The Role of Probiotics in Gastrointestinal Disorders of Infancy and Childhood

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Healthy human intestine is a complex and diverse microbial ecosystem, hosting more than 400 bacterial species (1), concentrated mostly in the terminal ileum and the colon. On the basis of many previous observations, microflora considered to be beneficial include the genera *Lactobacillus* and *Bifidobacterium*. These species—as well as others such as *Enterococcus faecium*, *Streptococcus thermophilus*, *Bacillus subtilis*, some strains of *Escherichia coli*, and even a yeast, *Saccharomyces boulardii*—have in recent years been studied in various ways for their possible beneficial effects in a wide range of clinical conditions in both adult and pediatric practice. They are defined as “probiotics,” a term first introduced in veterinary medicine in 1965 (2). Fuller (3) defined a probiotic as a “live microbial feed supplement, which beneficially affects the host animal by improving its microbial balance.” The definition most fitting the current understanding of the subject was provided more recently by Schaafsma (4), who defined probiotics as “living organisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition.”

Understandably, in the pediatric age group, probiotics have been most thoroughly investigated in recent years in gastrointestinal diseases, both infectious and noninfectious. Probiotics have been proposed or studied for efficacy in the prevention and treatment of a large and diverse spectrum of gastrointestinal disorders. These include acute infectious diarrhea, antibiotic-associated diarrhea (*Clostridium difficile* associated or not), small bowel bacterial overgrowth, diarrhea induced by continuous enteral feeding (5), *Helicobacter pylori* gastritis (6), food allergy (7), lactose intolerance (8,9), sucrase-isomaltase deficiency (10), and inflammatory bowel disease.

As the field is so ample, we focus on the most-explored areas, and in particular on those that appear to be best documented or most promising.

**ACUTE DIARRHEA**

Acute-onset diarrhea continues to be a major problem for child health worldwide. Although in developed countries, the prevalence and severity of acute diarrhea have declined in recent years, it remains an extremely common problem. In developing
countries, an average of three episodes per child per year in children younger than 5 years is reported, but there are regions with six to eight episodes per child per year. In these settings, malnutrition plays an important additional role by exposing the child at a higher risk of acute and prolonged diarrhea (11). Childhood mortality associated with diarrhea therefore remains high, with ~3 million deaths a year according to a World Health Organization (WHO) estimate (12). In the United States, there are one to two episodes per child per year in children younger than 5 years, with 220,000 hospital admissions (or ~10% of all admissions for children in this age range), and ~400 deaths per year (13). There is therefore general agreement that agents that could prove to be safe and effective in reducing the duration of an episode or preventing it from becoming protracted would be a valuable therapeutic resource.

Lactobacillus GG (ATCC 53103) is among the most thoroughly investigated probiotics in this area. Lactobacillus GG (L-GG) was first isolated from human intestine in 1985 by two Boston investigators, Gorbach and Goldin. L-GG adheres well to intestinal epithelial cells (14), it is resistant to acid and bile, and it is able to colonize the human gastrointestinal tract transiently (15,16). L-GG produces antimicrobial substances active against various bacteria including E. coli, streptococci, clostridia, bacteroides, and salmonellae (17).

In 1991 Isolauri et al. (18) showed the beneficial effects of L-GG in the treatment of acute childhood diarrhea (in 82% of their cases, this was caused by rotavirus). In this pioneer investigation—one of the first to approach the study of probiotic efficacy in a placebo-controlled fashion—involving 71 well-nourished children between ages 4 and 45 months, it was shown that L-GG reduced the mean duration of diarrhea by 1 day in comparison with controls (p < 0.001).

The same group later showed (19) that after a rotavirus infection, children who received L-GG developed a specific increase in circulating immunoglobulin A (IgA)-secreting cells much more often than did children who received placebo. Other reports, mostly from the same group but in different settings, consistently showed the efficacy of this probiotic in reducing the duration of the watery phase of diarrhea in children admitted to hospital with rotavirus diarrhea (20-24). In a randomized, controlled, but not blinded study of 100 children with mild diarrhea, Guarino et al. (25) found that diarrhea (as assessed by the parents) lasted on average for 3 days in patients receiving L-GG versus 6 days in controls (p < 0.02). In the same article, it also was shown that duration of viral shedding (61% of patients had rotavirus) was significantly shortened in children receiving L-GG.

