Immunogenetics of infection and inflammation of the urogenital and gastrointestinal tracts and probiotics

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Chapter 12

Probiotics in Clinical Practice as Therapeutics against Enteric Disorders

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Introduction

Probiotics are useful in mild enteric disorders that are limited to the presence of individual symptoms such as diarrhea, constipation, and bloating. More controversial is their use in colonic diverticular disease or in one the most common disorders, namely the irritable bowel syndrome. With few exceptions and in a limited well defined patients' group no evidence exists that probiotics induced and maintain remission in patients with inflammatory bowel disease. In this chapter we review the current evidence of the value of probiotics in clinical practice of the following enteric disorders as well as the formulations and compositions of specific probiotics. It will be clear that most of the compositions used are not classified as medicaments, and most of them are free to obtain as ¨over-the-counter¨ preparations administered without medical prescriptions. Therefore, their use as therapeutics is very limited at present. This situation is likely to change in coming years. As shown in other chapters of this book a multidisciplinary approach is bringing scientific protocols to the field of probiotics and new technology to study the complicated field of gut microbiology and ecology is being applied to the study of functional and inflammatory diseases of the gut. These advances will permit the design of specific, tailor-made probiotics to be used in specific clinical situations. Antibiotics are known to disrupt the normal gut microbiology of the individual. For example, patients who received antibiotic treatment during initial acute infectious diarrhea had significantly more and longer lasting IBS compared to those with a natural course[1-3] and patients with the so-called postinfectious irritable syndrome treated with antibiotics during initial acute infectious have an increased severity and duration of diarrhea. Thus demonstrating that during antibiotic treatment the intestinal microbiota is damaged additionally. In some cases patients suffer from overgrowth of intestinal flora and in this case the antibiotics are effective in reducing the overgrowth.[4] In many of these clinical situations probiotics may turn out to be the appropriate agents to restore the normal microbiota.

The current indications of probiotics in the management of the following intestinal disorders are briefly reviewed with an emphasis on data obtained in clinical trials: The established indications such as probiotics in diarrhea as in acute episodes following bacterial, fungus or viral infections; possibly indications under study, such as in traveler diarrhea or in antibiotic-associated, and diarrhea and complications produced by *Clostridium difficile*. We also review studies of the usefulness of probiotics in constipation and bloating, in irritable bowel syndrome, and finally in inflammatory bowel disease, ulcerative colitis, pouchitis and in Crohn's disease.

Probiotics in acute diarrhea

Multiple studies in children have shown that *Lactobacillus*, administered orally, may have antidiarrheal properties. To determine the effect of *Lactobacillus* GG on the course of acute diarrhea in hospitalized children, a prospective, and placebo-controlled, triple-blind clinical trial was carried out in Pakistan. Forty children (mean age, 13 months) received either oral *Lactobacillus* GG (n = 21) or placebo (n = 19) twice daily for 2 days, after rehydration in addition to the usual diet. The clinical course of diarrhea was followed during the treatment period. The features for admission into the study groups were similar and were characterized by severe diarrhea, malnutrition and inappropriate management before presentation. Response was evident on day 2, when the frequency of both vomiting and diarrhea was less in the *Lactobacillus* group. In those patients with acute non-bloody diarrhea (n = 32), the percentage of children with persistent watery diarrhea at 48 hours was significantly lower in the *Lactobacillus* group (31% versus 75%). No significant difference was observed after 48 hours in those with bloody diarrhea.[5]

Van Niel *et al.*[6] conducted a meta-analysis of randomized, controlled studies to assess whether treatment with *Lactobacillus* improved clinical outcome in children with acute infectious diarrhea. They conducted a search in bibliographic databases of traditional biomedical as well as complementary and alternative medicine literature published between 1966 and 2000. The original search yielded 26 studies, nine of which met the criteria. A reduction of 0.7 days in diarrhea duration and a reduction of 1.6 stools for diarrhea frequency were attained on day 2 of treatment in the participants who received *Lactobacillus* compared to those who received placebo. A preplanned subanalysis suggested a dose-effect relationship. The results of this meta-analysis suggested that *Lactobacillus* is safe and effective as a treatment for children with acute infectious diarrhea.

Campylobacter jejuni (*C. jejuni*) is an important cause of bacterial-induced enterocolitis in humans in the developed world, caused by consuming infected food.[7] Ternhag *et al*. studied 101,855 patients who had an episode of microbiologically confirmed gastrointestinal infections in the period of 1997−2004, in Sweden. In 56% of cases of acute diarrhoea *C. jejuni* was isolated.[8] Acute intestinal infection with this pathogen may involve extraintestinal manifestations and lead to complications and in some cases chronic disease, like reactive arthritis and Guillain–Barré syndrome[9], irritable bowel syndrome and chronic inflammatory bowel diseases.[10-11] Some studies show that pathogen virulence and disease severity determine the ability of *C. jejuni* to invade the cytosol of human cells has been demonstrated.[12-13] According to Wine et al. *C. jejuni* disruption of monolayers is mediated by invasion. The ability of this pathogen to invade epithelial cells is cell-type dependent.[14] These findings provide new insight in the pathogen-host epithelial barrier interaction and offer potential mechanisms of intestinal injury and chronic immune stimulation. Wine *et al*. determined the

ability of lactobacilli to inhibit *C. jejuni* invasion. *Lactobacillus helveticus* adheres to both T84 and intestine 407 cells. Protection of cells from *C. jejuni* invasion by lactobacilli seems to depend on strain specificity of both pathogen and probiotics.[15]

