Probiotics: future directions[1–3]

Jon A Vanderhoof

ABSTRACT Clinical studies have shown that certain probiotics may be useful in treating a variety of diarrheal disorders, including rotavirus diarrhea, antibiotic-associated diarrhea, *Clostridium difficile* diarrhea, and traveler's diarrhea. New data suggest that probiotics might be useful in controlling inflammatory diseases, treating and preventing allergic diseases, preventing cancer, and stimulating the immune system, which may reduce the incidence of respiratory disease. Different modes of administering probiotics are currently being investigated, which may ultimately lead to the widespread use of probiotics in functional foods. It is important that such practices be directed by carefully controlled clinical studies published in peer-reviewed journals. *Am J Clin Nutr* 2001;73(suppl):1152S–5S.

KEY WORDS Probiotics, *Lactobacillus* GG, diarrhea

INTRODUCTION

Just a few years ago, this entire supplement could have been entitled Probiotics: Future Directions. In a very short period of time, many studies have been conducted to validate the concept of probiotics as a viable therapeutic modality in the treatment of gastrointestinal disease. Some known beneficial effects of probiotics include the following: *1*) reduction in the severity and duration of rotavirus diarrhea, *2*) reductioin in the risk of traveler's diarrhea, *3*) reduction in the risk of relapsing after the occurrence of *Clostridium difficile*–associated diarrhea, and *4*) reduction in the risk of antibiotic-associated diarrhea in children. Although the number of organisms studied is small, the list is growing and it is likely that many more probiotic organisms with a variety of different therapeutic benefits will be discovered. Additional organisms may eventually be developed through genetic engineering.

KNOWN BENEFITS OF PROBIOTICS

There is unequivocal evidence that probiotics may be useful in the treatment of viral diarrheal disorders. *Lactobacillus* GG was shown to be efficacious in reducing both the severity and duration of rotavirus diarrhea. An initial study by Isolauri et al (1) was corroborated in an extensive study by Guandalini (2) in which children with gastroenteritis throughout Europe were given either *Lactobacillus* GG or placebo. There was a statistically significant reduction in both the severity and duration of diarrhea in children given *Lactobacillus* GG; however, it did not appear efficacious in ameliorating the clinical course of nonviral diarrhea.

Probiotics were shown also to be efficacious in reducing the incidence of or preventing diarrheal illness. Saavedra et al (3) reduced the dissemination of diarrhea in hospitalized infants by adding *Bifidobacterium* and *Streptococcus thermophilus* to infant formula; *Lactobacillus* GG was shown to reduce the incidence of diarrheal illness in formula-fed toddlers, but not in breast-fed infants in Peru (4); and preliminary evidence from Ribeiro and Vanderhoof et al (5) showed that *Lactobacillus plantarum* reduces the incidence of diarrheal illness in daycare centers, even when administered to only one-half of the children (5).

The occurrence of *C. difficile* diarrhea can also be significantly reduced by administering probiotics. In uncontrolled studies, Gorbach et al (6) and Biller et al (7) showed that small numbers of children and adults, respectively, with *C. difficile* responded well to treatment with probiotics. Pochapin et al (8) more recently confirmed in double-blind, placebo-controlled studies the efficacy of probiotics in preventing recurrence after an initial episode of diarrhea (8). The biotherapeutic agent, *Saccharomyces boulardii*, although not a true probiotic because it is not of human origin, is likewise capable of reducing the recurrence of *C. difficile* (9).

Two recently published studies showed that the coadministration of antibiotics and *Lactobacillus* GG in children significantly reduces the incidence of non–*C. difficile* antibiotic-associated diarrhea (10, 11). In our recently published study, *Lactobacillus* GG or placebo was given to 200 children at the initiation of a broad spectrum antibiotic therapy for a variety of minor infectious processes, which were usually respiratory (10). The parents were questioned every 3 d by telephone about the number and consistency of stools and about numerous other gastrointestinal symptoms. Only patients assigned to a 10-d course of antibiotic therapy were considered for this study and probiotics were continued throughout the course of antibiotic therapy. Older children were given 2 capsules/d of *Lactobacillus* GG containing $\geq 10^{10}$ organisms and children who weighed <12 kg were given only 1 capsule/d. The incidence of diarrheal stools was 24% in

[1] From the Department of Pediatric Gastroenterology and Nutrition, University of Nebraska/Creighton University, Omaha.

