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Probiotics in Clinical Conditions

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Abstract

Probiotics are nonpathogenic microorganisms which, when ingested, exert a positive influence on the health or physiology of the host. Their mechanisms of action and effects are now studied using the same pharmacological approach as for drugs. This article summarizes and comments on evidence for the positive effects of probiotics in various clinical situations. Substantial evidence can be achieved when randomized controlled trials or meta-analyses show positive results. The clinical situations studied include prevention or treatment of antibiotic-associated disorders, gastroenteritis, and diarrhea, lactose intolerance, intestinal infections and colonization by pathogenic bacteria (including *Helicobacter pylori* and *Clostridium difficile*), traveler's diarrhea, irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), colonic cancer, urogenital infections and tumors, allergy (especially atopic eczema), vaccination, and cholesterol lowering. Current probiotics have an excellent safety record—another topic discussed in this article.
Key words: Probiotics, *bifidobacterium*, *lactobacillus*, intestinal infections, antibiotic-associated diarrhea, gastroenteritis, traveler's diarrhea, intestinal flora, inflammatory bowel disease, colonic cancer, allergy, atopic eczema, oral vaccination, safety of probiotics.

Probiotics can be defined as nonpathogenic microorganisms which, when ingested, exert a positive influence on host health or physiology *(1)*. They consist of either bacteria, especially lactic-acid bacteria (LAB), or yeast (*Saccharomyces*), and are increasingly used in foods—especially fermented milks—and pharmaceutical products. Physicians have long been skeptical about their "real" efficacy *(2,3)*, and have raised three basic questions: are probiotic products stable, do they sur-

vive in the gastrointestinal tract, and what is the level of evidence for their efficacy? A pharmacological approach was used to assess the positive effects and potential side effects of the products as well as their pharmacokinetics *(2,4)*. The level of evidence for efficacy can be assessed with the rules of evidence-based medicine. We now know that the stability of many products (but not all) is good *(5)*, that many strains (but not all) survive in the gastrointestinal tract *(4)*, and that many double-blind randomized controlled trials (RCT) reported that some probiotics were more efficient than a placebo or a control treatment in specific clinical situations. The effects can be direct or indirect through modulation of the endogenous flora or of the immune system *(2)*. Some effects have clearly been demonstrated, and the level of evidence is still lower for other effects. The article summarizes the present knowledge on the clinical applications of probiotics in humans.

Gastrointestinal Diseases

Probiotics do not cross the intestinal barrier, and the first step of their effects occurs either in the lumen or in the wall of the gastrointestinal tract, where they can interact with the endogenous flora, luminal substrates, the mucus, the enterocytes, and the intestinal immune cells (gut-associated lymphoid tissue).

Antibiotic-Associated Intestinal Disorders

Intestinal disorders, especially diarrhea, occur frequently in patients who receive antibiotics. They result from a decrease in two fundamental physiological properties of the endogenous flora: colonization resistance and fermentation capacity (microbial imbalance). Colonization with *Clostridium difficile* or *Klebsiella oxytoca* may induce colitis. Several attempts have been made to determine whether administration of probiotics would prevent antibiotic-associated intestinal disorders (mainly diarrhea). The RCT which demonstrate a significant effect with a sufficient statistical power are shown in Table 1. The level of evidence is high for *Saccharomyces boulardii*, *Lactobacillus rhamnosus* GG, and *Enterococcus faecium* SF68. Three randomized, double-blind, placebo-controlled studies have demonstrated that oral administration of *S. boulardii* can decrease the risk of diarrhea (*6–8*; Table 1). Another trial failed to demonstrate a therapeutic benefit of the probiotic in elderly subjects *(9)*. And finally, another study showed that *S. boulardii* significantly shortened the duration of diarrhea *(10)*. The dose used in the most recent (and most convincing) studies was 1 g/d, and the evidence for the efficacy of lower doses is lower. The mechanism involved is unclear, because multiple biological effects of the yeast have been demonstrated which may contribute to the clinical efficacy (i.e., effects

Table 1
RCT Showing a Significant Therapeutic Effect of Probiotics to Prevent Antibiotic-Associated Diarrhea

