Probiotic use in clinical practice: what are the risks?[1–3]

Robert J Boyle, Roy M Robins-Browne, and Mimi LK Tang

ABSTRACT

Probiotics have been advocated for the prevention and treatment of a wide range of diseases, and there is strong evidence for their efficacy in some clinical scenarios. Probiotics are now widely used in many countries by consumers and in clinical practice. Given the increasingly widespread use of probiotics, a thorough understanding of their risks and benefits is imperative. In this article we review the safety of probiotics and discuss areas of uncertainty regarding their use. Although probiotics have an excellent overall safety record, they should be used with caution in certain patient groups—particularly neonates born prematurely or with immune deficiency. Because of the paucity of information regarding the mechanisms through which probiotics act, appropriate administrative regimens, and probiotic interactions, further investigation is needed in these areas. Finally, note that the properties of different probiotic species vary and can be strain-specific. Therefore, the effects of one probiotic strain should not be generalized to others without confirmation in separate studies. Careful consideration should be given to these issues before patients are advised to use probiotic supplements in clinical practice. *Am J Clin Nutr* 2006;83:1256–64.

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INTRODUCTION

Probiotics in the form of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in fermented milk have been ingested by humans for thousands of years in the belief that they have health benefits. For example, Persian tradition has it that Abraham owed his fertility and longevity to the regular ingestion of yogurt. In the early 20th century, the Russian immunologist Elie Metchnikoff proposed that lactic acid bacilli may have beneficial health effects and attributed his own longevity to regular probiotic ingestion. The proposed health benefits of probiotics have undergone increasingly rigorous scientific evaluation in recent years, and there is now strong evidence for their use in treating and preventing some human diseases. However, community use of probiotics is much wider than these specific indications, and probiotics have become an important commercial commodity. Given the increasingly widespread use of probiotics in both community and healthcare settings, clinicians need to have an understanding of the risks and benefits of probiotic treatment. In this article we review the known risks of probiotic treatment and explore areas of uncertainty regarding their use.

DEFINING PROBIOTICS

Probiotics are defined as "live microorganisms that when administered in adequate amounts confer a health benefit on the host" (1). It is believed by many that the ideal probiotic should remain viable at the level of the intestine and should adhere to the intestinal epithelium to confer a significant health benefit. Some evidence supports the importance of viability in human studies, with viable bacteria having greater immunologic effects than nonviable bacteria and killed bacteria being associated with adverse effects in some instances (2, 3). Some of the best characterized probiotics have also been shown to adhere strongly to intestinal epithelium in both in vitro and in vivo studies (4). Probiotics must also be resistant to gastric acid digestion and to bile salts to reach the intestinal intact, and they should be nonpathogenic. Most probiotics are strains of *Bifidobacterium* or *Lactobacillus* species. Some are derived from the intestinal microbiota of healthy humans, and others are nonhuman strains used in the fermentation of dairy products. Species from other bacterial genera such as *Streptococcus, Bacillus*, and *Enterococcus* have also been used as probiotics, but there are concerns surrounding the safety of such probiotics because these genera contain many pathogenic species, particularly *Enterococcus* (1). Nonbacterial microorganisms such as yeasts from the genus *Saccharomyces* have also been used as probiotics for many years.

EFFICACY OF PROBIOTICS IN PREVENTING AND TREATING DISEASE

Probiotics have been advocated for the prevention and treatment of a diverse range of disorders, from acute gastroenteritis to intestinal neoplasia (reviewed in 5). The evidence for their efficacy in many such disorders is not strong, but there are well-established benefits in a small number of conditions. The strongest evidence for the use of probiotics is in the management of

