CLINICAL–ALIMENTARY TRACT

Lactobacillus and *Bifidobacterium* in Irritable Bowel Syndrome: Symptom Responses and Relationship to Cytokine Profiles

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See editorial on page 783.

Background & Aims: **The aim of this study was to compare the response of symptoms and cytokine ratios in irritable bowel syndrome (IBS) with ingestion of probiotic preparations containing a lactobacillus or bifidobacterium strain.** ***Methods:*** **Seventy-seven subjects with IBS were randomized to receive either *Lactobacillus salivarius* UCC4331 or *Bifidobacterium infantis* 35624, each in a dose of 1×10^{10} live bacterial cells in a malted milk drink, or the malted milk drink alone as placebo for 8 weeks. The cardinal symptoms of IBS were recorded on a daily basis and assessed each week. Quality of life assessment, stool microbiologic studies, and blood sampling for estimation of peripheral blood mononuclear cell release of the cytokines interleukin (IL)-10 and IL-12 were performed at the beginning and at the end of the treatment phase.** ***Results:*** **For all symptoms, with the exception of bowel movement frequency and consistency, those randomized to *B infantis* 35624 experienced a greater reduction in symptom scores; composite and individual scores for abdominal pain/discomfort, bloating/distention, and bowel movement difficulty were significantly lower than for placebo for those randomized to *B infantis* 35624 for most weeks of the treatment phase. At baseline, patients with IBS demonstrated an abnormal IL-10/IL-12 ratio, indicative of a proinflammatory, Th-1 state. This ratio was normalized by *B infantis* 35624 feeding alone.** ***Conclusions:*** ***B infantis* 35624 alleviates symptoms in IBS; this symptomatic response was associated with normalization of the ratio of an anti-inflammatory to a proinflammatory cytokine, suggesting an immune-modulating role for this organism, in this disorder.**

Irritable bowel syndrome (IBS) is a common functional disorder usually defined by the coexistence of abdominal pain or discomfort and an alteration in bowel habit.[1–3] IBS may lead to impaired social and personal function and can diminish quality of life to a degree usually associated with major organic diseases such as hypertension and diabetes.[4,5] IBS represents a significant therapeutic challenge; currently available therapies provide symptomatic relief at best, and none have been shown to alter the natural history of the disorder.[1,2,6–11] While the precise pathophysiology of IBS remains to be elucidated,[12] dysmotility and altered visceral perception/sensation are currently the most popular hypotheses.[13] More recently, roles for enteric infection and intestinal inflammation have been proposed. Thus, both retrospective and prospective studies have documented the new onset of IBS following bacteriologically confirmed bacterial gastroenteritis[14–21] and others have provided evidence of low-grade mucosal inflammation[22–24] and immune activation[25–27] in patients with IBS. The enteric flora has also been implicated; there has been a suggestion that some patients with IBS may harbor bacterial overgrowth and that their symptoms may be ameliorated by its eradication.[28–31] Despite these observations, our ever-increasing understanding of gut flora-mucosa interactions,[32] and the existence of a significant body of basic research to support a role for inflammatory and immune processes in contributing to enteric neuromuscular dysfunction,[33] the role of lumen-mucosa interactions in IBS remains largely unexplored.

Probiotics, defined as live or attenuated bacteria or bacterial products that confer a significant health benefit to the host,[34] have the potential to provide a clinical tool to explore these interactions. There are several reasons why these agents might, in theory, prove of therapeutic

Abbreviations used in this paper: AUC, area under the curve; IBS, irritable bowel syndrome; IL, interleukin; PBMC, peripheral blood mononuclear cell; VAS, visual analogue scale.

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benefit in IBS. Firstly, many probiotic organisms exert antibacterial and antiviral effects and could thereby prevent or modify the course of postinfective IBS.[35,36] Secondly, probiotics have been demonstrated to exert anti-inflammatory effects at mucosal surfaces[37,38]; by reducing mucosal inflammation, probiotics could decrease immune-mediated activation of enteric motor and sensory neurons and modify neural traffic between the gut and the central nervous system. Thirdly, probiotics could alter the composition of the gut flora. While the status of the gut flora in IBS remains a source of some controversy,[12,39,40] probiotic-related changes in the enteric flora could directly (through the augmentation of commensal lactobacilli or bifidobacteria or the elimination of pathogens) or indirectly (through a reduction in either pathogen-related inflammation or bacterial fermentation[41]) influence gut function. Finally, probiotics could alter the volume and/or composition of stool and gas[42] or increase intestinal mucus secretion,[43] effects that could influence intestinal handling of its contents and thus modulate symptoms such as constipation and diarrhea.

A small number of studies have evaluated the response of IBS to probiotic preparations; while results between studies are difficult to compare because of differences in study design, probiotic dose, and strain, there has been some, but by no means consistent, evidence of symptom improvement.[44–53] The overall impact of probiotics in IBS remains unclear.[54–58] Several of these studies have involved either lactobacilli or bifidobacteria, although none have involved a direct comparison of these strains.[55]

Patients and Methods

Study Population

Patients were recruited from gastroenterology clinics at Cork University Hospital and by direct advertisement on the university campus and in a local newspaper. Individuals aged between 18 and 75 years who satisfied Rome II criteria for the diagnosis of IBS[3] and in whom organic gastrointestinal diseases, including inflammatory bowel disease, and clinically significant systemic diseases had been excluded were considered for inclusion in the study. Pregnant women, individuals with known lactose intolerance or immunodeficiency, and individuals who had undergone any abdominal surgery, with the exception of hernia repair and appendectomy, were excluded.

Trial Protocol

Each potentially eligible patient was evaluated by a full review of clinical history and performance of a physical examination as well as full blood count, serum chemistry, and quantitative serum immunoglobulin levels. Clinically significant abnormalities in any of the latter test results led to exclusion from randomization. Eligible subjects then entered a 4-week run-in period during which they recorded symptoms, as well as stool frequency and form, each day on a diary card. During this time and throughout the rest of the study, subjects were instructed not to take any medications that could influence gut motor or absorptive function, including laxatives and antidiarrheal agents, as well as any preparation that could alter the enteric flora, including antibiotics and commercially available probiotic preparations.

At the end of the run-in period, subjects were randomized to receive either a lactobacillus or bifidobacterium, each delivered in a dose of 1×10^{10} live bacterial cells in a malted milk drink, or the malted milk drink alone as placebo. All preparations were identical in color, taste, and consistency. Randomization was performed by picking a card from a pack of prerandomized identical cards in the presence of a study coordinator; all other investigators, as well as patients, remained blinded to the randomization process until completion of the trial. Subjects were instructed to ingest the preparation once a day, in the morning, for 8 weeks and record symptoms and stool characteristics on a daily basis throughout the study period. Compliance was assessed by direct questioning at clinic visits and by fecal flora analysis. On completion of the 8-week treatment phase, subjects continued to complete the daily symptom cards for a further 4-week washout period while off all therapy.

