in the BFM group and in 9 out of 10 in the control group. Statistical analysis of the cumulative exacerbation rates showed a significant reduction in exacerbations for the BFM group ($P = 0.0184$). A significant reduction in the relative proportion of *B. vulgatus* in *Bacteroidaceae* and butyrate concentration was observed after supplementation

with BFM in comparison with before.

Recently, Oliva *et al*[107] published a prospective, randomized, placebo-controlled study comparing the effectiveness of *Lactobacillus reuteri* ATCC 55730 enema and placebo in children with active distal UC. A total of 40 patients (median age 7.2 years; range 6-18 years) were enrolled. They received an enema solution containing 10^{10} CFU of *L. reuteri* or placebo for 8 wk, in addition to oral mesalazine. Clinical, endoscopic, and histological scores, as well as rectal mucosal expression levels of pro- and anti-inflammatory cytokines, were evaluated at the beginning and at the end of the trial. Mayo score (including clinical and endoscopic features) as well as histological score decreased significantly in the *L. reuteri* group ($P < 0.01$), but not in the placebo group. Moreover, the evaluation of cytokine mucosal expression levels revealed that IL-10 significantly increased ($P < 0.01$), whereas IL-1β, TNFα, and IL-8 significantly decreased ($P < 0.01$) only in the *L. reuteri* group.

In a small non-controlled pilot study, Guslandi *et al*[108] treated 25 patients with mild to moderate clinical flare-up of ulcerative colitis with *Saccharomyces boulardii* 250 mg three times a day for 4 wk during maintenance treatment with mesalazine. Of the 24 patients who completed the study, 17 attained clinical and endoscopic remission.

Furrie *et al*[109] explored the efficacy of a synbiotic combining a probiotic strain of B*ifidobacterium longum* and a prebiotic (Synergy 1), a preferential inulin-oligofructose growth substrate for this probiotic strain. Treatment was used in a double-blinded randomized controlled trial in 18 patients with active UC for a period of one month. Although the subsequent sigmoidoscopy score decrease in the synbiotic group was not statistically significant compared with placebo ($P = 0.06$), they found that biopsies in the test group had reduced inflammation, and increased regeneration of epithelial tissue and mRNA levels for beta defensins 2, 3 and 4 (which are strongly up-regulated in active UC), tumor necrosis factor alpha and interleukin-1 alpha were also significantly reduced in the test group after treatment ($P = 0.016$, 0.038, 0.008, 0.018 and 0.023, respectively).

In another study by Ishikawa *et al*[110], the investigators examined the effects of a live *Bifidobacterium breve* strain Yakult and GOS as synbiotic in active UC. Forty-one patients with mild to moderate UC were assigned to two groups; one was treated with the synbiotic (1 g of the probiotic powder (10^9 CFU/g) three times a day and 5.5 g of GOS once a day) and the other was not (control group). After one-year treatment with the synbiotic, the clinical status of the UC patients as assessed by colonoscopy significantly improved, and the amount of myeloperoxidase in the lavage, a marker of inflammation, decreased. The synbiotic also significantly reduced the fecal counts of *Bacteroidaceae* and fecal pH.

Several reviews and meta-analyses have been performed over recent years concerning the induction of remission in ulcerative colitis by probiotics. In a Cochrane Collaboration review from 2007, the authors assessed the efficacy of probiotics compared to placebo or standard medical treatment with 5-aminosalicylates, sulfasalazine, or corticosteroids[111]. Only 4 randomized controlled trials met the criteria, and a formal meta-analysis could not be performed because of heterogeneity in methodology, probiotic strains, and outcomes. The authors concluded that combining conventional therapy with probiotics did not improve overall remission rates in patients with mild to moderate UC. However, they found limited evidence that the addition of probiotics might provide modest benefits in terms of disease activity. The negativistic opinion shared in this early review can be at least partially attributed to the low number of high quality studies published at the time. In a meta-analysis later performed by Sang *et al*[112] and published in 2010, both the induction of remission and maintenance were compared between probiotic and non-probiotic treatment in ulcerative colitis. Thirteen randomized controlled studies met the selection criteria. Seven studies evaluated the remission rate, 8 the recurrence rate, and 2 both remission and recurrence rates. The remission rate for probiotics compared with non-probiotics therapy was 1.35 (95%CI: 0.98-1.85), while when compared with the placebo it was 2.00 (95%CI: 1.35-2.96). Although these differences were not statistically significant, the authors concluded that these results were probably subject to heterogeneous bias. Regarding maintenance of remission, the recurrence rate of ulcerative colitis in patients who received probiotics was 0.69 (95%CI: 2.47-1.01) and 0.25 (95%CI: 0.12-0.51) in patients with mild to moderate UC compared with the non-probiotic group. The group who received *Bifidobacterium bifidum* treatment had a recurrence rate of 0.25 (95%CI: 0.12-0.50) compared with the non-probiotics group. The authors concluded that probiotic treatment was more effective than placebo in maintaining remission in ulcerative colitis.

In contrast with these reviews, a meta-analysis performed by Zigra *et al*[113] showed a significant benefit of probiotic use for UC remission induction with pooled relative risk 2.27 (95%CI: 1.00-5.14, $P = 0.049$).

