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Inflammatory bowel disease, intestinal microflora, prebiotics and probiotics

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ABSTRACT:
Inflammatory bowel disease (IBD) is a group of intestinal conditions characterized by chronic relapsing course of uncontrolled inflammation within the gastrointestinal tract. The two main types of IBD are ulcerative colitis (UC) and Crohn's disease (CD).
Both genetic and environmental factors are involved in etiopathogenesis. According to most recent theories, IBD is probably a consequence of abnormal mucosal immune response to antigens of gut bacterial microflora in geneticaly susceptable individuals. If tolerance to commensal bacteria is lost, an immune response may be elucidated against non-pathogenic bacteria, leading to increased production of inflammatory cytokines and chemokines. Consequently different subsets of inflammatory cells are activated.
Growing knowledge about implication of gut microflora into the pathogenesis of IBD encouraged scientific word to search for new therapeutic strategies concentrated on changing the microenvironmental factors. Nutritional therapy has been advocated in CD patients, especially for children and adolescents.
The rationale behind prebiotic use is to elevate the populations of certain beneficial bacteria and thereby quantitatively changing the composition of microflora. Although several prebiotic compounds possess promising properties to have beneficial effect on IBD, only few of them (Plantago ovata, germinated barley foodstuff) have been clinically tested.
Multiple mechanisms of action have been suggested to explain the effect of probiotics in IBD. A great number of basic, animal model and human studies have revealed the great potential of probiotic use in treatment of IBD patients. However, clinical use of probiotics has been proved effective only in a therapy of pouchitis and maintenance of remission in ulcerative colitis, while their effectiveness in a therapy of Crohn's disease is not firmly proved.

KEY WORDS:
Inflammatory bowel disease, Crohn's disease, Ulcerative colitis, Pouchitis, Intestinal microflora, Prebiotics, Probiotics

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INTRODUCTION

Inflammatory bowel disease (IBD) is a group of idiopathic intestinal conditions characterized by chronic relapsing course of uncontrolled inflammation within the gastrointestinal tract. The two main types of IBD are ulcerative colitis (UC) and Crohn's disease (CD). UC and CD share many characteristics, but they also differ in certain aspects. UC is characterised by mucosal inflammation of large bowel while small bowel mucosa is spared. Rectal mucosa is regularly inflamed and inflammation spreads continuously to the proximal parts of the colon. On the contrary in Crohn's disease not only colon, but any part of gastrointestinal tract from mouth to anus can be involved. Inflammation spreads through all layers of intestinal wall and inflamed and healthy parts of intestine can follow each other (so called skip lesions). Moreover, CD can present not only with intestinal inflammation but also with penetrating lesions such as intestinal fistulas to other parts of intestine, adjacent hollow organs such as urinary bladder or vagina and skin, and with fibrozating course resulting in intestinal stenoses.

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Abdominal pain and tenderness, diarrhoea often containing blood and mucus in the stools, as well as fatigue, low grade fever, weight loss and growth retardation in the case of early onset disease in the childhood are most frequent symptoms and signs of IBD. Although IBD are primary diseases of the intestinal tract, involvement of many extraintestinal organs such as joints, skin, eyes, liver, biliary tract or urinary organs is not unusual.

The prevalence of IBD varies in different populations across the world. The prevalence of CD and UC are high in the most developed countries of Northern and Western Europe and North America, reaching 214 and 243, and 198 and 229 per 10^5, respectively, but they are low in the developing countries of Asia, Africa and South America [1]. Moreover, the incidences of UC and CD are still growing. IBD manifests during childhood and adolescence in approximately 25% to 40% of all patients [2,3].

Etiopathogenesis of IBD is not completely understood. Both genetic and environmental factors are involved. Increased sanitation and the lifestyles within developed countries appear to increase the risk of IBD. It has been proposed that the exposure to unhygienic conditions during childhood can prime the intestinal environment which will lead to optimal mucosal immune development and regulation, preventing a future immune response [4]. According to most recent theories, IBD is probably a consequence of abnormal mucosal immune response to antigens of gut bacterial microflora in genetically susceptible individuals.