Probiotic	Antibiotic	No. of subjects	% of diarrhea: probiotic/control	Reference
Saccharomyces boulardii	β-lactamins or tetracyclins	388	4.5 vs 17.5	*(6)*
Saccharomyces boulardii	Miscellaneous	180	9.5 vs 21.0	*(7)*
Saccharomyces boulardii	β-lactamins	193	7.2 vs 14.6	*(8)*
Lactobacillus rhamnosus GG	Miscellaneous	188	8 vs 26	*(13)*
Lactobacillus rhamnosus GG	Miscellaneous	119	5 vs 16	*(14)*
Enterococcus faecium SF68	Antituberculous	200	5 vs 18	*(15)*
Enterococcus faecium SF68	Miscellaneous	45	8.7 vs 27.2	*(16)*
Lactinex*	Ampicillin	98	8 ?3 vs 21	*(17)*
Lactinex*	Neomycin	39	20 vs 42	*(18)*
Lactinex*	Amoxicillin clavulanate	27	Positive effect	*(19)*

*Association of *L. acidophilus* and *L. bulgaricus*

against the population levels of *C. difficile*, the toxins, and intestinal secretion *(11,12)*.

Two recent double-blind RCT with a high number of subjects showed that *L. rhamnosus* GG (here also at high doses) was effective *(13,14)*. *E. faecium* SF 68 was more efficient than the placebo in two RCT *(15,16)*, and lactinex—a mixture of *L. acidophilus* and *L. bulgaricus*—was effective in three RCT *(17–19)*. The therapeutic efficacy of other probiotics is not established. Dose-response studies are lacking and the cost-effectiveness of systematic prescription of probiotics has not been assessed. However, many physicians consider (at least in countries where efficient products are available) that probiotic prevention with an active strain is indicated in subjects with a high risk, such as elderly subjects or patients receiving several antibiotics or those who had previous episodes of antibiotic-associated intestinal disorders *(20)*.

Gastroenteritis

Gastroenteritis, the main cause of acute diarrhea, is a frequent disorder which heals usually spontaneously within a few days. The use of oral rehydration solutions is the main treatment, especially in infants and elderly people, but it does not reduce the occurrence of diarrhea.

Table 2
RCT Showing a Significant Therapeutic Effect of Probiotics to Shorten the Duration of Acute Gastroenteritis

Probiotic	Situation	No. of subjects	Reference
Lactobacillus rhamnosus GG	Rotavirus diarrhea in infants	71	*(21)*
Lactobacillus rhamnosus GG	Rotavirus diarrhea in infants	39	*(22)*
Lactobacillus rhamnosus GG	Rotavirus diarrhea in infants	49	*(23)*
Lactobacillus rhamnosus GG	Rotavirus diarrhea in infants	42	*(24)*
Lactobacillus rhamnosus GG	Gastroenteritis in infants	32	*(25)*
Lactobacillus rhamnosus GG	Gastroenteritis in infants	26	*(26)*
Lactobacillus rhamnosus GG	Gastroenteritis in infants	100	*(27)*
Lactobacillus rhamnosus GG	Gastroenteritis in infants	123	*(28)*
Lactobacillus rhamnosus GG	Gastroenteritis in infants	287	*(29)*
Enterococcus faecium SF68	Gastroenteritis in infants	104	*(30)*
Enterococcus faecium SF68	Gastroenteritis in adults	56	*(31)*
Enterococcus faecium SF68	Gastroenteritis in adults	78	*(16)*
Enterococcus faecium SF68	Gastroenteritis in adults	211	*(32)*
Saccharomyces boulardii	Gastroenteritis in infants	38	*(33)*
L. casei Shirota	Rotavirus diarrhea in infants	32	*(34)*
L. reuteri	Gastroenteritis in infants	66	*(35)*

Curative Treatment

Several RCT have demonstrated a beneficial effect of some, but not all, probiotics in medicines or fermented dairy products in infantile or adult gastroenteritis *(14,21–35)*. *L. rhamnosus* GG repeatedly shortened diarrhea to about one-half in infants with rotavirus diarrhea (*21–24*; Table 2). It also proved effective in the treatment of acute diarrhea in children in Asia *(25,26)*. In one of the last RCT *(29)*, the probiotic was added to an oral rehydration solution. Two hundred and eighty-seven children of 1–36 mo of age with acute diarrhea were enrolled; they received the oral solution plus *L. rhamnosus* GG (at least 10^9 colony-form-

ing units per 250 mL) or placebo. The duration of diarrhea was significantly reduced by the probiotic in the children with rotavirus infection: 56 ± 17 h vs 77 ± 42, but not in those who were rotavirus-negative (n = 186). *L. rhamnosus* GG administration also shortened the duration of hospital stay *(29)*. Heat-inactivated *L. rhamnosus* GG was clinically as effective as the living lactobacillus on diarrhea in one study, although the living probiotic had a more pronounced effect on the rotavirus-specific IgA response *(36)*. Another trial showed that heat-killed lactobacilli (Lacteol fort®, Lactéol du Dr Boucard, France) shortened gastroenteritis in adults *(37)*. *E. faecium* strain SF 68 significantly shortened diarrhea in four RCT (*14,30–32*; Table 2). Other probiotics are probably also effective, but the evidence is lower (Table 2).