In a more recent review by Jonkers *et al*[56], only subgroup-specific meta-analyses per probiotic were performed. The only probiotic with several published randomized controlled studies for induction of remission in adult patients with UC was VSL#3. The calculated pooled RR for VSL#3 was 1.69 (95%CI: 1.17-2.43), indicating a significant benefit of VSL#3 over control in inducing remission in active UC.

Interestingly, in the 2011 recommendations for probiotic use from the 3rd Yale Workshop, both VSL#3 and *Escherichia coli* Nissle 1017 were rated B, meaning that recommendation of their use for induction of remission in UC is based on positive controlled studies, but with the presence of some negative studies that did not support the primary outcome[114].

In conclusion, the results of several clinical studies suggest that the addition of specific probiotics to conventional therapy in active UC may be beneficial. The

strongest evidence exists for multispecies preparation VSL#3, with several studies both in adults and children supporting its efficacy.

MAINTENANCE OF REMISSION IN ULCERATIVE COLITIS

There have been several published studies in which efficacy of the probiotic strain of *Escherichia coli* Nissle 1917 was compared to either placebo or standard therapy for maintenance therapy in UC. In a double-blind, double-dummy study by Kruis *et al*[115], 120 patients with inactive ulcerative colitis were randomized to mesalazine 500 mg three times daily or to an oral preparation of *E. coli* Nissle treatment for 12 wk to compare their efficacy in preventing a relapse of the disease. Study objectives were to assess the equivalence of the two therapeutic modalities by comparing the clinical activity index (CAI), relapse rates, relapse-free times, and global assessment. The start and end CAI scores demonstrated no significant difference ($P = 0.12$) between the two treatment groups. Relapse rates were 11.3% under mesalazine and 16.0% under *E. coli* (N.S.), and the relapse-free time was similar for mesalazine and *E. coli* (103 +/- 4 d and 106 +/- 5 d, respectively). Global assessment was also similar for both groups. Tolerability of the treatment was excellent in both groups. Conclusions of this study were that probiotic treatment with *E. coli* Nissle 1917 offered another option for maintenance therapy of ulcerative colitis. Subsequently, the same group performed another, albeit larger, double-blind, double dummy trial to confirm the equivalent efficacy of *Escherichia coli* Nissle 1917 and mesalazine in the maintenance of remission in UC[116]. Patients received either the probiotic drug 200 mg once daily ($n = 162$) or mesalazine 500 mg three times daily ($n = 165$) for 12 mo, and were assessed by clinical and endoscopic activity indices (Rachmilewitz) as well as by histology. The per-protocol analysis revealed relapses in 40/110 (36.4%) patients in the *E. coli* group and 38/112 (33.9%) in the mesalazine group (significant equivalence $P = 0.003$). Subgroup analyses showed no differences between the treatment groups in terms of duration and localization of disease or pretrial treatment. Safety profile and tolerability were very good for both groups. By the end of this second study the authors concluded that *E. coli* Nissle 1917 showed the same equivalent efficacy and safety as mesalazine in maintaining remission in patients with ulcerative colitis.

In another trial by Rembacken *et al*[104], both the capacity of induction and maintenance of remission by *E. coli* Nissle 1917 were evaluated. In this single-center, randomized, double-dummy study, patients were maintained on either mesalazine ($n = 59$) or *E. coli* ($n = 57$) and followed up for a maximum of 12 mo. A comparable percentage of patients relapsed in the mesalazine (73%) and *E. coli* groups (67%), and the mean duration of remission was practically similar in both (206 and 221 d, respectively). Again, the authors came to the conclusion that treatment with non-pathogenic *E. coli* was as equivalently efficient as mesalazine in maintaining remission of ulcerative colitis.

Zocco *et al*[117] studied the efficacy of a probiotic strain of *Lactobacillus rhamnosus* GG for maintenance therapy in UC. They randomized patients into three groups: *Lactobacillus* GG 18×10^9 CFU/d ($n = 65$), mesalazine 2400 mg/d ($n = 60$), or a combination of *Lactobacillus* GG and mesalazine ($n = 62$). Overall analysis of UCDAI scores and endoscopy and histology results showed no difference in relapse rate at 6 and 12 mo among the three groups. However, treatment with *Lactobacillus* GG alone or in combination seemed to be more effective than standard treatment with mesalazine in prolonging relapse-free time ($P < 0.05$).

A non-controlled trial using multispecies preparation VSL#3 in 20 UC patients in remission, intolerant, or allergic to 5-aminosalicylates for 12 mo was performed by Venturi *et al*[118]. They reported that 15 out of 20 patients remained in remission during the study, 4 relapsed, and one was lost to follow-up. They suggested that VSL#3 might be useful in maintaining remission in UC patients intolerant to standard therapy.

In the previously mentioned pediatric study by Miele *et al*[102], the investigators observed that only 21.4% of patients treated with VSL#3 (compared to 73.3% patients from the placebo group) relapsed within 1 year of follow-up ($P = 0.014$). They also found significantly lower endoscopic and histological scores in the VSL#3 group than in the placebo group ($P < 0.05$). The results of this study confirmed the efficacy of VSL#3 in the maintenance of remission in pediatric UC patients.