Most clinical trials with L-GG were done in patient populations in which the overwhelming majority had rotavirus infection (18-25). In studies in which a significant portion of the children were probably infected with bacterial pathogens—that is, patients with bloody diarrhea or dysentery (26,27), or in whom there was bacteriologic evidence of bacterial infection (24)—the efficacy of the probiotic was doubtful.

To assess this further, as well as to verify the efficacy of a novel way of giving the probiotic [dissolved in oral rehydration solution (ORS) so as to provide it during the early rehydration phase], one of us (S.G.) recently led a multicenter trial involving 11 centers (nine in Europe, one in Egypt, one in Israel) (28). We gathered the largest
reported series of children with acute-onset diarrhea in whom the efficacy of a probiotic was studied in a double-blind, placebo-controlled fashion (287 children, aged 1 month to 3 years). Diarrhea was caused by rotavirus in 35% of cases, by bacteria or protozoans in 32.6%, and was of undetermined etiology in 32.4%. Overall, 140 children received placebo, and 147 received L-GG [1010 colony-forming units (CFU)/250 ml of ORS, given *ad libitum*]. There were no differences at admission between the groups with regard to age, sex, previous duration of diarrhea, previous types of feeding, use of antibiotics, weight, height, weight/height centile, prevalence of fever, overall status, degree of dehydration, or percentage of inpatients compared with outpatients.

The duration of diarrhea after enrollment (Table 1) was 71.9–35.8 hours in controls versus 58.3–27.6 hours in the L-GG group (mean ± SD, *p* < 0.03). We confirmed that the children who benefited the most from the intake of L-GG were those affected by rotavirus. For these, diarrhea lasted 56.2–16.9 hours compared with 76.6–41.6 hours in the controls (*p* < 0.008).

**TABLE 1. Effect of Lactobacillus-GG in oral rehydration solution on diarrhea duration**

<table>
<thead>
<tr>
<th>Study group</th>
<th>Duration of diarrhea before admission (hours, mean ± SD)</th>
<th>Duration of diarrhea after admission (hours, mean ± SD)</th>
<th>Total duration of diarrhea (hours, mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (all etiologies, n = 147)</td>
<td>51.0 ± 30.9</td>
<td>71.9 ± 35.8</td>
<td>122.9 ± 33</td>
</tr>
<tr>
<td>L-GG (all etiologies, n = 140)</td>
<td>52.1 ± 31</td>
<td>58.3 ± 27.6</td>
<td>110.4 ± 28.1</td>
</tr>
<tr>
<td>Control (Rotavirus only, n = 45)</td>
<td>59.7 ± 33.5</td>
<td>76.6 ± 41.6</td>
<td>136.3 ± 29.0</td>
</tr>
<tr>
<td>L-GG (Rotavirus only, n = 56)</td>
<td>58.5 ± 30.3</td>
<td>56.2 ± 16.9</td>
<td>114.7 ± 16.2</td>
</tr>
<tr>
<td>Control (&quot;Invasive&quot; etiologies only, n = 21)</td>
<td>48.8 ± 30.5</td>
<td>72.0 ± 32.4</td>
<td>120.8 ± 44.1</td>
</tr>
<tr>
<td>L-GG (&quot;Invasive&quot; etiologies only, n = 22)</td>
<td>50.7 ± 35.1</td>
<td>73.3 ± 29.3</td>
<td>124.0 ± 46.4</td>
</tr>
<tr>
<td>Control (no pathogen identified, n = 54)</td>
<td>47.9 ± 29.9</td>
<td>64.2 ± 30.5</td>
<td>112.1 ± 29.5</td>
</tr>
<tr>
<td>L-GG (no pathogen identified, n = 45)</td>
<td>45.7 ± 29.2</td>
<td>53.2 ± 32.4</td>
<td>98.9 ± 37.2</td>
</tr>
</tbody>
</table>

From Guandalini et al. (28).
It was of interest (see Table 1) that whereas the children with proven bacterial diarrhea did not benefit from L-GG, those in whom no cause was found showed a response in terms of diarrheal duration. Of even greater interest was the observation (Fig. 1) that the diarrhea lasted >6 days in 10.7% of controls versus only 2.7% of the L-GG patients \((p < 0.01)\). Such a dramatic reduction in the risk of running a prolonged course (almost fourfold) shows the ability of the probiotic to help prevent a protracted course, one of the most feared events for acute-onset diarrhea in children. Although we do not yet know the mechanism of this preventive effect, it is tempting to speculate that the improved gut-barrier function shown with the use of L-GG in an animal model (29), as well as its known antiinflammatory effect in patients with food allergy (7), could have been contributing factors (Fig. 1).