diarrheal diseases. For example, a meta-analysis of randomized controlled trials has shown that many probiotics are effective in preventing antibiotic-associated diarrhea (6), including the yeast *Saccharomyces boulardii* and the bacterium *Lactobacillus acidophilus* in combination with *L. bulgaricus*, *L. rhamnosus* strain GG [American Type Culture Collection (ATCC) 53103; LGG], and *Enterococcus faecium* strain SF68. A separate meta-analysis of randomized controlled trials has shown a variety of probiotics (including *Lactobacillus* species, *Enterococcus* species, and *S. boulardii*) to be effective in the treatment of infective diarrhea in both adults and children (7). In this analysis, probiotics were found to reduce the mean duration of diarrhea by >30 h. There is also support from randomized controlled trials for the efficacy of a probiotic mix (containing 3×10^{11} CFU *L. bulgaricus*, *L. casei*, *L. plantarum*, *L. acidophilus*, *Bifidobacterium longum*, *B. breve*, *B. infantis*, and *S. thermophilus*) in preventing flares of chronic pouchitis in patients with inflammatory bowel disease and for the use of a different probiotic mix [*B. lactis* Bb12 and *Lactobacillus reuteri* (ATCC 55730) at 1×10^7 CFU/g in a cow milk formula] to prevent diarrheal illness in infants attending childcare (8, 9). Probiotic therapy has also been explored in nongastrointestinal diseases, including the treatment and prevention of atopic eczema (10, 11). Nevertheless the evidence to date suggests that the major clinical effects of probiotics are seen in gastrointestinal disorders. Below we review current concerns and areas of uncertainty regarding the use of probiotics and the limitations of such in disease management.

RISKS ASSOCIATED WITH PROBIOTIC TREATMENT

Probiotics are often regulated as dietary supplements rather than as pharmaceuticals or biological products. Thus, there is usually no requirement to demonstrate safety, purity, or potency before marketing probiotics. This can lead to significant inconsistencies between the stated and actual contents of probiotic preparations, as shown in a recent South African study (12). In Europe, those dietary supplements intended for use by infants and young children do have specific compositional legal requirements (13). In the United States, although dietary supplements do not generally require premarket review and approval by the Food and Drug Administration, those that are marketed specifically for the treatment or prevention of a disease are classified as biological products and do need review and approval by the Food and Drug Administration. Similarly, in Australia, those probiotics marketed for specific health benefits require premarket review by the Therapeutic Goods Administration and are usually regulated as complementary medicines. In Japan, those probiotic products marketed for a specified health use also require formal premarket review by the Health Ministry (14). Although most commercially available probiotic strains are widely regarded as safe, there are significant concerns with respect to safety in particular populations.

Infection

The most important area of concern with probiotic use is the risk of sepsis. Probiotics have been widely used in food processing for many years, and overall have an excellent safety record, as supported by reviews (15, 16). Many small studies also support the safety of particular probiotic strains in particular high-risk populations. For example, different *Lactobacillus* strains have been fed to adults and children infected with HIV, to term

infants, and to premature infants with no significant adverse effects (17–19). In Finland, there has been a marked increase in the use of the probiotic LGG since its introduction into the country in 1990. In 1992 alone, 3×10^6 kg of products containing LGG was sold in Finland (20). Despite this increased use, no significant increase in *Lactobacillus* bacteremia or bacteremia attributable to probiotic strains has been observed in southern Finland (20, 21). Thus, there is a body of evidence that supports the safety of some probiotics, particularly *Lactobacillus* strains.

One theoretical concern with the safety of probiotics is that some have been designed or chosen to have good adherence to the intestinal mucosa, and this is considered important for their mechanism of action. Adherence to the intestinal mucosa may also increase bacterial translocation and virulence. The most potent probiotics, therefore, may have increased pathogenicity. The relation between mucosal adhesion and pathogenicity in *Lactobacillus* spp. is supported by the finding that blood culture isolates of *Lactobacillus* spp. adhere to intestinal mucus in greater numbers than do isolates from human feces or dairy products (22). Murine experiments have also shown the potential for probiotics to cause sepsis. For example, Wagner et al (23) colonized athymic mice with human isolates of *L. reuteri*, *L. acidophilus*, *Bifidobacterium animalis*, or LGG. Although athymic adult mice were not adversely affected by the probiotics, colonization with the probiotics *L. reuteri* and LGG did lead to death in some athymic neonatal mice. This finding suggests that the presence of immune deficiency in neonates may put them at particularly high risk of probiotic sepsis. These theoretical concerns are highlighted by recent case reports of probiotic sepsis in humans.