Prevention

Saavedra et al. *(38)* demonstrated for the first time that feeding some probiotics to infants admitted to a hospital could significantly reduce the risk of diarrhea and shedding of rotavirus. In a double-blind placebo-controlled trial, 55 children admitted to a chronic medical-care unit were randomized to receive a standard formula or the same plus *Bifidobacterium bifidum* and *S. thermophilus*. During follow-up, diarrhea occurred in 7% of the children who received the probiotics vs 31% of the controls. The shedding rotavirus was also significantly reduced: 10% vs 39% *(38)*. In a double-blind RCT performed in Poland *(39)*, 81 children aged 1 to 36 mo who were hospitalized for reasons other than diarrhea received either *L. rhamnosus* GG 6×10^9 CFU or a placebo twice daily orally for the duration of their hospital stay. The probiotic reduced the risk of nosocomial diarrhea (6.7% vs 33.3%). The prevalence of rotavirus infection was similar in *L. rhamnosus* GG and placebo groups (20% vs 27.8%), but the risk of rotavirus gastroenteritis was reduced (2.2% vs 16.7%). Another double-blind RCT performed in Italy, which included 269 children, could not confirm the protective effect of the same strain against nosocomial infection with rotavirus *(40)*. In the RCT by Oberhelman et al., which included 204 undernourished Peruvian children, *L. rhamnosus* GG had no preventive effect against rotavirus and could not protect breastfed infants against diarrhea, but it reduced the risk of diarrhea in non-breastfed infants (4.7 episodes of diarrhea per infant per yr in the probiotic group vs 5.9 in the placebo group) *(41)*.

Despite the accumulation of positive studies, the practical use of probiotics in gastroenteritis is still limited, probably (at least partly) because available products (or formulations or doses) have not been properly assessed. Thus, efforts should be made to perform clinical studies with available products, formulations, and doses.

Lactose Intolerance

Lactose maldigestion is a frequent situation, especially in adults, and in subjects with acute or chronic enteritis or bowel resection. Alleviation of lactose intolerance has been the first demonstrated effect of probiotics *(2)*. The best evidence has been obtained with yogurt bacteria, which combine two advantageous properties: they are rich in lactase, and they are rapidly lyzed in the gastrointestinal tract—not only by acid in the stomach, but also by bile in the duodenum. The mechanisms leading to the better digestion of the lactose contained in yogurt than that contained in milk have been extensively studied. A role for viable LAB has been theorized, because the digestibility of lactose from yogurt is higher than that from pasteurized yogurt *(42,43)*. Two mechanisms, which do not exclude each other, have been demonstrated: digestion of lactose in the gut lumen by the lactase brought by the yogurt bacteria, and slower intestinal delivery or transit time of yogurt as compared to milk *(42,43)*. Other probiotics containing lactase—such as *L. acidophilus*—may also be active, but their higher resistance to bile probably explains why they are less efficient than yogurt bacteria *(43)*. In clinical practice, the replacement of milk by yogurt or fermented dairy products allows better digestion, and/or decreases diarrhea and other intolerance symptoms in subjects with lactose intolerance, in children with diarrhea and in subjects with short-bowel syndrome *(42–44)*.

Preliminary trials have suggested that sucrase or lipase could be delivered in the small bowel in subjects or animals with enzyme deficiency using natural or genetically modified probiotic vectors *(45,46)*, but this original and promising method of delivery must be studied in more detail.

Intestinal Infections and Colonization by Pathogenic Bacteria

The protective effects of probiotics against intestinal infections have been demonstrated in animal models *(47,48)*. The mechanisms which may be implicated include the production of acids, hydrogen peroxide, or antimicrobial substances, competition for nutrients or adhesion receptors, antitoxin actions, and stimulation of the immune system.