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Previous studies focused on identifying specific pathogenic infections responsible for IBD. *Mycobacterium paratuberculosis, Mycobacterium kansasii*, paramixovirus, *Listeria monocytogenes, Chlamydia trachomatis*, RNA reovirus and *Pseudomonas multophilia* infection were regarded associated with Crohn's disease, and *Escherichia coli*, diplostreptococcus, *Fusobacterium necrophorum, Shigella, Helicobacter hepaticus* and RNA viruses were linked to ulcerative colitis [5]. However, further studies have not confirmed the role of specific infections in the pathogenesis of IBD. Moreover, there is a growing evidence that normal bacterial microflora

Several observations in humans implicate microbial factors in the pathogenesis of IBD. Bowel lesions in IBD occur predominantly in areas with highest bacterial counts like terminal ileum and colon.

can trigger harmful immune reactions in susceptible hosts. The most convincing evidence supporting the role of enteric microflora in the pathogenesis of IBD comes from animal models. Animals with genetically engineered dysregulation of the immune response develop spontaneous colitis when grown in normal conditions resembling IBD in humans. However, they do not develop intestinal inflammation when grown in germ free environment, indicating that bacterial exposure and colonisation are essential for the development of colitis [6-8]. Interleukin-10 deficient mice displayed a significant higher number of mucosal adherent bacteria and lower levels of protective bacteria like *Lactobacillus* compared with healthy mice. Both the proportion of mucosal adherent bacteria and the development of colitis were significantly decreased by nutritional supplementation with lactose or enema delivery of *Lactobacillus reuteri* [9]. Similarly, *Lactobacillus plantarum* when given in feedings to the IL-10 knock-out mice attenuated established colitis, corresponding to reduction in intestinal permeability and anti-endotoxin core antibody levels [10,11].

Several observations in humans implicate microbial factors in the pathogenesis of IBD [12]. Bowel lesions in IBD occur predominantly in areas with highest bacterial counts like terminal ileum and colon [13]. Diversion of faecal stream is associated with distal improvement in patients with CD and relapse occurs after restoration of faecal stream [14]. UC patients who undergo ileal pouch-anal anastomosis surgery develop mucosal inflammation after bacterial colonisation of the pouch [15]. Early IBD lesions can be induced in susceptible individuals by the direct installation of faecal material into non-inflamed excluded loops of intestine [16]. Antibiotic treatment appears to provide clinical benefit in patients with CD and inflammation of ileal pouch [17].

In the case of IBD however, genetically predisposed individuals appear to lose the normal tolerance to commensal bacteria, leading to elevated inflammatory response.

Pathogenic events in IBD may be associated with different alterations in the intestinal flora in the ileum and colon. More bacteria were detected on the mucosal surface of IBD patients than on those of healthy controls and bacterial invasion of mucosa was evident in up to 83% of biopsies from IBD patients but no bacteria were detected in tissue of controls [18]. Moreover, IBD patients have altered composition of commensal enteric bacteria with increased *Bacteroides*, adherent/invasive *Escherichia coli, Enterococci*, and decreased *Bifidobacterium* and *Lactobacillus* species [19].

INTESTINAL MICROFLORA, IMMUNE SYSTEM, GENES AND INFLAMMATION

Optimal development of intestinal immune system is determined in part by environmental contact with the commensal gut microflora [20]. Changes in the microbial flora may alter mucosal immune development. In the healthy gut, there is a symbiotic relationship between the host and the commensal bacteria in which exposure leads to down-regulation of inflammatory genes, inhibiting the immune response of the gut (4). In the case of IBD however, genetically predisposed individuals appear to lose the normal tolerance to commensal bacteria, leading to elevated inflammatory response. The microbiota then provides a constant stimulus for the host immune system [21].

Majority of genes found to be associated with the increased risk for development of IBD are encoding proteins functioning in preservation of mucosal barrier function or in regulation of mucosal immune system. The major breakthrough of understanding a linkage between genetic predisposition and IBD development was made in 2001 by 3 independent groups which reported the identification of the first Crohn's disease susceptibility gene, NOD2, subsequently renamed CARD15 by the International Nomenclature Committee, on chromosome 16q12 [22-24]. The CARD15 gene encodes a protein that contains 2 caspase rich domains (CARDs), a central nucleotide-binding domain (NBD), and a leucine-rich repeat region [25]. There are 3 common genetic variants of CARD15 associated with CD, Arg702Trp, Gly908Arg, and Leu1007fsinsC, and many other less common putative variants [22,26]. 10% to 30% of CD patients are heterozygotes for one of these common mutations, whereas 3% to 15% of patients are either homozygotes or compound heterozygotes [27]. The relative risk of development CD associated with carriage of one CARD15 variants is between 1.5 and 3, increasing to 20 to 40 in people carrying two mutations [27]. CARD15 belongs to the family of pattern-recognition receptors which are responsible for microbial recognition, induction of anti-microbial genes and control of adaptive immune response [28]. While CARD15 is an intracellular pattern-recognition protein capable of recognizing peptidoglycans from gram-negative and gram-positive bacteria through the detection of muramyl dipeptide, the minimal motif in all peptidoglycans [29,30], other pattern-recognition receptors, named toll-like receptors (TLRs), recognize other microbial components like lipoproteins (TLR1, 2, 6), double-stranded RNA (TLR3), lipopolysaccharide (TLR4), flagellin (TLR5), single-stranded RNA (TLR7, 8), and CpG DNA (TLR9) [28,31]. Mutations in genes for toll-like receptors as well as for CARD4/NOD1 receptor may be also associated with increased susceptibility for IBD [32-35].