Reports of sepsis related to probiotic use

Lactobacillus species are a rare but well-recognized cause of endocarditis in adults (and other forms of sepsis in children) in the absence of probiotic supplementation. Several reports have directly linked cases of *Lactobacillus* and other bacterial sepsis to the ingestion of probiotic supplements. These case reports are discussed below and are summarized in **Table 1** and **Table 2**. Rautio et al (24) reported the case of a 74-y-old diabetic woman who developed LGG liver abscess and pneumonia 4 mo after commencing daily LGG supplementation. The infective and probiotic strains were indistinguishable by pulsed-field gel electrophoresis of chromosomal DNA restriction fragments. In a second case, Mackay et al (25) reported the development of *L. rhamnosus* endocarditis (strain not specified) after a dental extraction in a 67-y-old man with mitral regurgitation who was taking probiotic capsules daily. The authors found no differences between the probiotic and the infective *L. rhamnosus* with the use of standard API 50 CH (BioMerieux, Hazelwood, MI) biochemical analysis and pyrolysis mass spectrometry. These reports are highly suggestive of probiotic supplement–related sepsis, but it should be noted that LGG and other strains of *L. rhamnosus* can sometimes be found in the intestinal microbiota of healthy humans, so the source of infection in these cases is not conclusively proven. This point is emphasized by Presterl et al's (48) report of an adult with *L. rhamnosus* endocarditis, which was thought—after species identification with the use of API 50 CH—to be due to a probiotic strain but was found—after molecular typing with the use of randomly amplified polymorphic DNA—to be due to a different strain of unknown origin

TABLE 1
Cases of bacterial sepsis temporally related to probiotic use in humans[1]

Study	Age	Risk factors	Probiotic	Method of identification[2]	Form of sepsis
Rautio et al (24)	74 y	Diabetes mellitus	LGG	API 50 CH, PFGE of DNA restriction fragments	Liver abscess
Mackay et al (25)	67 y	Mitral regurgitation, dental extraction	*Lactobacillus rhamnosus,* 3×10^9 CFU/d	API 50 CH, pyrolysis mass spectrometry	Endocarditis
Kunz et al (26)	3 mo	Prematurity, short-gut syndrome	LGG	No confirmatory typing	Bacteremia
	10 wk	Prematurity, inflamed intestine, short-gut syndrome	LGG	PFGE of DNA restriction fragments	Bacteremia
De Groote et al (27)	11 mo	Prematurity, gastrostomy, short-gut syndrome, CVC, parenteral nutrition, rotavirus diarrhea	LGG, 1/4 capsule/d	rRNA sequencing	Bacteremia
Land et al (28)	4 mo	Cardiac surgery, antibiotic diarrhea	LGG, 10^{10} CFU/d	Repetitive element sequence-based PCR DNA fingerprinting	Endocarditis
	6 y	Cerebral palsy, jejunostomy feeding, CVC, antibiotic-associated diarrhea	LGG, 10^{10} CFU/d	Repetitive element sequence-based PCR DNA fingerprinting	Bacteremia
Richard et al (29)	47 y	Not stated	*Bacillus subtilis,* 8×10^9 spores/d	Antibiotic susceptibility	Bacteremia
	25 y	Not stated	*B. subtilis,* 8×10^9 spores/d	Antibiotic susceptibility	Bacteremia
	63 y	Neoplastic disease	*B. subtilis,* 8×10^9 spores/d	Antibiotic susceptibility	Bacteremia
	79 y	Not stated	*B. subtilis,* 8×10^9 spores/d	Antibiotic susceptibility	Bacteremia
Oggioni et al (30, 31)[3]	73 y	Chronic lymphocytic leukemia	*B. subtilis,* 10^9 spores/d	16S rRNA sequencing	Bacteremia

[1] Where no dose is given, there was no precise dose described in the original publication. CVC, central venous catheter; rRNA, ribosomal RNA; PFGE, pulsed-field gel electrophoresis; PCR, polymerase chain reaction; LGG, *Lactobacillus rhamnosus* GG; CFU, colony forming units.

[2] API 50 CH; BioMerieux, Hazelwood, MI.

[3] Fatal outcome not clearly related to probiotic sepsis.