Cui *et al*[119] randomized 30 patients with UC in remission achieved by treatment with sulfasalazine and glucocorticoids into two groups: one that received bifid triple viable capsule (BIFICO) (1.26 g/d) for 8 wk and the other an identical placebo group. The patients were evaluated clinically, endoscopically, and histologically after 2 mo of treatment or in the event of UC relapse. Only three patients (20%) in the BIFICO group relapsed during the 2-mo follow-up period compared with 14 (93.3%) the in placebo group ($P < 0.01$). Moreover, the microbiological and immunological analyses revealed that the concentration of fecal *Lactobacilli* and *Bifidobacteria* was significantly increased only in the BIFICO-treated group ($P < 0.01$). The expression of pro-inflammatory NFκB p65 and DNA binding activity of NFκB were significantly attenuated, and the mRNA expression of anti-inflammatory cytokines was elevated in the treatment group in comparison with the control group ($P < 0.05$). The authors concluded that oral administration of probiotic preparation BIFICO was effective in preventing flare-ups of chronic UC.

Shanahan *et al*[120] performed a double-blind, placebo-controlled study on 157 patients to compare the efficacy of *Lactobacillus salivarius* subspecies *salivarius* UCC118, *Bifidobacterium infantis* 35624 (1×10^9 CFU/d), or placebo for maintenance UC therapy. They found no difference

in relapse time between probiotics and placebo.

Wildt *et al*[121] performed a small double-blind placebo-controlled study using probiotic preparation Probio-Tec-AB-25 (containing the two probiotic strains *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subspecies *lactis* BB-12) or placebo in patients with left-sided UC in remission for 52 wk. 25% of patients on probiotics and 8% of those on placebo maintained remission after 1 year of treatment ($P = 0.37$). The median time to relapse was 125 d in the probiotic group and 104 d in the placebo group ($P = 0.683$). The authors concluded that no significant clinical benefit of Probio-Tec-AB-25 in comparison with placebo for maintaining remission in UC was demonstrated.

In the recent Cochrane Collaboration review of probiotic efficacy and safety for the maintenance of remission in UC by Naidoo *et al*[122], only 4 studies met the inclusion criteria. Three of those trials compared probiotics to mesalazine, and one to placebo. The pooled analysis was performed and revealed no statistically significant differences in the efficacy of probiotics over mesalazine. Relapse was reported in 40.1% of patients treated with probiotics and in 34.1% of those on mesalazine therapy. No statistical difference in the incidence of adverse events between the two groups was demonstrated. In only one placebo-controlled study was the relapse rate between probiotic and placebo groups considered non-significant. The authors concluded that, given the relatively small number of patients included in the clinical studies, the evidence was insufficient to make conclusions about the efficacy of probiotics for the maintenance of remission in UC.

A subgroup probiotic-specific meta-analysis by Jonkers *et al*[56] revealed that pooled relative risk for *E. coli* Nissle compared to mesalazine was 1.08 (95% CI 0.86-1.37), indicating that this strain of *E. coli* was not inferior to mesalazine in preventing relapses.

The American Recommendations for probiotic use from 2011 state very strong "A" recommendations for the use of the two specific probiotics *Escherichia coli* Nissle 1917 and multispecies mixture VSL#3 for the maintenance of remission in UC[114].

In conclusion, specific probiotics such as *Escherichia coli* Nissle 1917 and multispecies mixture VSL#3 are probably as efficient as standard maintenance therapy with mesalazine, and can therefore be used instead of mesalazine in patients intolerant or allergic to 5-aminosalicylates, or as adjunctive therapy to standard therapy, to potentially increase the duration of remission.

TREATMENT AND PREVENTION OF POUCHITIS

In some patients with UC in whom the disease does not respond to medical therapy or who develop dysplasia or cancer, proctocolectomy with the construction of ileal pouch-anal anastomosis (IPAA) is required. Inflammation of this ileal reservoir (pouch), referred to as pouchitis, develops in between 15% and 50% of such patients. Although the exact etiology of pouchitis is not clear, host genetic factors, fecal stasis, mucosal ischemia, and bacterial dysbiosis in the pouch seem to be involved[56,87]. Most patients develop pouchitis in the first year after the procedure. Antibiotic therapy is generally successful; however, discontinuation of antibiotics is often followed by recurrence of the disease. Treatment and prevention of pouchitis with probiotics has thus been studied extensively, and only a few studies addressing the use of probiotics for the treatment of active pouchitis were published.