[2] Presented at a symposium held at Experimental Biology 2000, in San Diego, April 2000.

[3] Address reprint requests to JA Vanderhoof, Department of Pediatric Gastroenterology and Nutrition, University of Nebraska Medical Center, 985160 NE Medical Center, Omaha, NE 68198-5160. E-mail: jvanderh@unmc.edu.

Am J Clin Nutr 2001;73(suppl):1152S–5S. Printed in USA.

the placebo group compared with 7% in the treatment group. Although typically mild, this nuisance form of diarrhea often results in the premature cessation of antimicrobial therapy and may constitute significant parental absenteeism from work because children with diarrhea often cannot be admitted to day-care centers. One could argue, therefore, that the coadministration of *Lactobacillus* GG and antibiotics to children might be routinely justified, at least in those children previously susceptible to antibiotic-associated diarrhea or taking antibiotics commonly associated with diarrhea.

Studies by Hilton et al (12) and Oksanen et al (13) both described the usefulness of *Lactobacillus* GG in reducing the risk of traveler's diarrhea. According to the results of these studies, travelers may expect a 25–50% reduction in the risk of diarrheal illness if they consume *Lactobacillus* GG when traveling to an area of high diarrheal risk.

Where does the road for probiotics lead from this point? We have already reviewed some of the material from Majamaa and Isolauri (14) regarding the potential use of probiotics in reducing the incidence of allergic disease. The potential of probiotics to reduce the incidence of allergic disease and to enhance the immune response to infections are probably the greatest arguments for widespread use of probiotics in healthy populations.

One of the first questions that needs to be answered is the appropriate means of administering probiotics. Probiotics can currently be administered in the form of sachets or capsules, or can be added to the food supply. Some data show that adequate colonization may be achieved at a lower dose if probiotics are administered in food (15, 16). More data is needed to firmly establish whether this is true and to establish the exact ratios indicating adequate colonization corresponding to these difficult vehicles of administration for each probiotic organism intended for prophylactic or therapeutic use. It is quite likely we will find that certain foods may be superior vehicles relative to others for disseminating probiotics. It is also possible that not all probiotics will be able to colonize the gastrointestinal tract when administered in food, whereas some strains may actually work best when administered in this fashion. All of these possibilities will require careful documentation.

It is quite likely that the beneficial effects of probiotics may be more important in infancy than in late childhood or adulthood. Recently, Vanderhoof et al (17) permanently colonized the gastrointestinal tracts of infants by administering probiotics to women beginning in their last trimester of pregnancy through childbirth. As beneficial organisms are identified, the administration of probiotics to mothers late in pregnancy might be better than lifelong administration of the organism to the child, at least from an economic standpoint. Further controlled studies are needed to determine whether any benefits are conferred by probiotics administered in this fashion. Additionally, as probiotics are added to the food supply, it is quite likely that pregnant women will consume these organisms and infant's gastrointestinal tracts will be colonized regardless of the intent of physicians. It is possible that a continuous administration or permanent colonization of probiotics may not be the best method of administration. It is possible that the immune enhancing properties of probiotics may require periodic pulse dosing to provide periodic immune stimulation. Again, there have been few studies in this area and further data are necessary to determine the best possible way to administer probiotics.

POTENTIAL USES OF PROBIOTICS

Preliminary data from several recent studies suggest the possibile wide-range beneficial effects of probiotics. Potential future uses of probioitcs include inflammatory disease control, the treatment and prevention of allergies, cancer prevention, immune stimulation, and a reduction in respiratory disease. Such effects could justify the addition of not one but potentially several probiotics to commonly consumed foods, which could achieve population-wide health benefits. Some of these data are discussed below.

The role of the intestinal flora in colon carcinogenesis and other forms of cancer is an important area for study. Bacterial metabolism of various dietary constituents results in the production of many compounds, some of which may be carcinogenic. It is likely that the composition of the intestinal flora may have a major effect on the production rate of such compounds. Altering the composition of the flora with probiotics may indeed change it enough to reduce the production of these carcinogenic compounds.