Bacterial sepsis related to probiotic use in children has also been reported. Kunz et al (26) described the cases of 2 premature infants with short gut syndrome who were fed via gastrostomy or jejunostomy and developed *Lactobacillus* bacteremia while taking LGG supplements In 1 of the 2 cases, pulsed-field gel electrophoresis of chromosomal DNA restriction fragments found the bacteremic strain and probiotic strain to be indistinguishable. De Groote et al (27) reported a similar case, confirmed with the use of pulsed-field gel electrophoresis and rRNA sequencing Recently, 2 definitive cases of probiotic sepsis due to LGG were reported in children; strain homology was confirmed by using repetitive element sequence-based polymerase chain reaction DNA fingerprinting (28). The authors reported the case of a 4-mo-old infant with antibiotic-related diarrhea after cardiac surgery, who developed LGG endocarditis 3 wk after commencing LGG at 10^{10} CFU/d. They also reported the case of a 6-y-old girl with cerebral palsy and antibiotic-associated diarrhea who developed LGG bacteremia on day 44 of treatment with LGG at 10^{10} CFU/d through a gastrojejunostomy tube. *Bacillus subtilis* bacteremia and cholangitis related to probiotic use have also been described. In one case, strain homology between the probiotic and pathogenic bacteria was confirmed by using molecular typing (29–31). Many cases of *Saccharomyces boulardii* fungemia in those taking *S. boulardii* supplements have now been described; in some cases, homology between the probiotic and infective organisms was confirmed by using molecular typing (32–45). Interestingly, 2 reports suggest that a probiotic supplement (*S. boulardii*) taken by one hospital inpatient may spread to neighboring patients, to whom it is not being directly administered, and lead to significant sepsis (33, 34). It has been suggested

that contamination of vascular catheters may be responsible for such cases (32). We are not aware of any reports of *Bifidobacterium* sepsis related to probiotic use, which is in keeping with animal studies that suggest its low pathogenicity (23). It may be that bifidobacteria have a better safety profile than other probiotics, but their infrequent association with sepsis may equally relate to the dominance of other genera such as lactobacilli in currently available probiotic preparations.

Risk factors for probiotic sepsis

All cases of probiotic bacteremia or fungemia have occurred in patients with underlying immune compromise, chronic disease, or debilitation, and no reports have described sepsis related to probiotic use in otherwise healthy persons. Most cases of probiotic sepsis have resolved with appropriate antimicrobial therapy, but in some cases patients have developed septic shock (32). In other cases the outcome has been fatal, but these fatalities were usually related to underlying disease rather than directly to probiotic sepsis (31, 35, 46). One exception is the report by Lestin et al (47) of a 48-y-old diabetic woman with diarrhea attributable to *Clostridium difficile* who died from multiorgan failure and septic shock in association with a toxic megacolon and probiotic fungemia. The case is suggestive of fatal probiotic sepsis, but molecular methods were not used to confirm homology between the probiotic and pathogenic fungi. Many case reports of probiotic sepsis describe persons with preexisting intestinal pathology, including diarrhea and short intestine. These may be common indications for probiotic use, but would also be expected to increase the risk of probiotic translocation through the intestinal mucosa. Some cases have occurred after probiotic strains were

TABLE 2
Cases of fungal sepsis temporally related to probiotic use in humans[1]