Probiotic Preparations

The probiotic preparations used in this study, *Lactobacillus salivarius* subspecies *salivarius* UCC4331 and *Bifidobacterium infantis* 35624, were originally isolated from the ileocecal region of an adult human undergoing reconstructive surgery. These strains were selected on the basis of the following probiotic properties: being of human origin, nonpathogenic, and resistant to intestinal acid and bile; demonstrating an ability to adhere to human epithelial cells; and demonstrating an ability to temporarily colonize and be metabolically active within the human gastrointestinal tract.[59,60] Furthermore, these organisms have been previously shown in volunteer studies to survive transit through the gastrointestinal tract, to be free of side effects, and to demonstrate anti-inflammatory activity in a number of models.[60,61] *L salivarius* UCC4331 was cultured in de Man/Rogosa/Sharp broth (Oxoid, Basingstoke, United Kingdom) at 37°C in an anaerobic environment for 24 hours. *B infantis* 35624 was cultured in de Man/Rogosa/Sharp broth enriched with cysteine at 37°C in an anaerobic environment for 48 hours.

Assessments

Throughout the entire study, subjects were seen on a weekly basis and diary cards collected. The following 3 cardinal IBS symptom clusters were assessed: (1) abdominal pain or discomfort, (2) bloating or distention, and (3) bowel movement difficulty. The latter could reflect either difficulty with

evacuation (ie, straining or a sense of incomplete evacuation) or urgency. Each symptom was evaluated using both an ordinal scale (Likert scale; maximum score, 7) and a 10-cm visual analogue scale (VAS; maximum score, 10).[62] A composite score, comprised of the sums of the 3 cardinal symptoms (pain/discomfort, bloating/distention, and bowel movement difficulty scores) was also calculated for each patient (maximum score: Likert scale, 21; VAS, 30).

Bowel movement frequency was recorded as number per day, and consistency was evaluated using the Bristol Stool Scale.[63]

Quality of life was assessed by administration of an IBS-specific questionnaire developed and validated by Drossman et al[64] at the time of randomization and at the end of the treatment and washout periods. The following 8 domains were assessed on each occasion in each patient: dysphoria, interference with activity, body image, health worry, food avoidance, social reaction, sexual function, and impact on relationships.

Blood samples for blood count, serum chemistry, and quantitative immunoglobulin levels were obtained at initial evaluation and at the end of the study and analyzed using standard laboratory methods.

Stool samples for fecal flora analysis were obtained at randomization and at the end of the treatment phase. Spontaneous rifampicin-resistant variants of both probiotic strains were isolated before initiation of this study to facilitate the differentiation of these bacteria from all other lactobacilli and bifidobacteria. Representative fecal suspensions were serially diluted and plated on de Man/Rogosa/Sharp agar containing rifampicin or de Man/Rogosa/Sharp agar containing cysteine and rifampicin to enumerate *L salivarius* UCC4331 or *B infantis* 35624, respectively.

Peripheral blood samples from patients with IBS was obtained both before and after treatment for cytokine levels and compared with that obtained from a group of age- and sex-matched healthy volunteers (n = 20). Peripheral blood samples were taken directly into sterile EDTA-containing Vacutainers (Econo-med, Long Sutton, United Kingdom). Mononuclear cells were isolated by Ficoll-Hypaque density centrifugation[65] and resuspended at 1×10^6 cells/mL in complete media/Dulbecco's modified Eagle medium containing 25 mmol/L glucose, 10% fetal calf serum, 1% nonessential amino acids, 50 U/mL penicillin, and 50 μg/mL streptomycin (Invitrogen, Paisley, Scotland). These mononuclear cells are termed peripheral blood mononuclear cells (PBMCs). PBMCs were incubated, nonstimulated, for 72 hours at 37°C in a 5% CO_2 humidified atmosphere. Nonstimulated PBMC cytokine production reflects the cytokine milieu from which the PBMCs were originally isolated. Cell-free supernatants were stored frozen at −70°C and analyzed for cytokine levels in batches. Interleukin (IL)-10 and IL-12p40 cytokine levels were measured using enzyme-linked immunosorbent assays (R&D Systems, Abington, United Kingdom).

Statistical Methods

All data were collected and analyzed independently of the investigators, who did not have access to the data or to its analysis until the latter had been completed. All of the efficacy analyses were summarized on data from all evaluative subjects and analyzed on an intention-to-treat basis. Baseline and demographic data were tested for balance across treatment groups using 1-way analysis of variance, χ^2 test, or Fisher exact test, as appropriate.

For each of the IBS symptoms and the composite score, efficacy data were analyzed in 2 ways. First, weekly individual symptom scores and composite scores were analyzed using a repeated-measures analysis of covariance model, with fixed effects for treatment and for the mean week −1 (baseline) symptom or composite score, as applicable. The variance/covariance matrix in this model was assumed to have compound symmetry. Second, to compare scores over the entire treatment phase, an area-under-the curve (AUC) analysis was performed for each symptom and the composite score. Symptom scores were first averaged within each week for each subject. AUCs were then calculated for each subject by using week 1 to week 8 scores in the treatment phase. AUCs were then analyzed using analysis of covariance using the baseline score at week 2 of the run-in phase as covariate and treatment as the factor in the model. Tukey's method for adjustment for multiple treatment comparisons was used to obtain the adjusted *P* values for between-treatments comparisons. Both adjusted and unadjusted *P* values were reported.

Quality-of-life measurements were analyzed using analysis of covariance with fixed effects for treatment group and baseline.

Results

Subjects

A total of 80 subjects were enrolled in the study. Two subjects (1 randomized to *B infantis* 35624 and 1 to placebo) were subsequently found to have taken antibiotics from the beginning of the treatment phase and were therefore determined to be nonevaluable. A further 3 subjects dropped out before the treatment phase. Therefore, 75 subjects provided some evaluable data. Of these, 3 subjects took an antibiotic during the course of the study; only data up to the commencement of antibiotic use were deemed to be evaluable. Another subject took a commercially available probiotic preparation during the washout/follow-up period (weeks 8–12); only data up to week 8 were determined to be evaluable for this subject. Four additional subjects failed to complete the washout phase; therefore, 67 subjects satisfactorily completed all phases of the study.

Baseline Characteristics

Among the 75 evaluable subjects, 64% were women and 36% were men. Subjects averaged 44.3 years in age (range, 18–73 years). Sex and age were both balanced across treatment groups. All subjects were white. Classification of subjects at baseline by predominant symptom indicated that 45% were alternators, 28% diarrhea predominant, and 26% constipation predomi-