Because colon tumors can be induced in rats with administration of dimethylhydrazine (DMH), they are considered to be a good animal model to represent human colon carcinogenesis. Tumors produced by this method closely resemble human disease in histologic type, distribution within the large bowel, metastasis, and cell turnover (18). To evaluate the possible effects of a probiotic species in the prevention of carcinogenesis in an animal model, Goldin et al (19) studied 3 groups of rats. One group of rats received a standardized diet with a relatively high fat content (corn oil diet), the second group received the corn oil diet plus *Lactobacillus* GG and DMH, and the third group received the corn oil diet and DMH but no *Lactobacillus* GG. The addition of *Lactobacillus* GG to the corn oil diet in the animals challenged with DMH resulted in significantly fewer small intestinal tumors than in animals who had not received DMH, provided that the bacteria were given early in the course of treatment. The incidence of colon tumors was also significantly lower in the rats given *Lactobacillus* GG.

Pool-Zobel et al (20) performed studies with several species of *Lactobacillus* and 2 carcinogens, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) and 1,2-DMH, which was used after MNNG administration; the induction of DNA damage was measured and the inhibition of this injury by several probiotic strains was evaluated. All organisms (*Lactobacillus gasseri, Lactobacillus confusus, Streptococcus thermophilus, Bifidobacterium breve, Bifidobacterium longum,* and *Lactobacillus acidophilus)* showed an antigenotoxic effect after MNNG administration. Subsequent studies examined the effect of various cell fragments of *L. acidophilus*. Metabolically active *L. acidophilus* was active in preventing MNNG-induced DNA damage. The inclusion of cytoplasm, cell wall skeleton, and cell wall had no antigenic activity, whereas the peptidoglycan fraction in whole freeze-dried cells was antigenotoxic.

Studies were conducted with the use of DMH to assay for DNA damage in the gastrointestinal tract of rats. Pretreatment with *L. acidophilus*, *L. confusus, L. gasseri, B. longum,* and *B. breve* inhibited the genotoxic effect of DMH, but only 1 of 4 *S. thermophilus* strains and only 1 of 3 *Lactobacillus delbrueckeii* ssp. *bulgaricus* strains were protective. Heat-treated *L. acidophilus* did not inhibit DMH-induced genotoxicity. The use of MNNG and DMH are well-established methods to detect potentially anticarcinogenic effects (21).

Several species of lactic acid bacteria appear to prevent carcinogenic compounds from inducing the first crucial steps of tumorigenesis that may ultimately activate protooncogenes or inactivate tumor-suppressor genes (22). Several species of *Lactobacillus* appear to exert a protective effect when administered orally in rats. Because the antigenotoxic substances are heat labile, it appears that they must be formed by viable multiplying bacteria, suggesting the importance of live culture administration for this probiotic effect. However, the antigenotoxic effects of the peptidoglycan fraction and freeze-dried cells also suggest the possibility that, when given in adequate quantities, probiotic bacteria may exert some beneficial effects, even when given in a nonviable form (22).

Probiotics might also be useful in the treatment and prevention of many inflammatory disorders in the gastrointestinal tract. Strains of *Lactobacillus reuteri* and *Lactobacillus plantarum* were used to prevent inflammatory changes associated with methotrexate-induced enterocolitis in rats (23). Administration of lactobacilli decreased the intestinal myeloperoxidase concentration, often associated with inflammation, and reduced bacterial translocation to extraintestinal sites. Plasma endotoxin concentrations were reduced by probiotics. There is speculation as to whether this animal model is a valid predictor of response in gastrointestinal tract inflammatory disorders, eg, ulcerative colitis and Crohn disease. However, studies that use an interleukin 10 knockout mouse model, considered a better animal model for inflammatory bowel disease, also showed the potential efficacy of certain strains of *Lactobacillus*, especially *L. plantarum*, in reducing inflammation (24, 25). Rath et al (26) showed in HLA-B27 transgenic rats that normal luminal bacteria predictably and uniformly can induce chronic inflammatory changes in the gastrointestinal tract. Bacterial species vary greatly in activity. It is quite likely that changing the milieu of the flora in the gastrointestinal tract through the use of probiotics may modulate the inflammatory process.

We also know that probiotics may be useful in treating inflammatory diseases associated with small bowel bacterial overgrowth. Vanderhoof et al (27) reported uncontrolled studies showing the efficacy of *L. plantarum 299v* and *Lactobacillus* GG in treating children with small bowel bacterial overgrowth, predominantly in patients with short-bowel syndrome. A similar disease, pouchitis or inflammation of an ileal pouch created after a total colectomy for ulcerative colitis, may also respond to probiotic therapy. Administration of multiple organisms, predominantly *Lactobacillus* strains, was shown to be effective in ameliorating pouchitis (28). Numerous studies are underway to investigate more thoroughly the potential role of probiotic therapy in inflammatory bowel disease.