Study	Age	Risk factors	Probiotic[2]	Method of identification[3]	Form of sepsis
Hennequin et al (32)	30 mo	Cystic fibrosis, CVC, poor nutritional state, intestinal surgery	*Saccharomyses boulardii,* 750 mg/d	PFGE of mitochondrial DNA restriction fragments	Fungemia
	36 y	HIV infection, CVC, diarrhea	*S. boulardii,* 1.5 g/d	PFGE of mitochondrial DNA restriction fragments	Fungemia
	47 y	Antibiotic-associated diarrhea, upper GI surgery for malignancy	*S. boulardii,* 2 g/d	PFGE of mitochondrial DNA restriction fragments	Septic shock
	78 y	Peptic ulcer, chronic renal failure, pneumonia, COPD	*S. boulardii,* 1.5 g/d	PFGE of mitochondrial DNA restriction fragments	Fungemia
Cassone et al (33)[4]	34 y	CVC, intensive care unit	No direct treatment	PFGE of undigested chromosomal DNA	Fungemia
	48 y	CVC, intensive care unit	No direct treatment	PFGE of undigested chromosomal DNA	Fungemia
	75 y	CVC, intensive care unit	No direct treatment	PFGE of undigested chromosomal DNA	CVC colonization
	35 y	Intensive care unit	Unclear	PFGE of undigested chromosomal DNA	Fungemia
Perapoch et al (34)	3 mo	CVC, diarrhea, parenteral nutrition	*S. boulardii,* 100 mg/d	PFGE of mitochondrial DNA restriction fragments	Fungemia
	Infant	Short-bowel syndrome, CVC, parenteral nutrition	Not received directly (no direct treatment)	PFGE of mitochondrial DNA restriction fragments PFGE of undigested chromosomal DNA	Fungemia
Lherm et al (35)[5]	50–82 y	Acutely unwell on intensive care unit with respiratory failure, CVC	*S. boulardii,* 1.5–3.0 g/d	PFGE of nuclear and mitochondrial DNA restriction fragments	Fungemia
Bassetti et al (36)	51 y	Immunosuppression, *Clostridium difficile*–associated diarrhea, CVC	*S. boulardii,* 1 g/d	PFGE of DNA restriction fragments	Fungemia
Riquelme et al (37)	42 y	Kidney and pancreas transplant, immunosuppression, *C. difficile*–associated diarrhea	*S. boulardii,* 1 g/d	PFGE of DNA restriction fragments	Fungemia
	41 y	HIV, diarrhea	*S. boulardii,* 750 mg/d	PFGE of DNA restriction fragments	Fungemia
Fredenucci et al (38)	49 y	Antibiotic-associated diarrhea, immunosuppression	*S. boulardii,* 200 mg/d	PFGE of undigested chromosomal DNA API 32C	Fungemia
Cesaro et al (39)	8 mo	Acute myeloid leukemia, CVC, neutropenia	*S. boulardii*	API 32C	Fungemia
Cherifi et al (40)	89 y	*C. difficile*–associated, colitis, gastrostomy	*S. boulardii,* 300 mg/d	No formal identification described	Fungemia
Henry et al (41)	65 y	Malignancy, immunecompromise, mucositis, diarrhea, parenteral nutrition	*S. boulardii*	No formal identification described	Fungemia
Niault et al (42)	78 y	Antibiotic-associated diarrhea, intensive care unit, intragastric feeding	*S. boulardii,* 1.5 g/d	No formal identification described	Fungemia
Viggiano et al (43)	14 mo	Burns, diarrhea, gastrostomy	*S. boulardii,* 200 mg/d	No formal identification described	Fungemic shock
Zunic et al (44)	33 y	Inflammatory bowel disease, intensive care unit, parenteral nutrition	*S. boulardii,* 1.5 g/d	No formal identification described	Fungemia
Pletincx et al (45)	1 y	Parenteral nutrition, antibiotic-associated diarrhea, CVC	*S. boulardii,* 600 mg/d	No formal identification described	Septicemia
Rijnders et al (46)[5]	74 y	Colitis, nasogastric feeding	*S. boulardii,* 600 mg/d	No formal identification described	Fungemia
Lestin et al (47)[6]	48 y	Diabetes, *C. difficile*–associated diarrhea	*S. boulardii,* 150 mg/d	API 32C	Fatal fungemia

[1] CVC, central venous catheter; COPD, chronic obstructive pulmonary disease; PFGE, pulsed-field gel electrophoresis; GI, gastrointestinal.
[2] 250 mg *S. boulardii* = 5.425×10^{13} live cells.
[3] API 32C; BioMerieux, Hazelwood, MI.
[4] Cases thought to be related to *S. boulardii* treatment of neighboring intensive-care-unit patients.
[5] Fatal outcome ($n = 3$) not clearly related to probiotic sepsis.
[6] Fatal fungemia in association with toxic megacolon; death thought to be related to probiotic sepsis.

