Journal of Pediatric Gastroenterology and Nutrition
48:126–141 © 2009 by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition and North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition

Invited Review

Clinical Evidence for Immunomodulatory Effects of Probiotic Bacteria

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ABSTRACT

Close, tightly orchestrated interactions between the intestinal epithelium and the mucosa-associated immune system are critical for normal intestinal absorptive and immunological functions. Recent data indicate that commensal intestinal microbiota represents a major modulator of intestinal homeostasis. This review analyzes the process of intestinal colonization and the interaction of microbiota with the intestinal epithelium and mucosal immune system, with special reference to the first years of extrauterine life. Dysregulation of the symbiotic interaction between intestinal microbiota and the mucosa may result in a pathological condition with potential clinical repercussions. Based on the concept that there is a beneficial and symbiotic relation between the host and endogenous microbiota, strategies aimed at directly modulating intestinal microbiota with regard to disease prevention or treatment have been developed. One strategy involves administering viable probiotic bacteria. Clinical evidence for the beneficial effect of probiotics in the prevention and/or treatment of necrotizing enterocolitis, infectious and antibiotic-associated diarrhea, allergic diseases, and inflammatory bowel disorders is reviewed herein. ***JPGN 48:126–141, 2009.*** **Key Words:** Microbial-epithelial crosstalk—Gut-associated immunity—Defensins—Probiotics—Necrotizing enterocolitis—Allergy.

The intestinal mucosa, with an area exceeding 300 m^2, is continuously exposed to a plethora of foreign antigenic molecules of both dietary and microbial origin. To ensure normal absorptive intestinal function, intestinal mucosal balance and homeostasis are necessary. These require close regulation of the intestinal epithelial barrier, enabling the efficient uptake of nutrients without eliciting an adverse immune reaction while concomitantly protecting the host from potentially harmful agents. To manage those conflicting tasks, an extremely complex control system has evolved within the intestinal mucosa, providing for immune tolerance of some antigens while ensuring protection against potential pathogens (1,2). A close interaction between the intestinal epithelium and mucosa-associated immune system is thus critical.

The concept of synergistic interaction between the intestinal epithelium and the immune system was recently expanded to include intestinal microbiota, which is now considered to be a third and major player that is indispensable for optimal intestinal function (1–6). In the present review, the interaction of the microbiota with the intestinal immune system and its modulation by the use of probiotics are discussed, with an exhaustive review of clinical evidence of potential effects of modulation of the microbiota by the use of probiotics on the prevention or treatment of diseases.

Received October 4, 2007; accepted April 23, 2008.

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Dr Marteau has received compensation from Danone, Rosel Lallemand, Biocodex, Merck Famille Santé, and PiLeJe for consultation and/or speaking engagements. The other authors report no conflicts of interest.

BACTERIAL COLONIZATION OF THE INTESTINAL TRACT

At birth, the neonate leaves a germ-free intrauterine environment and enters a highly contaminated extrauterine world. Within the first few hours of birth, the process

of intestinal colonization takes place. A huge variety of factors influences the initial colonization process, such as gestational age, the mode of delivery, the neonatal diet, and genetic factors (7,8). The maternal flora constitutes the predominant source of initial colonization. The first bacteria colonizing the neonatal colon are thus *Escherichia coli* and various *Enterococcus* species (9). Obligate anaerobes follow. In breast-fed babies, *Bifidobacterium* species predominate, whereas in formula-fed neonates *Bacteroides* species predominate and only a few *Bifidobacterium* sp. are present (10). The use of new molecular approaches will confirm these mainly culture-based analyses and may open the door for new major or dominant bacterial strains during the initial colonization process. Schwiertz et al (8) recently demonstrated the extent to which the environment can imprint on the composition of the colonic microbiota. In their longitudinal study using molecular techniques, they observed that the interindividual bacterial composition of the colonic flora in hospitalized preterm infants was markedly reduced compared with a large interindividual diversity in breast-fed full-term babies. The intestinal microbiota evolves during the first 2 years of life toward a stable and definite future adult pattern that presents in adulthood. As discussed, this process is influenced by environmental and lifestyle factors such as eating habits and infections. Given the relative instability of the intestinal colonization process during the first months of life, it is not surprising that any disturbance of this process may affect the microbiota, which may, in turn, have an impact on function and also potentially on the host's health.

The use of modern methods in molecular biology has provided further insight into the diversity of intestinal microbiota, which comprises hundreds of different species (11) that form a complex and highly interactive biomass (microbiome) of at least 10^{14} bacteria within the human gastrointestinal tract (Fig. 1). This microbiome contains more than 100-fold more genes than the human genome. This raises the question of the biological advantage of harboring such an enormous biomass within the human gut. One advantage could be that the microbial genome may contain coded information for functions that the human species have not developed during evolution. This genetic contribution may be a major ecological and biological advantage. It has long been established that in ruminants, the rumen flora contributes to fermentation of ingested nondigestible polysaccharides and resulting monosaccharides into short-chain fatty acids (4,12,13). The importance of this flora–host metabolic interaction was further highlighted by a recent comparison of germ-free and conventionally raised mice. Young adult conventionally raised mice had approximately 40% more total body fat than germ-free mice fed the same diet, even though the conventionally raised mice had lower energy intake (5,14,15). The microbiota thus provides an advantage in energy storage after a low intake of energy. Bacterial enzymes allow the host to salvage energy from

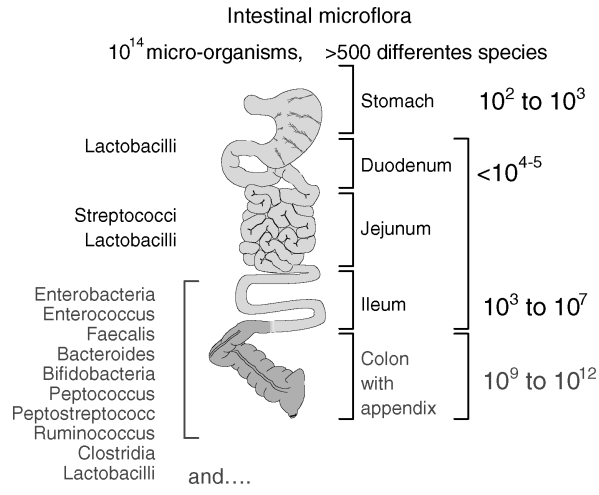

FIG. 1. Commensal intestinal microflora. Gradient along the gastrointestinal tract with $>10^{14}$ microorganisms and at least 500 different species. Lactobacilli are predominant in the upper gastrointestinal tract, whereas high concentrations of Gram-negative and Gram-positive bacteria are present in the colon.

otherwise indigestible dietary polysaccharides. Another example of beneficial symbiosis is the regulation of the production of different vitamins, particularly vitamins B and K, by intestinal microbiota (16). However, this microbiota is associated with advantages other than first nutritional advantages; for example, the complex microbiota competes with potential pathogens for the same ecological niche within the human intestine, thus imparting a degree of protection (1,17). Recent data also suggest that gut colonization by bacteria can be a major trigger for angiogenesis in the intestinal mucosa (18). In addition, there is increasing evidence that intestinal microbiota exerts positive stimulatory effects on the intestinal innate and adaptive immune systems (19,20). For instance, in response to intestinal colonization, the number of T lymphocytes and plasmocytes within the intestinal lamina propria clearly increases. Whereas immunoglobulin (Ig) A–producing cells are virtually absent in germ-free mice, high Ig levels are detectable within the mucosa upon bacterial colonization. Hooper and Gordon (6) developed this concept of a close bacterial host cross-talk. In addition to these direct stimulatory effects on the mucosal immune system, the intestinal microbiota is also a major source of regulatory metabolites, such as short-chain fatty acids (eg, lactic acid, butyrate). These metabolites can exert immunomodulatory or anti-inflammatory effects, such as those recently demonstrated for bacteria-derived butyrate, which is a strong inhibitor of the proinflammatory NK-κB pathway (21).