Open studies have suggested a beneficial role of *L. rhamnosus* GG, *S. boulardii*, and *L. plantarum* LP299v during *Clostridium difficile*-related infections. However, it is not possible to draw firm conclusions from open studies in such an unstable clinical situation. Two placebo-controlled RCT have demonstrated some efficacy of *S. boulardii* to decrease the risk of recurrence of *C. difficile* infection *(49,50)*. The first trial compared the efficacy of the standard antibiotic treatment combined either with *S. boulardii* (1 g/d for 28 d) or with a placebo. The risk of clinical

recurrence for the subjects with several episodes of *C. difficile* infection was significantly reduced by the probiotic: 34.6% vs 64.7% ($p = 0.04$) *(49)*. In the second study, a significant decrease in the risk of recurrence was observed in the subgroup of patients treated with a high dose of vancomycin plus *S. boulardii* vs those who received a high dose of vancomycin plus placebo *(50)*.

Colonization of the gastric mucosa by *Helicobacter pylori* is strongly associated with gastritis, duodenal and gastric ulcers, gastric carcinoma, and lymphoma. Such colonization is frequent (about one-half of the adults in the world), and eradication which requires two antibiotics and inhibition of gastric-acid secretion is only considered (because of its cost and side effects) for patients with ulcers or lymphoma. Antagonistic actions of some lactobacillus strains against *H. pylori* in vitro have been reported *(51)*. A significant reduction of the urease activity (of H. pylori) has been reported in patients treated with a supernatant of *L. johnsonii* LA1 (Nestlé, Switzerland) associated with omeprazole *(52)*. Two RCT have recently reported that the ingestion of a fermented dairy product containing this strain or a heat-killed *L. acidophilus* could help to decrease colonization by *H. pylori (53,54)*, yet confirmation is needed. Armuzi et al. *(55)* performed a RCT in 60 asymptomatic subjects who screened positive for *H. pylori* infection. All subjects received an usual treatment—i.e., rabeprazole, clarithromycin, and tinidazole (500 b.d.). One-half also received *L. rhamnosus* GG for 14 d, and the others received a placebo. The efficacy of the treatment did not differ between the two groups (83% vs 80%) but the tolerance was better in the probiotic group.

Traveler's Diarrhea

Acute diarrhea occurs frequently in travelers to high-risk areas. Antibiotics are effective prophylaxis, but are not recommended for widespread use because of their cost and side effects *(56)*. Several studies have failed to show any effect of lactobacilli *(2)*; however, three double-blind RCT suggested some efficacy of *L. rhamnosus* GG and S. *boulardii (57–59)*. Unfortunately, many subjects were lost for follow-up, and the statistical analysis of these studies is imperfect. A first trial reported a reduction of diarrhea by *L. rhamnosus* GG administration in subjects who traveled to one destination in Turkey *(69)*; however, the effect was not observed in subjects traveling to another destination. In another study, 400 American travelers were randomized to receive *L. rhamnosus* GG or a placebo *(70)*. More than one-third were excluded from the analysis because they did not take the medication. In subjects who took the capsules, the risk of diarrhea was 3.9% with the probiotic vs 7.4% with the placebo ($p = 0.05$). In the double-blind placebo-controlled trial which suggested the efficacy of *S. boulardii (68)*, only 1,016

of 3,000 Austrian travelers were compliant. Although the level of evidence is thus still too low to recommend any probiotic to prevent traveler's diarrhea, the evidence of a beneficial effect is strong, and further studies should be performed.

IBS and Various Conditions with Diarrhea

Some probiotics, including acidophilus or bifidus milks, have been reported to relieve constipation in short series of patients *(2)*; however, these studies were not controlled. An open study suggested that ingestion of propionibacteria may slow down the transit time in the left colon in healthy men, and two RCT showed that a milk fermented by *Bifidobacterium animalis* strain DN-173 010 shortened the colonic transit time in women *(60,61)*. In a randomized placebo-controlled study including only 34 patients, Maupas et al. observed that *S. boulardii* decreased functional diarrhea but did not affect other symptoms of IBS *(62)*. Halpern et al. *(63)* suggested in a randomized, double-blind, crossover trial that administration of heat-killed lactobacilli (Lacteol fort®) for 6 wk was more efficient than placebo to relieve symptoms of IBS. However, only 18 of 29 randomized subjects were studied, and this poor compliance is a weakness of the study. Hentschel et al. assessed the efficacy of two probiotic preparations containing lactobacilli and *E. coli* (Hylac® and Hylac N forte®, Germany) in 126 subjects suffering from non-ulcer dyspepsia, and no relief was observed *(64)*. IBS is a fluctuating disorder, and the placebo effect is often high. At the present time, the level of evidence that probiotics may help subjects with IBS is very low, but this is an area of potential interest.

