Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998[1–3]

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ABSTRACT We reviewed the evidence from human intervention studies for the health effects of probiotic bacteria, ie, live bacteria that survive passage through the gastrointestinal tract and have beneficial effects on the host. Of the 49 studies reviewed, 26 dealt with the prevention or treatment of diarrheal disease, 9 with the prevention of cancer or of the formation of carcinogens, 7 with the lowering of serum cholesterol, and 7 with the stimulation of the immune system. The most widely studied probiotic bacteria were *Lactobacillus* GG (22 studies), *Lactobacillus acidophilus* (16 studies), *Bifidobacterium bifidum* (6 studies), *and Enterococcus faecium* (7 studies). Intake of *Lactobacillus* GG consistently shortened the diarrheal phase of rotavirus infection by 1 d. However, evidence for the prevention by *Lactobacillus* GG and other probiotics of diarrhea due to viral or bacterial infections was less strong. Effects of probiotics on the immune system are inconclusive because of the variety of outcome variables reported. Cholesterol lowering by *L. acidophilus* was shown in some but not all studies; cholesterol lowering by *E. faecium* seems to be transient. Two studies of one research group showed a smaller recurrence of bladder tumors in patients after treatment with *Lactobacillus casei*; these results await confirmation. The production of mutagens after a meal might be reduced by the concomitant intake of probiotics, but the relevance of this finding is unclear. In conclusion, consumption of foods containing *Lactobacillus* GG may shorten the course of rotavirus infection. Other health effects of probiotic bacteria have not been well established. Well-designed placebo-controlled studies with validated outcome variables are needed to determine the health effects of probiotics. *Am J Clin Nutr* 2000;71:405–11.

KEY WORDS Probiotic bacteria, lactobacillus, enterococcus, rotavirus, cholesterol, diarrhea, lipid metabolism, carcinogenesis, review

INTRODUCTION

The term *probiotic* refers to live microorganisms that survive passage through the gastrointestinal tract and have beneficial effects on the host (1–3). Probiotic bacteria have been the focus of much scientific and commercial interest. This interest is due to a range of possible health effects of these bacteria. Probiotics are marketed as capsules, powders, enriched yogurts, yogurt-like products, and milks. Possible health effects include immune system stimulation, cholesterol lowering, and prevention of cancer recurrence (1, 3–7). Examples of probiotics are *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum* (1, 2, 8–12).

Several reviews describe criteria that should be met before a bacterial strain can be called a probiotic (3, 13, 14). Most importantly, probiotics should have a beneficial effect on human health. To meet this criterion, well-designed human studies with requirements similar to those for pharmaceutical studies are needed (3).

In this review, we evaluate human studies of probiotics. We did not perform a meta-analysis because not enough studies with probiotics have been conducted. Instead, we present an overview of studies on probiotic bacteria published between 1988 and 1998 that used *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* as probiotics. Health effects of traditional yogurt—ie, milk fermented with the starter cultures *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp *bulgaricus*—will not be reviewed here; several reviews have already been published on this topic (9, 15). Studies with the probiotic yeast *Saccharomyces boulardii* were reviewed by Elmer et al (7) and are not included here.

PREVENTION OF DIARRHEAL DISEASES

Probiotics can prevent or ameliorate diarrhea through their effects on the immune system. Moreover, probiotics might prevent infection because they compete with pathogenic viruses or bacteria for binding sites on epithelial cells (12, 16, 17). Probiotics might also inhibit the growth of pathogenic bacteria by producing bacteriocins such as nisin (18). Reviewed here are the effects of probiotics on 3 types of diarrhea: acute diarrhea,

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mainly due to rotavirus infection; traveler's diarrhea; and antibiotic-associated diarrhea.

Effects of probiotics on the immune system

Several probiotics are claimed to stimulate the immune system. Their modes of action appear to be nonspecific, resulting in increased immune responsiveness to a wide variety of antigens. In most studies, intermediary endpoints instead of disease symptoms were studied.

Spanhaak et al (19) tested the effect of *Lactobacillus casei* Shirota on various aspects of the immune system in a placebo-controlled study in 20 healthy men. The men were given a controlled diet for 8 wk. On weeks 3–6, 10 men received 100 mL fermented milk/d supplemented with 1×10^{12} colony-forming units (CFU) *Lactobacillus casei* Shirotas/L and 10 other men received unfermented milk. The treatment had no effect on natural killer cell activity, phagocytosis, or cytokine production.