The effect of the yeast *Saccharomyces boulardii* on acute diarrhea was described by Szajewska et al. in a metaanalysis showing clinical benefits in those using probiotics above the control group by shortening the duration of diarrhea. However, all trials included had methodological limitations.[16] The same group conducted a metaanalysis for the effect of LGG for treating diarrhea in children. They found moderate clinical benefits for LGG in the treatment of acute diarrhea in children. Also this metaanalysis discusses several methodological limitations and heterogeneity.[17]

Rotavirus was discovered in children with gastroenteritis by Bishop *et al.* in 1973.[18] This agent causes widespread morbidity and 870,000 deaths worldwide each year. Bishop said: "after doing a lot of background reading, it became clear that there probably was an infectious agent but we could not get anything to grow in culture". Bishop *et al.*[18] participated in the development of vaccines against rotavirus, the first of which was licensed for use in the USA in 1998.

The effect of orally administered *lactobacilli* on acute rotavirus diarrhea was tested by Isolauri *et al.*[19] in 42 well-nourished children aged 5–28 months. After oral rehydration, the patients received human *L. casei* strain GG 10^{10}cfu twice daily for 5 days. The control group was not given *lactobacilli*. *Lactobacillus* GG was found in the feces of 83% of the group with *L. casei* strain GG. The diarrheal phase was shortened in that group. The dietary supplementation with *lactobacilli* significantly influenced the bacterial enzyme profile. Urease activity during diarrhea transiently increased in the control group but not in the group receiving *L. casei* strain GG. No intergroup differences were found in a-glucuronidase, a-glucosidase, and glycocholic acid hydrolase levels. Therefore, Isolauri *et al.* suggested that rotavirus infection gives rise to biphasic diarrhea, the first phase being an osmotic diarrhea and the second associated with overgrowth of specifically urease-producing bacteria. Oral bacteriotherapy appears to be a promising means to counteract the disturbed microbial balance.

To evaluate the ingested strain's adherent properties and ability to inhibit murine rotavirus infection, Duffy *et al.*[20] administered human *Bifidobacterium* sp. strain *bifidum* to BALB/c lactating mice (n = 58) and their litters (n = 327 pups). ELISA and anaerobic bacteriologic techniques were used to measure murine rotavirus shedding and colonization of *Bifidobacterium* in the small intestine. At 1316 days of gestation, pregnant dams (and their expected litters) were randomly assigned to one of four experimental groups as follows: normal controls; *B. bifidum*-treated only; murine rotavirus-infected only; and *B. bifidum*-treated plus murine rotavirus-infected dams and litters. During the acute phase of diarrhea, 80% of small-intestine cultures in *B. bifidum*-treated litters were positive for the ingested *B. bifidum* strain compared to 24% of fecal cultures. The examination of tissue cross

sections under electron microscopy revealed the ingested *B. bifidum* strain survived passage through the upper gastrointestinal tract and adhered to the small-intestine epithelium. After the administration of the high dose of virus, diarrhea developed in all pups, but onset was significantly delayed in *B. bifidum*-treated plus murine rotavirus-infected litters compared to litters infected with murine rotavirus only. *B. bifidum*-treated plus murine rotavirus-infected pups demonstrated a significant reduction in murine rotavirus shedding compared with litters challenged with murine rotavirus only at day 2-10 after inoculation. More direct studies are needed to assess the mechanisms by which this anaerobe may modify the course of murine rotavirus infection at the level of gut epithelium.

Qiao *et al*.[21] evaluated the potential synergistic effects of *Bifidobacterium* spp. (*B. bifidum* and *B. infantis*), with or without prebiotic compounds (arabino-galactan, short-chain fructo-oligosaccharide, iso-malto-dextrins), on modulating the course of rhesus rotavirus infection, as well as their ability to mediate the associated mucosal and humoral immune responses. Therefore, they fed these species orally to Balb/c pups. Rotavirus-specific IgA and IgG in serum, rotavirus antigen, and specific IgA in feces were measured by ELISA. Mucosal total IgA and IgG levels were determined in Peyer's patches by flow cytometry. Significantly delayed onset and early resolution of diarrhea were observed in bifidobacteria-treated, rhesus rotavirus-infected mice compared with rhesus rotavirus-infected control mice. They saw that supplementation with prebiotic compounds did not shorten the clinical course of diarrhea more than that observed with bifidobacteria treatment alone. Rotavirus-specific IgA in feces was elevated 16-fold on day 5 postinfection in bifidobacteria-treated, rhesus rotavirus-infected mice compared with the rhesus rotavirus-infected only group. In addition, the level of rotavirus-specific IgA in serum was fourfold higher in bifidobacteria-treated, rhesus rotavirus-infected litters versus mice challenged with rhesus rotavirus alone on 28 and 42 days postinfection. They found no enhancement of the immune response in rhesus rotavirus-infected mice that were treated with both bifidobacteria and prebiotic compounds over those treated with bifidobacteria alone. These findings suggested that bifidobacteria may act as an adjuvant by modulating early mucosal and strong humoral rotavirus-specific immune responses, and mitigate the severity of rotavirus-induced diarrhea.[21]