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Binding of specific microbial components to the pattern-recognition receptors in most cases, including CARD15, leads to intracellular signalling pathways which result in most occasions to nuclear factor-κB (NF-κB) activation [30,36,37]. NF-κB is a key intracellular signalling molecule in a variety of inflammatory pathways and its elevated in IBD tissues [38]. However, common mutations of CARD15 gene lead to a decrease in NF-κB activation and not to its overactivation [30]. It has been proposed that failure to trigger protective pathways by bacterial components mediated by CARD15 results in defective bacterial eradication [39] resulting in a NF-κB activation by CARD15 independent mechanisms. CARD15 receptors originally thought to be confined to monocytic and dentritic cells [40] have been recently found also in intestinal epithelial cells [41] and Paneth cells [42]. Paneth cells play an important antibacterial role in the gut, secreting potent antimicrobial substances such as lyzozyme, phospholipase A2 and α and β defensins [27,43].

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Pattern-recognition receptors are required to discriminate between pathogenic and commensal microorganisms. If tolerance to commensal bacteria is lost, an immune response may be elucidated against non-pathogenic bacteria, leading to increased production of inflammatory cytokines and chemokines (4). Consequently different subsets of inflammatory cells are activated. Mucosa of patients with CD is dominated by CD4+ lymphocytes with a type 1 helper-T-cell (Th 1) phenotype, characterized by

the production of interferon-γ and interleukin-2, while the mucosa of patients with UC is dominated by CD4+ lymphocytes with an atypical type 2 helper-T-cell (Th 2) phenotype, characterized by production of transforming growth factor β (TGF-β) and interleukin-5 [44]. Th1 cytokines activate macrophages to produce a potent mix of broadly active cytokines as interleukin-12, interleukin-18, macrophage migration inhibitor factor, tumour necrosis factor (TNF), interleukin-1, and interleukin-6 [45].

In conclusion, intolerance to intestinal microorganisms because of genetical susceptibility in addition to possible dysbiosis of gut microflora may together lead to broad spectrum inflammation of the gut.

INFLUENCING GUT MICROFLORA – THERAPEUTIC OPTION IN IBD

Traditionally, therapy of IBD has been directed against inflammatory response of gut immune system. Corticosteroids, 5-aminosalicylates, immunosuppressive and immunoregulatory agents have been used for over a half of a century to treat active disease and to maintain remission. In the last decade new biologic agents, such as anti-TNF antibodies, emerged as therapeutical options targeting immune system components most important for intestinal inflammation. However, growing knowledge about implication of gut microbial environment into the pathogenesis of IBD encouraged scientific word to search for new therapeutic strategies concentrated on changing the microenvironmental factors that play an important role in the pathogenesis.

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Not surprisingly, nutritional therapy has been advocated in IBD patients, especially for children and adolescents, for many years, as the disease often results in weight lost, poor growth and development and numerous nutrient deficiencies [46-49]. However, early studies using enteral nutrition with so called elemental formulas, in which proteins were degraded to amino acids, showed that they were not efficacious only in restoring patient's nutritional status but also in reducing activity of intestinal inflammation in CD patients [50-52]. Because of their bitter taste and high cost, elemental formulas have been gradually replaced by semielemental formulas, using short peptides instead of amino acids, and by polymeric formulas containing whole protein molecules. Interestingly, several meta-analyses revealed that therapy with enteral nutrition has efficiency comparable of that of corticosteroid treatment in active CD, especially in childhood population and that polymeric formulas are as efficient as the elemental ones [53-57]. Although several mechanisms of an action could be important in therapeutical use of enteral nutrition, its influence on the intestinal microflora may play a crucial role [58].

