# Beneficial Effects of Probiotics, Prebiotics, Synbiotics, and Psychobiotics in Inflammatory Bowel Disease

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**Abstract:** Inflammatory bowel disease (IBD) is a group of diseases characterized by inflammation of the small and large intestine and primarily includes ulcerative colitis and Crohn's disease. Although the etiology of IBD is not fully understood, it is believed to result from the interaction of genetic, immunological, and environmental factors, including gut microbiota. Recent studies have shown a correlation between changes in the composition of the intestinal microbiota and IBD. Moreover, it has been suggested that probiotics and prebiotics influence the balance of beneficial and detrimental bacterial species, and thereby determine homeostasis versus inflammatory conditions. In this review, we focus on recent advances in the understanding of the role of prebiotics, probiotics, and synbiotics in functions of the gastrointestinal tract and the induction and maintenance of IBD remission. We also discuss the role of psychobiotics, which constitute a novel class of psychotropic agents that affect the central nervous system by influencing gut microbiota.

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**Key Words:** Crohn's disease, intestinal microbiota, probiotics, prebiotics, ulcerative colitis

The gastrointestinal tract (GIT) is colonized by a wide variety of microorganisms, which constitute gut microbiota. Microorganisms begin to settle in the GIT at birth, but the development of the microflora and the formation of intestinal barrier is a gradual process.[1] The relationship between the host and the gut microbiota is most commonly referred to as commensalism, where one organism benefits from the other without affecting it.[2] This specific microsystem evolved over several million years. The bacterial flora is involved, among others, in the renewal of intestinal epithelial cells, metabolism of food ingredients, and the modulation of the immune system, yet equally important is the influence of bacterial flora on peristalsis.[3]

GIT of adults is colonized by approximately $10^{14}$ different kinds of bacterial cells (i.e., 10 times more than the total number of cells constituting the human body), representing about 500 strains that belong to 40 to 50 families.[4] Gut microbiota of adults is dominated by 4 main groups of bacteria belonging to the genera *Bacteroidetes* (23%), *Firmicutes* (64%), *Proteobacteria* (8%), and *Actinobacteria* (3%).[2,5] Of note, bacteria, which are part of the microflora, have the ability of rapid growth and adhesion to the intestinal wall, and thus they can avoid leaching out of the body.[6,7]

Bacteria that have the ability to produce enzymes facilitating the distribution and absorption of nutrients are the most advantageous. Also important are the species forming the so-called "useful environment," such as *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Streptococcus salivarius*, which have the ability to defend against bacteriophages and to weaken acute immune response. These microorganisms also have a large genetic variation, allowing them to survive in everchanging environment and adapt to new conditions.[6,7]

Microbiota plays a number of functions within GIT. The immunomodulatory role, among others, relies on their influence on cytokine levels and interaction with gut-associated lymphoid tissue, which is the biggest lymphatic organ in the human body that produces 70% to 80% of the immune cells.[8] Intestinal bacteria also play protective functions, through competition with pathogenic bacteria for receptors on the surface of the intestinal epithelium and nutrients in the environment. Moreover, gut microbiota produce a number of antimicrobial agents (e.g., bacteriocins).[9] Of note, they also inhibit the growth of bacteria synthesizing carcinogens, such as *Citrobacter rodentium*, *Streptococcus bovis*, and *Bacteroides spp*., and some of them are even able to metabolize dietary carcinogens. Finally, gut microbiota plays an important structural role, which strengthens the tightness of the intestinal barrier by affecting the expression of some structural proteins constituting tight junctions between enterocytes and induces the synthesis of protective immunoglobulin A. In addition, intestinal bacteria exhibit many metabolic functions, such as affecting proliferation and differentiation of intestinal epithelial cells by supplying energy source (such as butyrate and short-chain fatty acids [SCFAs]) to the epithelium. They are also

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involved in the transformation of steroids and fatty acids, as well as in fermentation of dietary fiber and ions. In addition, intestinal bacteria synthesize several B-group vitamins and vitamin K.[9]

## GUT MICROBIOTA VERSUS IBD: FRIENDS OR FOES?

Inflammatory bowel diseases (IBD) refer principally to 2 chronic diseases that manifest with intestinal inflammation: ulcerative colitis (UC) and Crohn's disease (CD). The incidence of IBD is increasing, in particular, in developed countries. Although the etiology of these inflammatory disorders is not fully understood, there is a growing body of evidence that IBD morbidity is associated largely with genetic predisposition.[10] However, additional factors may be involved, such as diet, tissue damage associated with disturbance in the immune system, and abnormal intestinal microflora (quantitatively and qualitatively), what has been confirmed in murine models of IBD.[10] Noteworthy, genetic and microbiota-related backgrounds of IBD may be linked. Studies have shown that people with *CARD15/NOD* gene mutations, which rely on decreased activation of NF-κB, reduction of proinflammatory cytokine production, and defensin secretion are predisposed to CD development.[11] It has been shown that these mutations promote an increase in the number of bacteria in the distal ileum and the development of inflammation.[12]

However, gut microflora may trigger changes leading to IBD. One hypothesis suggests that there is an excessive activation of gut-associated lymphoid tissue, in response to subject's own intestinal microflora; this has been supported by experiments performed on laboratory animals.[13] More specifically, transgenic mice lacking IL-2 and IL-10 housed in sterile conditions did not show the development of such inflammatory disorders. In contrast, after the introduction of physiological and nonpathogenic microflora to the environment, the inflammatory process occurred.[14] Other studies found that the intervention in the intestinal microbiota by means of antibiotics significantly reduces inflammation in CD.[15] Furthermore, it has been shown in patients with CD that the transfer of the contents of the ileostomy to a healthy segment of the intestine of the same patient leads to the development of inflammation, what suggests a considerable share of microbiota in the etiology of IBD.[16]

The microorganism widely suspected to be involved in the initiation and development of IBD is *Mycobacterium paratuberculosis*. It regularly occurs in the intestinal biopsies of patients with IBD and the milk of nursing mothers diagnosed with CD; the antibodies against *Mycobacterium avium paratuberculosis spp*. have been detected in more than 83% subjects with CD.[17] Moreover, *Mycobacterium* is involved in the initiation of John's disease occurring in ruminants and symptomatically similar to CD.[17,18] Of note, *Mycobacterium* often exists within GIT in humans without any IBD symptoms. However, there have been no studies so far that would use *Mycobacterium* as a diagnostic tool for early detection of IBD or clearly link these bacteria with changes within the immune system and the development of the disease.