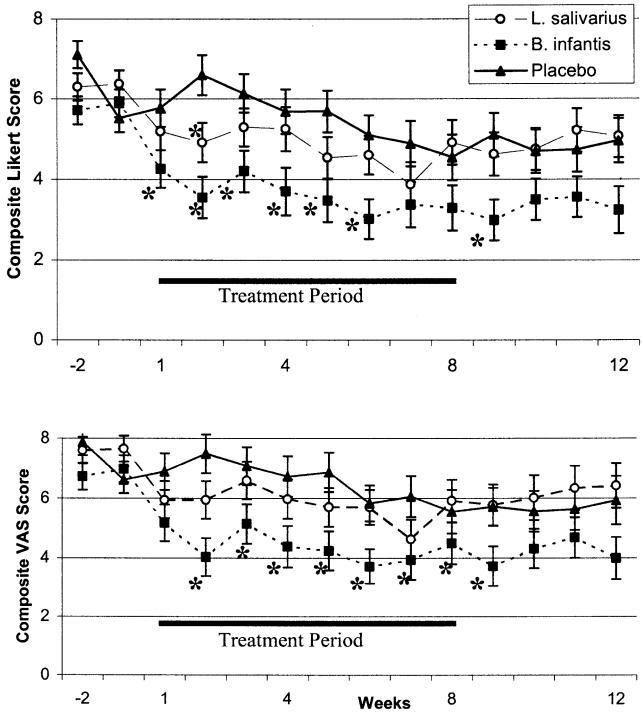

Figure 1. Composite Likert scale and VAS scores. Comparison of the effects of placebo, *L salivarius* UCC4331, and *B infantis* 35624 on a composite score of IBS symptoms. The scores are derived from the sum of scores for abdominal pain/discomfort, bloating/distention, and bowel movement difficulty. Note the significant reduction in composite scores throughout the treatment period and into the washout phase for subjects treated with *B infantis* 35624 but not with *L salivarius* UCC4331 or placebo. *$P < .05$ vs placebo; all comparisons adjusted for any differences in baseline symptom score.

nant. In addition, 25% of the subjects were smokers and 88% drank alcohol. Although subjects were balanced across treatment groups with respect to use of alcohol, there was imbalance detected with regard to smoking status; of the subjects responding, 72% of the *L salivarius* UCC4331 group did not smoke, compared with 92% of the *B infantis* 35624 group and 58% of the placebo group ($P = .03$).

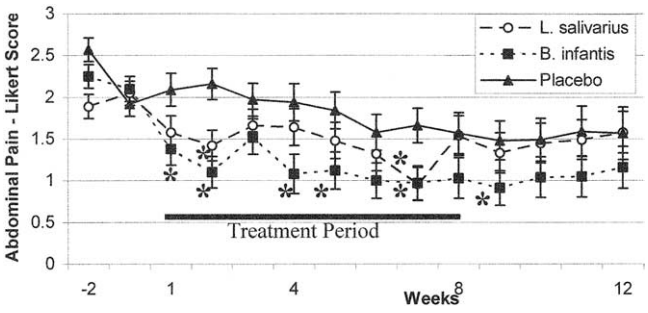

Figure 2. Abdominal pain scores on the Likert scale. Comparison of the effects of placebo, *L salivarius* UCC4331, and *B infantis* 35624 on abdominal pain/discomfort in IBS. Note the significant reduction in pain/discomfort score during most weeks of the treatment phase and into the washout phase among patients treated with *B infantis* 35624; for those randomized to *L salivarius* UCC4331, a significant benefit was evident at week 2 alone. *$P < .05$ vs placebo; all comparisons adjusted for any differences in baseline symptom score.

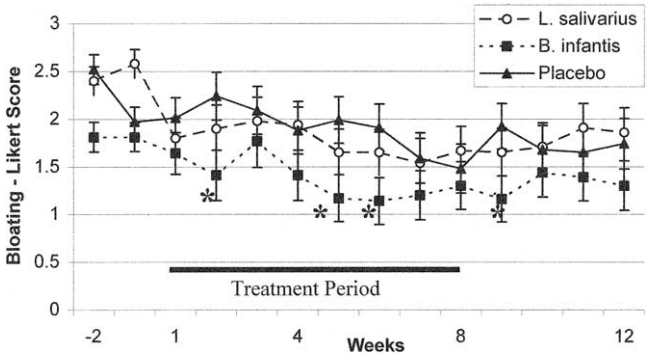

Figure 3. Bloating scores on the Likert scale. Comparison of the effects of placebo, *L salivarius* UCC4331, and *B infantis* 35624 on bloating/distention in IBS. Note the significant reduction in bloating/distention during weeks 2, 5, and 6 for those randomized to *B infantis* 35624; there was no significant benefit for those randomized to *L salivarius* UCC4331. *$P < .05$ vs placebo; all comparisons adjusted for any differences in baseline symptom score.

The 3 treatment groups were not quite balanced for baseline symptom scores. Statistically significant imbalance was detected at the $\alpha = .05$ level for the following baseline scores: abdominal pain/Likert score (week −2), bloating/Likert score (week −2), bloating/Likert score (week −1), bloating/VAS score (week −2), bloating/VAS score (week −1), and the composite Likert score (week −2). In consideration of these baseline differences, efficacy analyses were performed using baseline, as calculated by the average of the week −1 symptom scores, as a covariate.

Response to Treatment

Figures 1–4 summarize the least-squares means and standard errors for composite score and each of the primary symptom efficacy measurements (abdominal pain/discomfort, bloating/distention, and bowel movement difficulty) according to treatment and for each week of the 8-week treatment period and the 4-week washout period. Because similar results were obtained for all parameters studied using both the Likert scale and the VAS, results for both scales are presented for composite score alone; elsewhere, only the Likert scale results are presented. Table 1 presents results of the AUC analyses.

A comparison of scores for each week showed that subjects treated with *B infantis* 35624 had lower composite scores than those receiving placebo for all weeks in the treatment phase and the entire washout phase. Of the VAS scores for each of these 12 weeks, 10 were significantly lower than those for placebo. The only 2 weeks in which scores were not significantly lower than those for

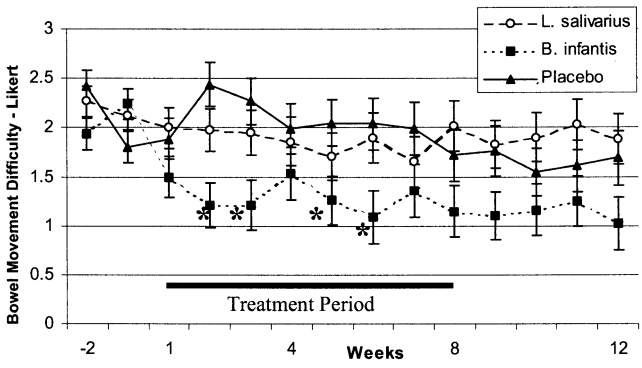

Figure 4. Bowel movement difficulty scores on the Likert scale. Comparison of the effects of placebo, *L salivarius* UCC4331, and *B infantis* 35624 on bowel movement difficulty in IBS. Note the significant reduction in bowel movement difficulty during weeks 2, 3, and 5–7 for those randomized to *B infantis* 35624; there was no significant benefit for those randomized to *L salivarius* UCC4331. *$P < .05$ vs placebo; all comparisons adjusted for any differences in baseline symptom score.

placebo were weeks 10 and 11, during the washout phase (Figure 1). In comparison, those randomized to *L salivarius* UCC4331 achieved a statistically significant reduction in composite symptom score during the second week of the treatment period alone (Figure 1). Furthermore, on at least 1 of the scales, composite scores for those treated with *B infantis* 35624 were significantly lower than for those treated with *L salivarius* UCC4331 during the second, fourth, sixth, and eighth weeks of the treatment phase and for 3 of the 4 weeks of the washout phase (Figure 1).

Comparison of AUCs for the treatment phase showed significantly lower Likert scale and VAS composite scores for the group randomized to *B infantis* 35624 compared with the placebo group, even after controlling for multiple between-treatments comparisons (Table 1).