Some preliminary data are now beginning to arise in regard to the usefulness of probiotics in extraintestinal disease. Guarino (29) described a significant reduction in the severity of pneumonia in children with cystic fibrosis treated with *Lactobacillus* GG compared with a placebo group. Ribeiro and Vanderhoof (5) also showed that the introduction of probiotics to children who attended daycare centers reduced the incidence of respiratory disease. Insight into the possible mechanisms for these findings are beginning to surface. Mack et al (30) showed up-regulation of mucin genes in cell culture systems by *L. plantarum. Lactobacillus* GG appears to selectively stimulate the antibody reaction to both rotavirus and rotavirus vaccine, a property not shared by most other species of lactobacilli. Finally, Jung (31) showed that *Lactobacillus* GG produced a better antibody response to typhoid vaccine in adults treated with *Lactobacillus* GG than in a placebo group (31).

It is speculated that the inflammation associated with rheumatoid arthritis might be modulated by consuming probiotics (32). Normal processing of antigens absorbed through an inflamed and permeable gastrointestinal tract might serve as a link between inflammatory diseases of the gut and extraintestinal inflammatory disorders. Modulation of the immune system or changed gut permeability as a result of consuming probiotics might eventually become an important primary or adjunctive therapy in some of these disorders.

Lactic acid bacteria are known to have a wide range of effects on the immune system. They may have general immune-enhancing effects, which include augmentation of phagocytic function, ie, neutrophils, monocytes, macrophages, and natural killer cells. Specific immune responses, both humoral and cellular, can also be enhanced by lactobacilli (33). Perhaps some of the modulation of the inflammatory response may be more related to reregulating or modulating the immune system.

The efficacy of probiotics in the treatment of gastrointestinal disease is well established. As more probiotic organisms are discovered or engineered and more data are accumulated it is likely that probiotics may be used to treat and prevent other infectious disorders, allergic diseases, and even cancer. However, one cannot overemphasize the importance of carefully conducted double-blind, placebo-controlled studies to document the individual efficacy of each specific organism for each potential clinical application. The success of one species of *Lactobacillus* in a certain application does not imply that all related strains of this species will be capable of producing a comparable response. Probiotics should be administered carefully and cautiously, and only on the basis of strong scientific evidence. Such evidence should direct the cautious, deliberate addition of clinically proven probiotics to commonly consumed food products to allow consumers to conveniently benefit from these organisms.

REFERENCES

1. Isolauri E, Juntunen M, Rautanen T, Sillanaukee P, Koivula T. A human *Lactobacillus* strain (*Lactobacillus casei* sp strain GG) promotes recovery from acute diarrhea in children. Pediatrics 1991;88:90–7.
2. Guandalini S. Probiotics in the treatment of diarrhoeal disease in children. Gastroenterol Intest 1998;11(suppl):87–90.
3. Saavedra JM, Bauman NA, Oung I, Perman JA, Yolken RH. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhea and shedding of rotavirus. Lancet 1994;344:1046–9.
4. Oberhelman RA, Gilman RH, Sheen P, et al. A placebo-controlled trial of *Lactobacillus* GG to prevent diarrhea in undernourished Peruvian children. J Pediatr 1999;134:15–20.
5. Ribeiro H, Vanderhoof JA. Reduction of diarrheal illness following administration of *Lactobacillus plantarum 299v* in a daycare facility. J Pediatr Gastroenterol Nutr 1998;26:561 (abstr).
6. Gorbach SL, Chang TW, Goldin BR. Successful treatment of relapsing *Clostridium difficile* colitis with *Lactobacillus* GG. Lancet 1987;2:1519.
7. Biller JA, Katz AJ, Flores AF, Buie TM, Gorbach SL. Treatment of *C. difficile* colitis with *Lactobacillus* GG. J Pediatr Gastroenterol Nutr 1995;21:224–6.
8. Pochapin MB, Oltikar A, Pringe-Smith R, Schreiber C. A prospective randomized placebo-controlled trial of Lactobacillus GG in combination with standard antibiotics for the treatment of *Clostridium difficile* infection. Am J Gastroenterol 1998;93:1697.