INTESTINAL EPITHELIAL BARRIER FUNCTION

A prerequisite for a homeostatic symbiosis between intestinal microflora and the host is a fully functional

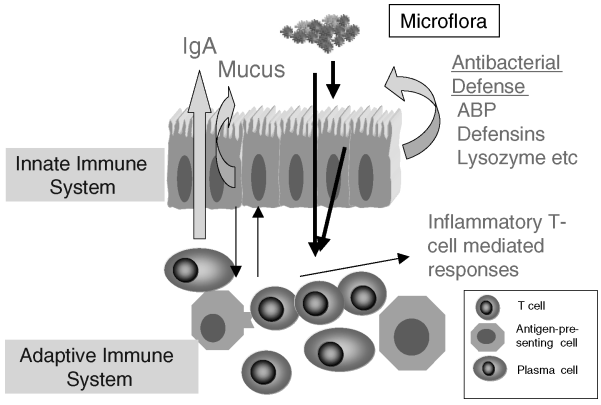

FIG. 2. Epithelial barrier. The intestinal epithelium and mucosal immune system form a physical, chemical, and immunological barrier protecting against microbial structures in the gut. The secretion of viscous mucus and highly bactericidal peptides and enzymes prevents direct contact between bacteria and intestinal epithelial cells. The secretion of immunoglobulin A produced by plasma cells in the lamina propria constitutes a potent immunological defense. ABP = antibacterial peptide.

intestinal epithelial barrier. To further elucidate the interplay between the various partners, it is important to consider the intestinal epithelial barrier as a highly dynamic system and not simply as a static, mechanical structure. The epithelial barrier, a complex physicochemical and biological system, is composed of a tight intestinal epithelium, overlaid by a mucous gel composed of mucin glycoproteins, defensins, and other antibacterial or repair peptides. It also contains high concentrations of pIgA (Fig. 2).

Intestinal Epithelial Layer

The intestinal epithelium consists of a single layer of densely packed enterocytes along the villous axis of the crypt. Tight intercellular junctions prevent leakage through this layer. The special architecture of the densely packed intestinal epithelium consists of highly specific carrier molecules on the surface of enterocytes, which allows for control and sampling of substrates to be absorbed while maintaining an intact barrier to antigens (22,23). The primary importance of an intact epithelial barrier is further illustrated by the spontaneous development of colitis in mice expressing a dominant mutant of N-cadherin associated with disruption of interenterocyte tight junctions (24).

Mucous Gel

The viscous mucus layer is formed by highly glycosylated mucin glycoproteins, trefoil, and antibacterial peptides. These mucins, secreted by goblet cells, form a viscoelastic biofilm overlying the intestinal epithelium. The film is highly hydrophobic because of the effect of enterocyte-secreted surfactant lipids that coat the microvillus surface, ensuring physical separation of the lumen from the epithelium (25,26). In its association with the villous surface, the hydrophobic viscous mucus prevents direct contact between intestinal flora and enterocytes under physiological, homeostatic conditions. Under these normal conditions, the apical membrane of enterocytes is rarely, if ever, directly exposed to intestinal bacteria (27).

Antibacterial Defense Factors

In addition to the physical and chemical barrier, the production and secretion of antibacterial defense factors by the intestinal epithelium constitutes a further means of controlling effects of colonizing bacteria. The contributions of pIgA, abundantly secreted by plasma cells in the intestinal submucosa to host defense, has been investigated. Those secretory molecules, which are transported via intestinal epithelial receptors, are present at high concentrations in the intestinal mucus layer and enable capture of a large array of antigens in the intestinal lumen, thus inhibiting mucosal invasion and penetration by pathogens. Specific IgA-mediated immunity against pathogens is an important mechanism of adaptive immune responses. Most of the bacteria in human feces are coated with specific IgA molecules (28). Germ-free mice have no IgA-producing plasma cells in the intestinal submucosa and do not secrete IgA into the intestinal lumen, which suggests that the IgA-dependent adaptive immune response of the intestinal mucosa is turned on in response to bacterial colonization of the gut (29,30).

Another important defense mechanism is the production and secretion of endogenous antimicrobial molecules by epithelial cells. Various categories of cationic antibacterial peptides (Table 1) have been identified recently on the basis of size, cysteine pairing, and peptide structure. Three groups of those peptides are present in the intestinal lumen (31). Members of the first group, α-defensins (HD5 and HD6), are constitutively expressed by Paneth cells, located at the base of the crypts of Lieberkühn. HD5 and HD6 are densely packed in secretory granules and are rapidly released in response to bacterial, inflammatory, and other stimuli (32). It is interesting to note that Gram-positive and Gram-negative bacteria and their products, such as lipopolysaccharide, lipoteichoic acid, and peptidoglycan, trigger Paneth cell degranulation in a few minutes, whereas fungi and protozoa do not. Paneth cells release propeptides into the crypt lumen and these enzymes are then activated by the metalloproteinase, matrilysin (33). The primary role of the bactericidal α-defensins is considered to be protection of the intestinal stem cells, located at the base of the crypts, from bacterial invasion. Paneth cells have a broad-spectrum antibacterial action against both Gram-positive and Gram-negative bacteria. In addition to α-defensins, Paneth cells also secret high levels of lysozyme and other

TABLE 1. *Human antibacterial peptides*

α-Defensins		
HNP1-4	Human neutrophil peptides 1–4	Neutrophils
HD5	Human denfensin 5	Paneth cells
HD6	Human defensin 6	Paneth cells
β-Defensins		
HBD1	Human β-defensin 1	Epithelial cells (small bowel and colon)
HBD2	Human β-defensin 2	Epithelial cells (small bowel and colon)
HBD3	Human β-defensin 3	Epithelial cells (colon)
Cathelicidins		
LL-37	Synonym: Fall-39	Neutrophils, epithelial cells

antimicrobial peptides. The second group of defensins consists of the β-defensins, which are either constitutively (HBD1) or inducibly (HBD2 and 3) expressed. Enterocytes constitute the major intestinal source of β-defensins. The third group is composed of cathelicidins (ie, FALL39) or chemokines, such as CCL20, with a high degree of structural homology with HBD1 and HBD2, despite the fact that they share little sequence homology. HBD1 and 2 and CCL20 have a 3 β-pleated sheet core structure that is stabilized by cysteine bonds, a high degree of similarity of positively charged amino acids, and similar motifs at the N-terminal region (34). In response to bacterial stress, peptides are secreted at both the apical and the basolateral surfaces of enterocytes (35). Their antimicrobial activity is more limited than that of Paneth cell–derived α-defensins. The role of those antimicrobial peptides with regard to the composition of the intestinal microbiota and potential intestinal infections is subject of speculation. The relevance of antibacterial peptides was recently further highlighted by Iimura et al (36), who showed reduced mucosal bacterial clearance in cathelicidin-deficient mice ($Cnlp^{-/-}$) challenged with *Citrobacter rodentium*.