For each individual symptom, with the notable exceptions of bowel movement frequency and consistency, the group randomized to *B infantis* 35624 experienced a greater reduction in symptom scores during the treatment period (Figures 1–4 and Table 1). Thus, subjects randomized to *B infantis* 35624 achieved lower scores for pain/discomfort (AUC, $P < .05$ vs placebo for unadjusted scores, Table 1; Likert scale scores for individual weeks, $P < .05$ vs placebo for weeks 1, 2, 4, 5, and 7 of the treatment phase and week 1 of the washout phase, Figure 2), bloating/distention (AUC, $P < .07$ vs placebo for unadjusted scores, Table 1; Likert scale scores for individual weeks, $P < .05$ vs placebo for weeks 2, 5, and 6 of the treatment phase, Figure 3), and bowel movement difficulty (AUC, $P < .005$ vs placebo for adjusted scores, Table 1; Likert scale scores for individual weeks, $P < .05$ vs placebo for weeks 2, 3, 5, and 6 of the treatment phase and week 1 of the washout phase, Figure 4). In contrast, the only symptom improvement observed for those randomized to *L salivarius* UCC4331 was an improvement in abdominal pain during weeks 2 and 7 of the treatment phase (Likert scale, $P < .05$, Figure 2); none of the AUC comparisons showed a significant effect for *L salivarius* UCC4331 on an individual symptom (Table 1).

Direct comparisons between the 2 probiotic-treated groups showed significantly lower ($P \leq .05$) scores for bowel movement difficulty for those treated with *B infantis* 35624 (AUC, $P < .05$ *B infantis* 35624 vs placebo or *L salivarius* UCC4331, Table 1; Likert scale scores for individual weeks, $P < .05$ *B infantis* 35624 vs *L salivarius* UCC4331 for weeks 2, 3, and 6 of the treatment phase and weeks 1 and 4 of the washout phase, Figure 4).

The time course of the response to *B infantis* 35624 demonstrated a relatively rapid response; improvement was evident at the end of the first week and reached a maximum by the end of the second week of an 8-week course of therapy (Figures 1 and 4).

Subjects receiving the 3 treatments reported a similar number of bowel movements per week and similar bowel movement consistency scores across all 8 weeks of the treatment period.

Quality of Life

For most domains, quality-of-life scores were numerically lower than those for placebo for the patients randomized to the probiotics but reached statistical significance versus placebo during the treatment phase only for health worry for bifidobacterium (at the .05 level) and dysphoria for lactobacillus (at the .10 level).

Table 1. AUC Analysis of Therapeutic Response

	L salivarius UCC4331	*B infantis* 35624	Placebo
Abdominal pain			
Likert score	8.98 (1.36)	7.78 (1.36)[a]	12.21 (1.85)
VAS score	11.40 (1.82)	9.45 (1.69)[a]	14.92 (2.39)
Bloating			
Likert score	12.61 (1.68)	10.17 (1.67)[b]	14.39 (2.18)
VAS score	15.32 (2.44)	11.66 (2.35)[b]	17.04 (3.14)
Bowel movement difficulty			
Likert score	15.61 (1.85)	7.84 (1.91)[c]	16.79 (2.34)
VAS score	19.71 (2.18)[b]	11.16 (2.14)[c]	24.50 (2.83)
Stool consistency	22.22 (1.66)	25.51 (1.65)	22.98 (2.11)
Composite			
Likert score	34.64 (3.60)	24.56 (3.63)[c]	40.52 (4.68)
VAS score	42.35 (4.93)	30.15 (4.80)[c]	52.14 (6.39)

NOTE. Values are expressed as least-squares mean (SE).
[a]$P < .05$.
[b]$.05 < P < .10$, between-treatments difference without adjustment.
[c]$P < .05$ between-treatments difference after adjustment for multiple comparisons.

PBMC Cytokine Levels

In vitro production of IL-10 and IL-12 by PBMCs was dysregulated at baseline in patients with IBS compared with healthy volunteers (Figure 5). IL-10 levels were lower in patients with IBS (575 ± 108 pg/mL vs 968 ± 220 pg/mL), whereas IL-12 levels were increased in patients with IBS (15 ± 2 pg/mL vs 6 ± 4 pg/mL). The ratio of IL-10/IL-12 levels was significantly different between the 2 groups (IBS, 69 ± 15; healthy volunteers, 176 ± 31; $P = .003$).

Following treatment with *B infantis* 35624, PBMC cytokine levels returned to levels similar to those observed for the healthy volunteer group (Figure 5). In contrast, PBMC cytokine levels did not return to levels observed for healthy volunteers in the subjects with IBS who received *L salivarius* UCC4331 or placebo.

Adverse Events

Four subjects reported adverse events during the study; 1 developed an episode of epistaxis that resolved spontaneously, 1 was hospitalized with unstable angina and another with an episode of chest pain that was attributed to anxiety, and 1 was hospitalized with abdominal pain that was attributed to an exacerbation of IBS and constipation. No clinically significant changes in full blood count, serum chemistry, or serum immunoglobulin levels were recorded in any of the subjects during the study.

Stool Recovery of Probiotics

Growth was observed on rifampicin-containing media with samples obtained from probiotic-treated patients, thus confirming that the probiotics consumed had survived gastrointestinal transit in the patients with IBS. No growth was observed from fecal samples obtained before probiotic feeding or at any point in patients receiving placebo.

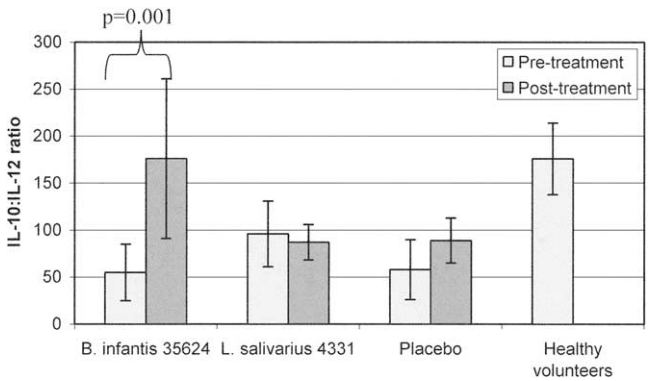

Figure 5. PBMC IL-10/IL-12 ratio. Comparison of PBMC IL-10/IL-12 ratios at baseline and following therapy with placebo, *L salivarius* UCC4331, and *B infantis* 35624 with that of a normal control period. Note the abnormal baseline ratio in subjects with IBS, with a normalization of this ratio following the administration of *B infantis* 35624 alone.

Discussion

In this study, we compared, for the first time, the effects of 2 probiotic strains on symptoms in patients with IBS. We have shown superiority for bifidobacterium over both a lactobacillus and placebo for each of the cardinal symptoms of IBS and for a composite score. These symptomatic benefits were associated with parallel trends in a quality-of-life measure developed specifically for IBS.[64] Furthermore, this therapy was well tolerated and free of significant adverse events. Interestingly, these benefits, in contrast to those observed with 2 newly approved therapies for IBS, namely alosetron[66] and tegaserod,[67,68] were observed independent of any change in stool frequency or form and cannot therefore be attributed to either a laxative or an antidiarrheal effect.

