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NUTRITIONAL IMPACT OF PRE- AND PROBIOTICS AS PROTECTIVE GASTROINTESTINAL ORGANISMS*

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■ **Abstract** The health benefits of pre- and probiotics have been the subject of increased research interests. These food supplements have been demonstrated to alter the pre-existing intestinal flora so as to provide an advantage to the host. This review focuses on the scientific evidence both for and against their role in promoting health and treating disease. Specific attention is turned to their effects on immunomodulation, lipid metabolism, cancer prevention, diarrhea, *Helicobacter pylori*, necrotizing enterocolitis, allergy, and inflammatory bowel disease.

CONTENTS

*Abbreviations: AC, aberrant crypts; ACF, aberrant crypt foci; AOM, azoxymethane; DMH, 1,2 dimethylhydrazine; FDA, Food and Drug Administration; FOS, fructooligosaccharide; IBD, inflammatory bowel disease; IFN, interferon; LGG, *Lactobacillus casei* GG; NEC, necrotozing enterocolitis.

INTRODUCTION

The mammalian intestinal tract contains a complex, dynamic, and diverse society of nonpathologic bacteria. Indeed, the number of bacteria that colonize the human body is so large that researchers have estimated that the human body contains 10^{14} cells, only 10% of which are not bacteria and belong to the human body proper (143). The majority of research to date has focused on the mechanisms by which pathogenic bacteria achieve their detrimental effects. However, more recently attention has turned to the indigenous nonpathogenic microorganisms and the ways in which they benefit the host. This is particularly relevant given the increasing incidence of bacterial resistance to antibiotics and the need to investigate alternative treatments for gastrointestinal diseases.

The normal colonization of the sterile newborn intestine is a complex process. Initial colonization is achieved with maternal vaginal and fecal bacterial flora. The first colonizers have a high reductive potential and include species such as enterobacter, streptococcus, and staphylococcus. These metabolize oxygen, thus encouraging the growth of anaerobic bacteria including lactobacilli and bifidobacteria. The microflora environment is regulated by the immune system with B cells secreting IgA to help control its volume and composition. In cesarean deliveries the normal contact the child would have with maternal flora is avoided and results in delayed colonization by typical flora such as lactobacilli and bifidobacteria by 10 days and one month, respectively (57). Similar delays are seen with the early use of antibiotics and sterile environments such as incubators. Studies have shown that early colonization of the intestinal tract is needed to activate the efferent limb of the mucosal immune response (69).

Further understanding of the beneficial effects of developing a normal bacterial flora is achieved by the analysis of germ free animal models. Germ free mice have small intestines that weigh less than their normal counterparts. This is at least in part due to the fact that the lymphoid constituents are underdeveloped and there are decreased numbers of immune cells. Specifically, plasma cells are lacking in the lamina propria and Peyer's patches, and subsequently there is little or no IgA expression. These changes "normalize" within 28 days of exposure to bacteria (106). Furthermore, antigen transport across the intestinal barrier increases in the absence of intestinal microflora (64).

A greater interest in understanding the importance of nonpathologic, or "good bacteria," has resulted in increased research efforts in this area. Accordingly, this has led to the concepts of probiotics and prebiotics as mediators of human health.

This paper discusses these entities and specifically reviews the literature that argues for and against their roles in the prevention and treatment of diarrhea, *Helicobacter pylori* infection, cancer, necrotizing enterocolitis, intestinal immunity, allergy, inflammatory bowel disease, lipid metabolism, and mineral absorption.

PROBIOTICS

Documentation of the health benefits of bacteria in food dates back to as early as the Persian version of the Old Testament (Genesis 18:8), which states "Abraham owed his longevity to the consumption of sour milk." Plinius, a Roman historian in 76 BC, recommended the use of fermented milk products for the treatment of gastroenteritis (149). In 1908 Nobel Prize–winning Russian scientist Elie Metchnikoff suggested that the ingestion of lactobacillus-containing yogurt decreases the number of toxin-producing bacteria in the intestine and thus contributes to the longevity of Bulgarian peasants (142).

It was these observations that led to the concept of a "probiotic," derived from the Greek, meaning "for life." The term was first used in 1965 in contrast to the word antibiotic and defined as "substances secreted by one microorganism, which stimulates the growth of another" (149). Since then the meaning of the word has evolved to apply to those bacteria that "contribute to intestinal balance." A more complete definition would be "a preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects on the host" (149). Current criteria for defining probiotics are found in Table 1.

Although probiotics are typically considered benign and without pathologic potential, there is a case report of a 1-year-old immunocompetent patient treated with *Sacchromyces boulardii* for gastroenteritis. Following bowel decontamination

TABLE 1 Defining criteria of microorganisms that can be considered probiotics

A probiotic should:
Be of human origin
Be nonpathogenic in nature
Be resistant to destruction by technical processing
Be resistant to destruction by gastric acid and bile
Adhere to intestinal epithelial tissue
Be able to colonize the gastrointestinal tract, if even for a short time
Produce antimicrobial substances
Modulate immune responses
Influence human metabolic activities (i.e., cholesterol assimilation, vitamin production, etc.)

with antibiotics, she had clinical evidence of septicemia and was found to be fungemic with *S. boulardii* (129). The Mayo Clinic reported eight patients who were immunocompromised after liver transplant who were found to have positive blood cultures for lactobacilli (123). The Food and Drug Administration (FDA) has no authority to establish a formal regulatory category for functional foods that include either probiotics or prebiotics (137). As such, there is variability among products, and some studies have found that certain preparations contain no viable bacteria (61).

Specific Probiotic Bacteria

Various bacteria have been identified as meeting the diagnostic criteria for probiotics; the more common ones are briefly discussed below.

Bifidobacterium is a major group of saccharolytic bacteria in the large intestine. It accounts for up to 25% of the bacteria in the adult colon and 95% of that in the breastfed newborn. They do not form aliphatic amines, hydrogen sulfide, or nitrites. They produce vitamins, mainly B group, as well as digestive enzymes such as casein phosphatase and lysozyme (50). Bifidobacteria produce acids as metabolic end products such as acetate and lactate to lower the pH in the local environment, which provides antibacterial effects. One study showed that the supplementation of bottle-fed infants with bifidobacteria successfully lowered the fecal pH to 5.38, which was identical to that of breast-fed infants, yet significantly lower than bottle-fed infants whose fecal pH was 6.38 (121). The survivability of the bifidospecies in various conditions has been investigated to determine if it can truly survive passage through the gastrointestinal system. The in vitro studies reveal that survivability depends on numerous factors, including the degree of stomach acidity, length of exposure to the acid, concentration and length of exposure to bile salts, and the level of bile salt hydrolase activity (14). Determination of survivability found that on average, approximately 30% of ingested *Bifidobacterium bifidum*, and 10% of *Lactobacillus acidophillus* can be recovered from the cecum (103). Fujiwara et al. identified a 1,000,000-kDa protein expressed by bifidobacteria that appears to inhibit adhesion of pathogenic *Escherichia coli* (45).

Lactobacillus casei GG (LGG) was originally selected because of its resistance to gastric acid and bile digestion, as well its ability to colonize human colon. Lactobacilli have no plasmids (thus, antibiotic resistance is stable) and make only L-lactic acid (not the D-isomer) (171). LGG expresses adhesive factors that permit interaction with human entrerocytes. Similarly, *L. acidophillus* was found to bind to a human enterocyte cell line (Caco-2) in a calcium independent fashion. The adhesion is thought to occur via an extracellular proteinaceous component. It inhibits other anaerobic bacteria in vitro including *Clostridium*, *Bacteroides*, *Bifidoacterium*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, and *Enterobacteriaceas* (154). *L. acidophillus* has also been shown to inhibit the growth of pathogenic bacteria including *Yersinea enterocolitica*, *Bacillus cereus*, *E. coli*, *Listeria monocytogenes*, and *Salmonella* (155). Lactobacilli generate hydrogen peroxide, decrease both intraluminal pH and oxygen concentration, and produce bacteriocins that can

inhibit the growth of pathologic bacteria (75). In general, colonization only lasts as long as the supplement is consumed. A study found that when LGG supplementation was stopped, the LGG disappeared from the feces in 67% of volunteers within 7 days (54).

S. boulardii is a patented yeast preparation that has been shown to inhibit the growth of pathogenic bacteria both in vivo and in vitro. It lives at an optimum temperature of 37°C, and has been shown to resist digestion and thus reach the colon in a viable state. *S. boulardii* appears to be unaffected by antibiotic therapy. However, once therapy is completed, they are rapidly eliminated (136).

HEALTH PROMOTING EFFECTS OF PROBIOTICS

Immunomodulation

In newborns the Th2 cytokine profile dominates over the Th1 cytokine profile, thus favoring allergic inflammation. Th2 cytokines include IL-4, which stimulates B cell differentiation into IgE producing cells, and IL-5, which plays an important role in the function of eosinophils. Intestinal bacteria help counterbalance this Th2 activity by promoting the Th1 subset and help downregulate IgE responses. This effect is multifactorial but thought to be significantly influenced by the CpG motif of certain bacterial DNA. The CpG motif is a six-base sequence which induces poly-clonal B cell activation and secretion of Th1 cytokines such as IL-6, IL-12, and interferon (IFN) (85). Intestinal bacteria may also modulate allergic inflammation via modification of antigen uptake (74), presentation (32), and degradation (160, 161).

Both human and rodent studies have documented an augmentation of sIgA production during probiotic treatment. Indeed, such an increase was previously discussed in relation to the use of probiotics in rotavirus diarrhea. Intestinal IgA is a dimer that binds antigens and thus prevents their interaction with the epithelial cell (41). Perdigon et al. demonstrated that *L. casei* and *L. acidophilus* enhanced IgA production from plasma cells in a dose-dependent fashion (125). *L. casei* was further shown to decrease IgE production by spleen cells and serum IgE to ovalbumin upon re-exposure to that protein (105).

Marin et al. investigated the effect of *Streptococcus thermophilus* on cytokine production utilizing both a macrophage and T-helper cell lines. The effects on the various cytokines appeared to be dose related. IL-6 production from the macrophage cell line was significantly increased (102). Conflicting studies in vivo found no effect of various probiotics on basal cytokine mRNA expression within mouse Peyer's patches, spleen, or lymph nodes after 14 days of exposure (163). However, a separate study of human circulating mononuclear cells found that exposure to lactobacilli resulted in increased expression of IL-1B, IL-6, and tumor necrosis factor (TNF) mRNA expression and cytokine excretion (109). Neumann et al. isolated *L. acidophilus* from a newborn human and inoculated it into germ free mice. After 7 days of colonization, there was improved macrophage phagocytic capacity as demonstrated by improved clearance of intravenously injected

E. coli (115). Furthermore, *B. bifidum* and *L. acidophilus* also doubled the number of white blood cells with phagocytic activity in 14 volunteers supplemented for 3 weeks (144, 145). However, Spanhaak et al. investigated the effects of *L. casei* on the immune system in 20 healthy volunteers. In a placebo controlled trial the probiotic had no effect on natural killer cell activity, phagocytosis, or cytokine production (156).

The ability of probiotics to affect the host's immune system remains ill defined. Good evidence exists for alterations in the humoral system, most notably IgA. However, effects on the cellular immune system and the production of cytokines warrants further investigation. Further evidence of their effect on the immune system is described below in reference to individuals with atopic disease.