THE INTESTINAL MUCOSAL IMMUNE SYSTEM

The intestine is an important immune organ. It harbors approximately 80% of B cells and more than 60% of T cells within the immune system (37,38). The intestinal mucosa thus houses the largest pool of immunocompetent cells in the body. Gut-associated lymphoid tissue is divided into organized (Peyer patches and lymph nodes) and diffuse lymphoid tissue (Fig. 3). Peyer patches are more permeable to antigens than are other parts of the intestinal epithelium because the overlying mucus is less densely packed (fewer goblet cells). In addition, specialized transporter cells, M cells, and dendritic cells close to the surface are responsible for the active uptake of soluble antigens and of microorganisms in the intestinal lumen (39). The close proximity of those cells to lymphoid follicles enables optimal antigen sampling and induction of an appropriate immune response (either a proinflammatory reaction or tolerance). Diffuse lymphoid tissue in the intestinal lamina propria consists of activated CD4+ and CD8+ T cells, some regulatory T cells, memory B cells, and IgA-producing plasma cells. Intraepithelial lymphocytes are mainly of the CD8+ phenotype.

Within the immune system, it is helpful to distinguish between closely interacting constituent innate and adaptive immune systems (40,41). The innate immune system discriminates between pathogens and harmless bacteria in colonizing intestinal microbiota. Pathogen recognition receptors, such as Toll-like receptors and NOD molecules, recognize a limited number of bacterial motifs (either microbe-associated molecular patterns or, in the

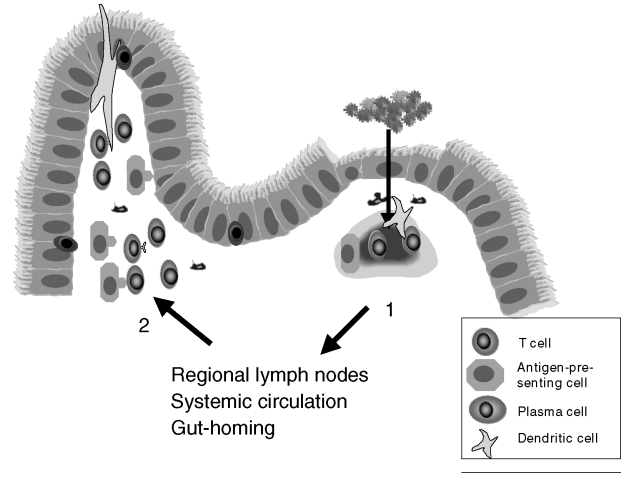

FIG. 3. The intestinal mucosa–associated immune system consists of (1) organized (Peyer patches and lymph nodes) and (2) diffuse lymphoid tissue. Peyer patches are more permeable to antigens than are other parts of the intestinal epithelium because the overlaying mucus is less densely packed. Highly specialized transporter cells, M cells, and dendritic cells close to the surface enable active uptake of soluble antigens and microorganisms from the intestinal lumen. The diffuse lymphoid tissue within the intestinal lamina is composed of mainly activated CD4+ and CD8+ T cells, some regulatory T cells, memory B cells, and IgA-producing plasma cells. Intraepithelial lymphocytes are mainly of the CD8+ phenotype.

case of pathogens, pathogen-associated molecular patterns) (42,43). Both types of pathogen recognition receptors are naturally expressed by intestinal epithelial and antigen-presenting cells, such as dendritic cells and macrophages, enabling those cells to readily detect bacterial motifs. Through this interaction at the intestinal epithelial cell level, an immediate innate immune response may be elicited within seconds or minutes (35,44–46). To prevent permanent and unwanted stimulation of the innate immune system, the intestinal epithelial barrier is protected by a highly viscous microfilm, as discussed above. The film prevents close contact between commensal bacteria and intestinal epithelial cells. However, when contact is made, the enterocyte is able to send ''alarm signals'' in the form of chemokines or cytokines to the mucosal innate and adaptive immune system while concomitantly secreting antibactericidal peptides into the lumen (35). This complex mechanism seems to be impaired in some patients with inflammatory bowel disease (IBD); early data indicate that the secretion of antibacterial peptides is impaired or insufficient in a subgroup of patients with IBD, the mucus layer is thinner and less effective as an antibacterial filter, and innate immune responses on the level of the intestinal epithelium seem to be pathologically increased. Proinflammatory signaling from enterocytes and antigen-presenting cells in the intestinal mucosa results in rapid upregulation of the homing receptors on endothelial cells of the intestinal vessels and chemoattraction of inflammatory cells to the site of invasion.

MODULATION OF INTESTINAL MICROBIOTA AND HUMAN DISEASE

The interaction between microbiota and the intestinal mucosa has only just begun to be elucidated. Scant information is available on how an intervention aimed at modulating the microbiota may be beneficial in terms of preventing, alleviating, or curing a disorder. One method of modulating the intestinal flora consists in administering live, viable bacteria via food or medicinal products. The term ''probiotic'' was recently introduced to explain this concept. A probiotic has been defined as living microorganisms which, upon ingestion in sufficient numbers, exert health benefits beyond basic nutrition. In other words, probiotics are live, viable bacteria or other microorganisms such as yeasts that have a clearly identifiable positive effect on health or disease. Nonviable bacteria or bacterial substrates are not considered to be probiotics. The most commonly used and studied species of probiotics belong to the genera *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces*. Different probiotic products and strains exist in a wide variety, and it is important to consider the term ''probiotics'' as a generic term for a whole range of completely different microorganisms endowed with different properties and effects. The term ''probiotics'' is comparable to the term ''antibiotics,'' which covers many different classes of drugs endowed with differing antibiotic activities. Thus, different antibiotics have different indications. If the term ''probiotics'' is used in a manner analogous to the way in which ''antibiotics'' is used, then it may prevent confusion with respect to the specific properties of probiotics. Some probiotics are used to prevent or treat infections, whereas others are of value in the prophylaxis or treatment of allergic or inflammatory disorders. What follows is a summary of recent progress and current knowledge in the field of probiotic research with regard to the benefits of probiotic prevention and treatment of human disorders, as evidenced by clinical trials. The use of probiotic approaches is particularly helpful in young pediatric patients because infants are particularly vulnerable to diseases and infancy is characterized by the delicate process of intestinal mucosa maturation and interaction with gut microbiota.

