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Supplement: Guidance for Assessing Probiotics Beneficial Effects—How to Fill the GAP

Guidance for Substantiating the Evidence for Beneficial Effects of Probiotics: Probiotics in Chronic Inflammatory Bowel Disease and the Functional Disorder Irritable Bowel Syndrome[1–3]

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Abstract

Ulcerative colitis and Crohn's disease, the 2 distinct idiopathic pathologies of inflammatory bowel diseases, are spontaneously relapsing, immunologically mediated disorders of the gastrointestinal tract. Selected probiotics strains have been proven to be clinically effective in maintaining remission in patients with ulcerative colitis. None of the probiotics thus far tested has been shown to be effective in induction of remission or in maintenance of remission in patients with Crohn's disease. The multispecies probiotics mixture of 8 strains seems effective in the maintenance of remission in pouchitis. Irritable bowel syndrome is a functional bowel disorder manifested by chronic, recurring abdominal pain or discomfort associated with disturbed bowel habit in the absence of structural abnormalities likely to account for these symptoms. Recently conducted appropriately powered studies with different (combinations of) probiotics show positive results on reduction of symptoms, although a considerable placebo effect is also found. Mechanistic studies aimed at pathophysiological mechanisms of inflammatory bowel diseases can identify new targets for probiotic bacteria. J. Nutr. doi: 10.3945/jn.109.113746.

Probiotic efficacy in inflammatory bowel disease clinical trials

A characteristic feature of the mucosal immune system in the normal host is that protective cell-mediated and humoral immune responses against enteropathogenic organisms are allowed to proceed, whereas defense mechanisms against commensal microorganisms of fermented food or the enteric microbiota are prevented. Environment-dependent disturbances of this tightly regulated intestinal balance contribute to the development of inflammatory bowel diseases (IBD)[10] in genetically susceptible hosts. It has become clear from numerous studies with IBD patients and animal models of experimental colitis that enteric bacteria are a critical component in the development and prevention/treatment of chronic intestinal inflammation of the genetically susceptible host. Thus, the genetic predisposition to deregulated mucosal immune responses and the concurrent prevalence of certain environmental triggers in developed countries are strong etiologic factors for the

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[3] Supplemental Tables 1–13 are available as Online Supporting Material with the online posting of this paper at jn.nutrition.org.

[10] Abbreviations used: CFU, colony-forming units; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; UC, ulcerative colitis.

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development of IBD (1–3). Ulcerative colitis (UC) and Crohn's disease, the 2 distinct idiopathic pathologies of IBD, are spontaneously relapsing, immunologically mediated disorders of the gastrointestinal tract. Polymorphisms associated with Crohn's disease have been reported for several genes including *NOD2/CARD15* (4,5) and *NOD1/CARD4* (6), *TLR4* (7) and *TLR9* (8), *SLC22A4* and *SLC22* (9,10), *ABCB1* (11,12), *ATG16L1* (13), *DLG5* (14), *TNFSF15* (15), and *IL23R* (16). On the other hand, and of considerable importance to understand the pathologic mechanisms of chronic intestinal inflammation, the low concordance rate in identical twins for Crohn's disease (~50%) and UC (~10%) confirm epidemiologic observations that environmental factors strongly contribute to disease progression (17). Both disorders affect people in approximately equal female/male proportion with a combined mean frequency of 5–200 cases per 100,000 European and North American inhabitants (18). The incidence of Crohn's disease is still increasing in Western societies, demonstrating the importance of mechanistic insights into the yet unknown etiology of disease pathogenesis.

An emerging therapeutic approach with rare adverse effects is the administration of specific bacterial strains that were shown to exert protective regulatory activities, so-called probiotics. This review is meant to provide new insights about the experimental and clinical relevance of probiotic activity in the context of IBD.