This study is not without its limitations. The small size of the study population may have failed to detect significant effects of either of the probiotics on some symptoms. Furthermore, the study was not powered to detect significant changes in quality of life. Symptoms were assessed on the basis of paper diaries, which are subject to recall bias and are therefore less accurate than electronic diaries. To capture the overall impact of IBS on the subject, we chose to use a composite score comprised of the cardinal features of IBS; an alternative approach to this same issue would have been to use some form of global assessment instrument. Assessments of individual symptoms also permitted the evaluation of the therapies on individual components of IBS. It needs to be stressed, however, that the small size of this study would, if anything, have mitigated against our ability to demonstrate differences. The fact that we did demonstrate efficacy for one probiotic strain over both placebo and another strain further supports the validity of these observations and suggests that the bifidobacterium strain used may have a selective and specific effect in IBS. It should also be noted that the placebo response rate observed in this study was similar to that recorded in many other IBS studies. As evidenced by their symptom scores at entry, the patients studied lay at the mild end of IBS and were more reflective of the type of patients with IBS seen in the community rather than in referral centers. Whether bifidobacterium would be similarly effective in the latter population remains to be seen. We chose to accept all eligible patients with

IBS for this study and did not exclude on the basis of sex or symptom predominance, an approach that has been adopted in studies of the serotonin agonists and antagonists.[66–68] In an attempt to score equally for symptoms related to defecation in a diarrhea- or constipation-predominant subject with IBS, we used the parameter of bowel movement difficulty, which could score for either urgency in the patient with diarrhea, or difficulty with evacuation (straining, incomplete evacuation), which is a symptom complex associated with constipation. Furthermore, the individual treatment groups were too small to permit a meaningful post-hoc subgroup analysis of response based on these or other parameters. The duration of the study was also similar to that of other recent studies but does not permit an assessment of the long-term effects of this therapy. Although this study did not involve a comparison with any other therapeutic modality and the study design differed in some aspects from recent large trials of serotonergic agonists and antagonists, the therapeutic gain observed for bifidobacterium over placebo (20%–25%) is certainly no less than that reported for tegaserod and alosetron (10%–20%).[66–68]

We chose *L salivarius* UCC4331 over several lactobacillus probiotic strains isolated and characterized in our laboratories, based on in vitro activity against several pathogens, tolerance to acid and bile, and our prior demonstration in a study conducted in 80 human volunteers that *L salivarius* UCC4331, delivered in either a milk drink or a yogurt, is well tolerated, successfully colonizes the gastrointestinal tract, and produces expected quantitative and qualitative changes in the gut flora.[59–61] Furthermore, the introduced lactobacilli were found to stimulate a mucosal but not a systemic immune response. Finally, related strains had been shown to be of benefit in the prevention of human diarrheal conditions such as toddlers' diarrhea and *Clostridium difficile*–related, antibiotic-associated diarrhea.[35,36,69–74]

B infantis 35624 was chosen from among several bifidobacterium probiotic strains developed in University College Cork based on tolerance to acid and bile and the demonstration in mouse models of inflammatory bowel disease that *B infantis* 35624 may prove highly effective in beneficially altering gut flora and in alleviating the inflammatory changes that characterize these models.[38,75]

What is the mechanism of action of this probiotic in IBS? This organism has been shown to exert potent immune effects, including the promotion of anti-inflammatory and the inhibition of proinflammatory cytokines. For example, oral administration of the bifidobacterium used in this study exerted a profound anti-inflammatory effect in the IL-10 knockout mouse, a potent model of inflammatory bowel disease that was associated with a suppression of the proinflammatory cytokines interferon gamma, tumor necrosis factor α, and IL-12 while preserving activity of the anti-inflammatory cytokine transforming growth factor β.[38] This is of particular interest given recent reports of low-grade inflammation and a similar pattern of cytokine activation among patients with IBS.[23–27] Our finding in this study of a cytokine ratio in IBS skewed toward a Th1, proinflammatory profile provides further support for this hypothesis. Based on these and other observations,[26] it is tempting to speculate that failure to adequately down-regulate a proinflammatory response following a precipitating event (eg, gastrointestinal infection) may sustain the IBS state. This study has taken this concept one step further; by demonstrating a normalization of the IL-10/IL-12 ratio in the bifidobacteria-fed subjects alone, and in parallel with symptomatic improvement, we provide the first evidence for efficacy for an anti-inflammatory approach in IBS.

While this study protocol did not include biopsies, thus precluding an evaluation of the effects of this therapy on colorectal mucosal histology or immune activation, others have recently shown potent anti-inflammatory effects at the mucosal level for probiotic preparations that contained bifidobacteria.[76,77] In this context, we must also acknowledge that the mucosa is functionally and operationally distinct from the systemic or peripheral immune systems and that direct relationships between these compartments have not been shown in humans. We are currently exploring these relationships in humans in IBS and in response to probiotic therapy; meanwhile, we would draw attention to studies from our group, albeit in a murine model, showing parallel mucosal and systemic cytokine responses to the same probiotic strains as used in this study.[38] The hypothesis that bifidobacterium is acting through an anti-inflammatory mechanism is indeed an attractive one; this effect could abrogate the induction of hypersensitivity, hyperalgesia, altered central perception, and dysmotility by inflammatory triggers.[78] There is indeed some preliminary evidence that probiotic administration may diminish visceral hypersensitivity in animal models.[79,80] Furthermore, effects on motility and perception could also go some way toward explaining the beneficial effects on bloating, given current concepts on the roles of altered gas transit and visceral hypersensitivity in the pathogenesis of this symptom.[81–83]

Of the other putative effects of probiotics, an effect on stool bulking would seem unlikely because we failed to observe any change in either stool consistency or frequency. This apparent independence of the effects of

bifidobacterium from any change in stool frequency or form has important clinical implications, implying that this therapeutic approach may be applicable to all patients with IBS, irrespective of stool pattern. Whether qualitative or quantitative changes in small intestinal or colonic flora accompany probiotic feeding and thereby alter flora-mucosal interactions to the benefit of the host cannot be determined from this study. We do know from the stool recovery studies that the organisms survived transit through the intestine, but we did not assess in this study interactions between the indigenous flora and the administered probiotics. These same studies from our group have shown continued recovery of orally administered probiotic organisms from stool for 3 or more weeks in 12.5% of healthy volunteers,[61] an observation that may explain the persistence of benefit from bifidobacterium in this study into the washout phase. Furthermore, others[84,85] have shown that probiotic organisms can adhere to the colonic mucosa and can continue to be recovered from colonic biopsy specimens long after the discontinuation of oral feeding and after they cease to be recovered from fecal samples.