Cholesterol Levels

The Maasai people of Africa consume large amounts meat, blood, and milk. Despite this atherogenic diet, the incidence of cardiovascular disease is low. It has been hypothesized that it is their consumption of milk that offers protection against disease. A study of these people (initially intended to investigate the role of surfactant) found that when one group consumed higher amounts of milk (up to an average of 8.3 liters/day) there was a decrease in cholesterol concentrations despite an increase in body weight. It was hypothesized that the milk inhibited cholesterol synthesis (101). A study of rats randomized to receive yogurt with or without bifidobacteria found that the total cholesterol of all rats fed yogurt decreased. The probiotic group had a notable increase in high density lipoprotein (HDL) cholesterol and a lowering of the low density lipoprotein (LDL) cholesterol by 21–27% compared with those rats fed whole milk (12). Gilliland et al. studied pigs on high-cholesterol diets and found that supplementation with *L. acidophilus* resulted in a smaller increase in total cholesterol compared with the unsupplemented group. The authors speculated that the bacteria modified the cholesterol within the lumen of the intestine, making it unavailable for absorption (51). Akalin et al., in a study of rats fed water, yogurt, or *L. acidophilus* yogurt, found that the probiotic group had lower total cholesterol concentration by day 28 of feeding, with levels 22% lower than those of controls. By day 56 the difference was 31%, with HDL and triacyglycerol being unaffected. Colonization of the animals with the acidophilus was documented by fecal culture (4). Finally, a group compared the cholesterol-lowering effects of a group of bacteria including bacilli, lactobacilli, streptococci, *C. butyrium*, *Sacchromyces cerevisiae*, and *Candida utilis* with those of *L. acidophilus*, or *Streptococcus faecalis*. The group receiving the mixture had a greater reduction in cholesterol concentration than did those receiving a single supplement (46). This concept of a "probiotic cocktail" in which multiple bacteria are used to complement each other's health promoting qualities has proven useful in other disease states as well (i.e., inflammatory bowel disease) and warrants future study.

Thompson et al. sought to investigate the cholesterol-lowering effects in healthy volunteers. Sixty-eight subjects were enrolled in a 10-week study that divided the

subjects into six groups. The groups included controls and others that were given various probiotic supplements. No differences were found in the cholesterol levels at the conclusion of the study. This was thought to be due to the fact that the subjects were healthy, with cholesterol concentrations less than 5.0 mmol/liter (164). This finding was supported by de Roos, who also found no change with supplementation in subjects with normal to borderline high cholesterol concentrations (35). Another study utilizing subjects with cholesterol concentrations between 5 and 6.5 mmol/liter randomized them to placebo or *Enterococcus faecium* supplementation. The supplemented group did have a 6% decrease in total cholesterol and a 10% decrease in LDL cholesterol at 6 weeks. A similar study found an 8% decrease in LDL in women supplemented with probiotic at one month. By 6 months, however, there were similar reductions in LDL concentrations among men provided placebo and probiotic. Other conflicting studies are those conducted by Lin et al., who using acidophilus found a decrease in serum cholesterol in one placebo controlled study but found no difference in a second study with more subjects (93).

The mechanism by which probiotics might lower serum cholesterol levels remains unclear. Observations that β-hydroxy-β-methylglutaryl-Coenzyme A reductase in the liver decreased significantly with the consumption of probiotics points towards a decrease in cholesterol synthesis. Further, increases in the amounts of fecal bile acids suggest that there is a compensatory increased conversion of cholesterol to bile acids (46). Wolever et al. found that rectal infusion of short chain fatty acid fermentation products, acetate or propionate, are absorbed into the blood. Acetate increased total cholesterol and decreased fatty acids, whereas propionate increased blood glucose and lowered the hypercholesterolemic response of acetate (174). It was thus suggested that the role of probiotics was to alter the proportion of these breakdown products produced during fermentation so as to have an effect on cholesterol. The cholesterol-lowering effect seen in culture media is thought to be secondary to precipitation of cholesterol with free bile acids formed by bacterial bile salt hydrolase (34). A final mechanism by which probiotics may have an effect is via hydrolysis of bile salts. Those bacteria that hydrolyze efficiently would lead to a faster rate of cholesterol conversion to bile acids and thus lower the serum cholesterol concentration. Studies that demonstrate that bile acids are eliminated faster in normally nourished rats than in germ free rats support these findings (42).

Although data on probiotics and serum cholesterol are somewhat mixed, there does appear to be a possible beneficial effect to the host. Thus, further studies are needed to define the role of probiotics and their mechanism of action in this regard.

Cancer Prevention

Numerous epidemiologic studies have been performed to determine risk factors for various forms of cancer, particularly those such as diet that can be easily altered.

Some of these studies have pointed to areas in which probiotics may play a role. Monique et al. (112) found an inverse relationship between frequency of yogurt ingestion and breast cancer in France. van't Veer et al. had similar results in the Netherlands (172), but this relationship was not seen by Kampman et al. (82). One study showed that supplementation with *L. casei* Shirota did decrease the rate of superficial bladder cancer (10). In this open labeled study 48 Japanese patients were enrolled after removal of one or more bladder tumors. They took *L. casei* for one year or until recurrence. Recurrence was documented in 83% of historical nonplacebo controls vs. 57% of the study group. When this study was repeated using 125 patients and a placebo control, overall there was no postponement of tumor recurrence. However, if the 47 patients who had more than one recurrent tumor were excluded, the tumor-free period was 79% with the probiotic and 55% without (11). Moore & Moore (114) studied the fecal flora in patients at high and low risk for cancer and found that the high-risk group had more bacteroides and bifidobacterium, whereas the low-risk group had more lactobacilli and *Eubacterium aerofaciens*. Peters et al. found yogurt to be a protective factor in a case controlled study of colon cancer (127).

Colon cancer is thought to arise as the end result of multiple initiating and activating steps that remain incompletely understood. Morphologically early changes begin as cell overgrowth within the colonic crypts, which are termed aberrant crypts (AC). These are considered preneoplastic structures. They are histologically identified as being enlarged and elevated when compared with normal crypts. Small fractions then progress to polyps and eventually tumors. Early studies investigated the role of fermented milk in tumor prevention. Takano et al. (162) found that fermented milk reduced the number of tumors caused by 1,2-dimethylhydrazine (DMH) in rats. Shackleford et al. (151) further studied these effects by examining placebo versus milk fermented by *Streptococcus thermophilis* or *Lactobacillus bulgaricus* in the same model of DMH colonic tumors. They found that although the numbers of colonic tumors was not different among the three groups, the survival time was greater for the rats given fermented milk. Abdelali et al. (1) examined the number of AC found in placebo versus bifidobacterium fed rats. The probiotic reduced the incidence of AC by nearly 50%. Cecal pH remained unchanged, but there was a reduction in β-glucuronidase activity. Tsuda et al. (167) found that *Bifidobacterium longum* decreased AC foci. Koo & Rao (87) provided mice with both *B. pseudolongum* and a prebiotic fructooligosaccharide (FOS) and demonstrated a near 50% reduction in AC in DMH-treated mice at 8 and 38 weeks. At the end, the number of bifidobacterium colonizing the study group was greater that that of the control.

Kulkarni & Reddy (90) fed bifidobacteria to 10-week-old rats 5 weeks prior to the induction of tumors with azoxymethane (AOM). AC development was approximately half in the study groups, and fecal β-glucuronidase was also decreased. Reddy further reported in 1998 that various markers of cell proliferation decreased in rats given *B. longum*. These included the colonic labeling index, ornithine decarboxylase activity, and *ras*-p21 oncogene activity (133). Gallaher et al.

(48) supported these findings by showing that bifidobacteria and FOS decreased AC by almost 50% in DMH-treated rats. However, a subsequent study using milk-fed animals as a control group found no change in AC numbers. Challa et al. (26) examined AOM-induced AC in rats consuming *B. longum* with or without lactulose. Alone or in combination, the numbers of AC were reduced. Rowland et al. showed that bifidobacteria with or without a prebiotic inhibited AOM-induced AC and resulted in a decrease in cecal β-glucosidase (138). Arimochi et al. (8) studied AOM-induced AC and found that bifidobacterium had no effect, whereas *L. acidophilus* and *Clostridium perfringens* decreased AC. The culture supernatants mediated the effect, and the authors speculated that butyrate was the metabolically active product.

The mechanism by which probiotics exert this anticancer effect is unclear. Investigators hypothesize that lactobacilli may bind to mutagenic compounds, and thus their excretion in urine would be diminished. Indeed, excretion of urinary mutagens after consumption of a hamburger, if supplemented with *L. acidophilus*, decreased about 50% (92). However, fecal excretion of mutagens was not significantly reduced. A similar study using LGG did not alter fecal mutagen excretion, but urinary excretion was not determined (88). A final study using patients as their own controls again showed reduction of urinary mutagens by 50% (62).

Another theory of the mechanism of cancer prevention is based on the ability of "bad bacteria" to convert procarcinogens into carcinogens using various enzymes such as β-glucuronidase, nitroreductase, and choloylglycine hydrolase. LGG has been shown to decrease fecal β-glucuronidase (54, 95), nitroreductase (95), and choloylglycine hydrolase (94, 95). Consumption of milk with *L. casei* Shirota for 4 weeks temporarily decreased β-glucuronidase and β-glucosidase in 10 healthy subjects compared with 10 healthy controls (136). Bifidobacterium decreased β-glucuronidase activity from baseline but not nitroreductase after 12 days (18). Another study using *L. acidophilus*, *B. bifidum*, *S. lactis*, and *Streptococcus cremoris* for 3 weeks demonstrated reduction of nitroreductase but not β-glucuronidase (104).

A final theory relating to the cancer protective effects of probiotics involves a deactivation of carcinogens. *L. bulgaricus* prevented DMH-induced DNA breaks in rats in vivo, whereas *Streptococcus thermophilus* did not. However, both strains prevented DNA damage in vitro when rats were exposed to N-methyl-N-nitro-N-nitrosoguanidine. Indeed extracts from the *S. thermophilus* were also effective in deactivating the N-methyl-N-nitro-N-nitrosoguanidine (175). The authors hypothesized it is thiol-containing breakdown products of proteins created by bacterial proteases that deactivate various colonic mutagens. In these experiments DNA damage was determined by the Comet assay, which detects damage via a microgel electrophoresis assay using plated colon cells.

Although many animal models of cancer described above do seem to offer encouraging results, long-term well-controlled studies of probiotics are needed to determine if these changes can be replicated in humans.

PROBIOTICS AND DISEASE

Diarrhea

Potential mechanism by which probiotics prevent or ameliorate diarrhea may be through stimulation of the immune system, competition for binding sites on intestinal epithelial cells (38, 39, 125), or through the elaboration of bacteriocins such as nisin (76). These and other mechanisms are thought to be dependent on the type of diarrhea being investigated, and therefore may differ between viral diarrhea, antibiotic-associated diarrhea, or traveler's diarrhea.

VIRAL DIARRHEA The effect of *Lactobacillus GG* (LGG) on the shortening of rotavirus diarrhea has been well documented. On average, the duration of diarrhea was shortened by one day in both hospitalized children (72, 73, 79, 80, 100, 122, 152) and in those treated at home (59). A multi-center European study investigated the use of LGG in acute diarrhea regardless of cause. There were 140 children in the placebo group and 147 in the LGG group. Supplementation with LGG was initiated during the rehydration phase. Overall LGG appears to shorten the duration of diarrhea from an average of 71.9 to 58.3 h, and with rotavirus (32–38% of those affected) from 76.6 to 56.2 h; here too the number of watery stools was decreased. Those with entero-invasive bacteria showed no difference. Prolonged diarrhea, defined as lasting longer than 7 days, was less common in the LGG group (2.7%) than the placebo group (10.7%). This resulted in shorter hospital stays for the LGG group (58).