Probiotic targets: what we learn from IBD animal models

The hypothesis that certain commensal bacteria from the indigenous microbiota accelerate and aggravate the disease pathologies of IBD is supported by clinical observations and studies in gnotobiotic animal models of experimental colitis (19,20). In at least 11 separate animal models with induced, spontaneous or genetically engineered disease, chronic intestinal inflammation is initiated and perpetuated in the presence of resident enteric bacteria, whereas germ-free (sterile) conditions prevent or dramatically attenuate the development of disease. The proposed mechanisms in the genetically susceptible host that lead toward aggressive cellular immune responses in response to commensal bacteria and the development of experimental colitis in mutant animals include loss of epithelial cell barrier function, overexpression of proinflammatory mediators in different effector T lymphocyte subsets (Th1 and Th17, Th2), deficient protective and regulatory signals, abnormal antigen presentation, and aberrant thymic education (19,20). Additional clinical studies suggested that luminal enteric bacteria penetrate the intestinal mucus layer and associate with the intestinal epithelium under conditions of chronic inflammation (21,22). Consistent with the observation that IBD patients fail to maintain immunologic tolerance and lack appropriate regulatory mechanisms to inhibit chronic immune activation toward bacterial antigens (23,24), the barrier function of the intestinal epithelium is abrogated under conditions of chronic intestinal inflammation (25–27). Considering the mechanistic approaches in the various IBD animal models and human studies, functional targets for probiotic activity include epithelial cell survival and cellular restitution targeting the intestinal barrier function as well as the modulation of innate and adaptive immune effector mechanisms (**Table 1 Supplemental Tables 1–13**) (28–80).

Crohn's Disease. Six studies are reported in patients with Crohn's disease during the years 1998–2008 (Table 1). In 2 studies, the patients had been brought into remission by pretreatment with steroids; in the other studies, the treatment was begun immediately or within 3 wk after intestinal surgery for Crohn's disease aiming to prevent postoperative recurrence of the disease. The probiotic microorganisms selected for these clinical trials included *Saccharomyces boulardii* (28), *Lactobacillus johnsonii* LA1 (2 studies) (32,33), and *Lactobacillus rhamnosus* GG (3 studies) (29–31). The dose of *Saccharomyces boulardii* was 1 g/d; the treatment with lactobacillus strains ranged between 10^9 to 10^{10} colony-forming units (CFU) twice daily. Clinical markers for the effectiveness of treatment were the Crohn's disease activity index, sustained remission, clinical and endoscopic signs of relapse, and size and severity of recurrent lesions. All studies reported no effects, and 1 study also mentioned poor compliance by patients. In addition, a recently published meta-analysis on 8 clinical trials with Crohn's disease patients confirmed the absence of probiotic efficacy (81).

Ulcerative colitis. Ten studies are reported in patients with UC (Table 1). In total, 861 patients were enrolled in the studies. The size of the patient groups varied between 18 and 327 patients. The study period also showed a great variance: 4 wk (1 study) (41), 6 wk (1 study) (42), 8 wk (2 studies) (38,40), 12 wk (1 study) (39), and 12 mo (5 studies) (34–37,43). Primary markers such as efficacy to attain remission, mean duration of remission, relapse of active disease, endoscopic and histological evaluation, clinical activity scores, disease activity scores, and endoscopic and histological scores were used. Most of the patients were pretreated with gentamycin, sulfasalazine, mesalamine, and/or corticosteroids before the start of the study. Comparison was made to patients treated with mesalazine in 3 studies, treatment with balzalazide in 1 study, or untreated controls. The 2 largest studies (116 and 327 patients) (34,37) were made with a nonpathogenic *E. coli* strain Nissle 1917 and demonstrated that the probiotic intervention was equivalent to the gold standard mesalazine in maintaining remission in patients with UC. The second largest study (187 patients) (43) used *Lactobacillus rhamnosus* GG and showed no effect, suggesting strain-specific effects for the probiotic intervention. Additional smaller studies with a preparation dominated by bifidobacteria and a commercial mixture of 8 strains (*Lactobacillus acidophilus*, *L. bulgaricus*, *L. casei*, *L. plantarum*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *B. infantis*, *B. longum*) (35–42) revealed efficacy in the maintenance therapy of UC patients. However, these results need to be replicated with larger study groups.