Malin et al (20) used the immune response of antigen-specific immunoglobulin A (IgA) as an intermediary endpoint in a study of 14 children with Crohn disease and of 9 children with juvenile chronic arthritis. The children were supplemented with *Lactobacillus* GG (2×10^{10} CFU/d) for 10 d. The immune response of IgA was measured ex vivo: lymphocytes from the patients were transferred into microtiter plates that were coated with casein, β-lactoglobulin, or gliadin. Lymphocytes that contained specific antibodies against these proteins showed up as colored spots. In the patients with Crohn disease, the number of lymphocytes with specific antibodies against casein and β-lactoglobulin, but not gliadin, was significantly higher after than before treatment with the probiotic. No effect was seen on clinical status in any of the patients. The authors did not discuss whether an increased formation of IgA antibodies against milk proteins was considered beneficial for these patients.

Lactobacillus GG ameliorated symptoms in infants with atopic eczema and cow milk allergy in a placebo-controlled study (21). Moreover, the concentration of tumor necrosis factor in feces decreased significantly from 709 to 34 pg/g feces in the infants receiving *Lactobacillus* GG but not in the infants receiving placebo.

The immune response after vaccination is an elegant tool with which to study the effects of probiotics. Link-Amster et al (22) tested whether the consumption of yogurt with *B. bifidum* and *L. acidophilus* La1 improved the immune response in 15 healthy volunteers after oral vaccination with *Salmonella typhimurium*; 15 other volunteers were asked not to consume fermented foods. Vaccination increased total IgA concentrations in serum 2.5 times in the control subjects compared with 4.1 times in the subjects who received the probiotic ($P = 0.04$). However, significantly more antibodies (per mg IgA) against *Salmonella* were found in the saliva of the control subjects than in the saliva of the treated subjects (22). Because no placebo yogurt was given to the control group, the effect seen may have been due to the probiotic but also to the consumption of yogurt as such.

These same probiotics, but given separately, doubled the number of white blood cells with phagocytic activity in healthy volunteers (23, 24). *B. bifidum* Bb12 (1×10^{10} CFU/d) and *L. acidophilus* La1 (7×10^{10} CFU/d) were each given to 14 volunteers for 3 wk. Phagocytic activity was measured before and after treatment; no control group was included in the study. The treatment period was preceded by a run-in period in which the volunteers consumed 360 mL nonfermented milk/L. The probiotics, however, were consumed as fermented milks. Again, the effect on phagocytosis may have been due to the probiotics but also to the consumption of fermented milk.

In another study, *L. acidophilus* (Infloran; Istituto Sieroterapico Berna, Como, Italy) and *B. bifidum* (both 8×10^6 CFU/d for 28 d) had hardly any effect on immune indexes in 15 elderly volunteers; only B lymphocytes increased significantly ($P < 0.01$), from 72 ± 29 to $119 \pm 31 \times 10^6$ cells/L serum (25). A control group with 10 subjects remained stable. All subjects underwent colonoscopy; consumption of the probiotic bacteria reduced signs of inflammation in the sigmoid and descending colon, whereas no changes were seen in the control group. In brief, several studies suggest that consumption of fermented milk enriched with probiotics increases the immune response. However, studies using nonenriched fermented milk as a placebo are needed to confirm this.

Acute diarrhea, mainly caused by rotavirus

Rotavirus is the main virus of interest in studies with probiotics. Rotavirus infection causes gastroenteritis, characterized by acute diarrhea and vomiting. Gastroenteritis is a leading cause of death and disease among children worldwide. There is ample evidence that probiotics reduce the duration and severity of rotavirus diarrhea. Consumption of *Lactobacillus* GG (10^{10}–10^{11} CFU/d) shortened the diarrheal phase from an average of 3.5 to 2.5 d in children hospitalized (26–32) or treated at home for rotavirus infection (33). Serum concentrations of IgA antibodies against rotavirus increased significantly more in children treated with probiotics than in untreated children (28–30), which might explain the effect on recovery. Moreover, Isolauri et al (34) showed that the immune response to vaccination with a live oral rotavirus vaccine was better in children receiving *Lactobacillus* GG than in control children.