Antibiotic-associated diarrhea

Diarrhea is a common side-effect of both the short- and long-term use of antibiotics. Several reports exist on the benefits of probiotics in this common complication. For example, in a multicenter study the value of a probiotic *Enterococcus* SF68 or placebo was assessed in the prevention of antibiotic-associated diarrhea. 45 patients treated with antibiotics were given, concurrently, one capsule of either *Enterococcus* SF68 or placebo for 7 days This probiotic

was effective in reducing the incidence of antibiotic-associated diarrhea compared to placebo (8.7% compared to 27.2%, respectively).[22] Unfortunately the *Enterococcus* SF68 has been withdrawn because of the risk of transfer of antibiotic resistance. Vanderhoof *et al*. showed a significant reduction in antibiotic associated diarrhea, using *Lactobacillus* GG in children.[23]

Clostridum difficile (C. difficile) is an anaerobic, spore-forming bacterium which can cause a primarily nosocomial disease ranging from mild diarrhoea to severe, life-threatening pseudomembranous colitis. Infections with *C. difficile* in hospitalised patients are an increasing worldwide problem. In a double-blind, placebo-controlled study, performed in a high-risk group of 193 hospitalized patients receiving a new prescription for a β-lactam antibiotic and having no acute diarrhea on enrollment it was shown that *Saccharomyces boulardii (S. boulardii)*1 g/day caused a significant decrease in antibiotic associated diarrhea. (7.2% in patients receiving *S. boulardii* compared to 14.6% with placebo; p = 0.02).[24] In another study McFarland *et al*. measured the recurrence of active *C. difficile*-associated disease using a combination of *S. boulardii* and standard antibiotics compared to placebo. Therapy with *S. boulardii* showed a significant efficacy in recurrent *C. difficile*-associated disease compared to placebo (recurrence rate 34.6% in probiotic group compared to 64.7% in the placebo group). However, no benefit was found when *S. boulardii* was used to treat primary infection with *C. difficile* (recurrence rate 19.3% compared to 24.2% respectively; P = 0.86).[25]

Lactobacillus GG was successfully used to treat a group of patients with recurrent diarrhea caused by *C. difficile*.[26]

In summary, probiotics can be utilized to restore the normal gut function and to reduce the duration of acute gastroenteritis.[27] Results so far are encouraging but the most effective dose and type of strain needs to be elucidated.

Probiotics in constipation and bloating

Constipation is a common heterogeneous gastrointestinal disease affecting up to 27% of the western population.[28-30] Although there is evidence supporting the fact that probiotics favorably modify the intestinal function, placebo-controlled studies on the possible treatment using probiotics are very rare.

De Paula et al.[31] investigated the effect of a probiotics in 266 females with functional constipation (according to Rome II criteria), randomized to receive either a mixture of *Bifidobacterium animalis* (DN-173 010) and prebiotic fructoligosaccharide (FOS) twice a day for 2 weeks or a lacteous dessert. The results show a 22% increase in the number of bowel movements per week and a slight increase in stool quality as assessed by the Bristol Stool Questionnaire when compared to placebo. Perception of pain and straining during defecation were significantly reduced in the probiotics group. Koebnick *et al.*[32] showed a significant improvement in self reported severity of constipation in 70 adults after the ingestion of

L.casei shirota versus placebo during a period of 4 weeks.
According to Ouwehand *et al.*[33] administering *L. rhamnosus*/ *Propionibacterium freudenreichii* supplemented juice increases the defecation frequency by 24%. Nevertheless, they observed no reduction in laxative use.

Probiotics in colonic diverticular disease

Colonic diverticulosis, characterized by sac-like protrusions, due to hernition of the colonic mucosa and sub-mucosa through defects in the muscular layer of the colon wall[34-35], it is highly prevalent in western countries, and rare in the developing world.[36-37]
Recent data have shown that chronic inflammation and abnormal colonic microflora play an important role in the pathogenesis of diverticular disease, suggesting that diverticular disease is an inflammatory mucosal disease, similar to inflammatory bowel diseases.[38-39] Therefore, normalizing the intestinal flora as well as administering an anti-inflammatory agent that has already proven effectiveness in IBD, may help treat the symptoms of diverticular disease, prevent the onset of acute diverticulitis and reduce the risk of symptomatic recurrence.
Tursy *et al.* studied the efficacy of *Lactobacillus casei* DG VSL#3 (VSL Pharmaceuticals, Inc., Fort Lauderdale, FL, USA) in combination with 5-ASA (a pH-dependent formulation of mesalazine (Pentacol, SOFAR S.p.A, Trezzano Rosa (MI), Italy) or balsalazide, respectively) in patients with symptomatic, uncomplicated diverticular disease in remission. This probiotic 5-ASA combination performed better in preventing disease relapses and improving symptoms than the single-agent regimens.[40]
In another study, nonpathogenic *E. coli* (Nissle strain) combined with antibiotic therapy (dichlorchinolinol) and an intestinal absorbent (active coal) resulted in greater symptomatic improvement and longer periods of remission than with the combination of an antibiotic and absorbent regimen alone.[41]