Pouchitis. Six studies have been reported in patients with pouchitis during the last 10 y. A total of 174 patients were studied. The study period varied between 4 wk and 12 mo, and all studies were limited to small group sizes (15–40). One study used *L. rhamnosus* GG (46); the other 5 studies used the commercial mixture of 8 strains (44,45,47–49). The comparison was made to placebo-treated control groups receiving maize starch (4 studies) or microcrystalline cellulose. The clinical efficacy of the probiotic intervention was evaluated based on clinical, endoscopic, histological, and microbiological criteria including the pouchitis disease activity index. Five studies demonstrated clinical efficacy of high doses (9×10^{11} to 3.6×10^{12} CFU/d) of the mixture of 8 different lactic acid bacteria. In contrast, the study with *Lactobacillus rhamnosus* GG observed alterations in intestinal microbiota but no other effects on clinical parameters (46).

Conclusions for the efficacy of probiotic action in clinical trials

There are 2 main probiotics that have been proven to be clinically effective in IBD, the commercial mixture of 8 strains and *E. coli* strain Nissle 1917 (*E. coli* Nissle). Two randomized

TABLE 1 Probiotics in IBD and IBS

Disease/marker and reference	Participants	Type of probiotic(s)	Duration	Type of study[1]	Outcome
Crohn's disease					
Guslandi et al. 2000 (28)	32 adults	*S. boulardii*	6 mo	R	Maintenance of remission (relapse in 6.25% of the probiotic group vs. 37.5% in the mesalamine reference group)
Prantera and Scribano 2002 (29)	55 adults	*L. rhamnosus* GG	12 mo	R, PC	No effect on postoperative recurrence
Schultz et al. 2004 (30)	11 adults	*L. rhamnosus* GG	6 mo	R, PC	No effect on moderate to active disease activity
Bousvaros et al. 2005 (31)	75 adults	*L. rhamnosus GG*	2 y	R, PC	No effect on the prevention of relapse in pediatric patients
Marteau et al. 2006 (32)	98 adults	L. johnsonii La1	6 mo	R, PC	No effect on postoperative recurrence
Van Gossum et al. 2007 (33)	70 adults	L. johnsonii,	1 y	R, PC	No effect on postoperative recurrence
UC					
Rembacken et al. 1999 (34)	116 adults	*E. coli* Nissle 1917	12 mo	R, PC	Maintenance of remission
Venturi et al. 1999 (35)	20 adults	Mixture of 8 strains, Open label	12 mo		Maintenance of remission
Ishikawa et al. 2003 (36)	21 adults	*B. breve, B. bifidum, L. acidophilus* (BFM),	12 mo	R	Maintenance of remission
Kruis et al. 2004 (37)	327 adults	*E. coli* Nissle 1917	12 mo	R	Maintenance of remission
Cui et al. 2004 (38)	30 adults	Mixture of 3 strains *(Enterococci, Bifidus, Lactobacillus*	8 wk	R, PC	Maintenance of remission