PROBIOTICS AND PREVENTION OF NECROTIZING ENTEROCOLITIS IN PREMATURE INFANTS

Neonatal necrotizing enterocolitis (NEC) is an extremely challenging clinical disease entity. NEC is a complication of very low birth weight (VLBW) infants and often is fatal. The pathophysiology of NEC is multifactorial and has only been partially elucidated (27). Several hypotheses have been advanced. They include the role of pathogens, the challenge of enteral feeding, and immaturity of the intestinal epithelial barrier function and the mucosal immune system. The normal intestinal protective functions are underdeveloped, and thus the newborn is incompletely equipped—to an extent dependent on gestational age—to deal with the challenges of dietary and microbial antigens (47). The normal neonatal intestinal colonization process may be markedly disturbed in premature infants, resulting in inappropriate colonization and a predisposition to intestinal inflammation. Theoretically, intervention that targets a positive modification in the intestinal flora could constitute an effective method of preventing the onset of NEC. Recent data generated using neonatal rat NEC models have shown that *Bifidobacterium infantis* supplementation significantly reduced the incidence of NEC (48). A clinical study of neonatal probiotic supplementation was conducted on 585 premature infants (49). This Italian multicenter, double-blind, placebo-controlled study showed a lower incidence of NEC in the group supplemented with *Lactobacillus* GG than in the placebo-supplemented control group (1.4% vs 2.7%). However, after 7 days of supplementation with this strain, the difference was not statistically significant. A first preliminary randomized trial including 208 VLBW or

extremely low birth weight infants given either *B breve* or placebo within 24 hours of birth (Y. Yamashiro et al, personal communication, 2006) suggested that probiotics may be of value in the prevention of NEC. No deaths from infection or sepsis occurred in the group supplemented with *B breve* compared with 13.5% mortality in the control group. *B breve* administration, which in another study had previously been shown to promote colonization by *Bifidobacterium* (50), may also stimulate mucosal immunological development of VLBW infants. These preliminary observations were recently confirmed by 2 independent, randomized, prospective, placebo-controlled studies. Both trials compared the incidence of NEC with or without probiotic supplementation. The study by Lin et al (51) included 367 VLBW (<1500 g) infants who received enteral nutrition and survived beyond postpartum day 7. All of the infants received breast milk. In the treatment group (n = 180), the breast milk was supplemented with *L acidophilus* and *B infantis*, 125 mg/kg per dose, twice daily until discharge. The incidences of NEC and mortality were significantly lower in the probiotic-supplemented group than in the control group (5% vs 12.8%, $P = 0.009$) of high-risk premature infants. Moreover, no case of severe NEC occurred in the treatment group, and 3% of cases of severe NEC occurred in the control group. The study by Bin-Nun et al (52) included 145 premature infants who were assigned to a control (nonsupplementation) group (n = 72) or a treatment group (n = 73). The treatment group received supplementation with a mixture of *B infantis, S thermophilus*, and *B bifidum*. The incidence of NEC was 4% in the probiotic-supplemented group and 16.4% in the nonsupplemented control group ($P = 0.03$). Moreover, the severity of NEC was lower in the probiotic-supplemented group (Bell criteria: 2.3 ± 0.5 vs 1.3 ± 0.5, $P = 0.005$). The molecular mechanisms underlying NEC prevention by probiotic supplementation remain unclear and need to be elucidated in the near future. With regard to the safety issues related to the use of viable bacteria in immunodeficient or immunocompromised patients, such as premature infants, it is important to draw attention to the fact that the studies by Lin et al (51) and Bin-Nun et al (52) had no evidence of any complications, in particular no increased risk of septicemia, related to the use of probiotics.

For the first time, the above studies have shown that the use of probiotics significantly reduces mortality. The recent meta-analysis of Deshpande et al (53) confirmed that the use of probiotics may reduce the risk for the development of both NEC and septicemia in newborns at risk. A total of 7 studies (including 1393 VLBW preterm infants) were analyzed using a fixed effects model. Despite heterogenicity in milk-feeding practices between the different studies, in the probiotic-supplemented group, the risk of NEC was markedly lower than in nonsupplemented infants (relative risk [RR] 0.36, 95% confidence interval [CI] 0.20–0.65), and the risk of death was also reduced in the probiotic group (RR 0.47, 95% CI 0.30–0.73). This evidence adds a completely new dimension to the concept of probiotic supplementation. It is important to confirm those findings and to generate a sound basis for the introduction of probiotics into routine neonatal care. However, additional studies need to determine the best bacterial strain for NEC prevention. Is a single strain or a combination of strains more effective in preventing NEC? What are the appropriate or optimal probiotic doses required to induce the effect, and how long should probiotic supplementation be maintained? At what time point postpartum should probiotic supplementation of the feed be introduced? Ongoing discussion is active with respect to the ethical feasibility of conducting further placebo-controlled studies in premature infants at high risk for NEC, given the observation that the administration of *Bifidobacterium* strains combined with *Lactobacillus* or *Streptococcus* significantly reduced both NEC and mortality. Including high-risk patients in a placebo group would therefore be questionable. A preferable strategy may be to compare different probiotic strains/mixtures/dosages in terms of their prophylactic potential with respect to NEC.

PROBIOTICS AND PREVENTION/TREATMENT OF INFECTIOUS AND ANTIBIOTIC-ASSOCIATED DIARRHEA

Infectious gastroenteritis is the most common infectious disease of young infants and children. Worldwide, rotavirus is the most common cause of severe diarrhea and mortality in children (54), but various other viral, bacterial, and parasitic agents induce enteritis and colitis in children and adults. To date, more than 50 studies have been conducted to determine the efficacy of probiotic strains in the treatment of various infectious enteritides, primarily in children. A few studies in adult cohorts have also been conducted. The results of recent randomized controlled trials and of meta-analyses support the overall notion that probiotics have a beneficial effect on the reduction in diarrhea risk and duration (55–58). Several strains of probiotics have been used in various settings. There is some evidence that certain probiotic strains, such as *L casei rhamnosus, L reuteri, B bifidum*, and *S thermophilus*, reduce the severity and duration of rotavirus-induced diarrhea. The recent Cochrane meta-analysis by Allen et al (58) included pediatric and adult studies that totaled 23 controlled trials with 1917 participants (1449 children and infants). The overall analysis indicated that the use of probiotics significantly reduced the risk that diarrhea would persist for more than 3 days, in comparison with placebo-treated patients, who experienced diarrhea for 3 or more days. The pooled data from 12 trials showed a reduction in mean diarrheal duration of

29.2 hours (95% CI 33–25 hours). One major weakness of this type of meta-analysis is that different strains are compared with regard to their effect on ''calming down'' diarrhea. Inasmuch as different strains of probiotics do not necessarily share the same mechanisms of action, it is difficult to compare these effects within a single meta-analysis.

In a meta-analysis restricted to adequately randomized and blinded studies on children using only *Lactobacillus* strains, Van Niel et al (56) recently reported that the use of those probiotics shortened the duration of diarrhea by 0.7 days (95% CI 0.3–1.2 days). On day 2 of probiotics use, diarrhea was reduced by 1.6 stools per day. These data indicate an efficacy for *Lactobacillus* in infectious diarrhea in children. A meta-analysis by Szajewska et al (59) focused on randomized placebo-controlled, double-blind studies of children and infants, with acute diarrhea lasting for 3 or more days. The use of probiotics reduced the risk of diarrhea lasting 3 or more days by 0.40 compared with placebo. Analysis of 8 trials that included 731 children showed that the duration of diarrhea was reduced by 18.2 hours (95% CI 9.5–26.9 hours). Given the marked variability in the studies, it is not surprising to observe differing results. However, *Lactobacillus* GG was once again reported to be particularly effective with respect to pediatric rotavirus diarrhea.