Kuisma *et al*[123] performed a double-blind placebo-controlled trial to investigate the efficacy of *Lactobacillus rhamnosus* GG supplementation as primary therapy for ileal pouch inflammation. Twenty patients with a previous history of pouchitis and endoscopic evidence of inflammation were randomized to *Lactobacillus* GG 0.5-1 $\times 10^{10}$ CFU twice daily or placebo for 3 mo. Clinical efficacy was assessed by a change in the pouchitis disease activity index (PDAI). In addition, quantitative bacterial cultures of fecal samples and biopsies taken from the pouch were performed before and after probiotic supplementation. No differences were observed between the groups with regard to the mean pouchitis disease activity index. *Lactobacillus* GG supplementation changed the pouch intestinal microbiota by increasing the ratio of total fecal *Lactobacilli* to total fecal anaerobes ($P = 0.03$) and enhancing the frequency of *Lactobacilli*-positive cultures in the pouch. The authors concluded that although probiotic supplementation with *Lactobacillus* GG changed pouch microbiota, it was clinically ineffective as primary therapy for active pouchitis.

In an open-label study, Laake *et al*[124] treated 51 UC patients with IPAA, 6 UC patients with ileorectal anastomosis without pouch, and 10 patients with IPPA because of familial adenomatous polyposis with a fermented milk product culture, containing probiotic strains *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subspecies *lactis* BB-12, in a dosage of 5×10^{10} CFU/d for 4 wk. Stool samples were cultured for examination of *Lactobacilli*, *Bifidobacteriae*, fungi, and pH before, during, and after intervention. In addition, before, during, and after intervention, symptom assessment and endoscopic evaluation was performed. Symptoms, such as involuntary defecation, leakage, abdominal cramps, fecal number and consistency, mucus, and urge to evacuate stools were significantly decreased during intervention in the UC/IPAA group. The median endoscopic score of inflammation also significantly decreased. The number of *Lactobacilli* and *Bifidobacteriae* significantly increased during intervention and remained significantly increased one week after intervention.

Gionchetti *et al*[125] evaluated the efficacy of high-dose VSL#3 in the treatment of mild active pouchitis in an open-label non-controlled study. Twenty-three patients with mild pouchitis were treated with VSL#3 (3.6×10^9 CFU/d) for four weeks. Symptomatic, endoscopic, and histologic evaluations were undertaken before and after treatment according to PDAI. Remission was defined as

a combination of a PDAI clinical score of ⩽ 2, an endoscopic score of ⩽ 1, and a total PDAI score of ⩽ 4. Patients in remission after initial treatment were treated with a maintenance dose of VSL#3 (1.8 10^9 CFU /d) for an additional six months. Sixteen out of 23 patients (69%) were in remission after treatment. The median total PDAI scores before and after therapy were 10 (range, 9-12) and 4 (range, 2-11), respectively ($P < 0.01$). The median Inflammatory Bowel Disease Questionnaire score also significantly improved ($P < 0.001$). All 16 patients who went into remission maintained remission during maintenance treatment. The authors conclude that high doses of the probiotic VSL#3 were effective in the treatment of mild pouchitis. As pouchitis is a recurrent state, many studies evaluated the potential of probiotics in preventing exacerbations. The most profoundly studied probiotic for this indication was multispecies preparation VSL#3. In a randomized, double-blind, placebo-controlled trial, the same group evaluated the efficacy of VSL#3 in the remission maintenance of chronic pouchitis compared with placebo[126]. Forty patients in clinical and endoscopic remission achieved by antibiotic therapy were randomized to receive either VSL#3 3 × 10^{12} CFU/d or placebo for 9 mo. Patients were assessed clinically every month, and endoscopically and histologically every 2 mo or in the event of relapse. In addition, bacterial stool cultures from fecal samples were performed before and after antibiotic treatment and each month during maintenance treatment. Only 3 patients (15%) in the VSL#3 group had relapses within the 9-mo follow-up period, in comparison with 20 (100%) in the placebo group ($P < 0.001$). In the VSL#3-treated group (but not in the control group), fecal concentrations of *Lactobacilli*, *Bifidobacteria*, and *S. thermophilus* increased significantly from baseline levels during treatment ($P < 0.01$). Therefore, the authors concluded that oral administration of VSL#3 is effective in preventing flare-ups of chronic pouchitis.

In another double-blind, placebo-controlled study, Gionchetti *et al*[127] evaluated the effectiveness of VSL#3 therapy in preventing the onset of pouchitis immediately and during the first year after ileal pouch-anal anastomosis. For this purpose, 40 patients who underwent IPAA for UC were randomized to receive either VSL#3 9 × 10^{11} CFU/d ($n = 20$) or placebo ($n = 20$) immediately after ileostomy closure for 1 year. The patients were assessed clinically, endoscopically, and histologically every few months, and health-related quality of life was assessed using the Inflammatory Bowel Disease Questionnaire (IBDQ). Only 2 (10%) patients from the VSL#3 group, compared to 8 (40%) from the placebo group, had an episode of acute pouchitis (log-rank test, $Z = 2.273$; $P < 0.05$). As expected, treatment with VSL#3 (but not placebo) produced a significant improvement in IBDQ score.