S. boulardii decreased the diarrhea induced by tube feeding in three trials *(65–67)*. In a double-blind RCT which included 128 critically ill tube-fed patients, *S. boulardii* 2 g/d significantly reduced the percentage of days with diarrhea from 18.9 to 14.2 *(67)*. Two open studies suggested that lactobacilli may have some efficacy against small-intestine bacterial overgrowth *(68,69)*, but *S. boulardii* was ineffective in the only RCT *(70)*. Diarrhea is a nearly constant side effect of irradiation of the pelvis. A preliminary study by Salminen et al. *(71)* reported a significant decrease in diarrhea in patients receiving *L. acidophilus* NDCO 1748 during pelvic irradiation. This potentially interesting therapeutic effect needs further study.

Inflammatory Bowel Disease (IBD)

Inflammatory bowel disease (IBD) is a group of disorders characterized by chronic or recurrent intestinal inflammation. These include Crohn's disease, ulcerative colitis, and pouchitis. The cause is unknown, but the main theory is that IBD results from an abnormal immunological response to some members of the endogenous flora *(72)*.

Table 3
Results of RCT Which Reported Efficacy of Probiotics in Patients With IBD

Probiotic	Situation	Control	No. of subjects	Duration	Relapse % probiotic vs control	Reference
E. coli Nissle 1917	Ulcerative colitis	5-ASA	120	4 mo	16 vs 11.3	*(74)*
E. coli Nissle 1917	Ulcerative colitis	5-ASA	120	12 mo	67 vs 73	*(75)*
E. coli Nissle 1917	Crohn's disease	placebo	28	12 mo	30 vs 70*	*(76)*
VSL#3	Pouchitis	placebo	40	9 mo	15 vs 100*	*(77)*
VSL#3	Prevention of pouchitis	placebo	40	12 mo	10 vs 40*	*(78)*
VSL#3	Crohn's disease**	5-ASA	28	12 mo	20 vs 40*	*(79)*
S. boulardii	Ulcerative colitis	5-ASA	31	12 mo	30 vs 35*	*(80)*
S. boulardii	Crohn's disease	5-ASA	28	6 mo	6.3 vs 37.5*	*(81)*

* $p < 0.05$
** Postoperative
5-ASA: 5-aminosalicylic acid

Several RCT have recently been performed with probiotics in various conditions of IBD (*73*; Table 3). The evidence for a relevant effect is already nearly strong enough to prescribe three probiotics in patients: VSL#3, *E. coli* Nissle 1917, and *S. boulardii* (*74–81*; Table 3). VSL#3 (CSL, Milan, Italy) contains 300 billion viable lyophilized bacteria per g of four strains of lactobacilli (*L. casei, L. plantarum, L. acidophilus, L. bulgaricus*), three strains of bifidobacteria (*B. longum, B. breve, B. infantis*), and one strain *of S. thermophilus* (77).

Ulcerative Colitis

Two RCT compared the efficacy of *E. coli* Nissle 1917 to mesalazine—i.e., the standard treatment for the maintenance of remission in ulcerative colitis *(74,75)*. Kruis et al. *(74)* included 120 patients with inactive ulcerative colitis in a double-blind double-dummy study. One-half received 1.5 g/d of mesalazine, and the other half received 200 mg/d of mutaflor (Ardeypharm GmbH, Herdecke, Germany) which contains 25×10^9 viable *E. coli* bacteria per 100 mg. After 12 wk, 11.3% of the subjects who received mesalazine and 16% of those who received the probiotic had relapsed, and this difference was not significant. In the second trial, *E. coli* strain Nissle 1917 was compared to mesalazine in 116 patients with ulcerative colitis *(75).* Patients with active colitis were randomized to receive 2.4 g/d of mesalazine or 200 mg/d of mutaflor. All patients were also given a 1-wk course of oral gentamycin 240 mg/d, and steroids. Remission was obtained in 75% of the patients in the mesalazine group vs 68% in the *E. coli* group (difference NS). When remission was reached, the steroids were stopped, and the dose of mesalazine was reduced to 1.2 g/d. After 1 yr, relapse occurred in 73% of the patients in the mesalazine group vs 67% in the *E. coli* group (difference NS). Despite the results of these two trials, the efficacy of *E. coli* Nissle 1917 to prevent recurrence of ulcerative colitis is still questionable. Indeed, no trial has compared this strain to placebo, the statistical power of the first study was too low to conclude that both treatment had equivalent efficacy, and the efficacy of mesalazine in the second study was far lower than the usual percentage. Ishikawa et al. *(82)* treated 21 subjects with 100 mL/d of fermented milk for 1 yr. The fermented milk contained a probiotic mixture with *B. bifidum* YIT 4007, *B. breve* YIT 4065, and *L. acidophilus* YIT 0168 in one-half of the subjects. The risk of relapse was significantly lower in the group who received the probiotic: 27% vs 90%.