Other potential etiologic factors for IBD may include *Chelonia Mycobacterium* species, *Mycobacterium fortuitum* and *Mycobacterium kansasii*[19] and pathogenic strains of *Escherichia coli*, primarily O157 and H7.[20] The latter produce enzymes that break down mucin, which forms a protective gel layer within GIT and constitutes a semipermeable barrier between the lumen and the epithelium. The changes in the mucus barrier increase permeability for bacteria and their metabolites that cause damage of epithelial cells and lead to an inflammatory process in patients with UC,[21] whereas hyperproduction of mucins and abnormal glycosylation is observed in patients with CD.[22,23] Moreover, hydrogen sulfide produced by these bacteria has a negative influence on the metabolism of SCFAs. It has been shown in patients with UC that the fatty acid metabolism disorder leads to impaired secretion of intestinal epithelial protective mucus and thus exacerbates inflammation.[24]

## PROBIOTICS

Probiotics, from the Greek "pro bios," meaning "for life," according to the definition of the World Health Organization and the Food and Agriculture Organization of the United Nations are living microorganisms which, when administered in adequate amounts, confer a health benefit to the host. The first observation of the therapeutic effect of bacteria was made by the "grandfather" of modern probiotics, Ilya Mechnikov, a Nobel laureate in Medicine in 1908. Mechnikov was the first to draw attention to the relationship between a very good general state of health and longevity of Bulgarian rural population and systematically ingested sour milk containing lactic acid bacteria (*Lactobacillales*), which he called "the Bulgarian bacillus."[25]

Presently, the mode of action of probiotics is not fully understood. Nevertheless, some of the most common uses for probiotics include the treatment of inflammatory disorders, such as arthritis,[26] radiation-[27] and NSAID-induced enteropathy,[28] antibiotic-induced diarrhea,[29] chemotherapy-induced mucositis,[30] pouchitis,[31] and UC[32,33] and CD.[34,35] Noteworthy, studies on the composition of intestinal microflora showed that patients with UC and CD had an increased number of aerobic bacteria, e.g., *E. coli*, and anaerobic bacteria of the genus *Bacteroides*, and a decreased number of microorganisms of the genus *Lactobacillus* and *Bifidobacterium*,[36] which suggests potential benefits of probiotics use in IBD therapy. However, application of a nonpathogenic *E. coli Nisle* 1917 strain in patients with CD did not cause any significant differences in the duration of remission compared with the control group. In contrast, *E. coli Nisle* 1917 application in patients with UC worked as effectively as mesalazine therapy alone[37] (Table 1). Moreover, administration of *Saccharomyces boulardii* strain with mesalazine in patients with CD significantly reduced the incidence of remission compared with mesalazine-treated group.[38]

In 2013, Shadnoush et al[47] reported that *Bifidobacterium* and *Lactobacillus*, administered in the form of probiotic yogurt exert anti-inflammatory effects. Two hundred ten adult patients in IBD remission and 95 controls received either probiotic or plain

**TABLE 1.** Effects of Probiotics in Patients with CD and UC

| Disease | Probiotic(s) Strain | Participants and Duration of Study | Dose of Probiotics | Main Findings | References |
|---|---|---|---|---|---|
| CD | *Saccharomyces boulardii* | 32 adults; 6 mo | 1 g daily | Maintenance of remission | 38 |
| | *Lactobacillus rhamnosus* GG | 45 adults; 12 mo | No data | No effect on recurrence | 39 |
| | *L. rhamnosus* GG | 11 adults; 6 mo | $2 \times 10^9$ CFU daily | No effect on moderate to active disease activity | 34 |
| | *L. rhamnosus* GG | 75 children; 2 yr | $1 \times 10^{10}$ CFU twice daily | No effect on recurrence in pediatric patients | 40 |
| | *Lactobacillus acidophilus (johnsonii) La1* | 98 adults; 6 mo | $2 \times 10^9$ CFU daily | No effect on postoperative recurrence | 41 |
| | *Lactobacillus acidophilus (johnsonii) La1* | 70 adults; 12 wk | $1 \times 10^{10}$ CFU daily | No effect on postoperative recurrence | 42 |
| UC | *Escherichia coli Nissle* 1917 | 116 adults; 12 mo | $5 \times 10^{10}$ CFU twice daily (4 capsules) | Maintenance of remission | 32 |
| | *Escherichia coli Nissle* 1917 | 327 adults; 12 mo | 200 mg once daily | Maintenance of remission | 37 |
| | *Enterococci*, *Bifidus*, *Lactobacillus* | 30 adults; 8 wk | 1.26 g daily | Maintenance of remission | 43 |
| | *Bifidobacterium brevis*, *Bifidobacterium bifidum*, *Lactobacillus acidophilus* | 21 adults; 12 mo | 100 mL daily | Maintenance of remission | 44 |
| | *L. rhamnosus* GG | 187 adults; 12 mo | $18 \times 10^9$ CFU daily | Maintenance of remission | 45 |
| | *Bifidobacterium longum* | 18 adults; 4 wk | $2 \times 10^{11}$ CFU daily | Decrease in inflammation | 46 |

CFU, colony-forming unit.

yogurt. The levels of IL-1β, TNF-α, and C-reactive protein in serum were significantly decreased in the group receiving probiotic yogurt after 8 weeks of administration, whereas IL-6 and IL-10 concentrations were significantly increased after the treatment when compared with the placebo group. These results suggest that probiotics may contribute to the maintenance of the homeostasis in GIT and regulate pro- and anti-inflammatory responses of the intestinal immunocytes. Another study that involved 21 patients with UC showed that *Bifidobacteria*-fermented milk administered once per day in a volume of 100 mL for 1 year has a possible preventive effect on recurrence of UC and helps maintaining its remission.[44] Furthermore, Zocco et al investigated the effect of *L. rhamnosus* GG, a strain of *L. rhamnosus* isolated in 1983, on IBD symptoms. In this study, executed on a group of 187 patients with UC, the effect of the administration of *Lactobacillus* GG and *Lactobacillus* GG in combination with mesalamine versus mesalamine alone have been compared. It has been shown that the combined treatment is more effective in prolonging the relapse-free time than the treatment with *Lactobacillus* GG and mesalazine alone.[45]