In 1994, Saavedra et al. (30) reported that probiotics were effective in prevention of diarrhea in children in hospital. In their double-blind, placebo-controlled investigation, infants aged 5–24 months received *Bifidobacterium bifidum* and *Streptococcus thermophilus* in their formula. Over a period of 17 months, in 31% of the control patients but only in 7% of those receiving the probiotics did diarrhea develop.

In a recently completed study, Szajewska et al. (31) assessed the efficacy of the oral administration of L-GG in the prevention of the hospital spread of diarrhea (most commonly due to rotavirus) in 1- to 36-month-old infants admitted for reasons other than diarrhea. In this double-blind, placebo-controlled, randomized trial, patients received either \(6 \times 10^9\) CFU of probiotic or a comparable placebo twice daily.

![Graph](image)

**FIG. 1.** Duration of diarrhea in patients receiving *Lactobacillus* GG (L-GG) or placebo. Patients are grouped according to the duration of diarrhea in 12-hour groups. Median duration of diarrhea in L-GG-treated patients is 51 versus 65.1 hours in control patients, as evident from the left-skewed curve. Significantly more patients from the control group had diarrhea that lasted >144 hours (6 days) from the start of the observations.
throughout their hospital stay. It was found that, although the incidence of rotaviral infections was not different between the groups, control patients developed rotaviral diarrhea significantly more often than did L-GG–treated patients: six episodes of rotavirus enteritis with diarrhea in 36 (16.7%) patients versus one episode in 45 (2.2%) patients. The prophylaxis also was effective against diarrheal episodes overall, as there were 12 (33.3%) episodes of diarrhea in controls versus only three (6.6%) in L-GG–treated inpatients ($p < 0.02$).

**ANTIBIOTIC-ASSOCIATED DIARRHEA**

Diarrhea is a common event after antibiotic use: 20–40% of children receiving broad-spectrum antibiotics experience this side effect, which can be induced by most antibiotics. As this is considered to be the result of an imbalance in the colonic microflora, it is only logical that one of the most common uses for probiotics should be to prevent or treat antibiotic-associated diarrhea. Although there are several published studies in adults, it is only recently that this problem has been approached in a scientifically sound way in the pediatric age range. In a double-blind, placebo-controlled trial of almost 200 children receiving antibiotics in an outpatient setting for acute infections, L-GG in doses of between 1 and $2 \times 10^{10}$ CFU/day was found to reduce the incidence of antibiotic-associated diarrhea from 26% in those receiving placebo to 8% in those receiving L-GG (32). L-GG overall significantly reduced stool frequency and increased stool consistency during antibiotic treatment by day 10 compared with the placebo group.

In a similar double-blind, placebo-controlled study on 119 patients (mean age, 4.5 years) undergoing antibacterial treatment for acute respiratory infections, Arvola et al. (33) found that the incidence of diarrhea within 2 weeks of the start of the antibacterial treatment was 5% in the group receiving $2 \times 10^{10}$ CFU of L-GG twice daily with their antibiotics and 16% in the placebo group—a highly significant difference.

**CLOSTRIDIUM DIFFICILE DIARRHEA**

*Clostridium difficile* infection can result in asymptomatic carriage, mild diarrhea, a less-common protracted enteropathy, or pseudomembranous colitis—a form that can be fulminant. Furthermore, the disease can occur in an annoyingly recurrent form. Although the reasons for the strikingly different courses are not clear, recent evidence suggests that after colonization, development of IgG antibodies against toxin A of *C. difficile* may play a role in protecting the host from subsequent clinical manifestations (34).