Probiotics in irritable bowel syndrome

Irritable bowel syndrome is a widespread and multifactorial functional disorder of the digestive tract.[42] It affects 8-22% of the population with a higher prevalence in women. It accounts for 20–50% of referrals to gastroenterology clinics and is characterized by abdominal pain, excessive flatus, variable bowel habit and abdominal bloating for which there is no evidence of detectable organic disease. Suggested etiologies include gut motility and psychological disorders as well as psychophysiological phenomena and colonic fermentation.[43]
A large proportion of patients have periods characterized by sudden and unforeseeable changes in the two main symptoms, constipation and diarrhea, even within a few days.[43] It is very likely that the syndrome represents different groups of patients with probably different

pathogenesis. Irritable bowel syndrome may follow gastroenteritis and may be associated with an abnormal gut flora and with food intolerance.[44] The fecal microflora in some of these patients has been shown to be abnormal with higher numbers of facultative organisms and low numbers of *lactobacilli* and *bifidobacteria*.[43] Bacteria are the major component of formed stools and are influenced by substrates arriving with the ileal affluent. Stool production is related to quantitative and qualitative aspects of the colonic microflora and nearly 80% of the fecal dry weight consists of bacteria, 50% of which are viable.

Although there is no evidence of food allergy in irritable bowel syndrome, food intolerance has been identified and exclusion diets are beneficial to many of these patients. Food intolerance may be caused by an abnormal fermentation of food residues in the colon, as a result of disruption of the normal flora.[43]

Some reports suggest that probiotics play a role in regulating the motility of the digestive tract.[42] This may result in improvements in pain and flatulence in response to probiotic administration.[43]

To assess whether preceding gastroenteritis or food intolerance were associated with colonic malfermentation, King *et al.*[44] conducted a crossover controlled trial with a standard diet and an exclusion diet matched for macronutrients in six female patients with irritable bowel syndrome and six female controls. In this study fecal excretion of fat, nitrogen, starch, and nonstarch polysaccharide was measured during the last 72 hours of each diet. The total excretion of hydrogen and methane were collected over 24 hours in purpose-built 1.4 m^3 whole body calorimeter. Breath hydrogen and methane excretion were measured for 3 hours after 20 g oral lactulose. The maximum rate of gas excretion was significantly greater in patients than in controls. The total gas production in patients was not greater than in controls, whereas hydrogen production was higher. After lactulose, breath hydrogen was greater on the standard than on the exclusion diet. This means that colonic-gas production, particularly of hydrogen, is greater in patients with irritable bowel disease than in controls, and both symptoms and gas production are reduced by an exclusion diet. This reduction may be associated with alterations in the activity of hydrogen-consuming bacteria. It was therefore concluded that fermentation may be an important factor in the pathogenesis of this syndrom.[44]

Spiller *et al.*[45] studied the intestinal permeability (lactulose/mannitol ratio) and histological and immunological features in rectal biopsy specimens in 21 patients who had acute *Campylobacter* enteritis, 10 patients with postdysenteric irritable bowel syndrome and 12 asymptomatic controls. They found that the increased enteroendocrine cell counts, T lymphocytes, and gut permeability, which may survive for more than a year after Campylobacter enteritis, contribute to post- dysenteric irritable bowel syndrome[45], thus offering a rationale to use probiotics for several months after the infectious episode.

To determine the efficacy of *Lactobacillus rhamnosus* GG (LGG) in the management of functional abdominal pain disorders in children, Gawronska et al performed a RCT in which 104 children with Rome-II criteria for functional dyspepsia, irritable bowel syndrome, or

functional abdominal pain were enrolled. Fifty two patients received LGG for 4 weeks whereas the other 52 subjects received a placebo. The results show a benefit for those receiving LGG in all groups.[46]

The effect of the probiotics was studied by Brigidi *et al.*[47] in a clinical trial in which 10 patients suffering from this syndrome were administered the VSL#3 probiotic preparation. The results indicated that the administration of VSL#3 improved the clinical picture and changed the composition and biochemistry of fecal microbiota. The exact mechanisms of the positive effects are not known. The selection of patients may have had an important role in detecting the positive effects. Whether the induction of a significant increase in *lactobacilli*, *bifidobacteria*, and *S. thermophilus* contributed to the regulation of the motility disorders or the increase in fecal beta-galactosidase with a decrease in urease content indicate that a good response requires further study. The importance of this study is that it showed that the measurement of specific parameters and changes in the specific microflora was possible.