Most of the presently published trials on probiotics were carried out using a preparation named VSL#3, containing 8 strains, namely *Lactobacillus casei*, *Lactobacillus plantarum*, *L. acidophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium longum*, *Bifidobacterium brevis*, *Bifidobacterium infantis*, and *Streptococcus thermophilus*. It has been found that the administration of VSL#3 greatly increased the secretion of IL-10, IL-1β, and inhibited the production of IL-12.[48] In the in vitro studies, stimulation of human lymphoid and myeloid dendritic cells led to the induction of IL-10 and inhibition of IFN-γ release, and the Th1-type cellular response. It was also found that the use of VSL#3 strengthens the integrity of the intestinal epithelial barrier by increasing the expression of proteins responsible for the formation of tight junctions and a reduction of the number of apoptotic epithelial cells.[49] In children with UC, administration of VSL#3 resulted in the induction and maintenance of remission (92.8%), compared with the placebo group.[50] Moreover, according to a recent study conducted by Shen et al.[51] VSL#3 has beneficial effect on the induction and the maintenance of UC remission in adults. To date, the role of VSL#3 in the treatment of CD has not been investigated.[52,53] However, VSL#3 was found to increase the variety of bacteria species in GIT of patients with IBD.[54]

## PREBIOTICS

Prebiotics are defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in GIT, and thus improve the host's condition.[55] These functional food components include oligosaccharides, which further divide into fructo-oligosaccharides (FOS) (oligofructose and inulin), galacto-oligosaccharides (lactulose), and gluco- and xylo-oligosaccharides. The main features of prebiotics are their resistance to digestive enzymes produced by the human body, while remaining susceptible to colonic microflora fermentation.

Long-chain oligosaccharides (e.g., inulin) and short-chain oligosaccharides (e.g., oligofructose) are neither digested nor absorbed by the upper GIT. However, they undergo fermentation in the colon by anaerobic bacteria, which metabolize these oligosaccharides to SCFAs, such as acetate, propionate, and butyrate, and selectively stimulate the growth of bifidobacteria. This leads to bifidogenic effects and a decrease in intraluminal pH.[56,57] The latter is particularly important for the prevention from diarrhea and inhibition of some strains of potentially pathogenic bacteria, e.g., *Clostridium* sp[56] (Tables 2 and 3).

Gibson et al[69] demonstrated that the administration of FOS in volunteers at a dose of 15 g per day for 15 days significantly increased the number of bifidobacteria in the feces, whereas the population of *Bacteroides*, *Fusobacteria*, and *Clostridium* decreased. The numbers of bacteria of the genus *Lactobacillus* and *E. coli* remained unchanged. In contrast, in a recent, randomized, double-blinded study in 103 patients with CD, the administration of FOS had no statistically significant effect compared with the control group.[66]

Muccioli et al[70] showed that the improvement of the structure and function of the intestinal barrier after the application of prebiotics is associated with a decreased activity of the endocannabinoid system (ECS) in the gut and an increased level of GLP-2, what stimulates synthesis of proteins forming tight junctions. However, the most important mechanism of prebiotic action involves SCFAs. It has been demonstrated that increasing the SCFAs concentration in the intestine (as a result of the consumption of prebiotics) enhances growth of protective bacteria (symbionts), while limiting the growth of pathobionts.[71] SCFAs improve mucosal barrier function, increase intestinal mucus synthesis, stimulate the production of regulatory T cells (Treg) and immunosuppressive cytokines (e.g., IL-10) and reduce the levels of proinflammatory mediators.[71,72] Noteworthy, there is also evidence that acetate, propionate, and butyrate administration alone or as a mixture results in an increased number of Treg cells and increased level of IL-10 within the colonic interstitium.[72] Smith et al[72] suggested that SCFAs, in particular propionate, exert their effects by inhibiting histone deacetylases 6 and 9 in a GPR43-mediated process.

## SYNBIOTICS

A combination of prebiotics and probiotics, named synbiotics, is believed to exert synergistic effects. Namely, synbiotics influence the development of beneficial intestinal microflora through probiotics, whereas prebiotics inhibit the growth of pathogenic bacteria.

Synbiotics help reduce the concentration of undesirable metabolites, including nitrosamines, inactivate carcinogens, and prevent constipation and diarrhea of various etiology.[73,74] For example, studies in rats whose diet included inulin, oligofructose, *L. rhamnosus*, and *Bifidobacterium lactis* showed an increased level of immunoglobulin A in the gut.[75] Because synbiotics are able to reduce cholesterol levels and blood pressure,[76] they are used in the treatment of patients with liver disease.[77] Moreover, most of the synbiotics improve absorption of calcium, magnesium, and phosphorus.[78]

Synbiotics also contribute to the reduction of harmful microflora, such as *Clostridium perfringens* and other endopathogens.[75] In line, administration of a combination of *Lactobacillus paracasei* and FOS led to an increase in *Lactobacillus* and *Bifidobacterium* and a decrease in *Clostridium* and *Enterobacterium*.[79] It has also been shown that the combination of *L. paracasei* and maltodextrin resulted in a decrease in *E. coli* colonization in the jejunum piglets.[79]

Several studies have been undertaken in patients with IBD. In a double-blind randomized controlled trial, Furrie et al[46]

**TABLE 2.** Main Benefits of Prebiotics and Potential Mechanisms of Their Action in Animal Models of IBD

| Animal Model | Prebiotics | Dose of Prebiotics | Effects | References |
|---|---|---|---|---|
| DSS-induced colitis in rats | Inulin | 1% in drinking water, or 400 mg/kg | Reduction of colitis severity | 58 |
| TNBS-induced colitis in rats | Galacto-oligosaccharides | 4 g/kg | No reduction of colitis; modification of gut microflora | 59 |
| DSS-induced colitis in rats | FOS | 63 g/kg | No reduction of colitis | 60 |
| HLA-B27 transgenic rats | Inulin and oligofructose | 5 g/kg | Reduction of colitis; decrease in proinflammatory cytokines | 61 |
| HLA-B27 transgenic rats | Inulin and fructo-oligosaccharides | 8 g/kg | Increase in *Bifidobacterium spp;* Reduction of chronic intestinal inflammation | 62 |
| DSS-induced colitis in rats | Goat's milk oligosaccharides | 20 g/kg | Reduction of colitis; recovery of damaged colonic mucosa | 63 |
| DSS-induced colitis in rats | Lactulose | 300–1000 mg/kg | Reduction of colitis in a dose-dependent manner | 64 |

DSS, dextran sulfate sodium; TNBS, 2,4,6-trinitrobenzenesulfonic acid.