TABLE 3
Proposed risk factors for probiotic sepsis[1]

Major risk factors
  1) Immune compromise, including a debilitated state or malignancy
  2) Premature infants
Minor risk factors
  1) CVC
  2) Impaired intestinal epithelial barrier, eg, diarrheal illness, intestinal inflammation
  3) Administration of probiotic by jejunostomy
  4) Concomitant administration of broad spectrum antibiotics to which probiotic is resistant
  5) Probiotics with properties of high mucosal adhesion or known pathogenicity
  6) Cardiac valvular disease (*Lactobacillus* probiotics only)

[1] The presence of a single major or more than one minor risk factor merits caution in using probiotics. CVC, central venous catheter.

given via jejunostomy tube, bypassing gastric acid, and this would be expected to increase the numbers of viable probiotic bacteria that reach the intestine. The presence of a central venous catheter is also a common finding in cases of probiotic sepsis and has been shown to be a possible source of sepsis (32). Premature infants appear to be overrepresented in case reports, as are those who are debilitated or immunocompromised. The increased susceptibility of premature infants and the immuncompromised to probiotic sepsis is supported by animal studies (23). On the basis of the characteristics of the cases reported to date, we propose a list of major and minor risk factors for probiotic sepsis (**Table 3**). We suggest that the presence of a single major risk factor or more than one minor risk factor merits caution in using probiotics.

Deleterious metabolic activities

The intestinal microbiota play an important role in many metabolic activities, including complex carbohydrate digestion, lipid metabolism, and glucose homeostasis (49). There is therefore a theoretical risk of adverse metabolic effects from manipulation of the microbiota with the use of probiotics, even if such manipulation is only temporary. The likelihood of significant adverse effects in this regard seems low however, and probiotic studies to date have not shown significant adverse effects on growth or nutrition (50).

Immune deviation or excessive immune stimulation

Murine experiments also showed that the intestinal microbiota is important in stimulating normal immune development, particularly the development of gut-associated lymphoid tissue. The presence of an intestinal microbiota is necessary for a range of immune functions, including antibody production, the development and persistence of oral tolerance to food antigens, and the formation of germinal centers within lymphoid follicles (49, 51). This crucial role of the intestinal microbiota in normal immune development suggests that manipulations designed to alter the microbiota may have significant immunomodulatory effects. The long-term effect of these manipulations on the host is difficult to predict, and adverse effects on immune development remain a possibility. This is particularly relevant in the field of neonatal probiotic supplementation, where medium- to long-term alteration of the microbiota or life-long modification of the immune response might be achieved. A second group that may be at increased risk of adverse immune stimulation is pregnant women. During pregnancy there is a bias in T cell responses toward a Th2 phenotype, which is thought to be important in maintaining fetal viability because Th1 cytokines are associated with pregnancy loss (52). Probiotic *Lactobacillus* species have been shown to suppress Th2 cytokine responses in vitro, and in some human studies were found to increase production of the Th1 cytokine interferon γ (53, 54). These effects may be detrimental to pregnancy viability. However, there is currently no direct evidence for this, and such a risk remains theoretical. At present there is little support for the hypothesis that probiotics cause adverse immune development from empirical studies, but this is an area that warrants further investigation.

Microbial resistance

In most circumstances the available data suggest that probiotics colonize the human intestine transiently. Nevertheless, concern exists regarding the possible transfer of antimicrobial resistance from probiotic strains to more pathogenic bacteria in the intestinal microbiota. Many *Lactobacillus* strains are naturally resistant to vancomycin, which raises concerns regarding the possible transfer of such resistance to more pathogenic organisms, particularly enterococci and *Staphylococcus aureus*. However, the vancomycin-resistant genes of *Lactobacillus* spp. are chromosomal and, therefore, not readily transferable to other species. Conjugation studies have not found the vancomycin-resistant genes of lactobacilli to be transferable to other genera (55).

AREAS OF UNCERTAINTY IN THE USE OF PROBIOTICS

When evaluating the risks and benefits of probiotic treatment, considerable uncertainty surrounds their use. This uncertainty arises from several areas, which are discussed below.