Experimental evidence of the central role of the luminal flora as an essential factor for the development of IBD provided an impetus to the development of alternative strategies to manipulate the intestinal flora by prebiotics and probiotics.

PREBIOTICS AND IBD

Prebiotics are non-digestible food constituents that beneficially affect the host by selectively stimulating the growth or activity of one, or limited

number of bacterial species in the gut, thus improving host health [59]. The rationale behind prebiotic use is to elevate the populations of certain endogene beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* and thereby quantitatively changing the composition of microflora. This change may act beneficially by causing luminal production of short chain fatty acids (SCFA), which are important nutrients for the intestinal cells and induce acidic environment, by preventing of pathogenic bacteria adherence and by production of anti-bacterial substances [60]. Some examples of prebiotics are dietary fiber and some types of oligosaccharides. Although several prebiotic compounds possess promising properties to have beneficial effect on IBD, only few of them have been clinically tested.

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Inulin and oligofructose are composed of multiple saccharide units, which are indigestible by the human enzymes. They stimulate the growth of lactic acid bacteria and the generation of SCFA [61]. In dextran sodium sulphate-induced colitis animal model, inulin attenuated gut inflammation [61].

Similarly, fructooligosaccharide was shown to decrease the severity of damage in experimental model of rat trinitrobenzene sulfonic acid induced colitis [62].

Psyllium, also called Ispaghula husk or Plantago ovata, is a water soluble dietary fiber [63]. Hallert and co-workers reported that Ispaghula husk significantly attenuates symptoms in patients with UC [64] and Spanish Group for the Study of Crohn's Disease and Ulcerative Colitis (GETECCU) found it as efficient as sulphasalazine in maintaining remission [65].

Germinated barley foodstuff (GBF) is derived from aleurone layer and scutellum fractions of germinated barley and consists mainly of dietary fiber and glutamine-rich protein [66]. It induces intestinal microflora to produce SCFA [67]. Treatment of rat experimental colitis with GBF led to improvement of the clinical and pathological signs of colitis and decrease serum IL-8 and alpha 1-acid-glycoprotein. GBF was comparable effective against mucosal inflammation and more effective against diarrhoea when compared with sulphasalazine [68]. The same Japanese group proved the effectiveness of GBF in several studies on patients with active UC and UC in remission [69,70]. Patients revealed both clinical and endoscopic improvement of colitis. Therefore, GBF is registered as a special foodstuff for UC by Japan's Ministry of Health, Labour and Welfare.

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PROBIOTICS AND IBD

Probiotics are defined as live microorganisms which, when consumed in adequate quantities, confer a health benefit on the host [21]. Multiple mechanisms of action have been suggested to explain the effect of probiotics in IBD. Potential mechanisms include suppression of growth or epithelial binding and invasion by pathogenic bacteria, production of antimicrobial substances, improved epithelial barrier function, and immunoregulation [60,71]. A great number of basic, animal model and human studies have revealed the great potential of probiotic use in treatment of IBD patients.

The effects of probiotic are probably both strain-dependent and dose dependent. For example, probiotic *Lactobacillus rhamnosus* GG (LGG) at-

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The effect of probiotics on barrier function was studied in T84 cell monolayers. It was demonstrated that VSL#3 prevented the decrease in trans-epithelial resistance following incubation with pathogenic bacteria.

tenuated the TNF-α induced IL-8 production at doses 10^{6-8} by the Caco-2 intestinal cell line, but on the contrary, at higher doses LGG actually increased IL-8 levels [72]. This finding indicated that determining the correct dose of probiotic for treatment is vital. The same study demonstrated that heat-killed LGG was also able to decrease IL-8 production, conflicting the paradigm that viability of probiotics is essential for their efficacy. Similarly, bacterial DNA from VSL#3, a high dose mixture of three strains of *Bifidobacteria*, four strains of Lactobacilli, and one strain of *Streptococcus salivarius* ssp. *thermophilus*, was able to decrease IL-8 secretion, delay NF-κB activation and stabilise IκB levels [73]. However, in another study using *Lactobacillus reuteri* on HT-29 and T84 cells only live but not deactivated bacteria reduced TNF-α induced IL-8 production and induced production of anti-inflammatory factors [74]. The effect of probiotics on barrier function was studied in T84 cell monolayers. It was demonstrated that VSL#3 prevented the decrease in trans-epithelial resistance following incubation with pathogenic bacteria [75].