Kato et al. 2004 (39)	20 adults	*B. breve, B. bifidum, L. acidophilus*	12 wk	R, PC	Clinical and endoscopic activity indices improved
Tursi et al. 2004 (40)	90 adults	Mixture of 8 strains (+balsalazide)	8 wk	R	Maintenance of remission
Furrie et al. 2005 (41)	18 adults	*B. longum*	4 wk	R, PC	Resolution of inflammation in active disease
Bibiloni et al. 2005 (42)	32 adults	Mixture of 8 strains, Open label	6 wk	R	Induction of remission
Zocco et al. 2006 (43)	187 adults	*L. rhamnosus* GG	12 mo	R	Maintenance of remission
Pouchitis					
Gionchetti et al. 2000 (44)	40 adults	Mixture of 8 strains	9 mo	R, PC	Maintenance of remission
Gionchetti et al. 2003 (45)	40 adults	Mixture of 8 strains	12 mo	R, PC	Prevention of acute disease after pouch-anal anastomosis
Kuisma et al. 2003 (46)	20 adults	*L. rhamnosus* GG	3 mo	R, PC	No effect
Mimura et al. 2004 (47)	36 adults	Mixture of 8 strains	12 mo	R, PC	Maintenance of remission in recurrent or refractory disease
Kuhbacher et al. 2006 (48)	15 adults	Mixture of 8 strains	2 mo	R, PC	Maintenance of remission
Gionchetti et al. 2007 (49)	23 adults	Mixture of 8 strains	4 wk	R, PC	Treatment of acute disease
IBS					
O'Sullivan and O'Morain 2000 (50)	24 adults	*L. casei* GG	4 wk	R, DB, PC	No effect of probiotic on global symptoms but a trend for improvement in the diarrhea subgroup
Nobaek et al. 2000 (51)	60 adults	*L. plantarum* DSM 9843	4 wk	R, DB, PC	Significant decrease in pain score and flatulence at study end and at follow-up
Niedzielin et al. 2001 (52)	40 adults	*L. plantarum* 299V	8 wk	R, DB, PC	*LP299v* significant reduction in pain in all patients, and tendency to improvement in constipation and bloating
Sen et al. 2002 (53)	60 adults	*L. plantarum* 299V	4 wk	R, DB, PC	No effect on symptom score and bloating but reduction in breath hydrogen excretion
Kim et al. 2003 (54)	25 adults	Mixture of 8 strains	4 wk	R, DB, PC	VSL significant reduction in bloating but no other symptoms; colon transit was retarded
Saggioro 2004 (55)	70 adults	*L. plantarum* LP01/ *B. breve* BR03 *or L. acid* LA02	4wk	R, DB, PC	Significant reduction of pain and global symptom severity score with both preparations
Tsuchiya et al. 2004 (56)	25 adults	SCM-III (unknown mixture)	8 wk	R, DB, PC	At 6 wk, significant improvement in pain and bloating
Niv et al. 2005 (57)	54 adults	*L. reuteri* ATCC 55730	4 wk	R, DB, PC	No effect of probiotic on IBS symptoms but a high placebo response
Bittner et al. 2005 (58)	25 adults	Prescript-Assist (*L. acid, L. helveticus)*, Mixture of 3 strains *(Enterococci, Bifidus, Lactobacillus*)	2 wk	R, DB, PC	Probiotic significantly reduced general ill feelings/nausea and indigestion/flatulence

(*Continued*)