Pouchitis

Pouchitis is an inflammation of the ileal pouch that is created by the surgeon during ileoanal anastomosis. An imbalance of the endog-

enous flora has been shown in such conditions, especially with reduced counts of bifidobacteria and lactobacilli *(83)*. Gionchetti et al. performed a double-blind RCT comparing the effect of VSL#3 and placebo to prevent recurrence of chronic relapsing pouchitis *(77)*. Forty patients with chronic relapsing pouchitis were studied. Remission was induced by 1 mo of ciprofloxacin and rifabutin, and the probiotic mixture (6 g/d) or the placebo were then prescribed for 9 mo. A relapse occurred in 15% of the subjects in the VSL#3 group vs 100% in the placebo group ($p < 0.001$). The authors showed that the fecal concentration of lactobacilli, bifidobacteria, and streptococci increased in the VSL#3 group, and in another study, that continuous treatment with VSL#3 increases the tissue levels of IL-10 in the inflamed pouch *(84)*. The same authors studied the effect of VSL#3 to prevent pouchitis in 40 patients who had colectomy and ileo-pouch anal anastomosis for ulcerative colitis *(78)*. Patients received either VSL#3 (3 g/d) or placebo for 1 yr after surgery. The risk of pouchitis was significantly lower in the probiotic group: 10% vs 40%.

Crohn's Disease

Several trials suggested that *S. boulardii* has some efficacy in the treatment of Crohn's disease. Plein & Hotz *(85)* performed a pilot double-blind RCT to test the efficacy of the probiotic on symptoms. Twenty patients with active disease were randomized to receive either *S. boulardii* or a placebo for 7 wk, together with the standard treatment. A significant reduction in the frequency of bowel movements and in the disease activity was observed only in the group receiving the probiotic. In a double-blind RCT, 32 patients with Crohn's disease in remission received either 1 g/d of *S. boulardii* plus mesalazine 2 g/d or mesalazine 3 g/d *(81)*. The risk of relapse at 1 yr was significantly lower in the probiotic group (1/16 vs 6/16). Campieri et al. compared the efficacy of a combination of rifaximin 1.8 g/g for 3 mo, followed by either VSL#3 or mesalazine 4 g/d to prevent postoperative recurrence of Crohn's disease *(79)* in 40 patients. After 1 yr, the risk of relapse was lower in the probiotic group (20% vs 40%). This trial has been published only as an abstract at the present time. In a double-blind RCT, Malchow treated 28 subjects suffering from Crohn's disease of the colon with *E. coli* Nissle 1917 or placebo *(76)*. The rate of relapse was lower in the probiotic group (33% vs 63%).

Although clearly promising, these trials must still be confirmed with a higher number of patients and by independent research teams. Many trials with the same strain and others are ongoing, and knowledge is progressing rapidly in this field *(73)*.

Colon Cancer

The endogenous flora and the immune system play a role in the modulation of carcinogenesis. As both may be influenced by probiotics, the efficacy of probiotics to prevent or cure tumors has been studied in various animal models *(86,87)*. Several human trials have shown that some probiotics may reproducibly decrease the fecal levels of enzymes, mutagens, and secondary bile salts which may be involved in colon carcinogenesis *(87)*. In addition, some epidemiological studies suggested that consumption of fermented dairy products may have some protective effect against large colon adenomas *(88)*. These results provide a good framework for future intervention trials.