Kim *et al.*[48] investigated the effects of VSL#3 on gastrointestinal transit and symptoms of patients with irritable bowel syndrome diagnosed with the criteria established by Rome II and with predominant diarrhea. Twenty-five patients with diarrhea-predominant irritable bowel syndrome were randomly assigned to receive VSL#3 powder (450 billion lyophilized bacteria/day) or matching placebo twice daily for 8 weeks after a 2-week run-in period. Pre- and post-treatment gastrointestinal transit measurements were performed in all patients. The patients recorded their bowel function and symptoms daily in a diary during the 10-week study, which was powered to detect a 50% change in the primary colonic transit endpoint. There were no significant differences in mean gastrointestinal transit measurements, bowel function scores or satisfactory global symptom relief between the two treatment groups, pre- or post-therapy. The differences in abdominal bloating scores between treatments were borderline significant. Abdominal bloating was reduced with VSL#3, but not with placebo. Furthermore, VSL#3 had no effects on individual symptoms such as abdominal pain, gas and urgency. VSL#3 was well tolerated by all patients, and thus it seems to relieve the abdominal bloating in patients with diarrhea-predominant irritable bowel syndrome.[48]

Probiotics in inflammatory bowel disease

The term chronic inflammatory bowel disease includes three disease types: ulcerative colitis, Crohn's disease, and an intermediate form (about 10%). Crohn's disease is defined as a chronic granulomatous inflammation of the digestive tract that most commonly involves the distal ileum, colon and anus. Less often, the disease affects the mouth, esophagus, stomach and duodenum. Occasionally, extraintestinal sites are affected and it is referred to as: "metastatic Crohn's disease". In ulcerative colitis, the colon is affected and the disease usually starts in the rectum and progresses proximally, although sometimes the first manifestation may be the involvement of the whole colon and rectum (panproctocolitis).

Ulcerative colitis is slightly more common than Crohn's disease. In Western Europe and North of America, there are 3000-5000 new cases of Crohn's disease and 8000-10000 new cases of ulcerative colitis. The incidence and prevalence of Crohn's disease have been increasing five times faster than that of ulcerative colitis. Young people are more likely to be more affected by inflammatory bowel disease than older people, with a peak incidence at the age of 15-30 years.

The etiology of this disease is unknown. An infectious hypothesis has been considered for years, and *Mycobacterium paratuberculosis* has been mainly isolated from patients with Crohn's disease. However, some patients with ulcerative colitis and controls harbor this pathogen. Viruses have also been involved in the pathogenesis. Several factors other than infectious agents have been postulated as the cause of the disease. These different factors are immunologic, genetic and psychological. The chronic inflammatory nature of these diseases may indicate the presence of an infectious cause or the presence of a dysregulatory abnormality in the control of inflammation.

An increasing number of both clinical and laboratory observations support the importance of the ubiquitous luminal bacteria in the inflammatory responses of these disorders.[49] Bacteria are present throughout the gastrointestinal tract but are not evenly distributed and their diversity and numerical importance vary in the different sections of the gastrointestinal tract.[48-50] In the stomach and duodenum there are facultative anaerobic bacteria (*Lactobacillus* spp. and *enterobacteriaceae*), with a small number of bacteria that are predominantly Gram-positive and aerobic.[49] In the lower distal part of the intestine there is a large variety of bacteria, mostly anaerobic bacteria belonging to *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Fusobacterium*, *Peptostreptococcus* and *Ruminococcus*.[50] There is a transition to higher concentrations of bacteria and increasing number of Gram-negative bacteria in the distal ileum. Across the ileocecal valve there is a dramatic increase in bacterial concentration and more anaerobes than aerobes.[49]

Enteric bacteria have been detected in patients with Crohn's disease and in those with pouchitis. These patients may be effectively treated with antibiotics. Purified bacterial products may initiate and perpetuate experimental colitis. The inflammation is due to loss of normal tolerance to the commensal flora.[49]

The onset of inflammation is associated with an imbalance in the intestinal microflora with relative predominance of "aggressive" bacteria and an insufficient concentration of "protective" species. Reconditioning the flora through either direct supplementation with protective bacteria or by indirect stimulation plays a protective role in inflammatory bowel disease.[49]

Antioxidant properties, the ability to increase prostacyclin and crampy in endothelial cell cultures and the ability to modulate adhesion molecule expression on human lymphocytes are all effects which are relevant for the use of probiotics in the treatment of immunological disorders such as inflammatory bowel disease.[51]

Probiotics in ulcerative colitis

Few data are available on the role of probiotics in human ulcerative colitis. Two studies have shown a significant decrease in lactobacilli concentration in colonic biopsies in patients with ulcerative colitis. Preventing or controlling the colitis is reported when the concentration of *Lactobacillus* was modulated through dietary supplementation with lactulose (prebiotic). This is a nondigestible food ingredient that affects the host by selectively stimulating the growth and activity of one or more "probiotic" bacteria, such as *Bifidobacterium* and *Lactobacillus* that have health-promoting properties.[52]

Ulcerative colitis is a chronic inflammation of the rectal and colonic mucosa, with a poorly defined etiology. Its characteristics are bloody diarrhea and mucus associated with a negative stool culture for bacteria, ova, or parasites. There is also fecal stasis with bacterial overgrowth and mucosal ischemia. The therapeutic role of probiotics is shown through two studies; in one of these, oral administration of *Lactobacillus* GG caused an increase in intestinal IgA immune response in patients with Crohn's disease. In the other study, exogenous administration of *L. reuteri* (pure bacterial suspension or as fermented oatmeal soup) prevented acetic acid-induced colitis or methotrexate-induced colitis in rats.