Various clinical trials on the prevention of community-acquired diarrhea have been conducted using home and day care center visits. *Lactobacillus* GG reduced the incidence of diarrhea in Peruvian babies (60) but not in Finnish infants 1 to 6 years old (61). Milk fermented with yogurt cultures and *L casei* DN-114 001, 125 g/day for 1 month, was shown to influence the intestinal flora of healthy infants (62). A multicenter, randomized double-blind clinical trial conducted on children attending day care centers in France showed that consumption of milk fermented with yogurt cultures and *L casei* DN-114 001 decreased the incidence of acute diarrhea in comparison with standard yogurt alone (63). Interestingly, a recent placebo-controlled study compared the effects of 2 different species, *L reuteri* and *B lactis* Bb12, as formula supplements (64). Although both reduced the number of days and number of episodes of diarrhea, *L reuteri* showed better results than Bb12. Children on Bb12 supplementation also had significantly fewer days of fever, fewer clinic visits, and less need for antibiotic prescriptions. Comparative studies like those cited, stratified on disease etiology, age group and setting (eg, rotavirus vs bacterial infection, pediatric vs adult cohort, outpatients vs hospital setting), may be helpful in further determining which bacterial strains should be recommended as cheap, effective prophylaxis against diarrhea in different settings and/or geographic populations (65–67).

The prevention of antibiotic-associated diarrhea (AAD) represents an additional clinical indication for probiotics. Data showing that probiotic prophylaxis may protect against AAD are available, but the number of clinical trials is limited. Sazawal et al (68) recently analyzed 34 pediatric and adult studies of probiotic prophylaxis of acute diarrhea after antibiotic use. The most impressive and significant result was a reduction of more than 50% (95% CI 35%–65%) reduction in the occurrence of acute AAD with probiotic prophylaxis.

A recent meta-analysis of pediatric studies (69) identified 6 randomized placebo-controlled trials in which 766 antibiotic-treated children were enrolled. The risk of AAD was reduced from 28.5% to 11.9% (all 6 studies pooled). The conclusion of the meta-analysis was as follows: for every 7 pediatric patients with AAD, 1 was spared by the use of probiotics. In studies of *Saccharomyces boulardii*, a reduction in the incidence of AAD in pediatric patients was observed in the groups receiving the yeast (70,71). A further meta-analysis of the data generated by 5 randomized clinical trials showed that *S boulardii* was moderately effective in preventing AAD in children and adults treated with antibiotics for any reason (mainly respiratory tract infections). In that analysis, 1 patient in 10 receiving *S boulardii* with antibiotic treatment was AAD free (72). A clinical trial involving a commercially available probiotic formula containing 10^7 viable cells of *B lactis* and 10^6 viable cells of *Streptococcus thermophilus* also reduced the frequency of AAD in infants (73). These observations are in contrast to the recent Cochrane database review of Johnston et al (74) based on 10 independent pediatric trials on the efficacy of probiotics in the prevention of AAD. Six studies used a single-strain probiotic agent, including lactobacilli, bifidobacteria, streptococci, or *S boulardii*, whereas combinations of 2 different strains were used in 4 studies. The overall results of this systematic review showed that in 9 of 10 trials reporting on the incidence of diarrhea, a statistically significant effect was observed that favored probiotic groups over control groups. However, an intention-to-treat analysis showed nonsignificant results (RR 0.90, 95% CI 0.50–1.63) because of a significant dropout. Therefore, the authors conclude that probiotics in general seem to have potential in the prevention of pediatric AAD; however, the current data must be confirmed by independent trials with validated primary outcome measures focusing on the most promising probiotics, such as *Lactobacillus* GG, *L sporogenes*, or *S boulardii* before any clear recommendations can be made.

The most frequent etiologic agent in AAD in older adults is toxin-secreting *Clostridium difficile*. A recent meta-analysis of 6 clinical trials (75) showed that only *S boulardii* was effective against *C difficile*–induced colitis in adults (RR 0.59, 95% CI 0.41–0.85). Those findings clearly show that different clinical settings require different treatments and that adult and pediatric studies cannot easily be compared.

Traveler's diarrhea and other (mainly infectious) forms of diarrhea were less readily prevented by probiotics. The beneficial and preventive effect of probiotics on diarrhea was dependent on age, inasmuch as the overall protective effect in children was close to 60% (35%–71%), whereas among adults only a 26% improvement (7%–49%) was reported. No significant differences were observed between different probiotic strains *S boulardii*, *L rhamnosus* GG, *L acidophilus*, *L bulgaricus*, and other strains used alone or in combinations of 2 or more strains.

The mechanisms by which probiotics are effective in preventing or shortening infectious diarrhea are not fully understood. Probiotics may compete with diarrheal pathogens for adhesion sites, strengthen the mucosal barrier and tight junctions between enterocytes, and/or enhance the mucosal IgA-mediated immune responses to pathogens (76). Secretion of antimicrobial substances and induction of intestinal mucin production may also contribute to the beneficial effects of probiotics. In vitro and in vivo animal studies have shown that certain probiotic strains have a particular efficacy against enteropathogenic *Escherichia coli*, *Salmonella*, and other pathogens. Genome sequence analyses have identified numerous bacterial cell surface–associated proteins with predicted intestinal cell- and mucus-binding functions (77,78). Homology searches have enabled identification of several *L acidophilus* adhesion factors (79). Genotype–phenotype matching has shown a mannose-specific adhesion of *L plantarum* that may impair the efficacy of enteropathogenic *E coli* infection via a competitive exclusion mechanism. This would constitute a molecular action mechanism for the probiotic strain (80).

Bacteria are known to secrete antimicrobial molecules against other bacteria. Bacteriocin is an example of such an antibacterial peptide. In an experimentally induced murine infection model, prefeeding with *L salivarius* UCC118, which produces a 2-component bacteriocin active against *Listeria monocytogenes* (81), before the oral administration of *Listeria* reduced pathogen levels 1000-fold in splanchnic and hepatic pathogen counts. Given that bacteriocins, however, often possess only a limited host range, it is of considerable interest to determine whether the effect is pathogen specific or reflects mechanisms other than direct antagonism. Further studies of this type will help to define the precise mechanisms of probiotic interference with the virulence of important gastrointestinal tract pathogens. Given that in vivo bacteriocins produced by Gram-positive probiotics do not affect Gram-negative organisms, other mechanisms may have been responsible for the positive and protective effect of *Lactobacillus murium* in a porcine experimentally induced *Salmonella* infection model. The protective effect seems to be related to probiotic interference with pathogen invasion of host cells (80,82).

PROBIOTICS AND PREVENTION/TREATMENT OF ALLERGIES IN INFANTS AND CHILDREN

The prevalence of atopic diseases has gradually increased in Western societies. Chronic allergic responses most commonly present as asthma, eczema, and atopic dermatitis. Those diseases may be the consequence of a dysequilibrium in the immune responses to environmental or food antigens (83). Many of the immune regulatory aberrations promoting sensitization instead of tolerance induction occur in early infancy. The intestinal mucosal immune system is an important organ in the development of tolerance toward dietary and harmless microbial and environmental antigens (84). As discussed above, during bacterial colonization of the ileum and colon after birth, appropriate microbiological stimulation is essential to correct the balance of a skewed T helper-2 immune response predominant in neonates. The normal interaction between neonates and microorganisms is thought to be compromised in the Western world, with a reduction in *Bifidobacteria* and an increase in *Clostridium* species, particularly in bottle-fed infants (84,85). In keeping with these hypotheses, the recent prospective Dutch birth cohort study, KOALA, by Penders et al (86) gave the first epidemiological evidence of a major impact of enteric pathogens on the development of atopic predisposition or allergic disorders. Based on the analysis of the gut microbiota composition in 957 infants at the age of 1 month Penders et al (86) identified the following risk factors. The presence of *E coli* was associated with a higher risk for the development of eczema (odds ratio [OR] 1.87, 95% CI 1.15–3.04). In addition, this risk clearly increased with increasing numbers of *E coli*. Another interesting finding of this study was that infants who were colonized with *C difficile* were at higher risk for the development of eczema (OR 1.40, 95% CI 1.02–1.91), recurrent wheeze (OR 1.75, 95% CI 1.09–2.80), and atopic dermatitis (OR 1.73, 95% CI 1.08–2.78). The fact that colonization with *E coli* was predominantly associated with eczema, but colonization with *C difficile* was associated with other atopic symptoms, indicates differing underlying molecular mechanisms in the development of atopic disorders.