In a study by Mimura *et al*[128], the researchers evaluated the effectiveness of a single daily high dose probiotic preparation of VSL#3 in maintaining antibiotic-induced remission. All patients included in this study had pouchitis at least twice in the previous year or required treatment with continuous antibiotics. After remission was induced within four weeks of combined metronidazole and ciprofloxacin therapy, the patients were randomized to receive either VSL#3 (9 × 10^{11} CFU) ($n = 20$) or placebo ($n = 16$) once daily for one year or until relapse. Symptomatic, endoscopic, and histological evaluations were made before and 2 and 12 mo after randomization or at the time of relapse. Remission was maintained for one year in 17 patients (85%) on VSL#3, but in only one (6%) on placebo ($P < 0.0001$). The IBDQ score remained high in the VSL#3 group but deteriorated in the placebo group ($P = 0.0005$). Therefore, the authors concluded that the once daily high dose probiotic VSL#3 was effective in maintaining antibiotic introduced remission in patients with recurrent or refractory pouchitis.

In an open-label trial by Pronio *et al*[129], 31 patients at different periods after surgery without signs or symptoms of pouchitis were randomized to VSL#3 9 × 10^{11} CFU/d or no treatment for 12 mo. Pouchitis activity was evaluated by PDAI, with different immunologic parameters being studied in peripheral-blood mononuclear cells and mucosal biopsies to reveal the mechanisms of probiotic action. During the study period, none of the patients from the probiotic group and only one from the placebo group developed active pouchitis. Because of the extremely low relapse-rate, even in the non-treated group, it was impossible to derive any firm conclusions regarding the efficacy of probiotic treatment from this study. However, a significant reduction in PDAI score was observed in VSL#3 treated patients.

In contrast with these studies, Shen *et al*[130] reported much more disappointing results. In an open-label uncontrolled trial, they gave VSL#3 9 × 10^{11} CFU/d for 8 mo to 31 patients after being treated for pouchitis with ciprofloxacin for 2 wk. Baseline PDAI scores were calculated and patient symptoms were reassessed at week 3 of VSL#3 therapy and at the end of the 8-mo trial. Some, but not all, patients underwent repeat pouch endoscopy at the end of the trial. At the 8-mo follow-up, only 6 patients were still on VSL#3 therapy while all others had discontinued the therapy due to either recurrence of symptoms ($n = 23$) or development of adverse effects ($n = 2$). All six patients who completed the 8-mo course had repeat clinical and endoscopic evaluation. Their mean PDAI scores were not statistically different to those before probiotic intervention ($P = 0.27$). However, this trial had several methodological drawbacks. The patients were pre-treated with only one antibiotic and the success of this therapy of acute pouchitis was not regularly evaluated by endoscopy. Therefore, it remains unclear whether all patients were really in remission before the start of maintenance therapy with VSL#3.

In an open-label study by Gosselink *et al*[131], 39 patients given a fermented milk product containing *Lactobacillus rhamnosus* GG in a dosage of 1-2 × 10^{10} CFU immediately after IPAA operation were compared to 78 patients without any maintenance treatment. The first

episodes of pouchitis were observed significantly less frequently in the *Lactobacillus* GG group than in the untreated group (cumulative risk at 3 years: 7% *vs* 29%, $P = 0.011$). Therefore, the authors concluded that daily intake of the fermented product containing *Lactobacillus* GG provided significant clinical benefits without side effects, and recommended its use for the primary prevention of pouchitis.

In the Cochrane Collaboration review by Holubar *et al*[132] published in 2010, different modalities for the treatment and prevention of pouchitis after ileal pouch-anal anastomosis for UC, including different antibiotics, probiotics, glutamine, butyrate, and budesonide, were meta-analyzed and reviewed. They concluded that *Lactobacillus* GG was not superior in effectiveness compared to placebo for the treatment of acute pouchitis, while VSL#3 was more effective than placebo in the maintenance therapy of chronic pouchitis (97% *vs* 3%, $P < 0.0001$). The number needed to treat with VSL#3 to prevent one additional relapse was 2. Similarly, in a strain-specific meta-analysis performed by Jonkers *et al*[56], the authors calculated the pooled relative risk for prevention of relapses of pouchitis for VSL#3 compared to placebo as 0.17 (95%CI: 0.09-0.33). As a result of these conclusions, the multispecies probiotic preparation VSL#3 was granted the A level recommendation for the primary prevention and maintenance of remission of pouchitis after IPAA according to the American Recommendations for probiotic use from 2011[115]. Furthermore, they suggested that there was some evidence (C level) supporting its use even for the therapy of active pouchitis.

Finally, clinical guidelines for the management of pouchitis from 2009 suggest the use of VSL#3 in patients with recurrence of pouchitis following antibiotic treatment or having several recurrences despite antibiotic therapy[133]. However, they do not suggest probiotics for the treatment of acute pouchitis.