TABLE 1 Continued

Disease/marker and reference	Participants	Type of probiotic(s)	Duration	Type of study[1]	Outcome
Kajander and Korpela 2006 (59)	103 adults	Mixture of: *L. rhamnosus* GG, *L. rhamnosus* LC 705, *P. freudenreichii shermanii* JS, *B. breve* Bb99	6 mo	R, DB, PC	Significant reduction in global symptom score and for bloating
O'Mahony et al. 2005 (60)	70 adults	*L. salivarus* UCC4331 or *B. infantis* 35624	8 wk	R, DB, PC	*B. infantis* reduced global symptom score, while *L. salivarus* reduced abdominal pain/ discomfort, bloating, and straining
Bausserman and Michail 2005 (61)	64 children	*L. rhamnosus* GG	6 wk	R, DB, PC	*L. rhamnosus* GG was not superior to treat abdominal pain but may help relieve bloating
Kim et al. 2005 (62)	48 adults	Mixture of 8 strains	4 wk (n+31) 8 wk (n+17)	R, DB, PC	Mixture of 8 strains significantly reduced bloating but no other symptoms; colon transit was retarded
Whorwell et al. 2006 (63)	363 adults	*B. infantis* 35624 (3 doses)	4 wk	R, DB, PC	With 10^8 CFU, improvement of IBS symptoms of abdominal pain, bloating, bowel dysfunction, incomplete evacuation, straining, passage of gas
Colecchia et al. 2006 (64)	636 adults	*B. longum* W11	$>$ 36 d	No control	Significant increase in stool frequency in IBS-constipation and reduction in pain and bloating
Fan et al. 2006 (65)	74 adults	3 strains *(Enterococci, Bifidus, Lactobacillus*	4 wk	No control	Significant improvement in global score and in abdominal pain/stool characteristics at wk 2
Fanigliulo et al. 2006 (66)	70 adults	Rifaximin with/without *B. longum* W11	2 mo	No control	Supplementation of the antibiotic therapy with a probiotic significantly reduced symptoms
Bittner et al. 2007 (67)	22 adults	Prescript-Assist (*L. acid, L. helveticus*, mixture of 3 species)	1 y	R, DB, PC	Significant reduction in remission at 1-y follow-up
Guyonnet et al. 2007 (68)	267 adults	*B. anim* DN-173 010, plus *S. thermophilus* and *L. Bulgaricus*	6 wk	R, DB, PC	Significant improvement of quality of life, bloating, and on stool frequency in constipated subjects
Gawronska et al. 2007 (69)	103 children	*L. rhamnosus* GG	4 wk	R, DB, PC	Moderate treatment for IBS, not for functional pain or for functional dyspepsia
Dughera et al. 2007 (70)	129 adults	*B. longum* W11	3 mo	No control	Significant improvement in symptoms and increase in stool frequency
Andriulli et al. 2008 (71)	267 adults	*L. paracasei* B21060 against a prebiotic	12 wk	No control	Only in IBS-predominant diarrhea, significantly reduced bowel movements, pain, and IBS scores
Enck et al. 2008 (72)	297 adults	*E. coli* DSM 17252 and *Enterococcus faecalis* DSM 16440 mixture	8 wk	R, DB, PC	Significant improvement in global symptom score and in pain score
Enck et al. 2009 (73)	298 adults	*E. coli* DSM 17252	8 wk	R, DB, PC	Significant improvement in global symptom score and in pain score
Drouault-Holowacz et al. 2008 (74)	116 adults	*B. longum* LA101, *L. acid* LA102, *L. lactis* LA103, *S. thermophilus* LA 104 mixture	4 wk	R, DB, PC	Not superior for overall symptom score but improved pain at wk 1 and 4
Williams et al. 2008 (75)	52 adults	LAB4 (mix): *L. acidphilus* CUL60 CUL21, *B. lactis* CUL34, *B. bifidus* CUL20	8 wk	R, DB, PC	Significant improvement in the symptom severity score and in scores for quality of life, days with pain, and satisfaction with bowel habit
Agrawal et al. 2008 (76)	34 adults	*B. lactis* DN-173–010	4 wk	R, DB, PC	Improvements in objectively measured abdominal girth and gastrointestinal transit, as well as reduced symptoms
Zeng et al. 2008 (77)	30 adults	*Strep. thermophilus, L. bulg, L. acid B. longum* mixture	4 wk	R, DB, PC	Significant decrease of the mean global IBS scores
Sinn et al. 2008 (78)	40 adults	*L. acidophilus* SDC 2012, 2013	4 wk	R, DB, PC	Significant difference in the proportion of responders between the probiotics and placebo
Barrett et al. 2008 (79)	18 adults	*L. casei* Shirota	6 wk	R, DB, PC	Moderate treatment for IBS, not for functional pain or for functional dyspepsia
Kajander et al. 2008 (80)	86 adults	Mixture of *L. rhamnosus* GG (ATCC 53103), *L. rhamnosus* Lc705 (DSM 7061), *P. freudenreichii shermanii* JS (DSM 7067), *B animalis* Bb 12 (DSM 15954)	5 mo	R, DB, PC	Significant decrease in overall bowel symptoms, as well as in abdominal pain and bloating, Stabilization in colonic microbiota