These studies showed a significant decrease in *lactobacilli* concentration in patients with active ulcerative colitis. The results showed that *L. plantarum* was more effective in methotrexate-induced colitis, and *Lactobacillus* treatment prevented development of spontaneous colitis in IL-10 gene-deficient mice.

In an open label study with 20 patients, intolerant or allergic to 5-aminosalicylic acid (5-ASA), a treatment consisting of 6g VSL#3 (1800 billion bacteria)/day for 12 months was instituted. Clinical, endoscopic assessment and stool culture and fecal pH determination were recorded.[53] Nineteen patients completed the trial and 15 were in remission for the whole year. Fecal concentrations of *bifidobacteria*, *lactobacilli*, and *S. salivarius spp. Thermophilus* were significantly increased in all patients and remained stable throughout the study. No changes were noted in the concentrations of total aerobic this suggesting that the beneficial effects of VSL#3 were not related to suppression of endogenous luminal flora. The treatment was well-tolerated with no reported significant side effects like those seen in the treatment with 5-ASA oral compounds. This shows that the probiotic preparation was able to colonize the intestine and suggested its possible usefulness in maintaining remission in ulcerative colitis patients intolerant or allergic to 5-ASA.[53] The hypothesis from these studies is that the intestinal environment may contribute to the pathophysiology of ulcerative colitis.

Guslandi *et al.*[54] studied the efficacy of *S. boulardii* in ulcerative colitis patients. Twenty-five patients with a mild to moderate clinical flare-up of ulcerative colitis received additional treatment with *S. boulardii* 250 mg three times a day for 4 weeks during maintenance treatment with mesalamine (mesalazine, 5-ASA). These patients were unsuitable for

steroid therapy. Rachmilewitz's clinical activity index was calculated before and after the treatment. Of the 24 patients who completed the study, 17 attained clinical remission; this was endoscopically confirmed. The preliminary results suggested that *S. boulardii* may be effective in the treatment of ulcerative colitis.

Kruis *et al.*[55] compared the efficacy of the probiotic preparation *Escherichia coli* Nissle 1917 and established therapy with mesalazine in maintaining remission in patients with ulcerative colitis. Three hundred and twenty seven patients received either probiotics 200 mg once daily (n = 162) or mesalazine 500 mg three times daily (n = 165) during a period of 12 months. Assessment was performed by clinical and endoscopic activity indices (Rachmilewitz) and histology. The results show relapses in 40 out of 110 (36.4%) and 38 out of 112 (33.9%) patients in the in the probiotic and mesalazine group respectively (p=0.003). Hereby, they show efficacy and safety of *E. coli Nissle* in maintaining remission equivalent to the gold standard mesalazine in patients with ulcerative colitis.

Probiotics in pouchitis

Pouchitis is a nonspecific inflammation of the ileal reservoir that may appear after surgery for ulcerative colitis, and results in various clinical symptoms. It is a well-recognized long-term complication of restorative proctocolectomy. The risk of pouchitis increases in patients with a history of extraintestinal manifestations, primary sclerosing cholangitis, positive serology for perinuclear antineutrophil cytoplasmic antibodies, and backwash ileitis.[56]

Pouchitis is associated with bacterial overgrowth and dysbiosis, and antibiotics represent the first-choice treatment. The distal ileum and the large bowel, the sites with the highest bacterial concentration, are the most frequently affected by inflammation. Enteric bacteria or their products have been detected within the inflamed mucosa. A significant decrease of *lactobacilli* and *bifidobacteria* concentrations has been found in ulcerative colitis, Crohn's disease and pouchitis. *Lactobacilli* as maintenance showed less frequent relapses of pouchitis than those using placebo. Diversion of the fecal stream in the small and large intestine reduces the activity of the inflammation. The luminal contents and purified bacterial products added to isolated intestinal loops trigger systemic and local signs of inflammation.

In a study by Campieri *et al.*[49], seven patients, after clinical, endoscopic, and histological diagnoses of inflammation of the ileal pouch anal anastomosis with a pouchitis disease activity index (PDAI) > 7, were treated with 2 g/day of rifaximin (a non absorbable antibiotic) and 1 g/day of ciprofloxacin for 1 month. All patients went into remission during this month, as judged by clinical, endoscopic and histological examination. After remission, all seven patients were treated with the highly concentrated probiotic mixture VSL#3 (a mixture of four strains of *lactobacilli* (*Lactobacillus casei, L. plantarum, L. acidophilus, L. delbrueckii subsp. bulgaricus*), three strains of *bifidobacteria* (*Bifidobacterium longum, B. breve, B.*

infantis) and one strain of *Streptococcus salivarius subsp. thermophilus*) for nine months. No patient had a relapse in this period. All patients who received a placebo had a relapse.