Although the therapeutic effects of *Lactobacillus* GG have been well established, it has not been reported whether it can prevent rotavirus infection. However, oral administration of 2 other probiotics, *B. bifidum* and *S. thermophilus*, reduced the incidence of diarrhea in a double-blind, placebo-controlled trial in 55 hospitalized infants (35). Of the 10 cases of diarrhea, 7 were due to rotavirus.

L. acidophilus has been tested for its therapeutic effects on acute diarrhea in 2 studies (36, 37). In the study by Bin (36), 50 Chinese children with acute diarrhea were randomly assigned to receive either *L. acidophilus* ($n = 30$) or the standard treatment ($n = 20$). The recovery from diarrhea was not significantly different between the 2 groups. Rotavirus was the main causal agent of the diarrhea in the *L. acidophilus* group (25 of 30) but not in the control group (7 of 20). In the study by Boulloche et al (37), 103 infants and children with acute diarrhea were randomly assigned to receive *L. acidophilus* (heat-killed, strain LB), loperamide, or placebo. Recovery times were not significantly different between the groups. However, in 71 children who received oral rehydration, *L. acidophilus* decreased the diarrheal period by ≈20 h ($P < 0.05$). Because of the different strains of *L. acidophilus* used, no firm conclusions can be drawn from these studies.

Another strain tested for its therapeutic effect on acute diarrhea was *Enterococcus* SF68, a strain that is also known as *Enterococcus faecium* SF68 or *Streptococcus faecium* SF68. In one study (38), patients with acute diarrhea were randomly assigned to treatment with 2.3×10^8 CFU *Enterococcus* SF68/d ($n = 40$) or placebo ($n = 38$). After 1 d, 5 patients in the experimental

group were cured compared with zero in the control group. Life-table analysis showed that patients in the experimental group were cured sooner than were patients in the control group. However, no effect of *Enterococcus* SF68 was seen in a placebo-controlled study of 183 Bangladeshi adults with acute watery diarrhea caused by *Vibrio cholerae* (n = 114), enterotoxic *Escherichia coli* (n = 41), or unknown causes (n = 28) (39). The placebo consisted of heat-inactivated *Enterococcus* SF68. This study did not rely on reported diarrhea but measured stool output in all patients. However, a large placebo-controlled study of 211 adults with acute diarrhea showed that intake of 15×10^7 CFU *E. faecium* SF68/d shortened the diarrheal phase from 2.8 d in the placebo group to 1.7 d in the treatment group (40). The effects of this probiotic on diarrhea are therefore inconclusive but tend to be positive.

We conclude that consumption of *Lactobacillus* GG can shorten the diarrheal phase of rotavirus infection by 1 d. No consistent evidence exists for other probiotics or for the prevention, as distinguished from treatment, of rotavirus-induced diarrhea.

Diarrhea caused by antibiotic use

Diarrhea due to the growth of pathogenic bacteria is the most common side effect of antibiotic use. Probiotics might inhibit this growth by releasing inhibitory substances, as indeed has been shown in vitro for some strains (41–45). So far, only one study has shown that *Lactobacillus* GG can prevent antibiotic-induced diarrhea (46). This study was done in 16 healthy men aged 18–24 y who received erythromycin for 7 d. Half of the men consumed 125 mL *Lactobacillus* GG–enriched yogurt/d, whereas the other half received placebo yogurt. The total number of days with diarrhea was 2 d in the *Lactobacillus* GG group and 8 d in the placebo group.

Two studies investigated the therapeutic effect of *Lactobacillus* GG in patients with recurrent *Clostridium difficile* infection (47, 48), an infection that causes severe diarrhea and colitis. The conclusion from both studies was that *Lactobacillus* GG cured the recurrent infection, but no control groups were included in these studies and the number of patients was small (n = 5 and 4, respectively).

Several strains of *L. acidophilus* have been tested as a prophylactic against diarrhea caused by antibiotics in 2 studies in patients (49, 50) and in 1 study with healthy volunteers (51). None of these studies provided conclusive evidence that intake of *L. acidophilus* prevents diarrhea caused by antibiotics. However, because the group sizes were small, significant effects could have been missed. Intake of *Enterococcus* SF68 (1.5×10^8 CFU/d) for 7 d reduced the incidence of diarrhea caused by antibiotics (38): 2 of 23 patients who received *Enterococcus* SF68 came down with diarrhea compared with 6 of 22 patients who received a placebo.