As to why LGG appears to be effective for viral but not bacterial diarrhea, the authors speculate that this is due to LGG enhancement of the expression of the elaboration of intestinal mucins. These glycoproteins appear to be protective during intestinal infections. However, the protective qualities are overcome by mucinase-producing bacteria (97). A study of the use of LGG in acute diarrhea (82% rotavirus) in 71 well-nourished children found that the duration of diarrhea was shortened from 2.4 to 1.4 days (72). Saavedra et al. examined chronically hospitalized children and demonstrated that supplementation with a combination of *B. bifidum* and *S. thermophilus* reduced the incidence of diarrhea and shortened the duration of rotavirus shedding (140). *L. casei* was used in a placebo-controlled manner in 42 children with acute rotavirus diarrhea. Eighty-three percent of the study group shed the probiotic bacteria in their stool. Supplementation occurred after the initial rehydration phase. Duration of diarrhea was reduced from 2.3 to 1.5 days. The placebo group had an increase in urease-producing bacteria not seen in the control group. The authors thus hypothesized that rotavirus causes a biphasic diarrhea, the first phase being osmotic, and the second phase owing to overgrowth of urease-producing bacteria. This latter phase is prevented by the probiotic (73).

Other investigators studied the immune-modulating effects of probiotics as a means of decreasing diarrhea. Majamaa et al. studied 49 children with acute rotavirus diarrhea. They were randomized to receive LGG, *L. casei* subsp rhamnosus (lactophilus) or a combination of *S. thermophilus* and *Lactobacillus delbruckii*

(Yalacta). The mean duration of diarrhea was 1.8 days for children in the LGG group, 2.8 in the lactophilus group, and 2.6 in the Yalacta group. Only LGG was shown to have significantly increased the number of rotavirus-specific IgA secreting cells and serum IgA in the convalescent stage (100). Similarly Kaila et al. assigned 39 children with acute rotavirus infection to either placebo or LGG. The LGG group had a shorter duration of illness, 1.1 days compared with 2.5 days for the placebo group. Also, the acute phase revealed greater numbers of immunoglobulin secreting cells in the circulation of the LGG group, and in convalescence a greater number of these patients had IgA-specific secreting cells to rotavirus (90% vs. 46%) (80). Two other studies support this finding of a significant increase in serum IgA specific to rotavirus (79, 128), suggesting that the humoral immune system plays a significant role in the probiotics' effect.

A study compared the efficacy of heat-inactivated LGG compared with viable bacteria in the treatment of rotavirus. The two seemed equally efficacious clinically in reducing the duration of diarrhea; however, the heat-inactivated strains did not result in an elevated IgA response at convalescence (100). Probiotics' role in immune modulation is extended to an improved immunologic response to live oral rotavirus immunization in children supplemented with LGG (71). Other studies demonstrated the role of probiotics in enhancing the immune response to enteric viruses. The ingestion of formula supplemented with *Bifidobacterium lactis* for 20 days significantly increased the levels of fecal total IgA and anti–polio-specific IgA in children between the ages of 15 and 31 months (47).

Another probiotic studied was *E. faecium*. Patients with acute diarrhea were assigned either this probiotic or placebo, and the study group was found to have a shorter duration of illness (176). A large study of 211 adults with acute diarrhea also demonstrated a shortened diarrheal phase, from 2.8 to 1.7 days in those taking *Streptococcus faecium* SF68 (23). Studies with *L. acidophilus* have not shown similar effects. A study by Bin on 50 Chinese children with acute diarrhea failed to demonstrate an effect of *L. acidophilus* in enhancing recovery (16). Boulloche et al. studied 103 children with acute diarrhea and randomized them to either *L. acidophilus*, loperimide, or placebo. No significant difference in recovery time was demonstrated (19).

From these numerous studies it is clear that probiotics do have a therapeutic role in viral diarrhea. However, the exact mechanism of action is not clear and is likely multifactorial. Finally, it appears as though not all strains of probiotics are equally effective in treating viral diarrhea.

ANTIBIOTIC-ASSOCIATED DIARRHEA The incidence of antibiotic-associated diarrhea is between 5 and 30%. The success of probiotics in reducing or preventing such diarrhea has been convincing and includes various probiotics, as well as various antibiotics. A study of 16 healthy volunteers taking erythromycin for one week found that co-administration of LGG yogurt reduced the number of days with diarrhea from 8 to 2, as well as decreasing associated side effects such as abdominal pain from 39% to 23%. Documentation of intestinal colonization via

collection of bacteria in the feces was seen in 75% (153). Arvola et al. studied 119 children with a respiratory illness requiring antibiotics and randomized them to receive either LGG or placebo. LGG resulted in a significant decrease in the incidence of diarrhea compared with placebo (5% verses 16%) (9). In a double-blind placebo controlled study Surawicz et al. (159) assigned 180 hospitalized patients on antibiotics to placebo or *S. boulardi*. Twenty-two percent of the placebo group and 9% of the probiotic group developed diarrhea. Large studies by Adam et al. (2) and McFarland et al. (96) using the same organism found similar results, with decreases in the incidence of diarrhea in the study and placebo groups of 13% and 7.2%, respectively. A multi-center study (176) looked at the same question using *E. faecium* or placebo for patients receiving antibiotics. The difference in incidence of diarrhea was 9% versus 27%, respectively. In a trial using a combination of *L. acidophilus* and *L. bulgaris* by Gotz et al. in 1979 (56), on 79 hospitalized patients on ampicillin, 14% of the placebo group had diarrhea compared with none in the probiotic group. Finally, 188 children taking a 10-day course of antibiotics were enrolled in a double-blind placebo controlled study using LGG. The LGG decreased the incidence of diarrhea from 26% to 8%, as well as the duration of diarrhea from 5.88 days to 4.7 days. Furthermore, the consistency of the stool was noted to be looser in the placebo group as determined by a visual scoring system. Colonization was not documented and *Clostridium difficile* was not checked, although the authors argue that the diarrhea was of rapid onset (171).

The use of probiotics for the treatment of *C. difficile* diarrhea is a logical step, particularly given the historical use of fecal enemas in the treatment of relapsing *C. difficile* (20, 150). Indeed this is supported by an early case report of four children with relapsing *C. difficile* that responded to supplement with LGG (15). *C. difficile* toxins A and B are cytotoxins, but toxin A has enterotoxic effects in rodent intestine. Toxin A in the ileum of rats increases intestinal fluid secretion, permeability, histologic damage, and mediators of inflammation. Toxin B can cause tissue damage. *S. boulardii* inhibits toxin A and B enteritis by releasing a 54-kDa protease that cleaves these toxins and their receptor for the brush border membrane (25). *S. boulardii* was used in conjunction with standard antimicrobial treatment in a placebo controlled study of 124 adult patients with *C. difficile*. Sixty-four patients were suffering from their initial presentation with this bacteria, whereas 60 had a prior episode of disease. Those with the initial episode had no difference in recurrence rate, but the probiotic significantly inhibited further recurrence in those patients with prior *C. difficile* disease (107).

BACTERIAL DIARRHEA, INCLUDING TRAVELER'S DIARRHEA Many studies have investigated the efficacy of LGG in preventing traveler's diarrhea. In one, 820 travelers to 2 resorts in Turkey failed to show an overall effect of the use of LGG as preventative, as 43% of the controls and 38% of the treatment group developed diarrhea. However, when the data from one resort was examined separately, the difference in diarrhea was 40% for controls and 24% for the treatment group (120). In a second study travelers were monitored through a hospital-based travel and immunization

center. The destinations were varied and compliance with the study drug (placebo or LGG) was good in 245 participants. Overall the LGG reduced the incidence of traveler's diarrhea to an average of 7.4%/day for the placebo group and 3.9%/day for the probiotic group (65). Tourists in Egypt given *S. thermophilus*, *L. bulgaricus*, *L. acidophilus*, and *B. bifidum* also had a lower incidence of diarrhea, decreased from 71% to 43% (17).

Whereas the above studies offer encouraging results, studies of traveler's diarrhea found no effect for *L. acidophilus* or *E. faecium* in Austrian tourists (86). Studies of *L. acidophilus* and *Lactobacillus fermentum* showed no effect in preventing traveler's diarrhea (83). Furthermore, healthy volunteers showed no benefit when given lactobacilli when exposed to enterotoxigenic *E. coli* (28). The effects of probiotics in decreasing the incidence of diarrhea caused by other pathogenic bacteria also appear to be mixed. A study of 183 Bangladeshi adults with acute diarrhea caused by *Vibrio cholera*, enterotoxic *E. coli*, or unknown organisms failed to demonstrate a positive effect of supplementation with *E. faecium* (111). Other studies of patients with infective bloody diarrhea do not demonstrate a benefit from probiotics (122, 131, 152). However, some studies with *L. acidophilus* have shown an effect against bacterial causes of enteritis (6, 55, 177). Also, there is a questionable benefit with *Bifidobacterium breve* against *Campylobacter* (165).

Overall the studies investigating probiotics for treatment or prevention of bacterial diarrhea, other than *C. difficile*, appear mixed. Indeed cost-benefit analysis studies need to be performed to investigate whether the marginal reduction in traveler's diarrhea warrants treatment of all travelers.

Helicobacter pylori

Although numerous studies exist to demonstrate probiotics' ability to alter the colonic bacterial flora, there is a paucity of data examining their role in affecting bacteria that can colonize the stomach. Some studies support the fact that certain probiotics can prevent the growth of *H. pylori* in vitro (3, 78, 108). Aiba et al. used an in vitro model and found that *L. salivarius* but not *L. casei* or *L. acidophilus* inhibited the growth of *H. pylori* in a mixed culture. It was thought that the unsuccessful organisms were unable to produce enough lactic acid to completely inhibit *H. pylori* growth. This finding was reproduced in vivo using an *H. pylori*–infected gnobiotic murine model (3). A recent study by Sakamoto et al. looked at 31 humans infected with *H. pylori*. They were provided yogurt with *Lactobacillus gasseri*. A [(13)C]urea breath test subsequently documented a suppression of *H. pylori* urease activity (141). Future research and well-controlled studies will need to be done to determine the true value of probiotics with respect to this organism.

Necrotizing Enterocolitis

Although necrotizing enterocolitis (NEC) accounts for a great deal of morbidity and mortality among premature infants, the mechanism of injury is still

incompletely understood. It is hypothesized that the intestinal injury is the result of synergy among the three major risk factors of NEC: prematurity, enteral feeding, and bacterial colonization. Together these factors result in an exaggerated inflammatory response that leads to ischemic bowel necrosis (27). Indeed one investigator described a case of 3-day-old twins in which one was mainly colonized with bifidobacteria and the other with *Clostridium* species. This latter child developed NEC (22). Such a finding was replicated in animal models; specifically a quail model exists in which exposure of the germ free quail to clostridia results in cecitis. In this model preexposure to bifidobacteria appears to be protective through inhibiting the growth of the clostridial species (22). Furthermore, after feeding the fecal flora of patients with NEC to germ free pups, one observes subsequent bowel injury (91). In another animal model of NEC using a combination of formula feeding and asphyxia in neonatal rats, Caplan et al. demonstrated that supplementation of bifidobacteria and subsequent colonization significantly reduced the incidence of NEC as compared with rats supplemented with *E. coli* (24). The injury caused by some NEC associated bacteria such as *E. coli* can be abrogated by coinfection with gram-positive organisms from non-NEC infants. In vitro studies using Caco-2 monolayers support this, as one sees a reduction in the transcytosis of *E. coli* across the monolayer when *E. faecium* is introduced (33). Other studies also support a link between enteric bacteria and NEC (13, 44, 66, 157). These studies demonstrate that a single pathogenic stain of bacteria such as *Klebsiella*, *Enterobacter*, or *E. coli* undergoes rapid growth prior to the clinical manifestations of NEC.

Based on the promising results of animal models demonstrating a protective effect of bifidobacteria in preventing NEC, Hoyos studied 1237 newborns in a neonatal intensive care unit in Columbia. Newborns were provided with 250 million live *L. acidophilus* and 250 million live *Bifidobacterium infantis* and their outcome was compared with historical controls from one year earlier. The historic control group had 85 newborns that developed NEC, and the probiotics group had only 34. There were 35 prior fatalities from NEC compared with 14 in the probiotic group (67). Clinical studies such as this are certainly encouraging and will no doubt become the basis for future studies in the use of probiotics in the prevention of NEC.