**TABLE 3.** Main Benefits of Prebiotics and Potential Mechanisms of Their Action in Clinical Studies

| Disease | Prebiotics | Participants and Duration of Study | Dose of Prebiotics | Effects | References |
|---|---|---|---|---|---|
| CD | FOS | 10 adults; 3 wk | 15 g/d | Increase in fecal *Bifidobacterium*; decrease in disease activity | 65 |
| | FOS | 103 adults; 4 wk | 15 g/d | No clinical effects | 66 |
| | Lactulose | 14 adults; 4 mo | 10 g/d | No clinical effects | 67 |
| UC | Inulin and FOS | 19 adults; 2 wk | 12 g/d | Reduction of fecal calprotectin | 68 |
| | Inulin and FOS | 18 adults; 4 wk | 6 g/twice daily | Improvement of clinical symptoms | 46 |
| | Lactulose | 14 adults; 4 mo | 10 g/d | No clinical effects | 67 |

indicated that the prebiotic Synergy 1, in combination with *Bifidobacterium longum*, led to an improvement of sigmoidoscopy scores and a decrease in β-defensin, TNF-α, and IL-1α in biopsy samples from patients with UC. The study provides strong preliminary evidence that synbiotic administration may be beneficial in IBD treatment. In another trial,[80] 35 patients with CD were divided into 2 groups, those who received a combination of *Bifidobacterium longum* and a prebiotic Synergy 1 (containing FOS/inulin mix) and a placebo group. A significant histological improvement was observed in the synbiotic group compared with controls (tissue samples for histological evaluation were collected at initiation of the study and after 3 and 6 mo). Although the synbiotic had little effect on mucosal IL-18, IFN-γ, and IL-1β, there was a significant decrease in TNF-α expression after 3 months ($P = 0.041$). Interestingly, the level of TNF-α did not change further after 6 months of the symbiotic treatment. These studies show the potential beneficial effect of synbiotics, but their role in anti-IBD therapy remains to be determined.

## PSYCHOBIOTICS

Frequent coexistence of intestinal disorders, such as irritable bowel syndrome or IBD and mental disorders, especially depression and anxiety,[81] suggests a specific connection between GIT and the central nervous system (CNS), often termed the gut–brain axis.[82] The importance of the microflora in the regulation of GIT function reflects the need to extend this concept to a term microbiota–gut–brain axis (MGBA).[83] MGBA constitutes a bidirectional communication pathway including neural, endocrine, and immune mechanisms. Neuronal mechanisms include the enteric nervous system with several neurotransmitters and neuromodulators, such as serotonin, acetylcholine, and corticotropin-releasing factor (Fig. 1). The latter is particularly noteworthy because of its participation in the increase of the permeability of the intestinal barrier under stress conditions.[84,85] The autonomic nervous system, which is another component of MGBA, consists of sympathetic and parasympathetic branches. Several studies have shown that proinflammatory cytokines may have a direct effect on the CNS through the activation of afferent nerve fibers, which transmit impulses to the specified regions of the brain, e.g., the solitary tract nucleus.[86] In turn, efferent innervation can mediate the inflammatory response, affecting inter alia the α-7 nicotinic receptor in immune cells, thereby reducing cytokine secretion.[87] A crucial role is played here by the vagus nerve, a parasympathetic branch of autonomic nervous system, which constitutes a vital line of communication between the gut microbiota and CNS, as described below.

The endocrine factors regulating MGBA include, among others, cortisol. Its secretion is regulated by the hypothalamic–pituitary–adrenal axis under stress conditions. Cortisol may affect immune cells by modulating the secretion of cytokines, as well as the composition and functions of the microbiota. However, intestinal bacteria have the ability to produce a number of neurohormones, such as serotonin, melatonin, γ-aminobutyric acid (GABA), catecholamines, histamine, acetylcholine, and SCFAs. All of these likely participate in the communication between the gut microbiota and may also exert peripheral and systemic action and affect behavior and brain function.[88]

GABA, which is the main inhibitory neurotransmitter in the CNS, seems to play the most important role in physiological processes within MGBA. Changes in the expression of GABA receptors are associated with the pathogenesis of anxiety and depression that often co-occur with functional GI disorders. Noteworthy, the effect of a prebiotic *L. rhamnosus* (JB-1) on the expression of GABA receptors in the CNS has been demonstrated in the animal model of depression. This modulation occurs through the vagus nerve. In addition, the administration of the probiotic resulted in the reduction of the content of corticosterone and restricted behaviors associated with depression and anxiety, hence the term psychobiotic has been applied. Importantly, neurochemical and behavioral influences of JB-1 were absent in mice after vagotomy, indicating that the vagus nerve is a key element of communication between intestinal microbiota and CNS.[89]

In another study, a mouse model of colitis induced by dextran sulfate sodium has been used to assess the influence of the strain *Bifidobacterium longum* NCC 3001 on animal behavior.[90] Administration of the probiotic decreased anxiety behavior in dextran sulfate sodium–treated mice but did not affect intestinal inflammation or the expression of brain-derived neurotrophic factor mRNA.[91] As in the previous case, the behavioral changes were