TREATMENT OF ACTIVE CROHN'S DISEASE

Clinical studies investigating the treatment of active Crohn's disease with probiotics were scarce. Gupta *et al*[134] reported a very small open-label pilot study of four children with mildly to moderately active Crohn's disease who were treated with entero-coated tablets containing *Lactobacillus rhamnosus* GG (10^{10} CFU) twice daily for 6 mo. Clinical activity was monitored by pediatric Crohn's disease activity index (PCDAI) and changes in intestinal permeability were measured by a double sugar permeability test. A significant improvement in clinical activity was observed 1 wk after starting *Lactobacillus* GG. Median PCDAI scores at 4 wk were 73% lower than baseline. Intestinal permeability improved in an almost parallel fashion. The authors concluded that the findings of this pilot study showed that *Lactobacillus* GG might improve clinical status and gut barrier function in children with mildly to moderately active Crohn's disease. Schultz *et al*[135] performed a small randomized, placebo-controlled trial to determine the efficacy of *Lactobacillus rhamnosus* GG in the induction or maintenance of medically-induced remission. Eleven patients with moderate to active Crohn's disease were enrolled to receive either *Lactobacillus* GG (2×10^9 CFU/d) or placebo for six months. In all patients, a tapering steroid regimen was applied for the induction of remission, and all received antibiotics the week before probiotic/placebo intervention was initiated. The primary end point was sustained remission; defined as freedom from relapse after 6 mo. Only 5 patients finished the study, with 2 patients in each group in sustained remission. The median time to relapse was 16 +/- 4 wk in the probiotic and 12 +/- 4.3 wk in the placebo group ($P = 0.5$). In contrast with the results of Gupta *et al*[134], this study did not demonstrate any benefit of *Lactobacillus* GG in inducing or maintaining medically-induced remission in CD.

Although Butterworth *et al*[136] in the Cochrane Collaboration review concluded that there was insufficient evidence to make any conclusions about the efficacy of probiotics in inducing remission in CD because of a lack of well-designed clinical studies, the two studies using synbiotics that were not included in this review revealed very promising results[137,138]. Fujimori *et al*[137] performed an open-label uncontrolled trial using a synbiotic for the therapy of active refractory Crohn's disease. Ten active CD patients who had failed to achieve remission via an initial therapeutic regimen of aminosalicylates and prednisolone were given synbiotic therapy consisting of two probiotic preparations that both contained *Bifidobacterium breve* 3×10^{10} CFU, *Lactobacillus casei* 3×10^{10} CFU, and *Bifidobacterium longum* 1.5×10^{10} CFU, as well as a prebiotic comprised of 3.3-9.9 g of psyllium (*Plantago ovata*). Patients were free to adjust their intake of probiotics or prebiotics throughout the trial. For the assessment of disease activity, Crohn's disease activity index (CDAI), International Organization for the Study of Inflammatory Bowel Disease (IOIBD) score, and blood sample variables were evaluated and compared before and after the trial. The duration of the trial was 13.0 +/- 4.5 mo. Of the ten included patients, 6 had a complete response, one had a partial response, and three were non-responders. Two patients discontinued treatment and four decreased their corticosteroid therapy. Both CDAI and IOIBD scores were significantly reduced after therapy (255-136, $P = 0.009$; 3.5-2.1, $P = 0.03$, respectively), however the laboratory markers of inflammation did not change. With the exception of some abdominal bloating disappearing with discontinuation of psyllium ingestion, there were no adverse events. The authors concluded that a combination of high-dose probiotics and prebiotics could be safely and effectively used as a co-therapy for the treatment of active CD.

Recently, Steed *et al*[138] conducted a randomized, double-blind, placebo-controlled trial including 35 patients with active CD using a synbiotic therapy comprised of probiotic *Bifidobacterium longum* 4×10^{11} CFU and prebiot-

ic Synergy 1 (oligofructose and inulin) 12g daily. Patients were requested to continue on stable doses of conventional medication they were receiving at initiation of the trial. The patients' clinical status was scored by CDAI and endoscopies with biopsies were performed at the start, and at 3 and 6 mo of therapeutic intervention. Six patients from the synbiotic group and 5 from the placebo group were lost from follow-up. Upon comparing pre- and post-treatment CDAI, there was a significant clinical improvement in the synbiotic group (start 219 ± 74.6, finish 147 ± 74; $P = 0.020$) but not in the placebo group (start 249 ± 79.4, finish 233 ± 155; $P = 0.81$). Similarly, there was a significant improvement in mean histological scores in the synbiotic group (start 6 ± 5, finish 3 ± 4; $P = 0.018$) but not in the placebo group (start 6 ± 5, finish 5 ± 6; $P = 0.75$). A significant reduction of pro-inflammatory TNF-α and an increase of mucosal *Bifidobacteria* was also observed in the synbiotic group.

MAINTENANCE OF REMISSION IN CROHN'S DISEASE

Only a few high-quality studies have been performed to assess the efficacy of probiotics for the maintenance of remission achieved with standard medical therapy or surgical resection in Crohn's disease. Currently, the use of probiotics for the maintenance of remission in Crohn's disease is not recommended.

In a trial by Guslandi *et al*[139], 32 patients with Crohn's disease in clinical remission (CDAI < 150) were randomized to treatment with either mesalamine 1 g three times daily or mesalamine 1 g two times daily plus probiotic yeast *Saccharomyces boulardii* 1 g daily for six months. Clinical relapses were observed in 37.5% of patients receiving mesalamine alone but in only 6.25% of patients combining mesalamine with the probiotic ($P = 0.04$). The authors hence concluded that *Saccharomyces boulardii* might be useful in the maintenance treatment of Crohn's disease.