Probiotics in the maintenance of remission of chronic pouchitis

Gionchetti *et al.*[57] evaluated the efficacy of VSL#3 in the maintenance of remission of chronic pouchitis. Forty patients in clinical and endoscopic remission were randomized to receive either VSL#3 6 g/day, or an identical placebo for 9 months. The patients were assessed clinically every month and endoscopically and histologically every 2 months or in the event of relapse. Three patients (15%) in the VSL#3 group had relapses within the 9-month follow-up period, compared with 20 (100%) in the placebo group ($p<0.001$). In the VSL#3-treated group, the fecal concentration of *lactobacilli, bifidobacteria,* and *S. thermophilus* increased significantly from baseline levels. These results suggested that oral administration of this new probiotic preparation is effective in preventing flare-ups of chronic pouchitis (Fig. 6). In another RCT, 36 subjects were administered 6g VSL#3 daily or a placebo for 12 months. A relapse was observed in 15% of the patients receiving VSL#3 versus 94% of those on placebo ($p<0.0001$).[58]

Probiotics in preventing the onset of pouchitis

A positive effect of VSL#3 in the prevention of pouchitis has been reported by Gionchetti *et al.*[59], who compared probiotic therapy with VSL#3 versus placebo in the ability to prevent the onset of acute pouchitis during the first year after ileal pouch-anal anastomosis. Forty patients who underwent ileal pouch-anal anastomosis for ulcerative colitis were randomized to receive either VSL#3 or an identical placebo immediately after ileostomy closure for 1 year. Both groups consisted of 20 patients. The patients were assessed clinically, endoscopically, and histologically after 1, 3, 6, 9 and 12 months. Health-related quality of life was assessed using the Inflammatory Bowel Disease Questionnaire (IBDQ). Two of the 20 patients (10%) treated with VSL#3 had an episode of acute pouchitis compared with eight of the 20 patients (40%) treated with placebo ($p<0.01$). Treatment with VSL#3 determined a significant improvement in IBDQ score, which was not the case with placebo. During treatment with VSL#3, fecal concentration of *lactobacilli*, *bifidobacteria*, and *S. salivarius* increased significantly. The fecal concentration of *Bacteroides*, *clostridia*, *coliforms*, and *enterococci* were not modified. This suggested that the beneficial effect was not mediated by the suppression of the endogenous flora. The treatment with VSL#3 was effective in the prevention of the onset of acute pouchitis and improved the quality of life of patients with ileal pouch anal anastomosis.

As mentioned above, probiotic bacteria do not survive for long, and rapidly disappear as soon as the treatment is stopped. Therefore, prophylactic probiotic therapy of pouchitis might require long-term treatment and might not be indicated for all patients. For this reason, VSL#3 would be highly beneficial for patients at high risk of chronic pouchitis. In these cases prophylactic probiotics may be administered, pouch function improved and their quality of life after ileal pouch anal anastomosis could be maintained. Katz *et al.*[56] suggested that probiotics should be used for maintaining remission in chronic pouchitis and as prophylaxis against pouch inflammation in high-risk patients. Although preliminary results suggest that high doses VSL#3 may improve active pouchitis, probiotic therapy seems to be more effective to prevent mucosal inflammation than to treat it.

Kuisma *et al.*[60] investigated the efficacy of *Lactobacillus* GG supplementation as primary therapy for pouchitis and its effect on the microbial flora. Twenty patients, with a previous history of pouchitis and endoscopic inflammation were recruited for a prospective, randomized, double-blind, placebo-controlled trial of *Lactobacillus* GG supplementation. Ten patients received *Lactobacillus* GG and 10 placebo, in two gelatin capsules b.i.d. for 3 months. Quantitative bacterial culture of fresh fecal samples and biopsies taken from the pouch and afferent limb was performed before and after supplementation. *Lactobacillus* GG supplementation was found to change the pouch intestinal flora by increasing the ratio of total fecal *lactobacilli* to total fecal anaerobes and enhancing the frequency of *lactobacilli*-positive cultures in the pouch and afferent limb mucosal biopsy samples. Only 40% of patients were colonized with *Lactobacillus* GG, and no differences were observed between the groups with regard to the mean PDAI or the total anaerobes or aerobes of fecal or tissue biopsy samples. Thus, a single-strain probiotic bacterium supplement of *Lactobacillus* GG changed the pouch intestinal bacterial flora, but was ineffective as primary therapy for a clinical or endoscopic response. More clinical trials are needed to evaluate the right placement and dosage of probiotics within a treatment regimen for pouchitis.

Probiotics in Crohn's disease

The therapeutic role of probiotics in the prevention of postoperative recurrence of Crohn's disease has been reported in some studies. Campieri *et al.*[61] studied the effects of VSL#3 in a randomized, investigator-blind trial. Forty patients with curative resection randomized within 1 week post surgery were divided into two groups of 20 patients. One group received mesalazine 4 g/day for 1 year and the other group received rifaximin 1.8 g/day for 3 months followed by VSL#3 6 g/day for 9 months. The endoscopic activity was assessed after 3 and 12 months. In the mesalazine group, eight patients had severe endoscopic recurrence after 3 months as well as after 12 months, whereas in the group with rifaximin and VSL#3, two patients had a severe recurrence after 3 months and two patients after 12 months. These

results suggested the efficacy of the combination of a nonabsorbable antibiotic with a highly concentrated probiotic preparation in the prevention of severe endoscopic recurrence of Crohn's disease after surgical resection.