Extraintestinal Diseases

Urogenital Infections and Tumors

Lactic-acid bacteria are normal inhabitants of the human genital tract, which probably help to avoid colonization by pathogens. Uncontrolled studies have suggested some potential for probiotics (either oral or local) for urogenital infections *(89,90)*. Several preparations are largely used by patients, despite the absence of evidence for positive effects *(89)*. Colonization of the vagina by orally administered lactobacilli has been proven in women *(91)*. One RCT showed a significant effect of *L. acidophilus* therapy to prevent recurrence of candidal vaginitis *(92)*. The study was not blinded, and many subjects were required to be excluded from statistical analysis. Hallén et al. studied the effect of vaginal suppositories containing lactobacilli of human origin *(93)*. Sixty women with bacterial vaginosis were randomized to receive two vaginal suppositories per d for 6 d, which contained either lactobacilli or starch (as a placebo). Immediate cure of bacterial vaginosis following treatment was obtained in 57% of the patients in the probiotic group vs 0% in the placebo group. However, when the efficacy of treatment was checked again after the next menstrual period, the difference between groups was not significant (21% vs 0%). Clearly, more studies are needed before any recommendation can be made *(89,90)*.

Two RCT from the same Japanese team showed that oral administration of *L. casei* (biolactis powder) significantly decreased the risk of recurrence of superficial bladder tumors *(94,95)*. In the first study, which included 58 patients, the 50% recurrence-free interval after initial surgery was significantly increased in the probiotic group: 350 d vs 195 *(94)*. In the second study, the significant preventive property of the probiotic was confirmed in patients with primary multiple tumors and patients with recurrent single tumors, but not in those with recurrent multiple tumors *(95)*.

Clinical Immunology

Allergy

Erica Isolauri, together with Seppo Salminen and their group, recently published two intriguing double-blind RCT which strongly suggest the efficacy of probiotics, especially *L. rhamnosus* GG, to cure or prevent atopic eczema in infants *(96,97)*. In the first trial, 27 breastfed infants suffering from atopic eczema were randomized to be weaned either with probiotic supplemented, extensively hydrolyzed whey formulas, or with the same formula without probiotic *(96)*. Two probiotics were studied: *B. lactis* Bb12 and *L. rhamnosus* GG. Treatment efficacy on atopic eczema was assessed with the SCORAD score. After 2 mo of treatment, the score was significantly lower in both probiotic groups when compared to the placebo group (SCORAD 0, 1, and 13.4, respectively). The mechanism involved is not established, but the authors suggest that probiotics counteracted inflammatory changes beyond the intestinal environment. This interesting result led the authors to try *L. rhamnosus* GG to prevent atopic eczema. The probiotic was given prenatally to mothers who had at least one first-degree relative with atopic eczema, allergic rhinitis, or asthma, and then postnatally for 6 mo to their infants *(97)*. The end point was the occurrence of atopic eczema in the infants before the age of 2 yr. One hundred and fifty-nine subjects were included, and 132 completed the 2-yr study. The frequency of atopic eczema was reduced by one-half in the infants who received the probiotic (23% vs 46%, $p = 0.008$). Again, the mechanism is still not understood, yet the result is consistent with population-based studies which showed that increased exposure to bacteria are protective against allergy *(98)*.

Vaccination

Probiotics may influence the local or systemic immune response in animals and humans *(99)*. Two clinical applications have been imagined: the first is the adjuvant effect of probiotics during vaccination, and the second is the use of genetically modified probiotics to develop new oral vaccines. Link-Amster et al. *(100)* demonstrated an adjuvant effect of a fermented milk containing *L. johnsonii* LA1 and bifidobacteria in humans receiving an oral vaccination with attenuated *Salmonella typhi* Ty21a. Thirty volunteers were randomized to receive either the fermented milk or no fermented milk. Both groups received the *S. typhi* Ty21a orally, and the specific antibodies against *S. typhi* Ty21a were measured in blood. The probiotic group showed a >fourfold rise in antibody titer, whereas the control group had a 2.5-fold rise in titer ($p = 0.04$). Fang et al. used a similar protocol in 30 volunteers who received *L. rhamnosus* GG, *L. lactis* or a placebo as adjuvants either there was no

difference in the specific IgA response against *Salmonella* in the three groups *(101)*. In another study, infants received an oral live rotavirus vaccine together with either *L. rhamnosus* GG or a placebo for 5 d *(102)*. Rotavirus IgA conversion was higher in the probiotic group (93% vs 74%; $p = 0.05$). Some probiotics may thus behave as adjuvants, and may improve the immunogenicity of oral vaccines. However, this result should not be extrapolated to all probiotics and all vaccines. The use of genetically modified probiotics as live vectors for oral immunization has been recently proposed. Results obtained thus far demonstrate that lactobacilli are capable of delivering antigen to the mucosal and systemic immune systems following intranasal, intravaginal, or intrarectal immunization. The importance of colonization or adhesion in oral administration remains an open question *(103)*.