Bousvaros *et al*[140] conducted a randomized, placebo-controlled trial of the probiotic *Lactobacillus rhamnosus* GG (LGG) to see whether the addition of LGG to standard therapy prolonged remission in children with CD. Seventy-five children and adolescents from 5 to 21 years old with CD in remission were randomized to receive either LGG ($n = 39$) or placebo ($n = 36$), and followed for up to 2 years. Concomitant medications, including aminosalicylates, 6-mercaptopurine, azathioprine, and low-dose alternate day corticosteroids were allowed. The percentage of patients that relapsed did not significantly differ between the LGG and the placebo group (31% *vs* 17%; $P = 0.18$), neither did the median time to relapse (9.8 mo *vs* 11.0 mo; $P = 0.24$). In conclusion, LGG did not prove to be effective for maintaining remission in children with CD when given as an adjunct to standard therapy. The ineffectiveness of probiotic strain *Lactobacillus rhamnosus* GG for maintenance therapy in CD was also supported by a study by Prantera *et al*[141], who performed a randomized placebo-controlled study in patients operated for Crohn's disease in whom all of the diseased gut had been removed. The patients received 1.2×10^9 CFU of *Lactobacillus* GG or placebo for one year. Ileocolonoscopy was performed at the end of the trial or at the onset of symptoms. Clinical recurrence was ascertained in 16.6% in the LGG group and in 10.5% in the placebo group. Sixty percent of patients in clinical remission on LGG had endoscopic recurrence compared with 35.3% on placebo ($P = 0.297$). There were no significant differences in the severity of the lesions between the two groups. Marteau *et al*[142] studied the potential of *Lactobacillus johnsonii* LA1 for prevention of recurrence in operated CD patients. This was a randomized, double-blind, placebo-controlled study. Patients were randomized to receive *Lactobacillus johnsonii* LA1 4×10^9 CFU/d ($n = 48$) or placebo ($n = 50$) for six months. No other treatment was allowed. There were 4 clinical recurrences in the probiotic group and 3 in the placebo group. In patients with symptomatic remission, endoscopic recurrence was observed in 64% in the placebo group compared to 49% in the probiotic group ($P = 0.15$). Endoscopic score distribution did not differ significantly between the two groups. A similar double-blind placebo-controlled study was performed by Van Gossum *et al*[143], who randomized 70 patients who had underwent elective ileocecal resection for CD to daily treatment with either *Lactobacillus johnsonii* LA1 10^{10} CFU ($n = 34$) or placebo ($n = 36$) for 12 wk. The primary objective of this study was to assess the effect of probiotics on the endoscopic recurrence rate at 12 wk. Clinical relapse rate was 15% in the probiotic group and 13.5% in the placebo group ($P = 0.79$). The mean endoscopic score at 3 mo was not significantly different between the two groups ($P = 0.48$), nor was the percentage of patients with severe endoscopic recurrence ($P = 0.33$). According to the results of these studies, it seems that, like LGG, *Lactobacillus johnsonii* LA1 has no effect on remission in CD.

With the intention of preventing postoperative recurrence of CD, another two double-blind placebo-controlled trials were performed. Chermesh *et al*[144] investigated the use of a synbiotic cocktail of 4 probiotics and 4 prebiotics (Synbiotic 2000), and Madsen *et al*[145] used multispecies probiotic VSL#3, which proved to be efficient in the therapy of ulcerative colitis and pouchitis.

In the 2006 Cochrane Collaboration review regarding probiotics for maintenance of remission in CD by Rolfe *et al*[146], the authors identified 7 eligible studies. They found no statistically significant benefits of *E. coli* Nissle for reducing the risk of relapse compared to placebo, or for *Lactobacillus rhamnosus* GG after surgical or medically-induced remission. There was no statistically significant benefit of probiotics for reducing the risk of relapse compared to medical maintenance therapy employing aminosalicylates or azathioprine. Moreover, they found more adverse events in *Lactobacillus* GG treated patients. However, a small study using *Saccharomyces boulardii* demonstrated a difference in favor of its use combined with medical maintenance therapy in comparison with standard medical therapy alone, although the difference was

not statistically significant. They concluded that there is no evidence to suggest the use of probiotics for the maintenance of remission in CD. In the second Cochrane Collaboration review analyzing different interventions for the prevention of post-operative recurrence of Crohn's disease, the authors came to the same conclusion that probiotics were not superior to placebo[147].

Similarly, a meta-analysis performed by Rahimi *et al*[148] also failed to demonstrate the efficacy of probiotics in maintaining remission and preventing clinical and endoscopical recurrence in CD. Moreover, in a meta-analysis performed by Shen *et al*[149], researchers came to the conclusion that not only were *Lactobacilli* inefficacious, but also that administration of *Lactobacillus* GG might increase the relapse rate.

PREBIOTICS AND IBD

Compared to probiotics, there is considerably less clinical evidence regarding the use of prebiotics for IBD therapy.

In an early trial by Hallert *et al*[150], the ingestion efficiency of *Psyllium* (*Plantago ovata*, ispaghula husk) for 4 mo compared to placebo was studied for relieving gastrointestinal symptoms in patients with UC in remission. Regarding the symptom's score, ispaghula was consistently superior to placebo ($P < 0.001$) and was associated with a significantly higher rate of improvement (69% *vs* 24%; $P < 0.001$). Based on these results, the authors suggested that ispaghula could be helpful in the management of gastrointestinal symptoms in UC.