In a pilot study Guandalini *et al.*[62] investigated the possible effect of *Lactobacillus* GG in children with active Crohn's disease. Four male patients with a median age of 14.5 years (range 10–18) were enrolled. In terms of clinical outcome, the patients showed significant improvement. In three patients receiving *Lactobacillus* GG, it was possible to taper the dose of steroids.

In a third published study using *Lactobacillus* GG this effect could not be confirmed. Forty-five patients were randomized to receive *Lactobacillus* GG 12 billion cfu/day (23 patients) or placebo (22 patients). A clinical remission after 52 weeks was seen in 15 of the 23 patients with *Lactobacillus* GG (83.3%) and in 17 of the 22 patients with placebo (89.4%). Mild endoscopic activity was seen in nine of the 15 patients with remission in the *Lactobacillus* GG group (60%) and in six of the 17 patients with remission in the placebo group. This study failed to show effectiveness in the postoperative prevention on Crohn's disease.[63]

In a double blind RCT Marteau *et al.* administered 98 patients after surgery for CD *L. johnsonii* LA1 ($4x10^9$ cfu/day) or a placebo for 6 months. Endoscopic recurrence was observed in 49% of patients on Probiotics versus 64% of patients in the placebo group (64). The same strain was studied by van Gossum *et al.* in a higher dose 10^{10} cfu/day in 70 postoperative patients in a multi center study.[65] They found *L. johnsonii* LA1 not effective in the prevention of recurrence. Twenty one and 15% in the probiotics versus placebo group developed severe endoscopic lesions (p=0.33). The percentages with clinical relapse was 15 and 13% in the probiotics and the placebo group respectively (p=0.79).

The limited experience indicates that different probiotics have different capacity to prevent intestinal inflammation. More studies are therefore necessary.

Conclusions and perspectives

In this exploding era of clinical research, there is evidence for a strong link between the intestinal microbiota and intestinal disorders, both in the pathogenesis of inflammatory and regulatory pathways. Using probiotics as ecological treatment for these disorders have proven efficacy as is the case for specific forms of IBD as well as other intestinal diseases. Although the exact mechanisms by which probiotics exert their beneficial effects in vivo have not yet been fully clarified, the luminal bacterial flora appears to play a major role in the initiation and perpetuation of chronic inflammatory bowel diseases in animal and human models. Increasing evidence shows that probiotics modify the gastrointestinal microflora in such a way that the bacterial activities are advantageous to the health of the host without colonizing the gastrointestinal tract, suppress gastrointestinal inflammation, and modulate the inflammatory response.[50]

Probiotics appear to have multiple modes of action through the direct or indirect modulation of the endogenous flora or the immune system. This is based on cross-talk between luminal bacteria and bacteria and the epithelial cells. Inhibition of pathogens includes competition for colonization sites and nutrients, production of toxic compounds, and stimulation of the immune system. These mechanisms are not mutually exclusive, and inhibition may include one, several, or all of these mechanisms.[66] Modulation of the endogenous flora, enhancement of the intestinal barrier, and immunomodulator effects of down regulating inflammation are other mechanisms by which probiotics exert their effects.[67]

Current evidence provides support for the consideration of probiotics therapy for intestinal diseases, keeping in mind that efficacy of probiotics is strain and disease specific. The variety of studies carried out with distinct strains of probiotics bacteria has suggested heterogeneous and strain-specific effects.

Limitations of most studies conducted with probiotics, either with regard to the power of the study, deficit of human studies, randomization, use of different strains and lack of standardized methodology it remains difficult to draw firm conclusions from the current trials.

Furthermore, discordance between recent meta-analyses (16-17, 68-69) and systematic reviews (70-72) show important differences in the different trials.

Probiotics differ from one another, making results obtained with one strain, or a cocktail of strains, not easily extrapolated to another strain or cocktail of strains. The results from trials done on one probiotics strain in a specific patients group cannot be applied to other probiotics or other patient groups. Also the dose response studies are too few to establish the exact dose needed for optimal treatment. Investigation of probiotic application as therapeutic for different intestinal disorders increases the understanding of the role of gastrointestinal milieu in the pathogenesis of IBD and other intestinal diseases. Further validation of probiotic properties in humans and clarification of their mechanisms of action are needed to better understand the role of probiotics in promoting human health, and for better definition and application of the potential use of probiotics in different clinical settings. Therefore more and larger controlled randomized clinical trials are necessary to investigate the as yet unresolved issues with regard to efficacy based on immunological and microbiological analysis of colonic mucosa and stool, dose, and duration of use, and the exact strains for specific diseases and/or single or multistrain formulation are to prove the beneficial effects.

With regard to the described differences in the immunological and antipathogenic effects exerted by different probiotics, clinicians for the time being should advise those probiotic strains proven to be efficacious in relevant patient groups and encourage further clinical research studies in order to define the proper place in the management of infectious, functional and inflammatory disorders of the gastrointestinal tract.

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