*Clostridium difficile* is the leading cause of hospital-acquired gastrointestinal illness in adult inpatients. Although *C. difficile* has been well studied in adults, research on pediatric *C. difficile* disease is still in its infancy because of a widely held but erroneous belief that *C. difficile* is limited to adults and is not a problem for children. There are recent reports of many cases of pediatric *C. difficile* infection and of
outbreaks of *C. difficile* disease in pediatric populations. Pediatric cases of *C. difficile* disease also have been associated with severe complications and mortality. Treatment of pediatric *C. difficile* disease usually relies on metronidazole or vancomycin, but clinical guidelines have not been defined for the pediatric population. As with adults, some of the children treated with antibiotics develop recurrent *C. difficile* disease that does not respond to conventional treatment.

In 1993, Buts *et al.* (35) reported a series of 19 infants (aged 2–32 months; median, 8 months) with *C. difficile* disease. Fifteen had persistent diarrhea, and three with evidence of malnutrition, failure to grow, and poor appetite. All the patients had *C. difficile* toxin B–positive stools as the sole etiology for the diarrhea. The probiotic *Saccharomyces boulardii* was given orally for 15 days according to the age of the child (500 mg/day for children younger than 1 year, 750 mg/day for those aged 1–4 years, and 1 g/day for those older than 4 years). No antibiotics were given during the treatment part of the trial. Within 1 week, symptoms resolved in 18 (95%) of the children. Clearance of toxin B was observed within 15 days in 16 (85%) of 19 cases. No side effects were noted during the study. Two (11%) patients had subsequent relapse of disease, which resolved rapidly after a second treatment with *S. boulardii*. No other studies of *S. boulardii* in pediatric patients with *C. difficile* have been published, and this is evidently an area requiring randomized, placebo-controlled studies.

L-GG has also been investigated for the treatment of *C. difficile*. Gorbach *et al.* reported in 1987 (36) that this probiotic was successful in preventing relapse of *C. difficile* colitis in five adult patients. Since then, an additional 32 adult patients with relapsing *C. difficile* diarrhea have been studied, and L-GG was effective in curing 94% of these (37).

With respect to children, in another study (38), four children (mean age, 34 months) with a history of relapsing of *C. difficile* colitis and diarrhea were treated
with L-GG orally for 2 weeks. No antibiotics were given during the treatment part of the trial, but all patients had antibiotics in the follow-up period (median, 9.5 months). All four children responded clinically within 5–7 days and were asymptomatic by the end of the 2 weeks of treatment. No side effects were noted during the study. However, two of the children relapsed within 2 months after discontinuing L-GG.

We recently had the opportunity to use L-GG (10^{10} CFU twice daily) in a 13-year-old boy with ileal Crohn’s disease, who had already had three episodes of *C. difficile* diarrhea associated with abdominal cramps (Fig. 2). The episodes had recurred in spite of conventional treatment with metronidazole. Shortly after beginning the L-GG treatment, the symptoms started to improve, and 3 weeks into the treatment, his stool culture was clear of both *C. difficile* and its toxin A. Repeated clinical and microbiologic follow-up over a 6-month period failed to show any recurrence of the infection.

**SMALL-BOWEL BACTERIAL OVERGROWTH SYNDROME**

Small-bowel bacterial overgrowth is a common and annoying complication of short-bowel syndrome or intestinal pseudo-obstruction and is known to occur as well in the absence of anatomic or functional defects. The syndrome is characterized by abdominal distention, protracted diarrhea, and malabsorption, and is often controlled with antimicrobial treatment. In 1998, Vanderhoof *et al.* (39) reported patients with bacterial overgrowth who did not respond to conventional antimicrobial treatment but were successfully treated with alternative regimens, including probiotic therapy with *L. plantarum* 299V and L-GG.