Allergy

Although the exact pathophysiology of allergic disease is incompletely understood, it is thought to represent the end result of disordered function of the immune system. One thought is that the abnormal immune response is due in part to increased permeability of the intestinal barrier. A study of suckling rats documented that prolonged cow milk challenge results in increased gut permeability to intact proteins as demonstrated by absorption of horseradish peroxidase. This is abrogated by the introduction of LGG. The authors suggest that an observed increase in the secretion of antibodies directed against β-lactoglobulin, a major antigen of the cow milk protein, contributes to the stabilization of the mucosal barrier (74). Another study analyzed the fecal flora from healthy and allergic infants. The

allergic infants were found to have high levels of the adult type *Bifidobacterium adolescentis* as compared with the healthy infants, who had greater numbers of *B. bifidum*. Comparison of the adhesive properties of these two strains found that *B. bifidum's* adhesive abilities were significantly higher. These results suggest that the greater adhesive qualities may help to stabilize the mucosal barrier and prevent absorption of antigenic proteins (63).

Oral tolerance to food antigens is underdeveloped in infancy and is thought to require bacterial colonization to develop properly. A recent study found that the anaerobic intestinal flora of milk-hypersensitive adults resembles that of healthy adults (7). Despite this, clinical studies do support the use of probiotics in the treatment of atopic disease. A study by Majamaa & Isolauri investigating cow's milk–sensitive infants with atopic dermatitis assessed their response to a hydrolyzed whey formula alone or in combination with LGG. At the end of one month the probiotic group had a significantly greater improvement in the extent and intensity of their eczema. Furthermore, the amount of fecal alpha-1-antitrypsin, a marker of intestinal inflammation, was significantly lowered in the study group, as was the concentration of fecal TNF. Fecal eosinophilic cationic protein was unchanged. The authors speculated that the LGG generated enzymes that can act as a suppressor of lymphocyte proliferation and that the LGG also helped to generate protein breakdown products that result in IL-4 downregulation. Furthermore, the LGG-stimulated increase in sIgA helps increase antigen elimination. All this taken together results in the observed downregulation of hypersensitivity reactions (99). The same authors further studied this by investigating a group of 27 exclusively breast-fed infants with atopic dermatitis at an average age of 4.6 months. This group was weaned to a hydrolyzed whey formula alone, with supplemental *B. lactis* or with LGG. The children's eczema was scored in a blinded fashion using a SCORAD grading system. After 2 months the skin condition of the probiotic group was significantly improved to nearly normal. This improvement was paralleled by a reduction in the concentration of soluble CD4 in the serum and eosinophilic protein X in the urine. This reflects downregulation of the T-cell mediated inflammatory state and eosinophilic inflammatory activity, respectively. After 6 months all three groups had resolution of their eczema (70). A recent study by Kalliomaki et al. provided LGG in a double-blind placebo controlled fashion to pregnant mothers with a first degree relative that is atopic. The newborn infants were then treated postnatally for 6 months. At 2 years of age 23% of the LGG group and 46% of the placebo group were found to have atopic eczema (81).

Other studies have also examined alterations of the cytokine profile. LGG has immunomodulatory effects and has been demonstrated to stimulate in vitro mononuclear cells, thus generating proinflammatory cytokines such as IL-6, IL-12, IFN, TNF, and the antiinflammatory IL-10 (110). IL-10 is produced by monocytes, activated B cells, macrophages, mast cells, keratinocytes, and dendritic cells (113). It inhibits the production of IL-2, IL-4, IL-6, IL-12, TNF, and IFN (36, 113). It upregulates the growth of mast cells and B cells and downregulates IgE synthesis by promoting the switch to IgG4 synthesis (77, 113). In atopy IL-10 is thought to

have antiinflammatory effects via downregulation of IL-4 (126). LGG was given for 4 weeks to nine children with atopic dermatitis and cow's milk allergy while on an elimination diet. Eight weeks following supplementation there was a transient increase in IL-10 in seven out of the nine patients. At 2 weeks there was an increase in IFN and a subsequent decline in IL-4. There was no change in fecal sIgA. No mention was made of the clinical effect the supplementation had on the children, but the authors proclaimed that the induction of IL-10 by the LGG justified continued study of this probiotic for the treatment of atopic disease.

Inflammatory Bowel Disease

It has long been conjectured that bacteria or other infectious agents play a role in the pathogenesis of inflammatory bowel disease (IBD). Some hypothesize that IBD is due to an abnormal T cell response to commensal microflora, whereas others speculate that pathogenic organisms such as an atypical mycobacteria or co-infection with viral particles such as mumps and measles are causative. Indeed, it is well accepted that antibiotics are effective in the treatment of Crohn's disease, and certain animal models of colitis only have phenotypic manifestations when exposed to bacteria. Epidemiologic studies have found that bifidobacterium colony counts are decreased in numbers in the feces of patients with Crohn's disease (168). A recent study of patients with Crohn's disease found them to have decreased amounts of beta-galactosidase in their feces during periods of active disease. This decrease correlates with a decrease in bifidobacteria, which is thought to be the source of the beta-galactosidase (43). Similar findings can be found in animal models in which a study of the intestinal flora of IL-10 knockout mice revealed that as colitis develops between 2 and 8 weeks of age there is a decrease in the numbers of lactobacilli. Indeed, by repopulating the mice with supplemental lactobacillus the histologic severity of colitis can be attenuated. This also decreases the amount of mucosal adherence and invasion by aerobic bacteria (98). A separate strain of lactobacillus, *L. salvirius*, has been shown in that same model to diminish the progression of inflammation to dysplasia and to subsequent cancer (29).

Clinical studies of affected patients have demonstrated the efficacy of other probiotics, most notably certain strains of *E. coli*, in maintaining remission in ulcerative colitis at a rate equivalent to mesalamine. A study of 116 patients with active ulcerative colitis were randomized to receive either standard treatment with mesalamine or treatment with a nonpathologic strain of *E. coli*. Both groups achieved remission at similar rates, 75% and 68% for the mesalamine and *E. coli* groups, respectively. The mean time to remission was 44 days and 42 days for the two groups and relapse occurred during the 1-year study in 73% and 67%, respectively. The authors concluded that the probiotic has an equivalent effect to mesalamine in achieving and maintaining remission (134). A similar study examining 120 ulcerative colitis patients in remission found similar relapse rates for the mesalamine and *E. coli* groups at the end of 12 weeks, 11.3% and 16%, respectively (89). A study designed to investigate the effectiveness of

probiotics in maintaining remission in Crohn's disease randomized 32 patients in clinical remission to receive either mesalamine alone or in conjunction with *Saccharomyces boulardii*. The control group experienced clinical relapse at a rate of 37.5%, whereas only 6.25% of the supplemented group relapsed (60). Various probiotic bacteria have also been shown to be useful in the maintenance treatment of chronic pouchitis including *Bifidobacterium*, *Lactobacillus*, and *Streptococcus* (52). Despite this, a study of another probiotic, *Lactobacillus plantarum*, failed to reduce the severity of trinitrobenzenesulfonic acid/ethanol) colitis or improve gut permeability (84).

IL-10 has been the center of much attention owing to its role in downregulating inflammatory cascades; indeed the IL-10 knockout mouse is a commonly used animal model in IBD research. Without the antiinflammatory effects of IL-10, a Th1 profile predominates with increased concentrations of IFN and IL-12 (98). This model emphasizes the importance of microflora in the pathogenesis of IBD in that when reared in a sterile environment, the IL-10–deficient mice are asymptomatic, yet when colonized with typical bacteria, they develop colitis. Researchers used two mice models of IBD to demonstrate the therapeutic effects of IL-10 in reducing the mucosal inflammation of IBD. IL-10 has previously been investigated for its potential role in the treatment of IBD; however, owing to presumed difficulty in mechanisms of delivery, clinical trials have been less encouraging. These researchers applied the concepts of probiotics, most notably their ability to escape digestion and colonize the intestine, and developed recombinant strains of *Lactobacillus lactis* that were genetically engineered to produce IL-10. When introduced into a chronic colitis model induced by 5% dextran sulfate sodium, it reduced the degree of histologic colitis by nearly 50% as compared to control mice provided with wild type *L. lactis*, better than the results seen with anti-TNF antibody therapy. It also virtually prevented the onset of colitis in the IL-10 knockout model (158). This approach, although clearly not attributable to the effects of the wild-type bacteria, does advance the concept of probiotics to a new level. The use of enteric bacteria as a mode of local delivery of drugs is novel and encouraging but not without concern. Clearly we must consider the potential effects of contamination of normal hosts as well as dosing issues before such ideas are applied clinically.

The use of probiotics in IBD clearly will not provide a panacea, but it does offer hope as an adjuvant form of therapy, specifically in maintaining a state of remission. In addition it sheds further light on the pathophysiology of the disease. Finally it illustrates an exciting new direction in which the theories of probiotics can be applied as a means by which one can achieve local drug delivery.

PREBIOTICS

Evidence of the beneficial effects of certain nonpathologic enteric bacteria, probiotics, gave birth to the concept of prebotics. Gibson & Roberfroid defined a prebiotic in 1995 as a "nondigestible food ingredient which beneficially affects

the host by selectively stimulating the growth of and/or activating the metabolism of one or a limited number of health promoting bacteria in the intestinal tract, thus improving the host's intestinal balance" (50). Because this concept has only been recently defined, there is not as much data to support prebiotics' health promoting effects. Examples of prebiotics include the fructooligosaccharides and complex oligosaccharides in human milk. Each of these satisfies the defining criteria of prebiotics as outlined in Table 2.

Fructooligosaccharides (FOS) are short and medium chains of β-D fructans in which fructosyl units are bound by a β 2-1 osidic linkage. The FOS are classified based on the number of osyl units, which defines their degree of polymerization. Accordingly, oligofructose has a degree of polymerization <9 (average 4.8) and inulin has a degree of polymerization <60 (average 12). Inulin-producing plant species include several monocotyledonous and dicotyledonous families such as Liliaceae, Amaryllidaceae, Gramineae, and Compositae. Commercially inulin is the product of hot water extraction from chicory roots (*Cichorium intybus*), and oligofructose is prepared by the partial hydrolysis of inulin under controlled conditions. They are also contained in a number of common foods including garlic, onion, artichoke, and asparagus (50).

The β 2-1 osidic bond of FOS, including the first glucose-fructose bond, is not readily hydrolyzed by mammalian digestive systems. However, bifidobacteria possess β-fructosidases that allow them to digest these compounds (50). Studies in volunteers with ileostomies have shown that when they are fed either inulin or oligofructose, the average recovery of the fed prebiotic at the terminal ileum is 86–89%. Further proof of their indigestibility rests in the fact that there is no observed increase in serum glucose after they are ingested (31).

The ability of prebiotics such as oligofructose to selectively stimulate the growth of bifidobacteria has been supported by numerous studies (155). Gibson et al. provided male volunteers with oligofructose or inulin. Subsequent analysis of

TABLE 2 Defining criteria to classify a food ingredient as a prebiotic

A prebiotic should:
Neither be hydrolyzed or absorbed in the upper part of the gastrointestinal tract.
Be a selective substrate for one or more potentially beneficial commensal bacteria in the large intestine. As such, it should stimulate that bacteria to divide, become metabolically active, or both.
Alter the colonic microenvironment toward a healthier composition.
Induce luminal or systemic effects that are advantageous to the host.

their feces revealed an increase in bifidobacteria of $10^{0.7}$–$10^{0.9}$ cells, whereas other bacteria remained unaffected (49). Several studies also support the finding that the selective growth advantage can be at the expense of other bacteria such as bacteroides, clostridia, or other coliforms (173). The mechanism for this is thought to be in part due to the resultant lowering of the pH in the local milieu, but this is likely not the only mechanism. Several studies have shown that lactobacilli are also able to degrade inulin, but a documented stimulation by these substances that supports a selective growth advantage has not been as well established (155). A study by Alles et al. with 40 subjects found no change in fecal flora after supplementation with trans-galactosylated oligosaccharides (5). The effects of prebiotics appear to be limited to the time during which they are being consumed, so that when supplementation is stopped, their beneficial effect is lost (155). Despite the effects prebiotics have in manipulating the bacterial flora, they have not been shown to have health promoting effects to the same degree as probiotics.