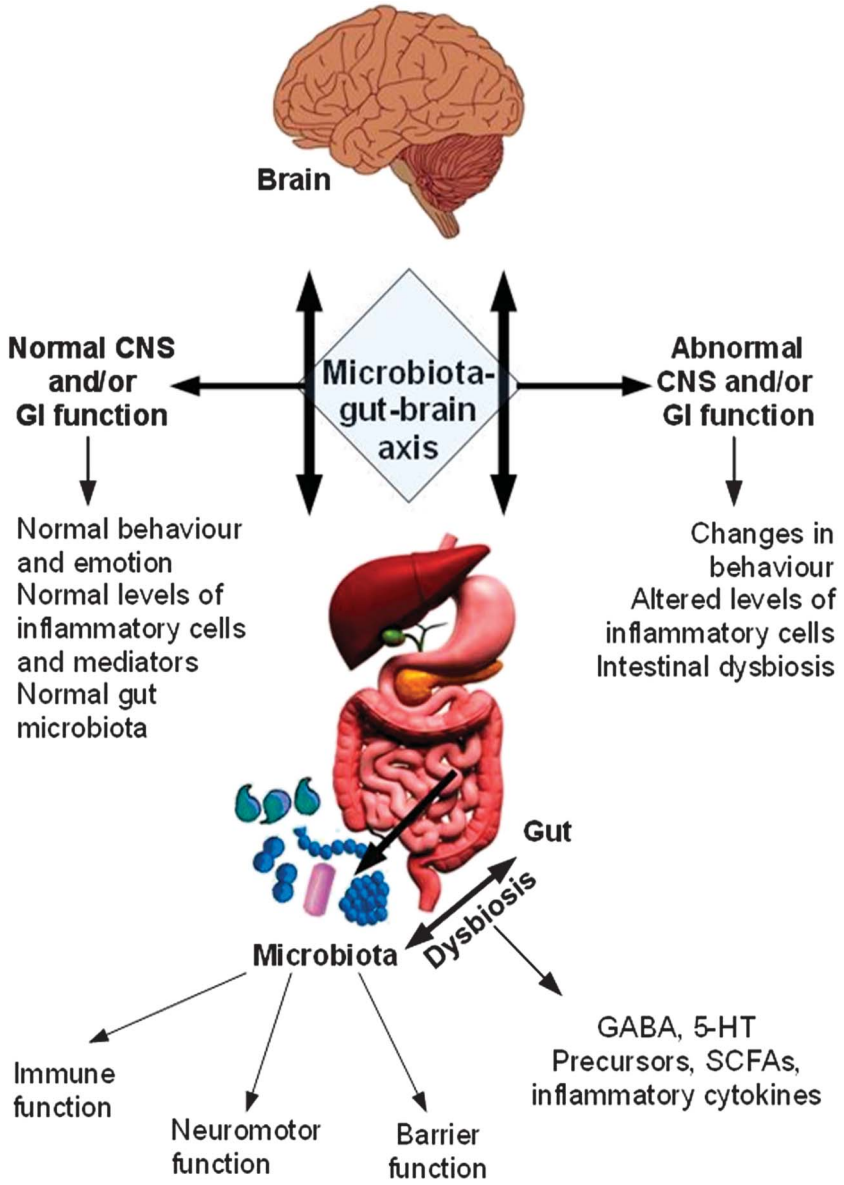


FIGURE 1. Bidirectional communication in the MGBA. Microbiota communicates with the gut–brain axis through a direct interaction with mucosal cells through immune cells and neural endings. Gut microbiota dysbiosis results in the synthesis of several microbial compounds, which gain access to the brain through the bloodstream, leading to incorrect gut–brain axis signaling and associated consequences for CNS functions that result in disease states. 5-HT, 5-hydroxytryptamine.

lost in mice after vagotomy. Thus, the anxiolytic effect of *Bifidobacterium longum* NCC 3001 involves vagal integrity and may involve MGBA but is independent of gut immunomodulation or brain-derived neurotrophic factor production.

Wall et al[92] demonstrated that the administration of *Bifidobacterium* strain to mice affects the fatty acid composition of the brain. Mice receiving *Bifidobacterium* for 8 weeks had a higher concentration of arachidonic acid and docosahexaenoic acid, compared with control group. Arachidonic acid and docosahexaenoic acid are important in brain development and play a role in neurotransmission and protection against oxidative stress.[93,94]

Microbiota and probiotics may exert an effect on MGBA through the immune system.[95,96] A number of studies have shown that intestinal bacteria can reduce the concentration of proinflammatory cytokines, such as TNF-α, IFN-γ, and IL-6 and modulate the concentration of anti-inflammatory cytokines, e.g., IL-10.[97] The proinflammatory cytokines play a key role in the activation of hypothalamic–pituitary–adrenal axis; namely IL-1β, IL-6, and TNF-α increase the permeability of the intestinal barrier and aggravate inflammation, whereas IFN-α, IFN-γ, and TNF-α activate an enzyme of the kynurenic pathway, indoleamine 2,3-dioxygenase, which transfers tryptophan from the serotonin

synthesis cycle to metabolism in the kynurenine pathway, reducing its concentration.[98–100] In addition, cytokines can exert a direct effect on the CNS through a variety of mechanisms, including passing through the permeable regions for some cytokines in the blood–brain barrier or by activating the afferent nerve fibers, e.g., the vagus nerve.[86]

The ECS has also been implicated in the MGBA. ECS consists of the endogenous arachidonate-based lipids, enzymes that synthesize and degrade the endocannabinoids and cannabinoid receptors. At present, it is clear that ECS is involved in maintenance of the gut homeostasis through modulation of GIT motility and anti-inflammatory actions (for review, see Refs. 101–103). Interestingly, it has also been shown that the *L. acidophilus* strain modulates expression of cannabinoid receptors in the spinal cord.[104] This may give a new insight into the anti-inflammatory actions in the GIT mediated by ECS and encourages more studies to fully understand the complex system linking intestinal microbiota, gut, and brain.

## SUMMARY

Numerous studies provide valuable information on the biology and function of the intestinal microbiota and the impact of microbiota changes on IBD. Despite available information, we still do not fully understand the mechanisms by which changes in the gut microbiota affect IBD. Clinical trials in humans are small in numbers, thus it is presently difficult to determine the full value of the administration of probiotics, prebiotics, and synbiotics in patients with CD/UC. However, presently available studies have demonstrated that certain bacterial species exert valuable effects on the GIT in IBD and are helpful especially in the maintenance of remission.