A rare complication of small-bowel bacterial overgrowth is the development of lactic acidosis. This complication results from the intraluminal metabolic activity of some bacterial species, mainly lactobacilli, which produce D-lactate. This metabolite is then absorbed into the systemic circulation, where it accumulates, as it cannot be metabolized, unlike its L-stereoisomer. We reported (40) a 4-year-old girl with small-bowel bacterial overgrowth secondary to short-bowel syndrome, who had multiple past episodes of acidotic coma due to D-lactic acidosis. Abundant lactobacilli producing D-lactate were identified in her stool cultures. As L-GG is a strain of lactobacillus that produces L-lactate and is unable to produce D-lactate, we hypothesized that continuous intake of this probiotic might result in effective displacement of the D-lactate–producing strains and thus prevent the recurrent episodes of acidosis. We thus gave her oral L-GG, 2 × 10^{10} CFU once daily. Since the start of treatment, no more episodes of acidosis have occurred in 18 months of follow-up. Thus it would appear that small-bowel bacterial overgrowth is definitely an area in which the use of probiotics is promising.

**INFLAMMATORY BOWEL DISEASE**

Although the cause of inflammatory bowel disease is unknown, there is increasing evidence that the endogenous bacterial flora plays an important role in the initiation
and perpetuation of the disease (41,42). It has been hypothesized that the intestinal inflammatory response is the result of an exaggerated intestinal host immune response to commensal enteric bacteria or their components in genetically predisposed individuals. A defect in mucosal barrier function could allow luminal bacterial antigens to initiate a chronic relapsing inflammation. The intestinal mucus layer from patients with inflammatory bowel disease contains a large number of bacteria compared with those in controls (43), and it was thus hypothesized that the intestinal mucus layer in this condition affords less protection against the endogenous microflora than does that in healthy individuals. The potential role of luminal bacteria in initiating the abnormal immune response seen in inflammatory bowel diseases is stressed by the observation that in mice deficient in interleukin (IL)-2 (44), IL-10 (45), and T-cell receptors (46), spontaneous colitis develops only in the presence of luminal bacteria, whereas this does not occur if mice are raised in germ-free conditions. Finally, metronidazole and ciprofloxacin are useful in treatment of Crohn’s disease (47,48).

Do probiotics have any protective role in this scenario? Recently, Madsen et al. (49) performed an elegant investigation that showed promising results. They reported that IL-10-deficient mice, in which a patchy, chronic colitis develops similar to that in human Crohn’s disease (50), have decreased levels of Lactobacillus species during the neonatal period and an increase in translocated bacteria and bacteria adherent to the colonic mucosa compared with those in normal controls. Normalizing the levels of Lactobacillus sp. by daily rectal delivery of L. reuteri or by oral lactulose reduced the numbers of colonic mucosa-adherent and translocated bacteria and markedly and significantly prevented the development of colonic inflammation (Fig. 3).

![Graph](image)

**FIG. 3.** Colonic histologic injury score in interleukin-10 (IL-10)-deficient mice [from Madsen (49), redrawn]. Lactobacillus reuteri repopulation and oral lactulose reduced injury significantly \( p < 0.01 \) when compared with that in untreated IL-10-deficient mice.
Very few data are so far available in humans, and those only for adults. Krus et al. (51), in a double-blind study, compared the effect of mesalazine with that of an oral preparation of nonpathogenic *E. coli* (strain Nissle, serotype 06: K5: H1) for 12 weeks for their efficacy in preventing relapse of ulcerative colitis. Relapse rates were not different with these two regimens. More recently, Rembacken et al. (52) studied the efficacy of oral administration of nonpathogenic *E. coli* (Nissle 1917) in the treatment of active ulcerative colitis and in preventing relapse of ulcerative colitis over a more prolonged period. In all, 116 patients were randomized to receive either mesalazine or *E. coli* and were followed up for a maximum of 12 months. Seventy-five percent of the patients in the mesalazine group attained remission, compared with 68% in the *E. coli* group. Mean time to remission was 44 days in the mesalazine group and 42 days in the *E. coli* group. In the mesalazine group, 73% of patients relapsed compared with 67% in the *E. coli* group. Mean duration of remission was 206 days in the mesalazine group and 221 days in the *E. coli* group. It was concluded that treatment with a nonpathogenic *E. coli* strain had an equivalent effect to that with mesalazine in maintaining remission of ulcerative colitis. It should, however, be noted that in both studies, the mesalazine doses given were rather low.