[1] Abbreviations: R, randomized; DB, double blind; PC, placebo controlled.

studies with an adequate number of study participants reported *E. coli* Nissle 1917 to be equivalent to mesalazine standard treatment of patients with UC in maintaining remission (34,37). Unfortunately, *E. coli* Nissle was not included in studies to treat Crohn's disease and pouchitis patients. The commercial mixture of 8 strains has been shown to be very effective in the maintenance of remission in pouchitis (44,45,47–49). Furthermore, the first small and open-label studies suggest that mixture to be effective in the maintenance therapy of UC patients (35,42). In addition, the commercial mixture of 8 strains in combination with a low dose of balsalazide was more effective than mesalazine or balsalazide treatment alone in the induction of remission in mild to moderate UC (40). However, these results require replication in higher-powered studies. The commercial mixture of 8 strains was also not included in the treatment of Crohn's disease patients. In addition, this mixture is not a single probiotic bacterial strain but a mixture of 8 different bacterial strains. None of the clinical studies addressed the question which of the 8 single bacterial strains is the effective one or whether the complex mixture is necessary for the reported probiotic effects. Some other probiotic compounds such as bifidobacteria-fermented milk, *L. rhamnosus* GG, and the yeast *S. boulardii* were rarely shown to be effective in the context of IBD. Crohn's disease appears for the time being to be totally resistant to probiotic therapy. The reason underlying the lack of success of probiotic treatment in Crohn's disease is unknown, raising the question whether this is related to disease or intestinal segment-specific mechanisms, for example, the discontinuous nature of the disease including ileal involvement.

Probiotics in irritable bowel syndrome

Irritable bowel syndrome (IBS) is a functional bowel disorder manifested by chronic, recurring abdominal pain or discomfort associated with disturbed bowel habit in the absence of structural abnormalities likely to account for these symptoms (82). The symptomatic array may include abdominal pain, discomfort, distension, cramping, distress, bloating, excess flatulence, and variable changes in frequency and form of stools. Such symptomatic episodes may be experienced by almost every individual, and to separate IBS from transient gut symptoms, experts have underscored the chronic and relapsing nature of IBS and have proposed diagnostic criteria based on the recurrence rate of such symptoms (83). IBS is 1 of the most common intestinal disorders in both industrialized and developing countries, and it is known to generate significant health care costs (82).

Pathogenesis

A precise etiology for IBS is not recognized. However, it has been shown that IBS patients have abnormal reflexes and perception in response to gut stimuli, and the individual symptoms may depend on the specific neural pathways affected. Several pathogenetic factors may play a role in these disturbances, including the following: genetic, early and environmental conditioning; cognitive /emotional adaptation; altered response to stress; inflammatory/postinfectious processes in the gut mucosa; and abnormalities in the composition of the gut microbiota (84).

This pathophysiological model explains the heterogeneity of IBS. Hypothetically, probiotics may correct or counteract some of these underlying disturbances because specific probiotic strains may modulate gut transit, visceral hypersensitivity, intestinal gas content, and inflammatory responses.

The recent interest in the role of inflammatory processes for the development of IBS revealed a possible link between low-grade inflammation and functional disturbances in the gut including constipation and/or diarrhea. In light of the circumstance that treatment of this condition is lacking in effective drugs, the success of probiotics in IBD has fostered the application of these compounds in IBS.

Outcomes from nutritional intervention trials with probiotics in IBS

Between 1989 and 2008, 5 uncontrolled (64–67,70,71) and 26 controlled studies (Table 1) have been reported in the literature with a total of 3,570 patients included. Case reports and clinical observations reported before 1989 were discarded because of a lack of adequate definition of the IBS study population. Since 1989, the Rome consensus defining IBS and other functional disorders has changed the clinical management of IBS patients substantially.