## REFERENCES


1. Savage DC. Microbial ecology of the gastrointestinal tract. *Annu Rev Microbiol.* 1977;31:107–133.
2. Backhed F, Ley RE, Sonnenburg JL, et al. Host-bacterial mutualism in the human intestine. *Science*. 2005;307:1915–1920.
3. de Moreno de LA, Dogi CA, Galdeano CM, et al. Effect of the administration of a fermented milk containing Lactobacillus casei DN-114001 on intestinal microbiota and gut associated immune cells of nursing mice and after weaning until immune maturity. *BMC Immunol.* 2008;9:27.
4. Hooper LV, Wong MH, Thelin A, et al. Molecular analysis of commensal host-microbial relationships in the intestine. *Science*. 2001;291:881–884.
5. Hattori M, Taylor TD. The human intestinal microbiome: a new frontier of human biology. *DNA Res.* 2009;16:1–12.
6. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*. 2006;124:837–848.
7. Tuomola EM, Salminen SJ. Adhesion of some probiotic and dairy Lactobacillus strains to Caco-2 cell cultures. *Int J Food Microbiol.* 1998;41:45–51.
8. O'Toole PW, Cooney JC. Probiotic bacteria influence the composition and function of the intestinal microbiota. *Interdiscip Perspect Infect Dis.* 2008;2008:175285.
9. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci.* 2012;13:701–712.
10. Shanahan F. Crohn's disease. *Lancet*. 2002;359:62–69.
11. Hugot JP, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature*. 2001;411:599–603.
12. Sartor RB. Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. *Gastroenterology*. 2004;126:1620–1633.
13. Chandran P, Satthaporn S, Robins A, et al. Inflammatory bowel disease: dysfunction of GALT and gut bacterial flora (II). *Surgeon*. 2003;1:125–136.
14. Sellon RK, Tonkonogy S, Schultz M, et al. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect Immun.* 1998;66:5224–5231.
15. Scribano ML, Prantera C. Antibiotics and inflammatory bowel diseases. *Dig Dis.* 2013;31:379–384.
16. D'Haens GR, Geboes K, Peeters M, et al. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. *Gastroenterology*. 1998;114:262–267.
17. Greenstein RJ. Is Crohn's disease caused by a mycobacterium? Comparisons with leprosy, tuberculosis, and Johne's disease. *Lancet Infect Dis.* 2003;3:507–514.
18. Romero C, Hamdi A, Valentine JF, et al. Evaluation of surgical tissue from patients with Crohn's disease for the presence of Mycobacterium avium subspecies paratuberculosis DNA by in situ hybridization and nested polymerase chain reaction. *Inflamm Bowel Dis.* 2005;11:116–125.
19. Chiodini RJ. Crohn's disease and the mycobacterioses: a review and comparison of two disease entities. *Clin Microbiol Rev.* 1989;2:90–117.
20. Lakatos PL, Fischer S, Lakatos L, et al. Current concept on the pathogenesis of inflammatory bowel disease-crosstalk between genetic and microbial factors: pathogenic bacteria and altered bacterial sensing or changes in mucosal integrity take "toll"? *World J Gastroenterol.* 2006;12:1829–1841.
21. Hinoda Y, Akashi H, Suwa T, et al. Immunohistochemical detection of MUC2 mucin core protein in ulcerative colitis. *J Clin Lab Anal.* 1998;12:150–153.
22. Buisine MP, Desreumaux P, Leteurtre E, et al. Mucin gene expression in intestinal epithelial cells in Crohn's disease. *Gut*. 2001;49:544–551.
23. Abraham C, Cho JH. Inflammatory bowel disease. *N Engl J Med.* 2009;361:2066–2078.
24. Liu Y, van Kruiningen HJ, West AB, et al. Immunocytochemical evidence of Listeria, Escherichia coli, and Streptococcus antigens in Crohn's disease. *Gastroenterology*. 1995;108:1396–1404.
25. Dixon B. Secrets of the Bulgarian bacillus. *Lancet Infect Dis*. 2002;2:260.
26. Baharav E, Mor F, Halpern M, et al. Lactobacillus GG bacteria ameliorate arthritis in Lewis rats. *J Nutr.* 2004;134:1964–1969.
27. Demirer S, Aydintug S, Aslim B, et al. Effects of probiotics on radiation-induced intestinal injury in rats. *Nutrition*. 2006;22:179–186.
28. Kamil R, Geier MS, Butler RN, et al. Lactobacillus rhamnosus GG exacerbates intestinal ulceration in a model of indomethacin-induced enteropathy. *Dig Dis Sci.* 2007;52:1247–1252.
29. Kim HJ, Camilleri M, McKinzie S, et al. A randomized controlled trial of a probiotic, VSL#3, on gut transit and symptoms in diarrhoea-predominant irritable bowel syndrome. *Aliment Pharmacol Ther.* 2003;17:895–904.
30. Tooley KL, Howarth GS, Lymn KA, et al. Oral ingestion of streptococcus thermophilus diminishes severity of small intestinal mucositis in methotrexate treated rats. *Cancer Biol Ther.* 2006;5:593–600.
31. Gionchetti P, Rizzello F, Venturi A, et al. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. *Gastroenterology*. 2000;119:305–309.
32. Rembacken BJ, Snelling AM, Hawkey PM, et al. Non-pathogenic Escherichia coli versus mesalazine for the treatment of ulcerative colitis: a randomised trial. *Lancet*. 1999;354:635–639.
33. Bibiloni R, Fedorak RN, Tannock GW, et al. VSL#3 probiotic-mixture induces remission in patients with active ulcerative colitis. *Am J Gastroenterol.* 2005;100:1539–1546.
34. Schultz M, Timmer A, Herfarth HH, et al. Lactobacillus GG in inducing and maintaining remission of Crohn's disease. *BMC Gastroenterol.* 2004;4:5.
35. Prantera C, Scribano ML, Falasco G, et al. Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn's disease: a randomised controlled trial with Lactobacillus GG. *Gut*. 2002;51:405–409.
36. Swidsinski A, Ladhoff A, Pernthaler A, et al. Mucosal flora in inflammatory bowel disease. *Gastroenterology*. 2002;122:44–54.