The most convincing evidence of probiotic efficacy and mechanisms came from animal studies. More than 20 different animal models of IBD are available [76]. In IL-10 knockout mice, *L. plantarum* 299V [77], *L. salivarius* subspecies *salivarius* 433118 and UCC118 [78,79] , B. infantis 35624 [78], and VSL#3 (80) have been shown to attenuate intestinal inflammation [76]. In HLA-B27 transgenic rats LGG prevented recurrent colitis after antibiotic treatment, whereas *L. plantarum* had no effect (81). Amelioration of inflammation was observed after administration of *L. salivarius* subspecies *salivarius* CECT5713 [82] and *L. plantarum* NCIMB8826 [83] in trinitrobenzene sulfonic acid-induced colitis, and after *L .reuteri* and oat fiber application in acetic acid and methotrexate induced colitis in rats [84,85]. Similarly, VSL#3 and LGG significantly improved sulphhydril-blocker iodoacetamide-induced colitis in rats, but had no effect on dinitrobenzene sulfonic acid-induced colitis [86]. These experiments clearly demonstrated that the effect of probiotic treatment depends both on probiotic strain and on type of inflammation.

The reviewers concluded that probiotics in combination with conventional therapy do not improve overall remission rates in patients with mild to moderate ulcerative colitis however; they may provide some benefits in terms of reduction of disease activity.

Not only live bacteria, but also soluble bacterial antigens extracted from *E. coli* in dextran sulphate sodium-induced colitis (DSS) model [87] and bacterial DNA from VSL#3 preparation in DSS and IL-10 knockout mouse models [73,88] showed the ability to reduce inflammation. Therefore, viability of probiotic bacteria was not proven to be a prerequisite for their effect.

Many clinical trials have been performed to investigate the efficacy of probiotics in achieving and maintaining remission of different forms of IBD. However, only four which investigated the efficacy of probiotics for induction of remission in ulcerative colitis met the criteria of randomised controlled trials and were reviewed in recent Cochrane Collaboration review [89]. The reviewers concluded that probiotics in combination with conventional therapy do not improve overall remission rates in patients with mild to moderate ulcerative colitis however; they may provide some benefits in terms of reduction of disease activity.

Rembacken et al. [90] reported that probiotics (*E. coli* Nissle 1917) with steroids had similar effectiveness to mesalazine with steroids in achieving

remission, however relapse rate was slightly higher in the mesalazine group compared to probiotic group (73% vs. 67%, $P < 0.05$).

Kato et al. [91] reported that probiotics (*Bifidobacterium breve, B. bifidum, Lactobacillus acidophilus*) with 5-aminosalicilates (5-ASA) were as effective as placebo with 5-ASA when they compared the proportion of patients achieving the remission. However, when they took into account not only remission data but also clinical improvement data, clinical activity index (CAI) was found to be significantly lover in the probiotic group compared to placebo group after 12 weeks of treatment (3.7+/-0.4 vs. 5.8+/-0.8, $P < 0.05$). They also found that mean endoscopic index score and mean histological score was significantly reduced in the probiotic group ($P < 0.01$) but not in the placebo group after therapy.

Although Tursi et al. [92] did not found statistical significant difference in proportion of patients who achieved remission in probiotic (VSL#3)+5-ASA group in compare with placebo+5-ASA group, the mean time to remission was significantly shorter for probiotic group (4 vs. 7 days, $P < 0.01$).

In the trial of Furrie and colleagues [93] no significant differences were found between synbiotic (probiotic *Bifidobacterium longum* and fructooligosaccharide) group and placebo group in numbers of patients improved, CAI, endoscopy and histology scores. However, synbiotic treatment in conjunction with standard therapy caused significant reduction in TNF-α and IL-1α compared to standard therapy and placebo.

Several controlled studies showed that some probiotics can be used in the maintenance therapy of UC.

Several controlled studies showed that some probiotics can be used in the maintenance therapy of UC. Rembacker et al. [94] randomized UC patients who entered remission with conventional therapy to receive mesalazine or probiotic (*E. coli* Nissle 1917) for maintenance treatment for one year. At the end of the trial 73% of 5-ASA-treated patients relapsed as compared with 67% of those assigned to the probiotic. Authors concluded that the two strategies were of equivalent efficacy.