Recently Venturi et al. (53) evaluated the effects on intestinal microflora and the clinical efficacy of a new probiotic preparation in 20 adult patients with ulcerative colitis in remission, intolerant or allergic to 5-amino-salicylic acid. The probiotic preparation studied (VSL#3, Yovis; Sigma-Tau, Pomezia, Italy) contained $5 \times 10^{11}$ cells/g of three strains of bifidobacteria, four strains of lactobacilli, and one strain of *S. thermophilus*. Two doses of 3 g were given daily for 12 months. It was found that fecal concentrations of *S. thermophilus*, lactobacilli, and bifidobacteria increased significantly in all the patients. Furthermore, 75% of the patients remained in remission during the study; one patient was lost to follow-up, and four relapsed. The same group had previously reported in abstract form (54) a study with the same probiotic preparation in the maintenance treatment of chronic relapsing pouchitis, one of the most frequent and annoying complications of the surgical management of patients with severe ulcerative colitis (55). Forty patients with chronic pouchitis who initially achieved remission after combination antibiotic treatment were randomized to placebo or VSL#3 for 9 months. All patients taking placebo relapsed. In contrast, 17 of 20 treated with VSL#3 were still in remission at 9 months.

Although the pathogenesis of pouchitis is not entirely clear, degradation of mucus overlying the small intestinal epithelium of the “pouch,” brought about by local bacterial overgrowth, is a likely contributing factor (56). In this regard, the use of probiotics such as L-GG, which do not possess mucus-degrading properties, also seems feasible.

As a pilot study to investigate the possible effect of L-GG in inflammatory bowel disease in children, we conducted an open-label small trial to assess the clinical efficacy of L-GG oral supplementation in children with Crohn’s disease (57). Children younger than 18 years with Crohn’s disease, diagnosed by established clinical, radiographic, and endoscopic criteria, were included in the study. Patients with mildly active disease, despite concomitant treatment with prednisone and immunomodulatory
drugs such as 6-mercaptopurine (6-MP), azathioprine, or methotrexate, were included in the study. Active disease was defined having as a pediatric Crohn's Disease Activity Index (PCDAI) score of >10 (58).

All patients were taking stable doses of immunomodulatory drugs including prednisone for ≥4 weeks before enrollment. Subjects were screened 1 week before the initiation of L-GG treatment. Clinical features and disease activity (PCDAI) were assessed at baseline and at 1, 4, 12, and 24 weeks taking L-GG. Stools were cultured at each follow-up visit to assess colonization by L-GG. We also assessed intestinal permeability by the cellobiose/mannitol (C/M) sugar permeability test at each visit. Intestinal permeability is thought to reflect disease activity (59), and we hypothesized that L-GG might have beneficial effect on this as well.

Four patients were enrolled. All were boys with a median age of 14.5 years (range, 10–18 years). Two patients had ileocolonic disease, and the other two had gastrocolonic disease. None had fistulas. Median duration of Crohn's disease was 3 years (range, 1–5 years). All patients were taking prednisone at entry, at a median dose of 22.5 mg, and also immunomodulatory drugs [either 6-MP or azathioprine (Imuran)]. Two patients also were taking metronidazole. Median PCDAI score at entry was 19 (range, 12–35). The C/M ratio also was high at baseline, reflecting increased intestinal permeability (median, 0.12; range, 0.023–0.17). There was successful intestinal colonization with L-GG in all patients—the probiotic was recovered in stool samples of all the patients at follow-up visits at fecal concentrations ranging from $10^7$ to $10^9$ CFU/g. Interestingly, concomitant treatment with metronidazole did not inhibit intestinal colonization with L-GG (Fig. 4). We found that L-GG intake improved intestinal permeability, as measured by the double sugar permeability test (Fig. 5). The decreased permeability was already evident at 1 week, and peaked at 12 weeks of follow-up (median, 0.021; range, 0.009–0.046; $p < 0.05$), although the improvement had started to reverse by the 24-week follow-up. In terms of clinical outcome, the