In conclusion, several studies have tested whether probiotics can prevent diarrhea due to antibiotic use. However, because of the small numbers of patients, the variety of antibiotics used, and, in many of the studies, the lack of a control or placebo group, this question remains unanswered.

Traveler's diarrhea

Traveler's diarrhea is defined as the passage of ≥3 unformed stools in a 24-h period in people who normally live in industrialized countries and who travel to tropical and semitropical areas (52). It affects 20–50% of travelers. The prevention of traveler's diarrhea by lactobacilli could be a safe alternative to antibacterial drugs.

Two studies investigated whether *Lactobacillus* GG can prevent traveler's diarrhea. In 820 travelers to 2 holiday resorts in Turkey, the incidence of diarrhea was 43% (178 of 418 participants) in the control group and 38% (153 of 402 participants) in the *Lactobacillus* GG group (53). The difference was not statistically significant when data from both resorts were combined. However, in one of the resorts, the treatment significantly reduced the incidence of diarrhea in the participants from 40% (30 of 76) in the placebo group to 24% (17 of 71 participants) in the *Lactobacillus* GG group. On the basis of this finding, the authors concluded that the use of *Lactobacillus* GG can diminish the risk of traveler's diarrhea. In another study with 245 travelers to developing countries, the risk of diarrhea on any given day was 3.9% in travelers who took *Lactobacillus* GG and 7.4% in control subjects who took a placebo (54). This study did not indicate how many travelers in each study group had diarrhea.

No effect of *L. acidophilus* strain LA (2×10^{11} CFU/d) or *Lactobacillus fermentum* strain KLD (2×10^{11} CFU/d) was seen in a double-blind, placebo-controlled study of 282 soldiers who were sent to Belize in Central America (55). The incidence of diarrhea was 24.5% in both treatment groups and in the placebo group. Compliance was >90% in 214 of the 282 volunteers. In the less-compliant volunteers, the incidence of diarrhea was higher but was not significantly different between the 3 groups. In conclusion, the effect of probiotics on the incidence of traveler's diarrhea seems to depend on the bacterial strain and the destination of the traveler and needs further study.

LOWERING OF SERUM CHOLESTEROL

Because in vitro studies have shown that bacteria can remove cholesterol from culture media (56–58), much attention has been given to the cholesterol-lowering potential of probiotics in humans. It is now thought that cholesterol removal from culture media was a result of precipitation of cholesterol with free bile acids, formed in the media because of the activity of the bacterial enzyme bile salt hydrolase (59, 60). Enhanced bile salt hydrolase activity in vivo would increase cholesterol excretion. However, consumption of a probiotic milk product did not increase cholesterol excretion in ileostomy subjects (61).

The cholesterol-lowering potential of *L. acidophilus* has been most widely studied. Lin et al (62) performed 2 studies: a pilot trial without a placebo and a large placebo-controlled trial. In the pilot trial, 23 subjects received tablets containing 3×10^7 CFU *L. acidophilus* (ATCC 4962) and *Lactobacillus bulgaricus* (ATCC 33409) daily for 16 wk, whereas 15 subjects received no tablets. Fasting blood samples were taken before and 7 and 16 wk after the start of the study. Serum cholesterol in the control group remained stable at 4.9 mmol/L; serum cholesterol in the experimental group decreased from 5.7 to 5.3 mmol/L after 7 wk ($P < 0.05$) and to 5.4 mmol/L after 16 wk ($P < 0.05$ compared with baseline and week 7). A second study with a double-blind, placebo-controlled and crossover design did not show a significant effect of lactobacilli on serum cholesterol (62). Two 6-wk study periods were separated by a washout period of 3 wk; 460 volunteers were enrolled and 334 completed the study. The mean serum cholesterol concentration after both treatments was 5.5 mmol/L.