Human milk is unique in the quantity of complex oligosaccharides it contains (5–8 g/liter). Only elephant milk has as many complex fucosylated oligosaccharides (53). In vitro studies exposed these compounds to human salivary amylase, porcine pancreatic amylase, and brush boarder membrane vesicles at various pHs. Overall less than 5% of human milk oligosaccharides were digested, supporting the fact that these compounds are not digested by the human intestinal tract (53).

Although health benefits are attributed to these compounds, as is summarized below, they do have potential side effects. When inulin was given at a dose of 14 g/day, women reported an increase in flatulence, borborygmi, abdominal cramping, and bloating (124). There also appears to be a laxative effect by which these compounds have been shown to increase the daily stool output from 136 g/day to 154 g/day (49).

Mineral Absorption

Evidence suggests that prebiotics improve the bioavailability of minerals such as calcium, magnesium, and iron for absorption. This increase has been thought to be clinically relevant in the treatment and/or prevention of diseases such as osteoporosis and anemia.

In a study by Scholz-Ahrens et al. an increase in calcium absorption was seen through 16 weeks of probiotic supplementation despite increased calcium excretion in the urine (146). Ohta et al. also demonstrated increased calcium absorption in young growing rats fed oligofructose (118). When ovariectomized rats, an animal model of postmenopausal women, were supplemented with oligofructose, femur calcium was significantly higher (147). However, bone density and stability are dependent on the trabecular network, and not necessarily correlated with mineral content. Therefore, the trabecular network of the distal tibia was analyzed by microradiography with computer supported image analysis. Such analysis found that oligofructose prevented ovariectomy-induced loss of trabecular bone (148).

The effect of prebiotics on calcium absorption appears to be most pronounced when the amount of dietary calcium is high (146). In general the duration of these effects appears to be short-lived, with statistical affects being seen only in the first few weeks. It is unclear if this is merely due to the fact that prebiotics themselves can create a transient effect, or if the changes in bacterial flora that they create are lost over time. Others have observed that the duration of effect on bone mineralization is dependent on the part of the skeleton that is studied. In the femur the effect of prevention of demineralization was most pronounced at 8 weeks but lost significance by 16 weeks. In the vertebrae this effect continued to be more evident with increased time of supplementation (146).

Human studies include that by Van den Heuvel, who investigated the effect of lactulose on calcium absorption in 12 healthy postmenopausal women. Intestinal calcium absorption was measured by dual calcium labeling. In a crossover design it was found that 10 g of lactulose did significantly result in increased calcium absorption above the nonsupplemented group (169). Another study by the same investigator asked 12 adolescent volunteers to consume oligofructose daily for one week. Calcium absorption increased significantly compared with placebo (170). Coudray et al., in a study of healthy male volunteers, documented increased calcium absorption with inulin (30).

Increased calcium absorption is most likely mediated by its increased solubility within the colon owing to fermentation of the prebiotic and the subsequent decrease in intraluminal pH. Indeed, Ellegard et al. found that when patients with ileostomies took inulin and oligofructose, there was no effect on calcium, magnesium, zinc, or iron (40). However, given that the effects on animals appear to be more pronounced than those found in humans, investigators have postulated that the effect is mediated through fermentation of fecal products to short chain fatty acids. This is particularly relevant given that such fermentation occurs at a significantly higher rate within animal models compared with humans (146). Indeed, one study found that the presence of short chain fatty acids is a prerequisite for stimulation of calcium resorption (166). Finally, some authors suggest that prebiotic-stimulated calcium absorption is mediated by an increased expression of calcium binding proteins such as calbindin-D9k (117).

Two studies have investigated the absorption of phosphorus with supplementation of prebiotics and neither showed effect (116, 146). However, there does appear to be a positive effect on magnesium absorption in both normal and deficient rats (116, 119, 146). This absorption, like that of calcium, occurred in the large intestine (118). Similar improvement in iron absorption was seen in both growing rats (37) and anemic rats (119). There does appear to be a limited dose-related effect of prebiotics to mineral absorption; however, it is unclear if this effect is directly related to increased numbers of bifidobacterium (146).

The effects of prebiotics on mineral absorption are encouraging, particularly in reference to calcium. However, the human studies have been of short duration and therefore have not addressed the more important question of effect on bone mineralization.

Cancer Prevention

Reddy et al. in 1997 (132) fed 10% oligofructose or inulin to rats given azoxymethane (AOM), a substance known to produce preneoplastic aberrant crypt foci (ACF) in the rat colon. At 7 weeks after the last dose of AOM there were fewer ACF in the study groups (inulin, 78 ACF; oligofructose, 92 ACF) compared with the placebo group (120 ACF). Similar studies support this finding (130, 133, 138). It was speculated that the result was due to the fact that bifidobacteria contain a relative paucity of enzymes such as β-glucuronidase, azoreductase, nitro, and nitrate reductase that can convert precancerous substances into carcinogens (68). Indeed, trans-galactosylated oligosaccharides and oligofructose were found to suppress fecal activities of carcinogen-metabolizing enzymes in humans and rats (21, 139). However, Bouhnik et al. fed healthy volunteers FOS (12.5 g/d) and found no change in their total fecal anaerobes, pH, nitroredyctase activity, azoreductase, β-glucuronidase, bile acids, or neutral sterols compared with controls after 12 days. The authors questioned if the duration of the study was too short to demonstrate an effect (18).

Prolonged feeding of a high-fat, low-fiber Western diet markedly reduces the number of colonic apoptotic cells and is associated with tumorgenesis (135). In a study by Hughes & Rowland, rats were fed either a high-fat diet alone, with oligofructans, or with inulin for 3 weeks. They were then exposed to 1,2-dimethylhydrazine, and it was found that the mean number of apoptotic bodies was higher in the oligofructans and inulin groups than control. No change was seen in fecal β-glucuronidase, ammonia, or glucosidase. It is speculated that the bulking effects of these prebiotics contribute to their antineoplastic effect by decreasing exposure to carcinogens (68).

These findings, especially when combined with those findings of anticancer effects of the probiotics whose growth prebiotics support, contribute to validation of these compounds as potential anticancer therapies. Future studies need to more clearly delineate prebiotics' role either separately or in conjunction with probiotics.

SYNBIOTICS

When Gibson introduced the concept of prebiotics he also speculated as to the additional benefits one might see if prebiotics were combined with probiotics to form what he called a synbiotic. He defined this as "a mixture of probiotics and prebiotics that beneficially effects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host wealthfare" (50). The name implies that the prebiotic should offer a selective advantage for the growth of the probiotic it is combined with to provide a synergistic effect. To date, there has been a limited amount of scientific research into this form of supplementation, and it is thus unclear whether this theoretical entity will provide

any additional health-promoting effects beyond those afforded by the prebiotic or probiotic alone.

CONCLUSION

Although pre- and probiotics might not be the "fountain of youth" that Metchnikoff had hoped, they do appear to have definable roles in the prevention and treatment of various disease entities and promotion of "health." Clearly we need more well-controlled studies to more firmly establish what these roles are. The studies need to be rigorous in their scientific approach, containing a placebo arm and documenting colonization by the various bacteria thought to afford a beneficial effect. The studies must also be of an appropriate duration to determine if the beneficial effects are sustained. More care must be taken to assure the viability of the bacteria provided, prior to ingestion. For the consumer, one would hope that the FDA continues their efforts to regulate these biologically active compounds to assure uniformity and safety. It is only by fulfilling these goals that this once "nontraditional" therapy can be accepted into the armamentarium of the more "traditional" practice of medicine. Finally, one can consider the recent use of probiotic theory and their use as novel means of delivering IL-10 locally, as to what the future might hold for this mode of therapy.

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LITERATURE CITED

1. Abdelali H, Cassand P, Soussotte V, Daubeze M, Bouley C, Narbonne J. 1995. Effect of dairy products on initiation of precursor lesions of colon cancer in rats. *Nutr. Cancer* 24:121–32
2. Adam J, Barret A, Barret-Bellet C. 1977. Essais cliniques controles en double insu de l'ultra-levure lyophilisee:etude multicentrique par 25 medicins de 388 cases. *Gaz. Med. Fr.* 84:2072–78
3. Aiba Y, Suzuki N, Kabir AM, Takagi A, Koga Y. 1998. Lactic acid-mediated suppression of *Helicobacter pylori* by the oral administration of *Lactobacillus salivarius* as a probiotic in a gnotobiotic murine model. *Am. J. Gastroenterol.* 93:2097–101
4. Akalin AS, Gonc S, Duzel S. 1997. Influence of yogurt and acidophilus yogurt on serum cholesterol levels in mice. *J. Dairy Sci.* 80:2721–25
5. Alles MS, Hartemink R, Meyboom S, Harryvan JL, Van Laere KM, et al. 1999. Effect of transgalacto-oligosaccharides on the composition of human intestinal microflora and on putative risk markers for colon cancer. *Am. J. Clin. Nutr.* 69:980–91
6. Alm L. 1983. The effect of Lactobacillus acidophilus administration upon survival of Salmonella in randomly selected human carriers. *Prog. Food Nutr. Sci.* 7:13–17
7. Apostolou E, Pelto L, Kirjavainen PV, Isolauri E, Salminen S, Gibson G. 2001. Differences in the gut bacterial flora of healthy and milk-hypersensitive adults, as measured by fluorescence in situ hybridization. *FEMS Immunol. Med. Microbiol.* 30:217–21
8. Arimochi H, Kinouchi T, Kataoka K, Kuwahara T, Ohnishi Y. 1997. Effect

of intestinal bacteria on formation of azoxymethane-induced aberrant crypt foci in the rat colon. *Biochem. Biophys. Res. Commun.* 238:753–57

9. Arvola T, Laiho K, Torkkeli S, Mykkanen H, Salminen S, et al. 1999. Prophylactic *Lactobacillus GG* reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. *Pediatrics* 104:64–68
10. Aso Y, Akazan H. 1992. Prophylactic effect of *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer. BLP study group. *Urol. Int.* 49:125–29
11. Aso Y, Akazan H, Kotake T, Tsukamoto T, Imai K, Group BS. 1995. Preventative effect of a *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer in a double-blind trial. *Eur. Urol.* 27:104–9
12. Beena A, Prasad V. 1997. Effect of yogurt and bifidus yogurt fortified with skim milk powder, condensed whey and loctose-hydrolyzed condensed whey on serum cholesterol and triacylglycerol concentrations in rats. *J. Dairy Res.* 64:453–57
13. Bell MJ, Shackelford P, Feigin RD, Ternberg JL, Brotherton T. 1979. Epidemiologic and bacteriologic evaluation of neonatal necrotizing enterocolitis. *J. Pediatr. Surg.* 14:1–4
14. Bezkorovainy A. 2001. Probiotics: determinants of survival and growth in the gut. *Am. J. Clin. Nutr.* 73:399S–405S
15. Biller JA, Katz AJ, Flores AF, Buie TM, Gorbach SL. 1995. Treatment of recurrent *Clostridium difficile* colitis with *Lactobacillus GG*. *J. Pediatr. Gastroenterol. Nutr.* 21:224–26
16. Bin LX. 1995. Controlled trial in infants and children comparing Lacteol Fort sachets with two antidiarrheal reference drugs. *Ann. Pediatr.* 42:396–401
17. Black FT, Andersen PL, Orskov J, Orskov F, Gaarslev K, Laulund S. 1989. Prophylactic efficacy of lactobacilli on traveller's diarrhea. In *Travel Medicine: Conference on International Travel Medicine I, Zurich, Switzerland*, ed. R Steffen, pp. 333–35. Berlin: Springer
18. Bouhnik Y, Flourie B, Andrieux B, Bisetti N, Briet F, Rambaud JC. 1996. Effects of *Bifidobacterium* sp fermented milk ingested with or without inulin on colonic bifidobacteria and enzymatic activities in healthy humans. *Eur. J. Clin. Nutr.* 50:269–73
19. Boulloche J, Mouterde O, Mallet E. 1994. Management of acute diarrhea in infants and toddlers. *Ann. Pediatr.* 41: 457–63
20. Bowden TAJ, Mansberger ARJ, Lykins LE. 1981. Pseudomembraneous enterocolitis: mechanism for restoring floral homeostasis. *Am. Surg.* 47:178–83
21. Buddington RK, Williams CH, Chen S-C, Witherly SA. 1996. Dietary supplement of Neosugar alters the faecal flora and decreases activities of some reductive enzymes in human subjects. *Am. J. Clin. Nutr.* 63:709–16
22. Butel MJ, Roland N, Hibert A, Popot F, Favre A, et al. 1998. Clostridial pathogenicity in experimental necrotizing enterocolitis in gnotobiotic quails and protective role of bifidobacteria. *J. Med. Microbiol.* 47:391–99
23. Buydens P, Debeuckelaerae S. 1996. Efficacy of SF68 in the treatment of acute diarrhea. A placebo controlled trial. *Scand. J. Gastroenterol.* 31:887–91
24. Caplan MS, Miller-Catchpole R, Kaup S, Russell T, Lickerman M, et al. 1999. Bifidobacterial supplementation reduces the incidence of necrotizing enterocolitis in neonatal rat model. *Gastroenterology* 117:577–83
25. Castagliuolo I, Riegler MF, Valenick L, LaMont JT, Pothoulakis C. 1999. *Saccharomyces boulardii* protease inhibits the effects of *Clostridium difficile* toxins A and B in human colonic mucosa. *Infect. Immun.* 67:302–7