Whether IBS is accompanied by quantitative or qualitative changes in the bacterial flora of the small or large intestine remains a contentious issue; while some have described bacterial overgrowth in the small intestine[28–30] and qualitative alterations in the fecal flora[39,40,47] and increased bacterial fermentation[41] in IBS, others have failed to replicate these findings.[12,31,81] A reduction in bacterial fermentation by a modulation of the composition of the flora could certainly contribute to the alleviation of the "gas-related" symptoms that are so common in IBS[82] and that seem to reflect a selective defect in intestinal gas transport.[83] Probiotics have also been shown to modulate enteroendocrine cell populations in the mouse intestine.[86]

Other studies have evaluated the response of IBS to a number of probiotic preparations. In a recent review, Hamilton-Miller, while drawing attention to the shortcomings of prior trials in terms of study design, concluded that there was, overall, sufficient evidence of efficacy to warrant further evaluation.[55] Most studies reviewed were small in size and almost certainly underpowered to show anything other than a very striking benefit. Several did not verify bacterial transit and survival by confirmatory stool studies. Many different organisms and strains were used, and dosages varied from as little as 10^5 to 10^{13}. Furthermore, some, including a recent study, used probiotic "cocktails" rather than single isolates, rendering it impossible to induce what, if any, were the active moieties.[53] Nevertheless, some positive results were noted. Niedzielin et al reported resolution of abdominal pain in all 20 patients treated for 4 weeks with *Lactobacillus plantarum* 299V, in contrast to only 11 of 20 patients who received a placebo,[48] and Halpern et al noted a significant reduction in an IBS symptom index with a capsule containing 5×10^9 heat-killed *Lactobacillus acidophilus*.[44] O'Sullivan and O'Morain, while failing to detect an effect of *Lactobacillus casei* GG on overall symptomatology, did note a trend toward reduction in bloating.[46] Nobaek et al, using *L plantarum* (DSM 9843),[45] described a similar benefit in terms of relief of bloating, as did Kim et al in their evaluation of the probiotic "cocktail" VSL#3.[53]

In contrast, the study reported herein provides, for the first time, clear evidence for a benefit in IBS for a clearly-defined single-organism probiotic preparation and thereby suggests that some strains, and bifidobacterium in particular, may be more effective that others for this indication. Furthermore, this symptomatic response was associated with a normalization of the ratio of an anti-inflammatory to a proinflammatory cytokine, suggesting an immune-modulating role for this organism, in this disorder. While the limitations inherent to the study mandate that its findings be interpreted with caution, it should at the very least prompt large randomized controlled trials of this bifidobacterium strain in IBS as well as detailed explorations of its mechanism(s) of action.

References

1. Brandt LJ, Locke GR, Olden K, Quigley E, Schoenfeld P, Schuster M, Talley N. An evidence-based approach to the management of irritable bowel syndrome in North America. Am J Gastroenterol 2002;97(Suppl):S1–S26.
2. Drossman DA, Camileri M, Mayer EA, Whitehead WE. AGA technical review on irritable bowel syndrome. Gastroenterology 2002; 123:2108–2131.
3. Thompson WG, Longstreth GF, Drossman DA, Heaton KW, Irvine EJ, Muller-Lissner SA. Functional bowel disorders and functional abdominal pain. Gut 1999;45(Suppl II):II43–II47.
4. Thompson WG, Heaton KW, Smyth GT, Smyth C. Irritable bowel syndrome in general practice: prevalence, characteristics, and referral. Gut 2000;46:78–82.
5. Hungin APS, Whorwell PJ, Tack J, Mearin F. The prevalence, patterns and impact of irritable bowel syndrome: an international survey of 40,000 subjects. Aliment Pharmacol Ther 2003;17: 643–650.
6. Jailwala J, Imperiale TF, Kroenke K. Pharmacologic treatment of the irritable bowel syndrome: a systematic review of randomized, controlled trials. Ann Intern Med 2000;133:136–147.
7. Jones J, Boorman J, Cann P, Forbes A, Gomborone J, Heaton K, Hungin P, Kumar D, Libby G, Spiller R, Read N, Silk D, Whorwell P. British Society of Gastroenterology guidelines for the management of the irritable bowel syndrome. Gut 2000;47(Suppl II):ii1–ii19.
8. Akehurst R, Kaltenthaler E. Treatment of irritable bowel syndrome: a review of randomised controlled trials. Gut 2001;48: 272–282.