A study performed in India showed that consumption of buffalo milk fermented with a specific strain of *L. acidophilus*

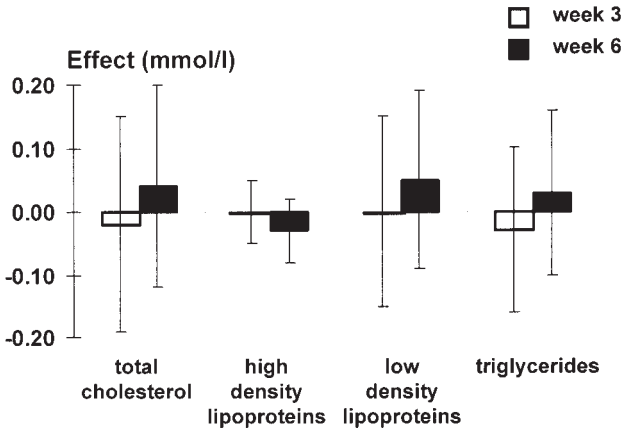

FIGURE 1. Net effects (means and 95% CIs) on blood lipid concentrations of the daily consumption of yogurt enriched with *Lactobacillus acidophilus* L-1 by healthy men and women after 3 (□) and 6 (■) wk. The net effects were calculated as changes in blood lipid concentrations in the *Lactobacillus acidophilus* group (n = 39) minus changes in the control group (n = 39). Reprinted with permission from reference 65.

reduced serum cholesterol by 12–20% after 1 mo (63). Serum cholesterol concentrations in the control group were not given, so no firm conclusions can be drawn from this study. A placebo-controlled crossover study with 30 volunteers found a 0.23-mmol/L (95% CI: 0.35, −0.11) lower serum cholesterol concentration after consumption of yogurt enriched with a specific strain of *L. acidophilus* and fructooligosaccharides than after consumption of a placebo yogurt (64). We found no effect of the consumption of yogurt enriched with *L. acidophilus* L-1 at a dose of 10^{10} CFU/d in a placebo-controlled study of 78 healthy men and women (65; **Figure 1**). Thus, the effects of *L. acidophilus* on serum cholesterol are inconclusive.

Two studies investigated the cholesterol-lowering effect of *E. faecium*. In the first study, 29 men received milk fermented with a human strain of *E. faecium* (10^8–10^{11} CFU/L) and 2 strains of *S. thermophilus* and 28 men received acidified milk as a control (66). Consumption of milk fermented with *E. faecium* decreased serum cholesterol by 0.37 ± 0.41 mmol/L after 6 wk, whereas consumption of the acidified milk had no effect. The authors then performed a second, larger study with 87 men and women and the same design (67). Serum LDL concentrations decreased throughout the study, with a significantly larger decrease in the *Enterococcus* group at weeks 4 and 12 than in the placebo-group ($P < 0.05$). At the end of the study period (week 24) and after the follow-up (week 30), serum LDL concentrations were no longer significantly different. The authors suggest that both chemically fermented milk and milk fermented with *E. faecium* lower serum cholesterol, with a more rapid effect by *E. faecium*. In conclusion, the effect of probiotics on serum cholesterol is inconclusive.

PREVENTION OF CARCINOGENESIS AND TUMOR GROWTH

Two studies have shown an effect of the consumption of probiotics on tumor growth; several other studies showed effects on markers of cancer risk. Daily intake of a viable *L. casei* strain postponed recurrence of bladder tumors in a randomized, controlled, multicenter study in 48 Japanese patients. Patients were enrolled within 2 wk after removal of one or more bladder tumors. *L. casei* was taken for 1 y or until tumor recurrence. After 1 y, tumors recurred in 19 of 23 (83%) patients in the control group compared with 12 of 21 (57%) patients in the *L. casei* group ($P < 0.01$); 4 patients were lost to follow-up. In a multivariate analysis including age and tumor characteristics, treatment with *L. casei* significantly ($P = 0.03$) postponed tumor recurrence (68). Because this study was not placebo controlled, the investigators conducted a second, larger, multicenter placebo-controlled study in 125 patients. Three subsets of patients were formed according to the number of tumors they had (one or more than one) and the nature of their tumors (primary or recurrent): 37 patients had one recurrent tumor, 41 patients had more than one primary tumor, and 47 patients had more than one recurrent tumor. *L. casei* did not significantly postpone the recurrence of tumors in the whole group of patients. However, when the 47 patients with more than one recurrent tumor were excluded from the analysis, the probiotic prolonged the tumor-free period ($P = 0.01$). The recurrence-free rate after 1 y was 79% in the 39 patients who took *L. casei* and 55% in the 39 patients who took the placebo (69). The results of these 2 studies suggest that consumption of *L. casei* might delay the recurrence of bladder tumors, but this finding awaits confirmation.