26. Challa A, Rao D, Chawan C, Shackleford L. 1997. *Bifidobacterium longum* and lactulose suppress azoxymethane-induced colonic aberrant crypt foci in rats. *Carcinogenesis* 18:517–21
27. Claud EC, Walker WA. 2001. Hypothesis: inappropriate colonization of the premature intestine can cause neonatal necrotizing enterocolitis. *FASEB J.* 15:1398–403
28. Clements ML, Levine MM, Black RE, Robins-Browne RM, Cisneros LA, Drusano GL. 1981. Lactobacillus prophylaxis for diarrhea due to enterotoxigenic *Escherichia coli*. *Antimicrob. Agents Chemother.* 20:104–8
29. Collins JK, Murphy L, O'Mahony L, Dunne C, O'Sullivan GC. 1999. A controlled trial of probiotic treatment of IL-10 knockout mice. *Gastroenterology* 116:G2931 (abstract)
30. Coudray C, Bellanger J, Castiglia-Delavaud C, Remesy C, Vermorel M, Rayssiguier Y. 1997. Effect of soluble or partly soluble dietary fibers supplementation on absorption and balance of calcium, magnesium, iron, and zinc in healthy young men. *Eur. J. Clin. Nutr.* 51:375–80
31. Cummings JH, Macfarlane GT, Englyst HN. 2001. Prebiotic digestion and fermentation. *Am. J. Clin. Nutr.* 73:415S–20S
32. Dahlgren UIH, Wold AE, Hanson LA, Midtvedt T. 1991. Expression of a dietary protein in *E. coli* renders it strongly antigenic to gut lymphoid tissue. *Immunology* 73:394–97
33. Dai D, Walker WA. 1998. Role of bacterial colonization in neonatal necrotizing enterocolitis and its prevention. *Acta Paed. Sin.* 39:357–65
34. de Roos N, Katan MB. 2000. Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. *Am. J. Clin. Nutr.* 71:405–11
35. de Roos N, Schouten EG, Katan MB. 1998. Yoghurt enriched with *Lactobacillus acidophilus* does not lower blood lipids in healthy men and women with normal to borderline high serum cholesterol levels. *Eur. J. Clin. Nutr.* 53:277–80
36. Del Prete G, DeCarli M, Almerigogna F, Giudizi MG, Biagiotti R, Romagnani S. 1993. Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones inhibits their antigen-specific proliferation and cytokine production. *J. Immunol.* 150:353–60
37. Delzenne N, Aertssens J, Verplaetse H, Roccaro M, Roberfroid M. 1995. Effect of fermentable fructo-oligosaccharides in mineral, nitrogen and energy digestive balance in the rat. *Life Sci.* 57:1579–87
38. Duffy LC, Zielezny M, Riepenhoff-Talty M, Dryja D, Sayahtaheri-Altaie S, et al. 1994. Effectiveness of *Bifidobacterium bifidum* in mediating the clinical course of murine rotavirus diarrhea. *Pediatr. Res.* 35:690–95
39. Duffy LC, Zielezny M, Riepenhoff-Talty M, Dryja D, Sayahtaheri-Altaie S, et al. 1994. Reduction of virus shedding by *B. bifidum* in experimentally induced MRV infection. *Dig. Dis. Sci.* 39:2334–40
40. Ellegard L, Andersson H, Bosaeus I. 1996. Inulin and oligofructose do not influence the absorption of cholesterol, Fe, Ca, Mg, and bile acids but increase energy excretion in man. *Eur. J. Clin. Nutr.* 51:1–5
41. Erickson KL, Hubbard NE. 2000. Probiotic immunomodulation in health and disease. *J. Nutr.* 130:403S–9S
42. Eyssen H. 1973. Role of the gut microflora in metabolism of lipids and sterols. *Proc. Nutr. Soc.* 32:59–63
43. Favier C, Neut C, Mizon C, Cortot A, Colombel JF, Mizon J. 1997. Fecal β-δ-galactosidase production and *Bifidobacteria* are decreased in Crohn's disease. *Dig. Dis. Sci.* 42:817–22
44. Frantz ID, L'Heureux P, Engel RR, Hunt

CE. 1975. Necrotizing enterocolitis. *J. Pediatr.* 86:259–63

45. Fujiwara S, Hashiba H, Hirota T, Forstner JF. 1997. Proteinaceous factor(s) in culture supernatant fluids of bifidobacteria which prevents the binding of enterotoxigenic *Escherichia coli* to gangliotetraosylceramide. *Appl. Environ. Microbiol.* 63:506–12
46. Fukushima M, Nakano M. 1996. Effects of a mixture of organisms, *Lactobacillus acidophilus* or *Streptococcus faecalis* on cholesterol metabolism in rats fed on a fat- and cholesterol-enriched diet. *Br. J. Nutr.* 76:857–67
47. Fukushima Y, Kawata Y, Hara H, Terada A, Mitsuoka T. 1998. Effect of a probiotic formula on intestinal immunoglobulin A production in healthy children. *Int. J. Food. Microbiol.* 42:39–44
48. Gallaher D, Stallings W, Blessing L, Busta F, Brady L. 1996. Probiotics, cecal microflora, and aberrant crypts in the rat colon. *J. Nutr.* 125:1401–12
49. Gibson GR, Beatty ER, Wang X, Cummings JH. 1995. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* 108:975–82
50. Gibson GR, Roberfroid MB. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* 125:1401–12
51. Gilliland SE, Nelson CR, Maxwell C. 1985. Assimilation of cholesterol by *Lactobacillus acidophilus*. *Appl. Environ. Microbiol.* 49:377–81
52. Gionchetti P, Rizzello F, Venturi A, Matteuzzi D, Rossi M, et al. 1998. Maintenance treatment of chronic pouchitis: a randomized placebo-controlled, double-blind trial with a new probiotic preparation. *Gastroenterology* 114:G4037 (abstract)
53. Gnoth MJ, Kunz C, Kinne-Saffran E, Rudloff S. 2000. Human milk oligosaccharides are minimally digested in vitro. *J. Nutr.* 130:3014–20
54. Goldin B, Gorbach SL, Saxelin M, Barakat S, Gualtieri L, Salminen S. 1992. Survival of *Lactobacillus* species (strain GG) in human gastrointestinal tract. *Dig. Dis. Sci.* 37:121–28
55. Gonzalez SN, Cardozo R, Apella MC, Oliver G. 1995. Biotherapeutic role of fermented milk. *Biotherapy* 8:126–34
56. Gotz V, Romankiewicz JA, Moss J, Murray HW. 1979. Prophylaxis against ampicillin-associated diarrhea with a lactobacillus preparation. *Am. J. Hosp. Pharm.* 36:754–57
57. Gronlund MM, Lehtonen OP, Eerola E, Kero P. 1999. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. *J. Pediatr. Gastroenterol. Nutr.* 28:19–25
58. Guandalini S, Pensabene L, Zikri MA, Dias JA, Casali LG, et al. 2000. *Lactobacillus* GG administered in oral rehydration solution to children with acute diarrhea: a multicenter European trial. *J. Pediatr. Gastroenterol. Nutr.* 30:54–60
59. Guarino A, Canani RB, Spagnuolo MI, Albano F, DiBenedetto L. 1997. Oral bacterial therapy reduces the duration of symptoms and of viral excretion in children with mild diarrhea. *J. Pediatr. Gastroenterol. Nutr.* 25:516–19
60. Guslandi M, Mezzi G, Sorghi M, Testoni PA. 2000. *Saccaromyces boulardii* in maintenance treatment of Crohn's disease. *Dig. Dis. Sci.* 45:1462–64
61. Hamilton-Miller JMT, Shah S, Smith CT. 1996. "Probiotic" remedies are not what they seem. *Br. J. Med.* 312:55–56
62. Hayatsu H, Hayatsu T. 1993. Suppressing effect of *Lactobacillus casei* administration on the urinary mutagenicity arising from ingestion of fried ground beef in the human. *Cancer Lett.* 73:173–79
63. He F, Ouwehand AC, Isolauri E, Hashimoto H, Benno Y, Salminen S. 2001. Comparison of mucosal adhesion