37. Kruis W, Fric P, Pokrotnieks J, et al. Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. *Gut*. 2004;53:1617–1623.
38. Guslandi M, Mezzi G, Sorghi M, et al. Saccharomyces boulardii in maintenance treatment of Crohn's disease. *Dig Dis Sci.* 2000;45: 1462–1464.
39. Prantera C, Scribano ML. Probiotics and Crohn's disease. *Dig Liver Dis.* 2002;34(suppl 2):S66–S67.
40. Bousvaros A, Guandalini S, Baldassano RN, et al. A randomized, double-blind trial of Lactobacillus GG versus placebo in addition to standard maintenance therapy for children with Crohn's disease. *Inflamm Bowel Dis.* 2005;11:833–839.
41. Marteau P, Lemann M, Seksik P, et al. Ineffectiveness of Lactobacillus johnsonii LA1 for prophylaxis of postoperative recurrence in Crohn's disease: a randomised, double blind, placebo controlled GETAID trial. *Gut*. 2006;55:842–847.
42. Van GA, Dewit O, Louis E, et al. Multicenter randomized-controlled clinical trial of probiotics (Lactobacillus johnsonii, LA1) on early endoscopic recurrence of Crohn's disease after lleo-caecal resection. *Inflamm Bowel Dis.* 2007;13:135–142.
43. Cui HH, Chen CL, Wang JD, et al. Effects of probiotic on intestinal mucosa of patients with ulcerative colitis. *World J Gastroenterol.* 2004; 10:1521–1525.
44. Ishikawa H, Akedo I, Umesaki Y, et al. Randomized controlled trial of the effect of bifidobacteria-fermented milk on ulcerative colitis. *J Am Coll Nutr.* 2003;22:56–63.
45. Zocco MA, dal Verme LZ, Cremonini F, et al. Efficacy of Lactobacillus GG in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther.* 2006;23:1567–1574.
46. Furrie E, Macfarlane S, Kennedy A, et al. Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut*. 2005;54:242–249.
47. Shadnoush M, Shaker HR, Mehrabi Y, et al. Probiotic yogurt affects pro- and anti-inflammatory factors in patients with inflammatory bowel disease. *Iran J Pharm Res.* 2013;12:929–936.
48. Lammers KM, Brigidi P, Vitali B, et al. Immunomodulatory effects of probiotic bacteria DNA: IL-1 and IL-10 response in human peripheral blood mononuclear cells. *FEMS Immunol Med Microbiol.* 2003;38:165–172.
49. Mennigen R, Nolte K, Rijcken E, et al. Probiotic mixture VSL#3 protects the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis in a murine model of colitis. *Am J Physiol Gastrointest Liver Physiol.* 2009;296:G1140–G1149.
50. Miele E, Pascarella F, Giannetti E, et al. Effect of a probiotic preparation (VSL#3) on induction and maintenance of remission in children with ulcerative colitis. *Am J Gastroenterol.* 2009;104:437–443.
51. Shen J, Zuo ZX, Mao AP. Effect of probiotics on inducing remission and maintaining therapy in ulcerative colitis, Crohn's disease, and pouchitis: meta-analysis of randomized controlled trials. *Inflamm Bowel Dis.* 2014; 20:21–35.
52. Dieleman L, Hoentjen F. Probiotics have a limited role in the treatment of inflammatory bowel disease [in Dutch]. *Ned Tijdschr Geneeskd.* 2012; 156:A5206.
53. Ruemmele FM, Veres G, Kolho KL, et al. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. *J Crohns Colitis.* 2014;8:1179–1207.
54. Kuhbacher T, Ott SJ, Helwig U, et al. Bacterial and fungal microbiota in relation to probiotic therapy (VSL#3) in pouchitis. *Gut*. 2006;55:833–841.
55. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr.* 1995;125: 1401–1412.
56. Cummings JH, Macfarlane GT. Gastrointestinal effects of prebiotics. *Br J Nutr.* 2002;87(suppl 2):S145–S151.
57. Roberfroid MB, Delzenne NM. Dietary fructans. *Annu Rev Nutr.* 1998; 18:117–143.
58. Videla S, Vilaseca J, Antolin M, et al. Dietary inulin improves distal colitis induced by dextran sodium sulfate in the rat. *Am J Gastroenterol.* 2001;96:1486–1493.
59. Holma R, Juvonen P, Asmawi MZ, et al. Galacto-oligosaccharides stimulate the growth of bifidobacteria but fail to attenuate inflammation in experimental colitis in rats. *Scand J Gastroenterol.* 2002;37:1042–1047.
60. Moreau NM, Martin LJ, Toquet CS, et al. Restoration of the integrity of rat caeco-colonic mucosa by resistant starch, but not by fructo-oligosaccharides, in dextran sulfate sodium-induced experimental colitis. *Br J Nutr.* 2003;90:75–85.
61. Hoentjen F, Welling GW, Harmsen HJ, et al. Reduction of colitis by prebiotics in HLA-B27 transgenic rats is associated with microflora changes and immunomodulation. *Inflamm Bowel Dis.* 2005;11:977–985.
62. Koleva PT, Valcheva RS, Sun X, et al. Inulin and fructo-oligosaccharides have divergent effects on colitis and commensal microbiota in HLA-B27 transgenic rats. *Br J Nutr.* 2012;108:1633–1643.
63. Lara-Villoslada F, Debras E, Nieto A, et al. Oligosaccharides isolated from goat milk reduce intestinal inflammation in a rat model of dextran sodium sulfate-induced colitis. *Clin Nutr.* 2006;25:477–488.
64. Rumi G, Tsubouchi R, Okayama M, et al. Protective effect of lactulose on dextran sulfate sodium-induced colonic inflammation in rats. *Dig Dis Sci.* 2004;49:1466–1472.
65. Lindsay JO, Whelan K, Stagg AJ, et al. Clinical, microbiological, and immunological effects of fructo-oligosaccharide in patients with Crohn's disease. *Gut*. 2006;55:348–355.
66. Benjamin JL, Hedin CR, Koutsoumpas A, et al. Randomised, double-blind, placebo-controlled trial of fructo-oligosaccharides in active Crohn's disease. *Gut*. 2011;60:923–929.
67. Hafer A, Kramer S, Duncker S, et al. Effect of oral lactulose on clinical and immunohistochemical parameters in patients with inflammatory bowel disease: a pilot study. *BMC Gastroenterol.* 2007;7:36.
68. Casellas F, Borruel N, Torrejon A, et al. Oral oligofructose-enriched inulin supplementation in acute ulcerative colitis is well tolerated and associated with lowered faecal calprotectin. *Aliment Pharmacol Ther.* 2007;25:1061–1067.
69. Gibson GR, Beatty ER, Wang X, et al. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology*. 1995;108:975–982.
70. Muccioli GG, Naslain D, Backhed F, et al. The endocannabinoid system links gut microbiota to adipogenesis. *Mol Syst Biol.* 2010;6:392.
71. Looijer-van Langen MA, Dieleman LA. Prebiotics in chronic intestinal inflammation. *Inflamm Bowel Dis.* 2009;15:454–462.
72. Smith PM, Howitt MR, Panikov N, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science*. 2013;341:569–573.
73. Bengmark S. Bioecologic control of the gastrointestinal tract: the role of flora and supplemented probiotics and synbiotics. *Gastroenterol Clin North Am.* 2005;34:413–436, viii.
74. Bengmark S, Martindale R. Prebiotics and synbiotics in clinical medicine. *Nutr Clin Pract.* 2005;20:244–261.
75. Arai S, Morinaga Y, Yoshikawa T, et al. Recent trends in functional food science and the industry in Japan. *Biosci Biotechnol Biochem.* 2002;66: 2017–2029.
76. Ooi LG, Ahmad R, Yuen KH, et al. Lactobacillus gasseri [corrected] CHO-220 and inulin reduced plasma total cholesterol and low-density lipoprotein cholesterol via alteration of lipid transporters. *J Dairy Sci.* 2010;93:5048–5058.
77. Malaguarnera G, Giordano M, Nunnari G, et al. Gut microbiota in alcoholic liver disease: pathogenetic role and therapeutic perspectives. *World J Gastroenterol.* 2014;20:16639–16648.
78. Rodrigues FC, Castro AS, Rodrigues VC, et al. Yacon flour and Bifidobacterium longum modulate bone health in rats. *J Med Food.* 2012;15:664–670.
79. Bomba A, Nemcova R, Gancarcikova S, et al. Improvement of the probiotic effect of micro-organisms by their combination with maltodextrins, fructo-oligosaccharides and polyunsaturated fatty acids. *Br J Nutr.* 2002;88(suppl 1):S95–S99.
80. Steed H, Macfarlane GT, Blackett KL, et al. Clinical trial: the microbiological and immunological effects of synbiotic consumption—a randomized double-blind placebo-controlled study in active Crohn's disease. *Aliment Pharmacol Ther.* 2010;32:872–883.
81. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*. 2010;464:59–65.
82. Fichna J, Storr MA. Brain-gut interactions in IBS. *Front Pharmacol.* 2012;3:127.
83. Rhee SH, Pothoulakis C, Mayer EA. Principles and clinical implications of the brain-gut-enteric microbiota axis. *Nat Rev Gastroenterol Hepatol.* 2009;6:306–314.