Although with few uncontrolled trials, the altogether positive picture may raise doubts, the large number of controlled trials confirms the interest of probiotics in alleviating IBS symptoms (Table 1).

As is shown in Table 1, an increasing number of patients have been included in these studies to account for a high variability of symptom severity, diffuse symptom changes, and a rather high placebo response with sample sizes up to 300. However, the appropriately powered studies (63,68,71–73) do confirm the overall positive results in 15 of the 19 studies. Two small-scale studies in children with functional bowel disorders report partial efficacy in IBS (69) or no effects (61), but further studies are needed.

These studies have been reviewed several times (85–88) and were subject to a recent meta-analysis (89) that concluded an overall improvement of global IBS symptoms [pooled relative risk 0.77 (0.62 to 0.94)] and reduced abdominal pain [relative risk 0.78 (0.69 to 0.88)] but without effects on specific IBS symptoms (diarrhea, constipation, and/or bloating).

Although more and more probiotic compounds have become available on the market or are seeking approval, their specific mechanisms of action in various clinical conditions remain obscure. Based on the reviewed and meta-analyzed data, bifidobacteria, *Lactobacillus*, *E.coli*, *Enterococcus faecalis*, and a mixture of different bacterial strains have been shown to be effective in IBS. This has cast some doubts on the overall rationale for their use in functional bowel disorders, as it is difficult to demonstrate a specific mechanism of action via the intestinal immune system, the enteric nervous system, or otherwise. A recent IBS trial (90) that attempted to do this was unable to identify a mechanism (e.g., via short-chain fatty acid modulation) but speculated that efficacy must result from factors other than the presence of induced microbiota itself (e.g., by eliciting its mechanisms of action via direct interaction with the immune system rather than via interaction with the local bacterial colony).

Recommendations for well-conducted intervention trials in IBS

Guidelines to design studies with IBS patients aiming at clinically meaningful endpoints were prepared by the European Agency for Evaluation of Medicinal Products (91) and by the Rome team of experts on functional bowel disorders (92). The double-blind, randomized, placebo-controlled, parallel group trial remains the preferred design. Potential disadvantages of cross-over or withdrawal (relapse rate after treatment) designs include

carryover effects, unblinding, and overestimation of the potential benefit for clinical practice. Given the chronic fluctuating nature of IBS, the following points should be taken into consideration:

- In studies with a clinical endpoint, treatment would be administered over months or years, on an intermittent basis.
- Minimum duration of active treatment would be 4 wk, and a duration of 6 mo is recommended for treatments intended for long-term use.
- Investigators should include as broad a spectrum of patients as possible and should report recruitment strategies and inclusion/exclusion criteria.
- Clinically meaningful endpoints include measures of patient-reported symptom improvement using psychometrically validated subjective global assessments or validated symptom severity questionnaires.

The problems associated with clinical trials are the variable course of IBS symptoms, the heterogeneity of this condition, and the very high placebo response (up to 50%). Data analysis should address all patients enrolled, using an intention-to-treat principle.

Mechanistic studies addressing specific physiological endpoints will overcome disadvantages associated with the subjective nature of patient-reported symptom scores and may provide further insights on the effects of specific bacteria on bowel functions. Gut stimuli induce specific reflexes and/or symptom perception. The tools for evaluating sensory and reflex pathways in the gut have been developed and may be applied to determine whether probiotics can correct abnormal responses in IBS patients or in various related disease models in healthy subjects, for example, in intestinal lipid or gas overload or stress (93). Furthermore, some physiological stimuli in the gut induce pleasant sensations, an effect commonly claimed for probiotics but so far unproven. The methodology developed to evaluate the pathogenesis of IBS symptoms can also be applied for the assessment of probiotics on gut comfort.

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