9. Camilleri M. Management of the irritable bowel syndrome. Gastroenterology 2001;120:652–668.
10. Talley NJ. Pharmacologic therapy for the irritable bowel syndrome. Am J Gastroenterol 2003;98:750–758.
11. Poynard T, Naveau S, Mory B, Chaput JC. Meta-analysis of smooth muscle relaxants in the treatment of irritable bowel syndrome. Aliment Pharmacol Ther 1994;8:499–510.
12. Quigley EMM. Current concepts of the irritable bowel syndrome. Scand J Gastroenterol 2003;38(Suppl 237):1–8.
13. Mayer EA, Collins SM. Evolving pathophysiological models of functional gastrointestinal disorders. Gastroenterology 2002; 122:2032–2048.
14. McKendrick MW, Read MW. Irritable bowel syndrome—post-salmonella infection. J Infect 1994;29:1–3.
15. Neal KR, Hebdon J, Spiller R. Prevalence of gastrointestinal symptoms six months after bacterial gastroenteritis and risk factors for development of the irritable bowel syndrome. BMJ 1997;314:779–782.
16. Gwee K-A, Leong Y-L, Graham C, McKendrick MW, Collins SM, Walters SJ, Underwood JE, Read NW. The role of psychological and biological factors in post-infective gut dysfunction. Gut 1999; 44:400–406.
17. Garcia Rodriguez LA, Ruigomez A. Increased risk of irritable bowel syndrome after bacterial gastroenteritis: cohort study. BMJ 1999;318:565–566.
18. Spiller RC. Estimating the importance of infection in IBS. Am J Gastroenterol 2003;98:238–241.
19. Spiller RC, Jenkins D, Thornley JP, Hebden JM, Wright T, Skinner M, Neal KR. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute Campylobacter enteritis and in post-dysenteric irritable bowel syndrome. Gut 2000;47:804–811.
20. Dunlop SP, Jenkins D, Neal KR, Spiller RC. Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. Gastroenterology 2003;125:1651–1659.
21. Cumberland P, Sethi D, Roderick PJ, Wheeler JG, Cowden JM, Roberts JA, Rodrigues LC, Hudson MJ, Tompkins DS; IID Study Executive. The infectious intestinal disease study of England: a prospective evaluation of symptoms and health care use after an acute episode. Epidemiol Infect 2003;130:453–460.
22. Collins SM. A case for an immunological basis for irritable bowel syndrome. Gastroenterology 2002;122:2078–2080.
23. Chadwick V, Chen W, Shu D, Paulus B, Bethwaite P, Tie A, Wilson I. Activation of the mucosal immune system in irritable bowel syndrome. Gastroenterology 2002;122:1778–1783.
24. Tornblom H, Lindberg G, Nyberg B, Veress B. Full-thickness biopsy of the jejunum reveals inflammation and enteric neuropathy in irritable bowel syndrome. Gastroenterology 2002;123:1972–1979.
25. O'Sullivan M, Clayton N, Breslin NP, Harman I, Bountra C, McLaren A, O'Morain CA. Increased mast cells in the irritable bowel syndrome. Neurogastroenterol Motil 2000;12:449–457.
26. Gonsalkorale WM, Perrey C, Pravica V, Whorwell PJ, Hutchinson IV. Interleukin 10 genotypes in irritable bowel syndrome: evidence for an inflammatory component? Gut 2003;52:91–93.
27. Gwee K-A, Collins SM, Read NW, Ranjnakova A, Deng Y, Graham JC, McKendrick MW, Moochhala SM. Increased rectal mucosal expression of interleukin 1beta in recently acquired post-infectious irritable bowel syndrome. Gut 2003;52:523–526.
28. Pimentel M, Chow EJ, Lin HC. Eradication of small bowel bacterial overgrowth reduces symptoms of irritable bowel syndrome. Am J Gastroenterol 2000;95:3503–3506.
29. Pimentel M, Chow E, Lin H. Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome: a double-blind, randomized, placebo-controlled study. Am J Gastroenterol 2003;98:412–419.
30. Tursi A, Brandimarte G, Giorgetti GM. High prevalence of small intestinal bacterial overgrowth in celiac patients with persistence of gastrointestinal symptoms after gluten withdrawal. Am J Gastroenterol 2003;98:839–843.
31. O'Leary C, Quigley EMM. Small bowel bacterial overgrowth, celiac disease and IBS: what are the associations? Am J Gastroenterol 2003;98:720–722.
32. Shanahan F. Therapeutic manipulation in gut flora. Science 2000;289:1311–1312.
33. Collins SM. The immunomodulation of enteric neuromuscular function: implications for motility and inflammatory disorders. Gastroenterology 1996;111:1683–1699.
34. Gorbach SL. Probiotics in the third millennium. Dig Liver Dis 2002;34(Suppl 2):S2–S7.
35. Isolauri E, Kirjavainen PV, Salminen S. Probiotics: a role in the treatment of intestinal infection and inflammation? Gut 2002;50: iii54–iii59.
36. Von Wright A, Salminen S. Probiotics: established effects and open questions. Eur J Gastroenterol Hepatol 1999;11:1195–1198.
37. O'Mahony L, Feeney M, O'Halloran S, Murphy L, Kiely B, Fitzgibbon J, Lee G, O'Sullivan G, Shanahan F, Collins K.. Probiotic impact on microbial flora, inflammation, and tumour development in IL-10 knockout mice. Aliment Pharmacol Ther 2001;15:1219–1225.
38. McCarthy J, O'Mahony L, O'Callaghan L, Sheil B, Vaughan EE, Fitzsimons N, Fitzgibbon J, O'Sullivan GC, Kiely B, Collins JK, Shanahan F. Double blind, placebo controlled trial of two probiotic strains in interleukin 10 knockout mice and mechanistic link with cytokine balance. Gut 2003;52:975–980.
39. Bradley HK, Wyatt GM, Bayliss CE, Hunter JO. Instability in the faecal flora of a patient suffering from food-related irritable bowel syndrome. J Med Microbiol 1987;23:29–32.
40. Balsari A, Ceccarelli A, Dubini F, Fesce E, Poli G. The fecal microbial population in the irritable bowel syndrome. Microbiologica 1982;5:185–194.
41. King TS, Elia M, Hunter JO. Abnormal colonic fermentation in irritable bowel syndrome. Lancet 1998;352:1187–1189.
42. Jiang T, Savaiano DA. Modification of colonic fermentation by bifidobacteria and pH in vitro: impact on lactose metabolism, short-chain fatty acid, and lactate production. Dig Dis Sci 1997; 42:2370–2377.
43. Ouwehand AC, Lagstrom H, Suomalainen T, Salminen S. Effect of probiotics on constipation, fecal azoreductase activity and fecal mucin content in the elderly. Ann Nutr Metab 2002;46:159–162.
44. Halpern GM, Prindiville T, Blankenburg M, Hsia T, Gershwin ME. Treatment of irritable bowel syndrome with Lacteol Fort: a randomized, double-blind, cross-over trial. Am J Gastroenterol 1996; 91:1579–1585.
45. Nobaek S, Johansson ML, Molin G, Ahrne S, Jeppsson B. Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. Am J Gastroenterol 2000;95:1231–1238.
46. O'Sullivan MA, O'Morain CA. Bacterial supplementation in the irritable bowel syndrome. A randomised double-blind placebo-controlled crossover study. Dig Liver Dis 2000;32:302–304.
47. Brigidi P, Vitali B, Swennen E, Bazzocchi G, Matteuzzi D. Effects of probiotic administration upon the composition and enzymatic activity of human fecal microbiota in patients with irritable bowel syndrome or functional diarrhea. Res Microbiol 2001;152:735–741.
48. Niedzielin K, Kordecki H, Birkenfeld B. A controlled, double-blind, randomized study on the efficacy of Lactobacillus plantarum 299V in patients with irritable bowel syndrome. Eur J Gastroenterol Hepatol 2001;13:1143–1147.