![FIG. 4. Fecal recovery of Lactobacillus GG from four children with Crohn's disease in the presence or in the absence of treatment with oral metronidazole.](image-url)
patients showed significant improvement, as documented by repeated PCDAI assessments ($p = 0.02$; Fig. 6). Median PCDAI score at the 4-week visit was 5 (range, 0–12.5), and the score at 12 weeks also was significantly lower than that at baseline (median, 7.5; range, 0–10). The median PCDAI at the 24-week follow-up (a time when intestinal permeability seemed to be deteriorating) was still lower than that at baseline, but not significantly so. In three patients, we were able to taper the dose of steroids with L-GG; the average reduction in steroid dose in these patients was 50% at 12 weeks. Finally, no patient reported any adverse effects during the study period (Figs. 4–6).
Thus although our data are obviously very preliminary, and a larger study done in a double-blind, placebo-controlled fashion is warranted and needed, L-GG appears to be effective in improving the clinical status of children with Crohn's disease. The interesting improvement in intestinal permeability suggests that L-GG may act by enhancing the gut mucosal barrier directly or by offering resistance to colonization by bacteria that modulate tight-junction permeability.

CONCLUSIONS

The theoretic potential advantages of probiotics and of prebiotics [the so-called colonic foods for probiotics (60)] over conventional treatment, including antibiotics, are many. They include low cost, a more physiologic approach, and the avoidance of hazardous side effects. Although in recent years, there has been an explosion in scientific interest in probiotics—a novel yet ancient way of preventing or treating a variety of gastrointestinal diseases—there is clearly a need for more information. We need basic studies on mechanisms of action (there may well be many, one or more for each specific strain in the context of each specific disorder); information on pharmacokinetics (the dosage is still empiric, and we know next to nothing about distribution, metabolism, and duration of effect); and well-designed, randomized, placebo-controlled investigations of well-characterized probiotics, either alone or in combination, aimed at understanding synergistic effects. Microorganisms can be friends as well as foes. So far we have been dealing with them only as enemies, but the road is now clear for appreciating and establishing links of cooperation with the many strains that can be our allies. We should learn to respect our minute friends who inhabited this planet for so many million of years before us!

REFERENCES


**DISCUSSION**

**Dr. Zoppi:** I have a question about possible mechanisms. There is a hypothesis that after an episode of gastroenteritis, the ecosystem in the intestine is washed out, and in this situation, it is possible that pathogenic organisms establish themselves in the bowel. Probiotics may block that process. Do you agree with that hypothesis?

**Dr. Guandalini:** I think it is entirely possible, although there are different causes of acute infectious diarrhea, and there may well be several different mechanisms of action of probiotics. One is certainly the one you speculate about—there are indeed changes in the microenvironment, and I think Isolauri has data on that also. Another mechanism that may be playing a role in the case of LGG and rotavirus infection is the enhanced immune response, which is probably responsible for preventing the next episode. It also is likely that there are local changes in the physical chemical nature of the glycocalix. For example, Mack et al. (1) has shown in an *in vitro* system that *Lactobacillus plantarum* and LGG would upregulate MAC2 and MAC3, which are mucoproteins of the glycocalix, and so these changes in the physical chemical environment also might play a role. So it may well be that probiotics are active in the small intestine, although traditionally we think of the colon as their site of action. Rotavirus infection is a small-intestinal infection, there is no doubt about that, so we have to think about mechanisms of probiotic action in the small intestine as well.

**Dr. Marini:** If you divide your patients with diarrhea according to clinical severity—let us say on the basis of pH or base deficit—do you find the same advantages with *Lactobacillus* GG in comparison with placebo?

**Dr. Guandalini:** Although our population was very large, I do not think I can answer that question. Most of our patients were typical of the current presentation of acute diarrhea in Europe. In other words, they were all well nourished, and dehydration was either absent or mild in most cases. Only a couple of patients among this entire group were severely dehydrated or in shock. So I do not really believe that any of these patients was sick enough to allow us to assess the effect of the probiotic, differentiating those who had acidosis and a base deficit from those who did not.

**Dr. Marini:** I also am interested in the prevention study of hospital-acquired disease. Is there any evidence that probiotics not only may in some way prevent colonization from rotavirus but also may help to prevent urinary tract infection in girls? These kinds of probiotics are now widely used in adult women to treat urinary infection, and there is some clinical evidence that they are effective.

**Dr. Guandalini:** Indeed this is an interesting new area. Although I do not have any personal experience of the subject, I am aware that three strains of lactobacilli have been shown to colonize the vagina and prevent the ascent of uropathogens. A recent concise review on this has just appeared (2).