9. Surawicz CM. *Clostridium difficile* disease: diagnosis and treatment. Gastroenterologist 1998;6:60–5.
10. Vanderhoof JA, Whitney DB, Antonson DL, Hanner TL, Lupo JV, Young RJ. *Lactobacillus* GG in the prevention of antibiotic-associated diarrhea in children. J Pediatr 1999;135:564–8.
11. Arvola T, Laiho K, Torkkeli S, et al. Prophylactic *Lactobacillus* GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. Pediatrics 1999;104:e64.
12. Hilton E, Kolakowski P, Smith M, Singer C. Efficacy of *Lactobacillus* GG as a diarrheal preventative in travelers. J Travel Med 1997;4:41–3.
13. Oksanen P, Salminen S, Saxelin M, et al. Prevention of traveler's diarrhoea by *Lactobacillus* GG. Ann Med 1990;22:53–6.
14. Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. J Allergy Clin Immunol 1997;99: 179–85.
15. Alander M, Satokari R, Korpela R, et al. Persistence of colonization of human colonic mucosa by a probiotic strain, *Lactobacillus rhamnosus* GG, after oral consumption. Appl Environ Microbiol 1999; 65:351–4.
16. Alander M, Korpela R, Saxelin M, et al. Recovery of *Lactobacillus rhamnosus* GG from human colonic biopsies. Lett Appl Microbiol 1997;24:361–4.
17. Vanderhoof JA, Iwen P, Hinrichs SH, Bilyeu DV, Young RJ. Colonization of an infant with the probiotic *Lactobacillus* GG by maternal transmission. Pediatr Res 1999;45:118A (abstr).
18. Rogers AE, Nauss KM. Rodent models for carcinoma of the colon. Dig Dis Sci 1985;30:875–1025.
19. Goldin BR, Gualtieri LJ, Moore RP. The effect of *Lactobacillus* GG on the initiation and promotion of DMH-induced intestinal tumors in the rat. Nutr Cancer 1996;25:197–204.
20. Pool-Zobel BL, Neudecker C, Domizlaff I, et al. *Lactobacillus*- and *Bifidobacterium*-mediated antigenotoxicity in the colon of rats. Nutr Cancer 1996;26:365–80.
21. Rafter JJ. The role of lactic acid bacteria in colon cancer prevention. Scand J Gastroenterol 1995;30:497–502.
22. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–67.
23. Mao Y, Nobaek S, Kasravi B, et al. The effects of *Lactobacillus* strains and oat fiber on methotrexate-induced enterocolitis in rats. Gastroenterology 1996;111:334–44.
24. Veltkamp C, Tonkonogy SL, Schultz M, Sartor RB. *Lactobacillus plantarum* is superior to *Lactobacillus* GG in preventing colitis in IL-10 deficient mice. Gastroenterology 1999;116:A838 (abstr).
25. Madsen KL, Doyle JS, Jewell LD, Tavernini M, Fedorak RN. *Lactobacillus* species prevents colitis in interleukin 10 gene-deficient mice. Gastroenterology 1999;116:1107–14.
26. Rath HC, Herfarth HH, Ikeda JS, et al. Normal luminal bacteria, especially bacteroides species, mediate chronic colitis, gastritis, and arthritis in HLA-B27/Human β_2 microglobulin transgenic rats. J Clin Invest 1996;98:945–53.
27. Vanderhoof JA, Young RJ, Murray N, Kaufman SS. Treatment strategies for small bowel bacterial overgrowth in short bowel syndrome. J Pediatr Gastroenterol Nutr 1998;27:155–60.
28. Gionchetti P, Rizzello F, Venturi A, et al. Maintenance therapy of chronic pouchitis: a randomized, placebo-controlled, double blind trial with a new probiotic preparation. Gastroenterology 1998;114: A4037 (abstr).
29. Guarino A. Effects of probiotics in children with cystic fibrosis. Gastroenterol Int 1998;11(suppl):91.
30. Mack DR, Michail S, Wei S, McDougall L, Hollingsworth MA. Probiotics inhibit enteropathogenic *E. coli* adherence in vitro by inducing intestinal mucin gene expression. Am J Physiol 1999;276:G941–50.
31. Jung LK. *Lactobacillus* GG augments the immune response to typhoid vaccination: a double-blinded, placebo-controlled study. FASEB J 1999;13:A872 (abstr).
32. Malin M, Verronen P, Mykkanen H, Salminen S, Isolauri E. Increased bacterial urease activity in faeces in juvenile chronic arthritis: evidence of altered intestinal microflora? Br J Rheumatol 1996;35:689–94.
33. Gill HS. Stimulation of the immune system by lactic cultures. Int Dairy J 1998;8;535–44.