49. Sen S, Mullan MM, Parker TJ, Woolner JT, Tarry SA, Hunter JO. Effect of Lactobacillus plantarum 299v on colonic fermentation and symptoms of irritable bowel syndrome. Dig Dis Sci 2002;47: 2615–2620.
50. Bazzocchi G, Gionchetti P, Almerigi PF, Amadini C, Campieri M. Intestinal microflora and oral bacteriotherapy in irritable bowel syndrome. Dig Liver Dis 2002;34(Suppl 2):s48–s53.
51. Adler SN, Jacob H, Eliakim R. The probiotic agent E-Coli strain ATCC20226 has a healing effect on proximal inflammation of the small bowel (abstr). Gastroenterology 2002;122:A527.
52. Parker P, McNaught CE, Anderson ADG, Mitchell CJ, MacFie J. Synbiotic in irritable bowel syndrome: a double blind prospective randomised controlled trial (abstr). Gut 2003;52:A11.
53. Kim HJ, Camilleri M, McKinzie S, Lempke MB, Burton DD, Thomforde GM, Zinsmeister AR. 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.
54. Barbara G, Corinaldesi R. Probiotics: could they turn out to be ineffective in irritable bowel syndrome? Dig Liver Dis 2000;32: 294–301.
55. Hamilton-Miller JMT. Probiotics in the management of irritable bowel syndrome: a review of clinical trials. Microb Ecol Health Dis 2001;13:212–216.
56. Thompson WG. Probiotics for irritable bowel syndrome: a light in the darkness? Eur J Gastroenterol Hepatol 2001;13:1135–1136.
57. Madden JA, Hunter JO. A review of the role of the gut microflora in irritable bowel syndrome and the effects of probiotics. Br J Nutr 2002;88(Suppl 1):S67–S72.
58. Floch MH. Probiotics, irritable bowel syndrome, and inflammatory bowel disease. Curr Treat Options Gastroenterol 2003;6:283–288.
59. Dunne C, O'Mahony L, Murphy L, Thornton G, Morrissey D, O'Halloran S, Feeney M, Flynn S, Fitzgerald G, Daly C, Kiely B, O'Sullivan GC, Shanahan F, Collins K. In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. Am J Clin Nutr 2001;73:886S–892S.
60. Dunne C, Murphy L, Flynn S, O'Mahony L, O'Halloran S, Feeney M, Morrissey D, Thornton G, Fitzgerald G, Daly C, Kiely B, Quigley EMM, O"Sullivan GC, Shanahan F, Collins JK. Probiotics: from myth to reality. Demonstration of functionality in animal models of disease and in human clinical trials. Antonie Van Leeuwenhoek 1999;76:279–292.
61. Collins JK, Dunne C, Murphy L, Morrissey D, O'Mahony L, O'Sullivan E, Fitzgerald G, Kiely B, O'Sullivan GC, Daly C, Marteau P, Shanahan F. A randomised controlled trial of a probiotic Lactobacillus strain in healthy adults: assessment of its delivery, transit, and influence on microbial flora and enteric immunity. Microb Ecol Health Dis 2002;14:81–89.
62. Veldhuyzen Van Zanten SJ, Talley NJ, Bytzer P, Klein KB, Whorwell PJ, Zinsmeister AR. Design of trials for functional gastrointestinal disorders. Gut 1999;45(Suppl 2):II68–II77.
63. Heaton KW, O'Donnell LJ. An office guide to whole-gut transit time. Patients' recollection of their stool form. J Clin Gastroenterol 1994;19:28–30.
64. Drossman DA, Patrick DL, Whitehead WE, Toner BB, Diamant NE, Hu Y, Jia H, Bangdiwala SI. Further validation of the IBS-QOL: a disease-specific quality-of-life questionnaire. Am J Gastroenterol 2000;95:999–1007.
65. Boyum A. Separation of leukocytes from blood and bone marrow. Scand J Clin Lab Invest Suppl 1968;97:7.
66. Cremonini F, Delgado-Aros S, Camilleri M. Efficacy of alosetron in irritable bowel syndrome: a meta-analysis of randomized controlled trials. Neurogastroenterol Motil 2003;15:79–86.
67. Muller-Lissner SA, Fumagalli I, Bardhan KD, Pace F, Pecher E, Nault B, Ruegg P. Tegaserod, a 5-HT receptor partial agonist, relieves symptoms in irritable bowel syndrome patients with abdominal pain, bloating and constipation. Aliment Pharmacol Ther 2001;15:1655–1666.
68. Kellow J, Lee OY, Chang FY, Thongsawat S, Mazlam MZ, Yuen H, Gwee KA, Bak YT, Jones J, Wagner A. An Asia-Pacific, double blind, placebo-controlled, randomised study to evaluate the efficacy, safety, and tolerability of tegaserod in patients with irritable bowel syndrome. Gut 2003;52:671–676.
69. Perdigon G, Alvarez S, Nader DE, Macias ME, Roux ME, de Ruiz Holgado AP. The oral administration of lactic acid bacteria increases the mucosal immunity in response to enteropathogens. J Food Protection 1990;53:404–440.
70. Rolfe RD. The role of probiotic cultures in the control of gastrointestinal health. J Nutr 2000;130(2S Suppl):396S–402S.
71. Duffy LC, Leavens A, Griffiths E, Dryja D. Perspectives on bifidobacteria as biotherapeutic agents in gastrointestinal health. Dig Dis Sci 1999;44:1499–1505.
72. Marteau P, Seksisk P, Jian R. Probiotics and intestinal health effects: a clinical perspective. Br J Nutr 2002;88(Suppl 1):S51–S57.
73. Fooks LJ, Gibson GR. Probiotics as modulators of the gut flora. Br J Nutr 2002;88(Suppl 1):S39–S49.
74. Cremonini F, Di Caro S, Santarelli L, Gabrielli M, Candelli M, Nista EC, Lupascu A, Gasbarrini G, Gasbarrini A. Probiotics in antibiotic-associated diarrhoea. Dig Liver Dis 2002;34:S78–S80.
75. Byrne F, Murphy LM, Binder S, Collins JK, O'Sullivan GC, Shanahan F, Aranda R. Prevention of wasting and colitis in SCID mice reconstituted with CD4+ and RB high cells by the administration of the probiotic Bifidobacterium longum infantis. (in press)
76. Rachmilewitz D, Katakura K, Karmeli F, Hayashi T, Reinus C, Rudensky B, Akira S, Takeda K, Lee J, Takabayashi K, Raz E. Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. Gastroenterology 2004;126:520–528.
77. Jijon H, Backer J, Diaz H, Yeung H, Thiel D, McKagney C, De Simone C, Madsen K. DNA from probiotic bacteria modulates murine and human epithelial and immune function. Gastroenterology 2004;126:1358–1373.
78. Barbara G, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, Pasquinelli G, Morselli-Labate AM, Grady EF, Bunnett NW, Collins SM, Corinaldesi R. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. Gastroenterology 2004;126:693–702.
79. Greenwood-van Meerveld B, Johnson AC, Kajs T, Charbonnealu D, Poehner R, Chen K-S, Carryl O. Probiotic bacteria normalize post inflammatory visceral hyperalgesia in rats (abstr). Gastroenterology 2002;122:A476.
80. Lamine F, Cauquil E, Eutamene H, Fioramonti J, Bueno L, Theodorou V. Lactobacillus farciminis treatment reduces sensitivity to rectal distension in rats: involvement of nitric oxide (abstr). Gastroenterology 2003;122:A476.
81. Quigley EMM. The role of gas in IBS. In: Camilleri M, Spiller RC, eds. Irritable bowel syndrome. Diagnosis and treatment. Philadelphia, PA: Saunders, 2002:77–84.
82. Quigley EMM. From comic relief to real understanding; how intestinal gas causes symptoms. Gut 2003;52:1659–1661.
83. Serra J, Azpiroz F, Malagelada JR. Impaired transit and tolerance of gas in the irritable bowel syndrome. Gut 2001;48:14–19.
84. Alender M, Satokari R, Korpela R, Saxelin M, Vilpponen-Salmela T, Mattila-Sandholm T, von Wright A. Persistence of colonization of human colonic mucosa by a probiotic strain, *Lactobacillus rhamnosus* GG, after oral consumption. Appl Environ Microbiol 1999;65:351–354.
85. Zoetendal EG, von Wright A, Vilpponen-Salmela T, Ben-Amor K, Akkermans ADL, de Vos WM. Mucosa-associated bacteria in the human gastrointestinal tract are uniformly distributed along the colon and differ from the community recovered from feces. Appl Environ Microbiol 2002;68:3410–3407.

86. Uribe A, Alam M, Johansson O, Midtvedt T, Theodorsson E. Microflora modulates endocrine cells in the gastrointestinal mucosa of the rat. Gastroenterology 1994;107:1259–1269.

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Wirsung of the Duct of Wirsung

Johann Georg Wirsung (1600–1643) was born in Augsburg, Bavaria. From there he emigrated to the famed school of medicine at Padua where he became a prosector in anatomy. It was there that one of his students Maurice Hoffman came across a pancreatic duct in a rooster and displayed the dissection to his instructor. Thereupon, Wirsung set out to demonstrate a counterpart in a human cadaver. This accomplished, Wirsung communicated his finding, together with a detailed illustration, in a letter to Jean Riolan (1577–1657), a senior colleague in Paris. In the following year 1643, Wirsung was fatally felled by a gunshot fired by an unknown assassin, presumably the culmination of a dispute over priority of the discovery. The lesson, if any, is perhaps to refrain from pressing one's claim to originality with undue vigor.

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