Same can be concluded from the studies of Kruis et al. They found that 11.3% of patients treated with 5-ASA relapsed in 12-week follow-up period as compared with 16% treated with *E. coli* Nisssle 1917 [95]. In their later study they found that during one year follow-up relapse occurred in 36.4% of the *E. coli* group and 33.9% of the mesalazine group [96].

Ishikawa et al. found out that bifidobacteria-fermented milk supplemented as a dietary adjunct was effective in maintaining remission of UC. After 1 year treatment, exacerbation of symptoms was observed in 27% of probiotic group and in 90% of the control group [97].

Zocco et al. [98] compared three groups of therapeutic regimen for maintenance of remission in UC patients. They found no significant difference in relapse rate at 6 and 12 month between the groups that received *Lactobacillus rhamnosus* GG or 5-ASA or both of them.

In an open uncontrolled study Venturi et al. [99] with VSL#3, 75% of patients with UC remained in remission during one-year follow-up.

Pouchitis, chronic inflammation of ileal pouch created after proctocolectomy, is usually treated by antibiotics. However, several controlled trials have revealed that probiotic use can be highly effective. Gionchetty et al.

[100] compared the efficacy of VSL#3 with placebo in maintenance of remission of pouchitis. The patients in the probiotic group relapsed in 15% as compared with 100% in the placebo group.

These results were practically replicated by Mimura et al. [101], who found that the relapse rate in one year after beginning of the therapy was 15% for VSL#3 group versus 94% for the placebo group.

Gionchetti et al. [102] studied prophylactic role of probiotic treatment in patients undergoing colectomy and pouch surgery. During the first year after operation only 10% of the patients in VSL#3 group but 40% of the patients in placebo group developed pouchitis.

In contrast to these results, probiotic *Lactobacillus rhamnosus* GG was ineffective in preventing relapses of chronic pouchitis.

In contrast to these results, probiotic *Lactobacillus rhamnosus* GG was ineffective in preventing relapses of chronic pouchitis [103].

Efficacy of probiotics for preventing recurrence of active disease has been studied in patients who rich the remission after medical or surgical therapy of CD. Malchow [104] who treated small number of patients with colonic CD in remission with either *E. coli* Nissle 1917 or placebo for 3 months, observed relapse rate 33% in the probiotic group and 63% in the placebo group.

Campieri et al. [105] reported that VSL#3 in combination with 5-ASA were more efficient than 5-ASA alone in prevention of post-operative recurrence of CD. They observed endoscopic recurrence in 40% of patients treated with 5-ASA alone but only in 10% of patients on combined therapy with 5-ASA and probiotics.

However, in two clinical studies using probiotic *Lactobacillus rhamnosus* GG, no differences were found in compare to placebo in CD relapse prevention [106,107]. French GETAID group [108] reported slightly lower endoscopic recurrence in a group of patients that was treated post-operatively with probiotic *Lactobacillus johnsonii* strain LA1 than in the placebo group (49% vs. 64%), but the difference was not statistically significant.

Guslandi et al. [109] reported that maintenance therapy with *Saccharomyces boulardii* and 5-ASA was significantly more effective in preventing relapse of CD than 5-ASA alone.

In conclusion, clinical use of probiotics has been proved effective in a therapy of pouchitis and maintenance of remission in ulcerative colitis, while their effectiveness in a therapy of Crohn's disease is not firmly proved.

In the meta-analysis by Rolfe et al. for Cochrane Collaboration [110] seven studies were identified to reach the inclusion criteria for review of maintenance therapy in CD. The authors concluded that there was no statistical significant benefits of *E. coli* for reducing the risk of relapse compared to placebo (RR 0.43, 95% CI 0.15 to 1.20), or *Lactobacillus* GG after surgically-induced remission (RR 1.58, 95% CI 0.30 to 8.40) or medically-induced remission (RR 0.83, 95% CI 0.25 to 2.80). There were no statistically significant benefits for reducing the risk of relapse compared to maintenance therapy employing aminosalicylates or azathioprine (RR 0.67, 95% CI 0.13 to 3.30). In children, there was no statistically significant difference between *Lactobacillus* GG and placebo for reducing the risk of relapse (RR 1.85, 95% CI 0.77 to 4.40).

In conclusion, clinical use of probiotics has been proved effective in a therapy of pouchitis and maintenance of remission in ulcerative colitis, while their effectiveness in a therapy of Crohn's disease is not firmly proved.

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