One hypothesis for the prevention or delay of tumor development by lactobacilli is that they might bind to mutagenic compounds in the intestine (70), thereby decreasing the absorption of these mutagens. Note that mutagenicity is mainly estimated as mutagenic potency in the in vitro Ames *Salmonella* test; effect of mutagens on cancer risk in humans can differ more than a thousandfold between humans (71).

If lactobacilli bind to mutagenic compounds, then the urinary excretion of mutagens will decrease. If the lactobacilli do not metabolize the compounds, the fecal excretion will increase. Indeed, excretion of mutagens in urine after a hamburger meal was ≈50% lower ($P < 0.05$) when the meal was supplemented with milk fermented with a strain of *L. acidophilus* (2.5×10^{11} CFU/d) than with unfermented milk (72). The fecal excretion of mutagens was not significantly reduced. In a similar experiment with a hamburger meal and *L. rhamnosus* GG, again no significant effect on the excretion of mutagens in the feces of 30 volunteers was found (73). Unfortunately, no information on the excretion of mutagens in urine was given. Intake of freeze-dried *L. casei* (Biolactis Powder, 3×10^8 CFU/d; Yakult Honsha Co, Tokyo) for 3 wk reduced the urinary excretion of mutagens after a test meal by 50% in 6 volunteers compared with pretreatment excretion (74). Thus, short-term studies suggest that intake of lactobacilli can reduce the absorption of mutagens from the intestine. Whether this will lead to a decreased incidence of cancer needs to be established.

Another possible explanation for the preventive effect of probiotics on carcinogenesis is their effect on other bacteria in the intestine. Probiotics might suppress the growth of bacteria that convert procarcinogens into carcinogens, thereby reducing the amount of carcinogens in the intestine. The activity of the enzymes that convert procarcinogens into carcinogens is often used as an indicator of the effect of probiotics on the intestinal microflora. Several studies showed an effect of consumption of probiotics on these enzyme activities, which are measured in feces.

Consumption of *L. rhamnosus* GG was shown to decrease the activity of β-glucuronidase (8, 75), nitroreductase (75), and choloylglycine hydrolase (75, 76). Consumption of milk enriched with *L. casei* Shirota for 4 wk temporarily decreased β-glu-

curonidase activity in 10 healthy men but not in 10 healthy control subjects and decreased β-glucosidase activity to a greater degree in 10 healthy men than in 10 healthy control subjects (19). Consumption of milk fermented with a *Bifidobacterium* species for 12 d decreased β-glucuronidase activity compared with baseline but had no effect on fecal pH or the activity of nitrate reductase, nitroreductase, and azoreductase (77). Consumption of a fermented milk with *L. acidophilus*, *B. bifidum*, *Streptococcus lactis*, and *Streptococcus cremoris* for 3 wk decreased the activity of nitroreductase from baseline but not that of β-glucuronidase and azoreductase (78). Thus, the results of these studies are inconclusive and apart from 2 clinical studies with *L. casei*, no conclusive evidence for an effect of probiotics on cancer risk or tumor recurrence exists. The relation between enzyme activity and cancer risk needs to be investigated further.

DISCUSSION

Probiotics are claimed to have beneficial effects on health. However, only few well-performed studies have looked at clearly defined health effects such as serum cholesterol concentrations or tumor recurrence. We based our conclusions predominantly on the results of placebo-controlled studies. Inconsistent findings of equally well-designed studies were regarded to indicate no effects. Only one consistent finding remains: *Lactobacillus* GG shortens the diarrheal phase of rotavirus infection in infants (26–31, 33).

In some studies, milk was given as a placebo whereas the probiotic was given as a fermented milk (23, 24, 72). This could have resulted in false-positive results in studies in which measures of immune status were the outcome variables. There is indeed some evidence that consumption of fermented milk stimulates the immune system (79, 80).

Probiotics are, by definition, live microorganisms and indeed most studies have been done with viable bacteria. Kaila et al (28) compared the effects of viable compared with those of heat-inactivated *L. casei* GG on diarrhea in 26 infants with acute diarrhea. The recovery time from diarrhea was not significantly different between the 2 groups. However, the patients that had received viable bacteria showed a higher specific IgA antibody response than did the patients who had received heat-inactivated bacteria. Viability might therefore be a prerequisite for effects on the immune system. In conclusion, the therapeutic effect of *Lactobacillus* GG on rotavirus diarrhea is well established. Other health effects, such as immune system stimulation, cancer prevention, and cholesterol lowering, need further investigation.

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