Cholesterol Lowering Effect

A few RCT have suggested that some probiotics, including lactobacilli, *S. thermophilus*, and *E. faecium*, may have moderate hypocholesterolemic properties. However, it is impossible to draw conclusions from these studies on the efficacy of the probiotics by themselves, because of the presence of confounders such as changes of fat contents in the diet, the absence of a proper placebo (as milk has also hypocholesterolemic properties), and the insufficiency of methodological details in some publications *(104)*. Many studies also used very large quantities of fermented milks, which would hardly be accepted by the general population. Early deconjugation of bile salt in the small bowel by ingested probiotics has been proposed as a potential mechanism which may help to increase the fecal excretion of bile salts (and thus that of cholesterol) *(104,105)*. However, others believe that effective bile-salt deconjugation in the small bowel has a high risk of inducing secretory diarrhea *(106)*. At the present time, there is no evidence that a commercialized product consumed in a reasonable quantity could have any relevant intrinsic hypocholesterolemic property.

Safety Aspects

The safety of the current products is excellent. However, probiotics as living microorganisms may theoretically be responsible for four types of side effects: systemic infections, deleterious metabolic activities, excessive immune stimulation in susceptible individuals, and gene transfer. Probiotics are not selected among pathogens, and the theoretical risk of infections is very low. Rare cases of local or systemic infections, including septicemia and endocarditis caused by lactobacilli, bifidobacteria, or other LAB, have been reported *(107)*. Most *Lactobacillus* strains isolated from clinical cases are members of to the species *L.*

rhamnosus, *L. casei* or *paracasei*, and *L. plantarum*. *E. faecium* and *E. faecalis* are more frequently involved in clinical infections, and there is concern over the emergence of vancomycin-resistant strains. In most cases of infection, the organism appeared to originate from the patient's own microflora. However, in a few cases, the recent use of probiotics by the subject was mentioned as a potential cause. Thirteen cases of fungemia have been reported in humans treated with *S. boulardii (108)*, and two case of infection have been traced back to food-borne *L. rhamnosus (109,110)*. All thirteen subjects who had a fungemia had an indwelling vascular catheter *(108)*. Contamination of the air, environmental surfaces, and hands of the nurses following the opening of the probiotic packets strongly suggested that catheter contamination was the source of infection. The case of infection caused by *L. rhamnosus* similar to the GG strain was observed in a 74-yr-old woman with non-insulin-dependent diabetes, who reported a daily intake of dairy drinks containing *L. rhamnosus* GG during the 4 mo before the onset of her symptoms *(109)*. She suffered from a liver abscess, associated with a right basal pneumonia and a right-sided pleural empyema. Hepatic abscess aspirate showed that the microorganism was a *L. rhamnosus*, which appeared to be indistinguishable from the GG strain. The other case included a 67-yr-old man with a mild mitral-valve regurgitation, and carious teeth which needed to be removed. This man was accustomed to chewing probiotic capsules containing a mixture of *L. rhamnosus, L. acidophilus*, *E. faecalis*. He received amoxicillin 1 h before the dental extraction, and suffered a few days later from an endocarditis. *L. rhamnosus* was isolated from several blood cultures, and further analysis showed that the *L. rhamnosus* cultured from the probiotic capsule was indistinguishable from that isolated from the blood *(110)*. Saxelin et al. *(111,112)* studied the prevalence of bacteriemia resulting from *Lactobacillus* species in Southern Finland during a 4-yr period and a 6-yr period, and compared the characteristics of the blood-culture isolates and of dairy strains. The studies included 3,317 and 5,912 blood-culture isolates respectively, and none of them corresponded to a dairy strain. One may therefore conclude that although a zero risk does not exist, the risk is extremely low. Furthermore, classical risk factors for opportunists, such as extremes of age, pregnancy, immunodeficiency, or digestive lesions, have not been identified as risk factors for probiotic infections *(107)*.

Conclusions

Strong evidence for the positive effects of some probiotics in some specific clinical situations supports further research. Extrapolation of positive (or negative) results from one probiotic to another, or from one situation to another, cannot be made. The development of probiotic use

in clinical practice will now depend on the availability of probiotic preparations, and of studies testing them in the real clinical conditions of products, formulations, and doses. When considering that the endogenous flora clearly plays a role in many diseases (even if it is not the cause of the disease), important developments in probiotic products can be expected.

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