**Dr. Read:** You mentioned in the study of acute disease that you were able to decrease diarrhea but not infection with rotavirus. Could you comment on the mechanism of that effect?

**Dr. Guandalini:** This is a very interesting question. However, we are talking of very small numbers of patients at present, so we may be drawing unjustified conclusions. Nevertheless, if you have a rotavirus infection and you do not have diarrhea, it is likely that the lesions are less
extensive. The mechanism of action of rotavirus is to destroy the mature enterocytes at the tip of the villi, and this results in loss of absorptive function on one side of the enterocyte, whereas on the other side, it results in unmasking of secretory activity, which goes on in the crypt cells. An enterotoxin also has been described that may contribute to secretion by increasing intracellular calcium (3), so there is an osmotic and a secretory component to the diarrhea in rotavirus patients. Even though you may have the ability to detect rotavirus in the stool, its presence does not necessarily mean that a large portion of the intestine is affected by the process. I think this is the explanation, although at a speculative level.

**Dr. Duvina:** First, what is a rectal infusion of feces? Second, although I found your study interesting, the statistics are poor, because two or three patients do not give any degree of statistical security. Third, did you give the probiotics with or without antibiotics?

**Dr. Guandalini:** In patients with recurrent *C. difficile* colitis, feces from one of the patients himself, or a mixture of bacteria, were infused into the patient's rectum by enema to repopulate the colon with bacteria. This was reported in *Lancet*; there was some further correspondence as well (4). I do not think there has been any follow-up on this since. With regard to your criticism of the small numbers, in terms of *Lactobacillus GG* in children with acute diarrhea, this is the largest study ever done with any probiotic in pediatric patients and was conducted in a randomized, double-blinded, placebo-controlled fashion, so I do not think the criticism of poor statistics is justified! If you are referring to the four patients I presented in relation to Crohn's disease, I agree with you, but I drew no conclusions; I stated repeatedly that this is a pilot open-label study, and we should not draw conclusion from it. I also stated that we are now in the process of starting a multicenter Food and Drug Administration (FDA)-approved project to evaluate these patients in a scientifically sound way. Finally, the question about antibiotics: No, we never give antibiotics to patients with acute diarrhea because that is not a recommended way of treating diarrhea. If you are referring to the Crohn's patients, some of them were taking metronidazole. We checked the colonization with L-GG in every patient on each occasion and found that the use or nonuse of metronidazole had no influence on colonization with L-GG. That was the only antibacterial agent used.

**Dr. Alliet:** In the prevention study, you showed that there was no difference in rotavirus infection, but there was a difference in rotavirus diarrhea. Were these data obtained in infants while they were in the hospital?

**Dr. Guandalini:** Yes, they were all hospitalized children, aged 1–36 months.

**Dr. Alliet:** What happened once they were discharged?

**Dr. Guandalini:** This is not my study. These are studies by Dr. Szajewska, who was kind enough to allow me to present them to you, even though they are not yet published. What I can say is that I do not think they followed up the patients after discharge. They were followed up throughout their hospital stay, and the duration of diarrhea was as usual—between 3 and 4 days—and there were no complications.

**Dr. Marini:** I think that any studies on prevention should include data on the age of the babies and how were they fed—whether they were receiving mother's milk or not. This is a very important point. I would like to ask a question about the Crohn's patients. Why do you think their gut permeability returned to the pretreatment level after 180 days, even though they were continuing with the treatment?

**Dr. Guandalini:** We were not happy about that, as we would have hoped to see a continuous steady decline. The disease activity index continued to be relatively quiescent, although there is a known correlation between intestinal permeability and disease activity in Crohn's patients, which was the reason we wanted to look at that. I do not know why there was this "washout" effect, even with continued L-GG treatment. However, although in three of the
patients, there was a clear trend toward increased intestinal permeability, in the fourth, the permeability remained low. We need more patients to explore this further, as there may be interindividual differences.

Dr. Marini: Did the patients remain on the same diet, or did you change their diet?

Dr. Guandalini: We did not do any dietary interventions or make any changes to the drugs they were taking. We did not want to introduce any further variables.

REFERENCES