and species identification of bifidobacteria isolated from healthy and allergic infants. *FEMS Immunol. Med. Microbiol.* 30:43–47
64. Heyman M, Corthier G, Petit A, Meslin JC, Morcau C, Desjeux JF. 1987. Intestinal absorption of macromolecules during viral enteritis: an experimental study on rotavirus-infected conventional and germ-free mice. *Pediatr. Res.* 22:72–78
65. Hilton E, Kolakowski P, Singer C, Smith M. 1997. Efficacy of *Lactobacillus GG* as a diarrheal preventative in travelers. *J. Travel Med.* 4:41–43
66. Hoy C, Miller MR, Mackay P, Godwin PGR, Langdale V, Levine MI. 1990. Quantitative changes in faecal microflora preceding necrotizing enterocolitis in premature neonates. *Arch. Dis. Child.* 65:1057–59
67. Hoyos AB. 1999. Reduced incidence of necrotizing enterocolitis associated with enteral administration of *Lactobacillus acidophilus* and *Bifidobacterium infantis* to neonates in an intensive care unit. *Int. J. Infect. Dis.* 3:197–202
68. Hughes R, Rowland IR. 2001. Stimulation of apoptosis by two prebiotic chicory fructans in the rat colon. *Carcinogenesis* 22:43–47
69. Insoft RM, Sanderson IR, Walker WA. 1996. Development of immune function in the intestine and its role in neonatal disease. *Pediatr. Clin. N. Am.* 43:551–71
70. Isolauri E, Arvola T, Sutas Y, Moilanen E, Salminen S. 2000. Probiotics in the management of atopic eczema. *Clin. Exp. Allergy* 30:1604–10
71. Isolauri E, Joensuu J, Suomalainen H, Luomala M, Vesikari T. 1995. Improved immunogenicity of oral D x RRV reassortant rotavirus vaccine by *Lactobacillus casei GG*. *Vaccine* 13:310–12
72. Isolauri E, Juntunen M, Rautanen T, Sillanaukee P, Koivula T. 1991. A human *Lactobacillus* strain (*Lactobacillus Casei* sp strain *GG*) promotes recovery from acute diarrhea in children. *Pediatrics* 88:90–97
73. Isolauri E, Kaila M, Mykkanen H, Ling WH, Salminen S. 1994. Oral bacteriotherapy for viral gastroenteritis. *Dig. Dis. Sci.* 39:2595–600
74. Isolauri E, Majamaa H, Arvola T, Rantala I, Virtanen E, Arviolmmi H. 1993. *Lactobacillus casei* strain GG reverses increased intestinal permeability induced by cow milk in suckling rats. *Gastroenterology* 105:1643–50
75. Itoh T, Fujimoto Y, Kawai Y, Toba T, Saito T. 1995. Inhibition of food-borne pathogenic bacteria by bacteriocins from *Lactobacillus gasseri*. *Lett. Appl. Microbiol.* 21:137
76. Jack RW, Tagg JR, Ray B. 1995. Bacteriocins of gram-positive bacteria. *Microbiol. Rev.* 59:171–200
77. Jeannin P, Lecoanet S, Delneste Y, Gauchat JF, Bonnefoy JY. 1998. IgE versus IgG4 production can be differentially regulated by IL-10. *J. Immunol.* 160:3555–61
78. Kabir AM, Aiba Y, Takagi A, Kamiya S, Miwa T, Koga Y. 1997. Prevention of *Helicobacter pylori* infection by lactobacilli in a gnotobiotic murine model. *Gut* 41:49–55
79. Kaila M, Isolauri E, Saxelin M, Arviolmmi H, Vesikari T. 1995. Viable versus inactivated lactobacillus strain GG in acute rotavirus diarrhea. *Arch. Dis. Child.* 72:51–53
80. Kaila M, Isolauri E, Soppi E, Virtanen E, Laine S, Arviolmmi H. 1992. Enhancement of the circulating antibody secreting cell response in human diarrhea by a human *Lactobacillus* strain. *Pediatr. Res.* 32:141–44
81. Kalliomaki M, Salminen S, Arviolmmi H, Kero P, Koskinen P, Isolauri E. 2001. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 357:1057–59
82. Kampman E, Goldbohm RA, van den Brandt A, van't Veer P. 1994. Fermented

dairy products, calcium, and colorectal cancer in the Netherlands cohort study. *Cancer Res.* 54:3186–90

83. Katelaris PH, Salam I, Farthing M. 1995. Lactobacilli to prevent traveler's diarrhea? *N. Engl. J. Med.* 333:1360–61
84. Kennedy RJ, Hoper M, Deodhar K, Kirk SJ, Gardiner KR. 2000. Probiotic therapy fails to improve gut permeability in a hapten model of colitis. *Scand. J. Gastroenterol.* 35:1266–71
85. Klinman DM, Yi AK, Beaucage SL, Conover J, Kreig AM. 1996. CpG motifs present in bacterial DNA rapidly induce lymphocytes to secrete interleukin 6, interleukin 12, and interferon γ. *Proc. Natl. Acad. Sci. USA* 93:2879–83
86. Kollaritsch H, Wiedermann G. 1990. Traveller's diarrhoea among Austrian tourists: epidemiology, clinical features and attempts at nonantibiotic drug prophylaxis. In *Proc. 2nd Int. Conf, Tourist Health*, ed. W Pasini, pp. 74–82. Rimini: World Health Org.
87. Koo M, Rao A. 1991. Long-term effect of *Bifidobacteria* and neosugar on precursor lesions of colonic cancer in CF1 mice. *Nutr. Cancer* 16:249–57
88. Korpela R. 1995. *Role of rye fibre and Lactobacillus GG in colonic metabolism.* PhD thesis, Kuopio Univ., Kuopio, Finland
89. Kruis W, Schotz E, Fric P, Fixa B, Judmaier G, Stolte M. 1997. Double-blind comparison of an oral *Escherichia* coli preparation and mesalazine in maintaining remission of ulcerative colitis. *Aliment. Pharmacol. Ther.* 11:853–58
90. Kulkarni N, Reddy B. 1994. Inhibitory effect of *Bifidobacterium longum* cultures on azoxymethane-induced aberrant crypt foci formation and fecal bacterial β-glucuronidase. *Proc. Soc. Exp. Biol. Med.* 207:278–83
91. Lawrence G, Bates J, Gaul A. 1982. Pathogenesis of neonatal necrotizing enterocolitis. *Lancet* 1:137–39
92. Lidbeck A, Nord CE, Rafter J, Nord C, Gustaffson JA. 1992. Effect of *Lactobacillus acidophilus* supplements on mutagen excretion in faeces and urine in humans. *Micobiol. Ecol. Health Dis.* 5:59–67
93. Lin SY, Ayres JW, Winkler W, Sandine WE. 1989. *Lactobacillus* effects on cholesterol: in vitro and in vivo results. *J. Dairy Res.* 72:2885–99
94. Ling WH, Hanninen O, Mykkanen H, Heikura M, Salminen S, von Wright A. 1992. Colonization and fecal enzyme activities after oral *Lactobacillus* GG administration in elderly nursing home residents. *Ann. Nutr. Metab.* 36:162–66
95. Ling WH, Korpela R, Mykkanen H, Salminen S, Hanninen O. 1994. *Lactobacillus* strain GG supplemetation decreases colonic hydrolytic and reductive enzyme activities in healthy female adults. *J. Nutr.* 124:18–23
96. McFarland LV, Surawicz CM, Greenberg RN, Elmer GW, Moyer KA, et al. 1995. Prevention of beta-lactam-associated diarrhea by *Saccharomyces boulardii* compared with placebo. *Am. J. Gatroenterol.* 90:439–48
97. Mack DR, Michail S, Wei S, McDougall L, Hollingsworth MA, et al. 1999. Probiotics inhibit enteropathogenic *E. coli* adherence in vitro by inducing intestinal mucin gene expression. *Am. J. Physiol. Gastrointest. Liver Physiol.* 276:G941–50
98. Madsen KL, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN. 1999. *Lactobacillus* species prevents colitis in interleukin 10 gene-deficient mice. *Gastroenterology* 116:1107–14
99. Majamaa H, Isolauri E. 1997. Probiotics: a novel approach in the management of food allergy. *J. Allergy Clin. Immunol.* 99:179–85
100. Majamaa H, Isolauri E, Saxelin M, Vesikari T. 1995. Lactic acid bacteria in the treatment of acute rotavirus

gastroenteritis. *J. Pediatr. Gastroenterol. Nutr.* 20:333–38

101. Mann GV. 1974. Studies of a surfactant and cholesteremia in the Maasai. *Am. J. Clin. Nutr.* 27:464–69
102. Marin ML, Tejada-Simon MV, Lee JH, Murtha J, Ustunol Z, Pestka JJ. 1998. Stimulation of cytokine production in clonal macrophage and T-cell models by *Streptococcus thermophilus*: comparison with *Bifidobacteria* sp and *Lactobacillus bulgaricus*. *J. Food Prot.* 61: 859–64
103. Marteau P, Minekus M, Havenaar R, Hiuis in't Veld JHJ. 1997. Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: validation and the effects of bile. *J. Dairy Sci.* 80:1031–37
104. Marteau P, Pochart P, Flourie B, et al. 1990. Effect of chronic ingestion of a fermented dairy product containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on metabolic activities of the colonic microflora. *Am. J. Clin. Nutr.* 52:685–88
105. Matsuzaki T, Yamazaki R, Hoshimoto S, Yokokura T. 1998. The effect of oral feeding of *Lactobacillus casei* strain Shirota on immunoglobulin E production in mice. *J. Dairy Sci.* 81:48–53
106. McCraken VJ, Lorenz RG. 2001. The gastrointestinal ecosystem: a precarious alliance among epithelium, immunity and microbiota. *Cell. Microbiol.* 3:1–11
107. McFarland LV, Surawicz CM, Greenberg RN, Fekety R, Elmer GW, et al. 1994. A randomized placebo-controlled trial of *Saccharomyces boulardii* in combination with standard antibiotics for *Clostridium difficile* disease. *JAMA* 271:1913–18
108. Midolo PD, Lambert JR, Hull R, Luo F, Grayson ML. 1995. In vitro inhibition of *Helicobacter pylori* NCTC 11637 by organic acids and lactic acid bacteria. *J. Appl. Bacteriol.* 79:475–79
109. Miettinen M, Matikainen S, Vuopio-Varkila J, Pirhonen J, Varkila K, et al. 1998. Lactobacilli and streptococci induce interleukin-12 (IL-12), IL-18, and gamma interferon production in human peripheral blood mononuclear cells. *Infect. Immun.* 66:6058–62
110. Miettinen M, Vuopio-Varkila J, Varkila K. 1996. Production of human tumor necrosis factor anpha, interleukin-6, and interferon-10 is induced by lactic acid bacteria. *Infect. Immun.* 64:5403–5
111. Mitra AK, Rabbani GH. 1990. A double-blind, controlled trial of Bioflorin (*Streptococcus faecium* SF68) in adults with acute diarrhea due to *Vibrio cholera* and enterotoxigenic *Escherichia coli*. *Gastroenterology* 99:1149–52
112. Monique G, Moulton LH, Hill C, Kramar A. 1986. Consumption of dairy produce and alcohol in a case control study of breast cancer. *J. Natl. Cancer Inst.* 77:633–36
113. Moore KW, O'Garra A, Waal Malefyt R, Vieira P, Mosmann TR. 1993. Interleukin-10. *Annu. Rev. Immunol.* 11: 165–90
114. Moore WE, Moore LH. 1995. Intestinal floras of populations that have a high risk of colon cancer. *Appl. Environ. Microbiol.* 61:3202–7
115. Neumann E, Oliveira MA, Cabral CM, Moura LN, Nicoli JR, et al. 1998. Monoassociation with *Lactobacillus acidophilus* UFV-H2b20 stimulates the immune defense mechanisms of germfree mice. *Braz. J. Med. Biol. Res.* 31:1565–73
116. Ohta A, Baba S, Takizawa T, Adachi T. 1994. Effects of fructooligosaccharides on the absorption of magnesium in the magnesium-deficient rat model. *J. Nutr. Sci. Vitaminol.* 40:171–80
117. Ohta A, Motohashi Y, Ohtsuki M, Hirayama M, Adachi T, Sakuma K. 1998. Dietary fructooligosaccharides change the intestinal mucosal concentration of calbindin-D9k in rats. *J. Nutr.* 128:934–39