84. Gareau MG, Jury J, MacQueen G, et al. Probiotic treatment of rat pups normalises corticosterone release and ameliorates colonic dysfunction induced by maternal separation. *Gut*. 2007;56:1522–1528.
85. Collins SM, Surette M, Bercik P. The interplay between the intestinal microbiota and the brain. *Nat Rev Microbiol.* 2012;10:735–742.
86. Irwin MR, Miller AH. Depressive disorders and immunity: 20 years of progress and discovery. *Brain Behav Immun.* 2007;21:374–383.
87. Pavlov VA, Tracey KJ. Neural regulators of innate immune responses and inflammation. *Cell Mol Life Sci.* 2004;61:2322–2331.
88. Iyer LM, Aravind L, Coon SL, et al. Evolution of cell-cell signaling in animals: did late horizontal gene transfer from bacteria have a role? *Trends Genet.* 2004;20:292–299.
89. Bravo JA, Forsythe P, Chew MV, et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci U S A.* 2011;108: 16050–16055.
90. Bercik P, Denou E, Collins J, et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology*. 2011;141:599–609.
91. Duman RS. Role of neurotrophic factors in the etiology and treatment of mood disorders. *Neuromolecular Med.* 2004;5:11–25.
92. Wall R, Marques TM, O'Sullivan O, et al. Contrasting effects of Bifidobacterium breve NCIMB 702258 and Bifidobacterium breve DPC 6330 on the composition of murine brain fatty acids and gut microbiota. *Am J Clin Nutr.* 2012;95:1278–1287.
93. Innis SM. Dietary (n-3) fatty acids and brain development. *J Nutr.* 2007; 137:855–859.
94. Maekawa M, Takashima N, Matsumata M, et al. Arachidonic acid drives postnatal neurogenesis and elicits a beneficial effect on prepulse inhibition, a biological trait of psychiatric illnesses. *PLoS One*. 2009;4:e5085.
95. Forsythe P, Bienenstock J. Immunomodulation by commensal and probiotic bacteria. *Immunol Invest.* 2010;39:429–448.
96. Duerkop BA, Vaishnava S, Hooper LV. Immune responses to the microbiota at the intestinal mucosal surface. *Immunity*. 2009;31:368–376.
97. Desbonnet L, Garrett L, Clarke G, et al. The probiotic Bifidobacteria infantis: an assessment of potential antidepressant properties in the rat. *J Psychiatr Res.* 2008;43:164–174.
98. Wichers MC, Maes M. The role of indoleamine 2,3-dioxygenase (IDO) in the pathophysiology of interferon-alpha-induced depression. *J Psychiatry Neurosci.* 2004;29:11–17.
99. Maes M, Leonard BE, Myint AM, et al. The new "5-HT" hypothesis of depression: cell-mediated immune activation induces indoleamine 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression. *Prog Neuropsychopharmacol Biol Psychiatry.* 2011;35:702–721.
100. Myint AM, Kim YK, Verkerk R, et al. Kynurenine pathway in major depression: evidence of impaired neuroprotection. *J Affect Disord.* 2007; 98:143–151.
101. Salaga M, Sobczak M, Fichna J. Inhibition of proteases as a novel therapeutic strategy in the treatment of metabolic, inflammatory and functional diseases of the gastrointestinal tract. *Drug Discov Today.* 2013;18: 708–715.
102. Salaga M, Mokrowiecka A, Zakrzewski PK, et al. Experimental colitis in mice is attenuated by changes in the levels of endocannabinoid metabolites induced by selective inhibition of fatty acid amide hydrolase (FAAH). *J Crohns Colitis.* 2014;8:998–1009.
103. Fichna J, Salaga M, Stuart J, et al. Selective inhibition of FAAH produces antidiarrheal and antinociceptive effect mediated by endocannabinoids and cannabinoid-like fatty acid amides. *Neurogastroenterol Motil.* 2014;26:470–481.
104. Rousseaux C, Thuru X, Gelot A, et al. Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. *Nat Med.* 2007;13:35–37.