A Spanish group performed a multicenter open-label, randomized clinical trial to assess the efficacy and safety of *Plantago ovata* seeds as compared with mesalamine in maintaining remission in UC[151]. A total of 105 patients with UC in remission were randomized into three groups treated with *Plantago ovata* (10 g twice daily), mesalamine (500 mg twice daily), or *Plantago ovata* plus mesalamine at the same doses for 12 mo. Three patients, all from the *Plantago ovata* group, were withdrawn because of adverse events (i.e., constipation and/or flatulence). After 12 mo, the treatment failure rate was 40% in the *Plantago ovata* group, 35% in the mesalamine group, and 30% in the *Plantago ovata* plus mesalamine group. The probability of continued remission was similar ($P = 0.67$). A significant increase in fecal butyrate levels was observed in the groups using *Plantago ovata* ($P = 0.018$). The authors concluded that *Plantago ovata* seeds might be as effective as mesalamine for maintenance therapy in UC patients in remission. Furthermore, Casellas *et al*[152] conducted a prospective, randomized, placebo-controlled pilot trial comparing the effect of oligofructose-enriched inulin 12 g/d ($n = 10$) and maltodextrin used as placebo ($n = 9$) for 2 wk in patients with mild to moderately active UC. Concomitant treatment with mesalazine (3 g/d) was allowed. A significant reduction of fecal calprotectin, a marker of intestinal inflammation, was observed in the group receiving oligofructose-enriched inulin (day 0: 4377 +/- 659 μg/g; day 7: 1033 +/- 393 μg/g, $P < 0.05$) but not in the placebo group (day 0: 5834 +/- 1563 μg/g; day 7: 4084 +/- 1395 μg/g, n.s.).

Hafer *et al*[153] investigated the clinical and histological efficacy of lactulose in patients with both UC and CD. In a pilot study, 14 UC and 17 CD patients, most of whom were in a clinically active state, were randomized either to receive 10 g lactulose daily or placebo, adjuvant to standard therapy for 4 mo. No significant improvement of clinical activity index, endoscopic score, or immunohistochemical parameters was observed in CD or UC patients receiving lactulose in comparison to the control group.

Several clinical trials were performed in Japan using germinated barley foodstuff (GBF), which mainly consists of dietary fiber and glutamine-rich protein, for the therapy of UC. Kanauchi *et al*[154] investigated the efficacy of long-term administration of GBF in the treatment of active UC in a multi-center open trial. Twenty-one patients with mild to moderate UC received 20-30 g of GBF while baseline treatment with 5-aminosalicylates and/or steroids was continued. After 24 wk of treatment, the GBF group showed a significant decrease in clinical activity index compared with the control group ($P < 0.05$). No side effects related to GBF were observed. The same group published results of another study in which GBS was used for maintenance therapy in UC[155]. Patients were randomized into two groups: GBF 20 mg/d ($n = 22$) and control ($n = 37$). Response to treatment was assessed by monitoring the clinical activity index (CAI) and endoscopic score. Significantly better CAI values and a significantly lower recurrence rate were observed in the GBF group at 3, 6, and 12 mo compared with the controls. No side effects related to GBF were observed. According to the results of both studies, GBF could reduce the clinical activity of active UC, and appeared to be effective as a maintenance therapy in patients with UC.

Moreover, a small open-label trial was performed by Lindsay *et al*[156] in which they treated 10 patients with active ileocolonic Crohn's disease with 15 g of FOS for three weeks. FOS induced a significant reduction in the disease activity index from 9.8 ± 3.1 to 6.9 ± 3.4 ($P < 0.01$). They also observed a significant increase in fecal *Bifidobacteria* concentration, in the percentage of IL-10 positive, and TLR2 and TLR4 expressing dendritic cells in mucosal biopsies.

In contrast to previous findings, the results from a randomized double-blind placebo-controlled trial performed by Benjamin *et al*[157] did not confirm the efficacy of FOS for therapy of active CD. In this study patients were randomized to 15 g/d FOS ($n = 54$) or placebo ($n = 49$) for 4 wk. More patients receiving FOS (26% *vs* 8%; $P = 0.018$) withdrew before the 4-wk end point and there was no significant difference in the number of patients achieving a clinical response between the FOS and placebo groups (22% *vs* 39%; $P = 0.067$).

Considering all the above facts regarding the use of prebiotics, there is very little evidence to support their use in IBD therapy. However, supplementation with germinated barley foodstuff, *Psyllium* (*Plantago ovata*, ispaghula

husk), or oligofructose-enriched inulin might provide some benefit in patients with active UC or UC in remission, but more high-quality clinical studies are needed to confirm their effectiveness.

CONCLUSION

Probiotics and prebiotics definitely have great potential for future therapeutic approaches in inflammatory bowel disease. However, further research is required to identify specific probiotic strains or their combinations and prebiotic substances that will be most efficient for therapies of different types and stages of activity of intestinal inflammation.

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