118. Ohta A, Ohtsuki M, Baba S, Adachi T, Sakaguchi EI. 1995. Calcium and magnesium absorption from the colon and rectum are increased in rats fed fructooligosaccharides. *J. Nutr.* 125:2417–24
119. Ohta A, Ohtsuki M, Baba S, Takizawa T, Adachi T, Kimura S. 1995. Effects of fructooligosaccharides on the absorption of iron, calcium, and magnesium in iron-deficient anemic rats. *J. Nutr. Sci. Vitaminol.* 41:281–91
120. Oksanen PJ, Salminen S, Saxelin M, Hamalainen P, Ihantola-Vormisto A, et al. 1990. Prevention of traveler's diarrhoea by *Lactobacillus GG*. *Ann. Med.* 22:53–56
121. Pahwa A, Mathur BN. 1987. Assessment of a bifidus containing infant formula. Part II. Implantation of *Bifidobacterium bifidum*. *Indian J. Dairy Sci.* 40:364–67
122. Pant AR, Graham SM, Allen SJ, Harikul S, Sabchareon A, et al. 1996. *Lactobacillus GG* and acute diarrhoea in young children in the tropics. *J. Trop. Pediatr.* 42:162–65
123. Patel R, Cockerill FR, Porayko MK, Osmon DR, Ilstrup DM, Keating MR. 1994. Lactobacillemia in liver transplant patients. *Clin. Infect. Dis.* 18:207–12
124. Pederson A, Sandstrom B, Van Amelsvoort JMM. 1997. The effects of ingestion of inulin on blood lipids and gastrointestinal symptoms in healthy females. *Br. J. Nutr.* 78:215–22
125. Perdigon G, Alvarez S, Rachid M, Aguero G, Gobbato N. 1995. Immune system stimulation by probiotics. *J. Dairy Sci.* 78:1597–606
126. Pessi T, Sutas Y, Hurme M, Isolauri E. 2000. Interleukin-10 generation in atopic children following oral *Lactobacillus rhamnosus* GG. *Clin. Exp. Allergy* 30: 1804–8
127. Peters RK, Pike MC, Garabrant D, Mack TM. 1992. Diet and colon cancer in Los Angeles County, California. *Cancer Causes Control* 3:457–73
128. Phuapradit P, Varavithya W, Vathanophas K, Sangchai R, Podhipak A, et al. 1999. Reduction of rotavirus infection in children receiving bifidobacteria-supplemented formula. *J. Med. Assoc. Thai.* 82:S43–48
129. Pletincx M, Legein J, Vandenplas Y. 1995. Fungemia with *Saccharomyces boulardii* in a 1-year-old girl with protracted diarrhea. *J. Pediatr. Gastroenterol. Nutr.* 21:113–15
130. Rao CV, Chou D, Simi B, Ku H, Reddy BS. 1998. Prevention of colonic aberrant crypt foci and modulation of large bowel microbial activity by dietary coffee fiber, inulin, and pectin. *Carcinogenesis* 10:1815–19
131. Raza S, Graham SM, Allen SJ, Sultana S, Cuevas L, Hart CA. 1995. *Lactobacillus* GG promotes recovery from acute nonbloody diarrhea in Pakistan. *Pediatr. Infect. Dis. J.* 14:107–11
132. Reddy B, Hamid R, Rao C. 1997. Effect of dietary oligofructose and inulin on colonic preneoplastic aberrant crypt formation. *Carcinogenesis* 19:1371–74
133. Reddy BS. 1998. Prevention of colon cancer by pre- and probiotics: evidence from laboratory studies. *Br. J. Nutr.* 80:S219–23
134. Rembacken BJ, Snelling AM, Hawkey PM, Chalmers DM, Axon AT. 1999. Non-pathologic *Escherichia coli* versus mesalamine for the treatment of ulcerative colitis: a randomised trial. *Lancet* 354:635–39
135. Risio M, Lipkin M, Newmark H, Yang K, Rossini FP, et al. 1996. Apoptosis, cell replication and Western style diet induced tomorigenesis in mouse colon. *Cancer Res.* 15:4910–16
136. Rolfe RD. 2000. The role of probiotic cultures in the control of gastrointestinal health. *J. Nutr.* 130:396S–402S
137. Ross S. 2000. Functional foods: the Food and Drug Administration perspective. *Am. J. Clin. Nutr.* 71:1735S–38S
138. Rowland IR, Rumney CJ, Coutts JT,

Lievense LC. 1998. Effect of *Bifidobacterium longum* and inulin on gut bacterial metabolism and carcinogen induced aberrant crypt foci in rats. *Carcinogenesis* 19:281–85

139. Rowland IR, Tanaka R. 1993. The effects of transgalactosylated oligosaccharides on gut flora metabolism in rats associated with a human faecal microflora. *J. Appl. Bacteriol.* 74:667–74
140. Saavedra JM, Bauman NA, Oung I, Perman JA, Yolken RH. 1994. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhea and shedding of rotavirus. *Lancet* 344:1046–49
141. Sakamoto I, Igarashi M, Kimura S, Takagi A, Miwa T, Koga Y. 2001. Suppressive effect of Lactobacillus gasseri OLL 2716 (LG21) on *Helicobacter pylori* infection in humans. *J. Antimicrob. Chemother.* 47:709–10
142. Sanders ME. 2000. Considerations for use of probiotic bacteria to modulate human health. *J. Nutr.* 130:384S–90S
143. Savage DC. 1977. Microbial ecology of the human gastrointestinal tract. *Annu. Rev. Microbiol.* 31:107–33
144. Schiffrin EJ, Brassart D, Servin AL, Rochat F, Donnet-Hughes A. 1997. Immune modulation of blood leukocytes in humans by lactic acid bacteria: criteria for strain selection. *Am. J. Clin. Nutr.* 66:515S–20S
145. Schiffrin EJ, Rochat F, Link-Amster H, Aeschlimann JM, Donnet-Hughes A. 1995. Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. *J. Dairy Sci.* 78:491–97
146. Scholz-Ahrens KE, Schaafsma G, van den Heuvel EGHM, Schrezenenmeir J. 2001. Effects of prebiotics on mineral metabolism. *Am. J. Clin. Nutr.* 73:459S–64S
147. Scholz-Ahrens KE, van Loo J, Schrezenenmeir J. 2001. Effect of oligofructose on bone mineralization in ovarectomized rats is affected by dietary calcium. *Am. J. Clin. Nutr.* 73:498S (abstract)
148. Scholz-Ahrens KE, van Loo J, Schrezenenmeir J. 2001. Long-term effect of oligofructose on bone trabecular structure in ovarectomized rats. *Am. J. Clin. Nutr.* 73:498 (abstract)
149. Schrezenenmeir J, deVrese M. 2001. Probiotics, prebiotics, and synbiotics—approaching a definition. *Am. J. Clin. Nutr.* 73:361S–64S
150. Schwan A, Sjolin S, Trottestam U, Aronsson B. 1984. Relapsing *Clostridium difficile* enterocolitis cured by rectal infusion of normal faeces. *Scand. J. Infect. Dis.* 16:211–15
151. Shackleford L, Rao D, Chawan C, Rulusani S. 1983. Effect of feeding fermented milk on the incidence of chemically induced colon tumors in rats. *Nutr. Cancer* 5:159–64
152. Shornikova A-V, Isolauri E, Burnakova L, Lukovnikova S, Vesikari T. 1997. A trial in the Karelian Republic of oral rehydration and *Lactobacillus GG* for treatment of acute diarrhea. *Acta Paediatr.* 86:460–65
153. Siitonen S, Vapaatalo H, Salminen S, Gordin A, Saxelin M, et al. 1990. Effect of *Lactobacillus GG* yoghurt in prevention of antibiotic associated diarrhoea. *Ann. Med.* 22:57–59
154. Silva M, Jacobs NV, Deneke C, Gorbach SL. 1987. Antimicrobial substance from a human *Lactobacillus* strain. *Antimicrob. Agents Chemother.* 31:1231–33
155. Simmering R, Blaut M. 2001. Pro- and prebiotics—the tasty guardian angels? *Appl. Microbiol. Biotechnol.* 55:19–28
156. Spanhaak S, Havenaar R, Schaafsma G. 1998. The effect of consumption of milk fermented by *Lactobacillus casei* strain Shirota on the intestinal microflora and immune parameters in humans. *Eur. J. Clin. Nutr.* 52:1–9
157. Speer ME, Taber LH, Yow MD, Rudolph AJ, Urteaga J, Waller S. 1976.

Fulminant neonatal sepsis and necrotizing enterocolitis associated with a "non-enteropathic" strain of *Escherichia coli*. *J. Pediatr.* 89:91–95

158. Steidler L, Hans W, Schotte L, Neirynck S, Obermeier F, et al. 2000. Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. *Science* 289:1352–55
159. Surawicz CM, Elmer GW, Speelman P, McFarland LV, Chin J, van Belle G. 1989. Prevention of antibiotic-associated diarrhea by *Saccharomyces boulardi*: a prospective study. *Gastroenterology* 96:981–88
160. Sutas Y, Hurme M, Isolauri E. 1996. Down-regulation of anti-CD3 antibody-induced IL-4 production by bovine caseins hydrolysed with Lactobacillus GG-derived enzymes. *Scand. J. Immunol.* 43:687–89
161. Sutas Y, Soppi E, Korhonen H, Syvaoja EL, Saxelin M, et al. 1996. Suppression of lymphocyte proliferation in vitro by bovine caseins hydrolyzed with *Lactobacillus casei* GG-derived enzymes. *J. Allergy Clin. Immunol.* 98:216–24
162. Takano T, Arai K, Murota I, Hayakawa K, Mizutani T, Mitsuoka T. 1985. Effect of feeding sour milk on longevity and tumorigenesis in mice and rats. *Bifid Microflora* 4:31–37
163. Tejada-Simon MV, Ustunol Z, Pestka JJ. 1999. Effects of lactic acid bacteria ingestion on basal cytokine mRNA and immunoglobulin levels in the mouse. *J. Food Prot.* 62:287–91
164. Thompson LU, Jenkins DJ, Amer MA, Reicher R, Jenkins A, Kamulsky J. 1982. The effects of fermented and unfermented milks on serum cholesterol. *Am. J. Clin. Nutr.* 36:1106–11
165. Tojo M, Oikawa T, Morikawa Y, Yamashita N, Iwata S, et al. 1987. The effects of *Bifidobacterium breve* administration on Campylobacter enteritis. *Acta Paediatr. Jpn.* 29:160–67
166. Trinidad TP, Wolever TM, Thompson LU. 1993. Interactive effects of calcium and short chain fatty acids on absorption in the distal colon of man. *Nutr. Res.* 13:417–25
167. Tsuda H, Sekine K, Nakamura J, Ushida Y, Kuhara T, et al. 1998. Inhibition of azoxymethane initiated colon tumor and aberrant crypt foci development by bovine lactoferrin administration in F344 rats. In *Advances in Lactoferrin Research*, ed. G Spik. New York: Plenum
168. Van de Merwe JP, Schroder AM, Wensinck F, Hazenberg MP. 1988. The obligate anaerobic fecal flora of patients with Crohn's disease and their first-degree relatives. *Scand. J. Gastroenterol.* 23:1125–31
169. Van den Heuvel EGHM, Muys T, van Dokkum W, Schaafsma G. 1999. Lactulose stimulates calcium absorption on postmenopausal women. *J. Bone Miner. Res.* 7:1211–16
170. Van den Heuvel EGHM, Muys T, van Dokkum W, Schaafsma G. 1999. Oligofructose stimulates calcium absorption in adolescents. *Am. J. Clin. Nutr.* 69:544–48
171. Vanderhoof JA, Whitney DB, Antonson DL, Hanner TL, Lupo JV, Young RJ. 1999. *Lactobacillus GG* in the prevention of antibiotic-associated diarrhea in children. *J. Pediatr.* 135:564–68
172. van't Veer P, Dekker JM, Lamers JWJ, Kok FJ, Schouten EG, et al. 1989. Consumption of fermented milk products and breast cancer: a case control study in the Netherlands. *Cancer Res.* 49:4020–23
173. Wang X, Gibson GR. 1993. Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. *J. Appl. Bacteriol.* 75:373–80
174. Wolever TM, Spadafora P, Eshuis H. 1991. Interaction between colonic acetate and propionate in humans. *Am. J. Clin. Nutr.* 53:681–87

175. Wollowski I, Ji ST, Bakalinsky AT, Neudecker C, Pool-Zobel BL. 1999. Bacteria used for the production of yogurt inactivate carcinogens and prevent DNA damage in the colon of rats. *J. Nutr.* 129:77–82
176. Wunderlich PF, Braum L, Fumagalli I, D'Apuzzo V, Heim F, et al. 1989. Double-blind report on the efficacy of lactic-acid producing *Enterococcus* SF68 in the prevention of antibiotic-associated diarrhoea and in the treatment of acute diarrhoea. *J. Int. Med. Res.* 17: 333–38
177. Zychowicz C, Surazynska A, Siewierska B, Cieplinska T. 1974. Effect of Lactobacillus acidophilus culture (acidophilus milk) on the carrier state of Shigella and Salmonella organisms in children. *Pediatr. Pol.* 49:997–1003