The efficacy of probiotic prevention of allergic disease has been demonstrated in studies using *L rhamnosus* GG. The seminal study by Kalliomäki et al (87) was based on prenatal prophylactic administration of *L rhamnosus* GG to future mothers with a history of allergy, followed by a 6-month postnatal supplementation period of administration to breast-feeding mothers and infants at high risk for atopy. After 2 years of follow-up, the prevalence of atopic dermatitis was 23% in the probiotic-treated children versus 46% in the children receiving placebo (87). Similar results were observed when follow-up was extended to 4 and 7 years, respectively, with 26% atopic dermatitis in the probiotic group versus 46% in the

placebo control group at 4 years (88) and 34% atopic dermatitis in the probiotic group versus 57% in the placebo group at 7 years (89). However, skin prick test reactivity was comparable in both groups, whereas allergic rhinitis and asthma tended to be more common in the probiotics group, indicating specific mechanisms for the protection of atopic dermatitis differing from those involved in asthma or allergic rhinitis.

Various clinical trials have shown significant alleviation of the clinical symptoms of children with atopic dermatitis who receive a probiotic-supplemented diet. Weston et al (90) included 56 infants 6 to 18 months old who had moderate or severe atopic dermatitis in a randomized double-blind placebo-controlled trial. Children received either a probiotic (1×10^9 *L fermentum* VRI-033 PCC) or an equivalent volume of placebo twice daily for 8 weeks. The final assessment at 16 weeks showed a significant reduction in the severity score (Scoring Atopic Dermatitis: SCORAD) over time in the probiotic group ($P < 0.03$) but not in the placebo group. Williams (91) recently criticized this study by reporting it showed a significant drop in the SCORAD index within the probiotic group but not within the placebo group. However, there was no statistically significant difference between both groups at the endpoint of the study. Therefore, the conclusion of a beneficial effect of probiotics compared with placebo are difficult to confirm because this study was intended to compare the differences in outcome between probiotic-treated versus placebo-treated patients with atopic dermatitis. In a larger cohort of 230 patients in a similar clinical setting, Viljanen et al (92) observed that only children with IgE-sensitized atopic dermatitis benefited from probiotic supplementation, and only from *L rhamnosus* GG supplementation. During the 4-week treatment period, the SCORAD score fell by 26.1 points in the probiotic group versus 19.8 in the control group ($P < 0.036$). Once again, this type of post hoc analysis was criticized (91) because the main primary outcome of this study was clearly negative. This positive tendency of probiotic strains on atopic dermatitis has not been reproduced in other studies. Brouwer et al (93), in a randomized double-blind, placebo-controlled study of 50 children, failed to demonstrate any significant effect on atopic dermatitis treated with probiotics. After 4 to 6 weeks of baseline and double-blind, placebo-controlled challenges for diagnosis of cow's milk allergy, infants younger than 5 months old with atopic dermatitis received a hydrolyzed whey-based formula as placebo (n = 17) or formula supplemented with either *L rhamnosus* (n = 17) or *Lactobacillus* GG (n = 16) for 3 months. No statistically significant differences between the groups with or without probiotic supplementation on SCORAD index, sensitization, inflammatory parameters, or cytokine production were found. Similarly, the prophylactic effect of prenatal and postnatal use of probiotic *Lactobacillus* GG with respect to allergic disease reported by Kalliomäki et al (87) still awaits confirmation. Abrahamsson et al (94) recently failed to reproduce the results of the Kalliomäki study using a similar study design; however, the authors used a different probiotic strain, *L reuteri*, at lower doses and for a prolonged postnatal period of 12 months. The overall cumulative incidences of eczema in the *L reuteri* group versus the placebo group were identical: 36% versus 34%. However, the authors observed a clear effect of *L reuteri* supplementation on the occurrence of IgE-associated eczema during the second year, with 8% in the probiotics group versus 20% in the placebo group, along with reduced skin prick test reactivity. This finding is rather surprising and raises once more the question of the specificity of the effect of different strains and of different doses. By contrast, Taylor et al (95) failed to reproduce these findings in a study that included a total of 178 infants who completed the study; 89 were treated by *L acidophilus* (LAVRI-A1), and 88 received placebo. The atopic dermatitis rate at 6 months of supplementation was 25.8% in the probiotic group compared with 22.7% in the placebo group. No significant difference could be observed at 12 months, and furthermore it was observed in this study that early *L acidophilus* supplementation was associated with an increased risk for subsequent cow's milk sensitization. This report clearly challenges the findings of Kalliomäki et al (87). However, the contrasting results may be related to differences between the Finnish and the Australian study designs in that different probiotic strains were used and supplementation was started at different time points (before delivery in the Finnish study and at birth in the Australian study). There is a clear need to have further comparable studies done before any general recommendation can be made.

Once again, the molecular basis of the effect of probiotics on allergy must be elucidated. The effects were initially attributed to normalization of intestinal permeability, enhanced immunological barrier functions, decreased intestinal inflammatory response, and reduced production of the proinflammatory cytokines characteristic of local and systemic allergic inflammation (96). However, recent studies indicate that *L rhamnosus* GG supplementation causes an initial inflammatory reaction at the intestinal mucosal and systemic levels (97). With regard to the fecal compartment, *L rhamnosus* GG supplementation of children with AD or cow's milk allergy resulted in increased IgA and reduced tumor necrosis factor-α levels in comparison with children receiving placebo (98). Moreover, there is some evidence that intestinal microflora strains contribute to the production of T helper-1 immune responses, which may in turn block or prevent T helper-2 allergic responses in atopic disease. This approach may help to create optimal conditions for orienting T helper-2 polarized neonatal immune responses toward a positive T helper-1/T helper-2 balance. Given the recent advances in the

understanding of immune tolerance in the intestinal mucosa, one may also speculate about the interaction between bacterial motifs or molecular patterns of specific probiotic strains and immune regulatory T cells, such as CD4+CD25+FOXP3+ regulatory T cells, in the intestinal mucosa (99).

PROBIOTICS AND PREVENTION/TREATMENT OF INFLAMMATORY BOWEL DISORDERS

Several lines of experimental and clinical evidence suggest that a loss of immunological tolerance of the intestinal microbiota (1,100,101) is a crucial component in the etiology of Crohn disease (CD) and perhaps also of ulcerative colitis (UC). The role of the intestinal microbiota is primordial in the onset of inflammation in various experimental animal models of Crohn colitis, including interleukin-10 knockout mice, adoptive transfer colitis models, and trinitrobenzene sulfonic acid–induced colitis (102–104). In animals raised under germ-free conditions, colitis either does not develop or develops only in an attenuated form. In this context, it is intriguing to note that patients with IBD have a greater number of bacteria attached to the intestinal mucosa than do healthy control individuals (105). The discovery that the intestinal microbiota plays an important role in the onset of clinical IBD led to major interest in developing a therapeutic modulation of the intestinal flora of these patients. Clinical trials of probiotics in patients with IBD were the logical extension of this interest, and numerous studies have been conducted recently, mainly in adult patients. Analysis of the clinical potential and efficacy of probiotics in those studies calls for careful distinction of conditions in the clinical settings.

The most convincing data generated in the use of probiotics to treat IBD was in the clinical context of pouchitis. The latter condition consists of a nonspecific inflammation in the ileal pouch used as a reservoir after colectomy for severe UC. The cause of this relatively frequent complication of ileoanal anastomosis remains unclear. However, recent studies have shown impairment of the luminal microbiota, with reduced *Lactobacillus* and *Bifidobacterium* counts (106). The efficacy of probiotics with respect to pouchitis has been investigated in 3 different clinical conditions: maintenance of antibiotic-induced remission, treatment of acute active pouchitis, and prophylaxis for postoperative pouchitis. Gionchetti et al (107) recently evaluated the potential of VSL#3, a cocktail of 4 strains of *Lactobacillus* (*L casei, L plantarum, L acidophilus*, and *L delbrueckii* subsp. *bulgaricus*), 3 strains of *Bifidobacterium* (*B longum, B breve*, and *B infantis*), and 1 strain of *Streptococcus salivarius* subsp. *thermophilus*. Forty patients were included in a randomized double-blind, placebo-controlled trial. In all 40 patients, remission was induced by 4 weeks of antibiotic therapy. Thereafter, the patients were randomized to either 6 g of VSL#3 (containing 1.8×10^{12} freeze-dried viable colony-forming units [CFU]) or placebo). All 20 patients receiving placebo experienced relapse in the 9-month follow-up period. By contrast, in the probiotic group, remission was maintained in 17 of 20 patients. The difference was highly significant ($P < 0.001$). Moreover, all of the patients experienced relapse within 4 months of cessation of VSL#3 supplementation. Recently, a second independent trial confirmed the efficacy of VSL#3 in maintaining antibiotic-induced remission of pouchitis (108). In all, 36 patients were included: 20 received VSL#3 and 16 received placebo. Remission was maintained at 1 year for 17 patients receiving VSL#3 and 1 patient (6%) receiving placebo, providing further evidence of the potential of this probiotic cocktail in maintaining remission in settings of severe active pouchitis. By contrast, a clinical study of the potential of the single strain *Lactobacillus* GG with respect to acute active pouchitis completely failed to demonstrate efficacy. Kuisma et al (109) conducted a randomized double-blind, placebo-controlled trial in which *L rhamnosus* GG or placebo was administered for 3 months. At the end of the treatment period, no between-group difference was seen. *Lactobacillus* GG alone was thus not effective with respect to acute active pouchitis. In a second open-label study using a combination of *L acidophilus* and *B lactis*, no beneficial effect of probiotic supplementation on acute pouchitis was observed (110). However, an Italian study of VSL#3 demonstrated the potential of the probiotic mixture with respect to postoperative pouchitis prevention (111): pouchitis had not developed after 1 year in 90% of the patients receiving VSL#3, versus only 60% of the patients receiving placebo.

In comparison with the pouchitis studies, only a few randomized controlled, clinical studies of CD have been conducted. The capacity of probiotics to induce remission in patients with active CD and their ability to maintain medically or surgically induced remission were investigated. Two open-label pilot studies of *Lactobacillus*, 1 in children (112) and 1 in adults (113) with CD, generated encouraging results. Unfortunately, those encouraging preliminary data were not validated by a randomized double-blind, placebo-controlled trial (114).

Similarly, we are unaware of any convincing data on a beneficial effect of probiotics in the maintenance of surgically induced remission or in the prevention of postoperative relapse in patients with CD. Prantera et al (115) included 45 patients, all in postresection remission, in a single-center, randomized, double-blind, placebo-controlled trial. The patients were randomized to *L casei* subsp *rhamnosus* GG (1.2×10^{10} CFU) or placebo for 1 year. At the end of the study, 15 patients in the *Lactobacillus* GG group (83%) and 17 patients in the placebo group (89%) were in complete remission,

indicating that *Lactobacillus* GG was not more effective than placebo. A recently published French multicenter, randomized, double-blind, placebo-controlled trial (the GETAID study) of *L johnsonii* LA1 included 98 patients after resection but failed to demonstrate a positive effect of LA1 supplementation (116). At 6 months, endoscopic recurrence was observed in 30 of 47 patients (64%) in the placebo group and 21 of 43 patients (49%) in the LA1 group ($P = 0.15$). In addition, 4 clinical relapses were observed in the probiotics group, compared with 3 in the placebo group. Van Gossum et al (117) studied the same strain but at the higher dose of 10^{10} CFU/day in 70 patients who had undergone surgery for CD (102). In this second randomized controlled trial also, LA1 was not effective in preventing recurrence. Indeed, the percentage of patients with recurrence of severe endoscopic lesions was 21% and 15% in the LA1 and placebo groups, respectively ($P = 0.33$), and the percentage of patients with clinical relapse was 15% and 13.5%, respectively ($P = 0.79$).

The ability of various probiotic strains to maintain medically induced CD remission has been investigated. An initial, randomized, double-blind, placebo-controlled pilot study of steroid-induced remission evaluated the remission-maintenance potential of *E coli* Nissle 1917 (118). Twenty-eight patients receiving prednisolone (60 mg/day) were randomized to *E coli* Nissle 1917 or placebo for 1 year. There was no between-group difference in the initial remission rate or in 1-year remission maintenance. A second study included 32 patients with CD with complete remission for at least 3 months (119). The patients were randomized to mesalamine alone (3 g/day) or to mesalamine (2 g/day) plus *Saccharomyces boulardii* (1 g/day) for 6 months. Clinical remission was observed in 10 of 16 patients receiving mesalamine maintenance and 15 of 16 patients receiving mesalamine plus *S boulardii*. Unfortunately, no further studies with *S boulardii* have been published as far as we are aware, despite the encouraging initial results. Bousvaros et al (120) investigated the potential of *L rhamnosus* strain GG to maintain remission in 75 children with CD in a multicenter, randomized, double-blind, placebo-controlled trial. Unfortunately, the duration of remission with *Lactobacillus* GG was not significantly greater than that with placebo.

An innovative approach using genetically modified *Lactococcus lactis* bacteria for mucosal delivery of anti-inflammatory cytokines was recently tested in 10 patients with CD in an open-label phase 1 trial (121) Daily oral ingestion of these genetically engineered probiotics producing recombinant human interleukin-10 was well tolerated and safe, with a beneficial effect on disease activity. Given the safety concerns with the use of genetically modified bacteria, further safety studies should be performed before efficacy can be tested in larger placebo-controlled trials. However, this pharmacobiotic approach opens new treatment strategies for pediatric and adult patients with IBD.

Three trials were conducted in patients with UC to investigate the potential of various probiotics in the treatment of active UC. Bibiloni et al (122) evaluated the efficacy of VSL#3 in an open-label study in which 34 patients with mild to moderate UC were included. After 6 weeks of VSL#3 administration (10^{12} CFU daily), remission was observed in 53% of the 32 probiotic-treated patients who completed the study. The preliminary data require confirmation in a randomized controlled trial. In a second study, 116 patients with acute active UC that had failed to respond to mesalamine therapy were included and treated with *E coli* Nissle 1917 (123). When remission had been induced, the patients received maintenance mesalamine or *E coli* for as long as 12 months. At the end of the study, 25% of the patients in the mesalamine group and 26% in the *E coli* group were in remission. The median remission durations were similar for the 2 groups. Given the fact that in both groups the 1-year remission rates were similar to the historical placebo rates, no conclusion can be drawn from the study. In the third study, which had an open-label design, *S boulardii* was tested in 25 patients with UC relapse (124). After 4 weeks of *S boulardii* administration to mesalamine-treated patients, remission was obtained in 17 of 25 patients. The yeast thus seems to be of potential interest in UC. However, an appropriately designed randomized clinical trial with sufficient statistical power is necessary before this approach can be recommended for routine clinical treatment.

The value of various probiotic strains in the maintenance of clinical remission of UC was investigated in randomized placebo-controlled trials. Kruis et al (125,126) conducted 2 studies comparing *E coli* Nissle and mesalazine. The first study included 103 patients. At 3 months, 89% of the patients receiving mesalazine and 84% of the patients receiving *E coli* Nissle were in clinical remission. Rembacken et al (123) published a second randomized controlled trial, again comparing *E coli* Nissle with mesalazine (at the low dose of 1.2 g/day) in 116 patients with UC treated for 1 year. Relapse occurred in 67% of the *E coli* Nissle group and in 73% of the mesalazine group. The percentage of relapse in both groups was surprisingly high. A total of 327 patients were included in an independent trial with a duration of 12 months (126). At the end of the study, clinical relapses were observed in 40 of 110 patients (36.4%) in the *E coli* Nissle 1917 group and 38 of 112 patients (33.9%) in the mesalazine group ($P = 0.003$). These 2 studies indicate that the probiotic *E coli* Nissle 1917 shows efficacy and safety in maintaining remission equivalent to well-established anti-inflammatory drugs, such as mesalazine, which is part of the first-line treatment in patients with UC. Ishikawa et al (127) randomized 21 patients in UC remission to receive either placebo

or fermented milk (Yakult) containing live bifidobacteria (*B breve* and *B bifidum*) and *L acidophilus* for 12 months. The clinical remission rates were 73% in the fermented milk–probiotic group versus 10% in the placebo group. However, endoscopic evaluation at the end of the 12-month treatment period did not show any between-groups difference, calling into question the validity of the clinical assessment criteria used in the study. In a pilot open-label study that included 20 patients with UC, the value of VSL#3 supplementation after steroid induction of remission was investigated (128). After steroid withdrawal, VSL#3 supplementation for 12 months maintained the remission in 15 of 20 patients.

MECHANISMS

The precise molecular mechanisms of probiotic strains in the prevention and treatment of IBD are still largely unknown. However, ongoing in vitro research and research using animal models point to effects on intestinal epithelial cells and on the mucosal immune system (129–137). Recent in vitro and in vivo studies have shown that *Lactobacillus* and *Bifidobacterium* exert direct effects on intestinal epithelial barrier function that are evidenced by decreased intestinal permeability and enhanced intestinal epithelial resistance (134–136). For instance, exposure of colonic epithelial T84 cells to a combination of *L acidophilus* and *S thermophilus* induced enhanced phosphorylation of actin and occludins, contributing to the formation of tight junctions. Probiotics have been shown to reverse the deleterious effects of tumor necrosis factor-α and interferon-γ on epithelial permeability and ion transport (137,138). Several studies have demonstrated enhanced antibody (IgA) production in response to *Bifidobacterium* supplementation, and changes in cell-mediated immunity, including antigen presentation, in response to both bifidobacteria and lactobacilli (139–142). Furthermore, *L casei* DN-114 001 has been shown to attenuate the proinflammatory intestinal epithelial response to pathogenic *Shigella flexneri* by decreasing NF-κB activation (143). Therefore, it is legitimate to speculate that probiotics down-regulate the inflammatory immune response, induce apoptosis of inflammatory T cells, and may suppress T cell clonal expansion. Current research is also investigating whether particular probiotic strains are able to alter the function of dendritic or other antigen-presenting cells. Fink et al (144) recently demonstrated that different gut-derived probiotic bacteria can distinctly imprint monocyte-derived dendritic cell functions in initiating T or NK cell responses. Dendritic cells treated with probiotic strains confer protection against the development of colitis in the experimental trinitrobenzene sulfonic acid-colitis model. Molecular analyses indicated that probiotics were able to induce regulatory T cells in this model (145). Another theoretical mechanism operates in probiotic-induced suppression of pathogen growth via the secretion of antimicrobial factors, such as lactic acid or bacteriocins. It is also possible that some probiotic strains may inhibit the interaction of pathogens with intestinal epithelial cells, as was recently shown with *L casei* DN-114 001, which decreased adhesion of and invasion by an adherent-invasive *E coli* strain isolated from patients with CD (146).

CONCLUSIONS

The concept of beneficial interaction (symbiosis) between the intestinal mucosa and the endogenous microflora is now firmly established. Any disturbances in endogenous (host control mechanisms that are crucial for homeostatic and symbiotic interaction) or exogenous (composition of the microflora during the colonization process or later in life) factors may cause acute or chronic disorders. It is therefore logical to develop new treatment strategies aimed at modifying the intestinal microflora. Those modifications may enable re-equilibration of intestinal flora, for instance in response to a pathogen, or after antibiotics treatment. However, changes in the intestinal microflora may also be sensed by the intestinal mucosal immune system and give rise to specific or nonspecific changes in endogenous inflammatory and immune responses. Probiotics are an excellent tool with which to achieve controlled modification of the intestinal microflora. We therefore consider that the term ''probiotics'' denotes a therapeutic strategy and not a specific ''microbial drug.'' In all discussions of probiotic interventions, it is important to define the specific clinical setting (prevention vs treatment) and disease (eg, infectious, immunoallergic, inflammatory, dysimmune, NEC) clearly. In this context, it seems crucial to analyze the effect of a specific strain on a specific indication, rather than to simplify the concept by considering probiotics as a whole and as a multipurpose response to a variety of disorders or diseases. At present, the knowledge that would enable selection of a specific strain for a specific condition remains fragmentary. On the basis of the various studies reviewed, various bifidobacteria, lactobacilli, and saccharomyces may be promising probiotics in certain clinical contexts. The optimum dosages for specific probiotic interventions (which will probably differ between probiotics) and the optimum durations of treatment have yet to be defined. Therefore, many well-designed comparative or placebo-controlled studies are required before clear recommendations can be formulated. Given the particular microbial nature of probiotics, various delivery systems may be envisaged, such as tablets (as with other drugs) or food additives, thus adding a completely different dimension to the use of probiotics.

Finally, given the fact that probiotics are living microorganisms, particular quality standards are mandatory to

ensure a safe and completely harmless approach. Such standards are elaborated at the European and North American levels (147).

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