Specificity of probiotic effects

Although pooled analyses have, in some cases, shown significant treatment effects for probiotics as a whole, different probiotics can have different effects in both in vivo and in vitro analyses. The clinical or laboratory effects of one probiotic cannot be assumed for another probiotic species or for different strains of the same species. *Bifidobacterium* species isolated from human feces were found, in a detailed study, to be genetically heterogeneous, and different strains had different properties in terms of acid and oxygen tolerance and growth requirements (56). This variation in properties is likely to lead to strain-to-strain variation in microbiological and clinical effects. Murine studies confirm this variation by showing a diversity of clinical effects between probiotics. Wagner et al (57) studied the effects of 4 different probiotic species (*L. reuteri, L. acidophilus,* LGG, and *B. animalis*) in preventing colonization and sepsis with *Candida albicans* in both athymic and euthymic mice. They found all strains to be protective, but there were significant differences in efficacy and a great diversity of immune effects in terms of antibody and proliferative responses to *C. albicans* and intestinal inflammatory cell infiltration. In vitro studies also support the diversity of actions of different probiotics. Indeed, one study showed different strains to have antagonistic effects. In a study of dendritic cell function with the use of 2 different *Lactobacillus* species, *L. reuteri* DSM12246 was found to specifically inhibit *L. casei* CHCC3139–induced interleukin (IL) 12, IL-6, and tumor necrosis factor α production by murine dendritic cells and to inhibit *L.*

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casei CHCC3139–induced up-regulation of dendritic cell co-stimulatory markers (58). Similarly, studies of the effects of *Bifidobacterium* species on dendritic cell function have shown marked variation between species (59). Studies in humans provide confirmation of the significance of these murine and in vitro findings. For example, LGG has specific effects in enhancing immunoglobulin A responses against rotavirus that are not found with other *Lactobacillus* species. Furthermore, in the treatment of infective diarrhea, a combination of *S. thermophilus* and *L. bulgaricus* was ineffective, whereas a combination of *L. acidophilus* and *L. bifidus* was particularly effective (7). It is therefore of the utmost importance to examine probiotic-specific effects when reviewing their clinical efficacy and to not generalize the effects of one probiotic strain to another, even within the same species. Nevertheless, in some clinical scenarios, a range of different probiotics appear to be effective—presumably by acting through a mechanism common to a range of nonpathogenic microbes. Additional work is needed to clarify the relative importance of strain-specific effects in different scenarios and the nature of probiotic-probiotic interactions.

Probiotic treatment schedules

Limited information is available about appropriate probiotic dosing regimens. Few dose-comparison studies have been undertaken; those that have investigated this issue have more commonly used fecal recovery as an outcome rather than clinical response. For example, fecal recovery of LGG after administration to adults in various doses has been studied using culture-based methods (60). A daily dose of $\geq 10^{10}$ CFU was needed to ensure reliable fecal recovery of LGG. However, fecal recovery is not an optimal outcome measure because fecal detection may not reflect clinical outcomes. The number of viable bacteria reaching or colonizing the intestine depends on many factors other than dose, particularly the probiotic formulation, coadministration of food or milk (which may protect the probiotic from gastric acid), and the person's gastric pH, intestinal motility, and prior composition of intestinal microbiota. The latter varies considerably from one person to another (61). Commercially available probiotic formulations generally contain $\geq 10^{6}$ CFU/g of viable organisms, but the doses of specific probiotics required for specific clinical effects are not well established.

Probiotic mechanisms of action

One of the difficulties in assessing the place of probiotics in clinical practice is our limited understanding of their mechanisms of action. However, some of the biological effects of probiotics have now been characterized, and it is important for clinicians using probiotics to have some knowledge of these microbiological and immunologic effects.

Microbiological mechanisms

The human intestinal microbiota contains hundreds of different species of bacteria as well as archaea and eukarya, and the bacterial density is particularly high in the large intestine (up to 10^{11} CFU/g). In adults, the species composition appears to be stable in a given person over time in the absence of pathologic states such as infective diarrhea or antibiotic use (62). However, studies indicate that probiotic bacteria can significantly influence the composition of the healthy intestinal microbiota. For example, Sepp et al (63) treated 15 newborns with 10^{10}–10^{11}

CFU LGG/d for the first 2 wk of life and monitored the development of intestinal microbiota in these infants and in an untreated control group. Using culture-based detection methods, they found that LGG persisted in the stool at 1 mo of age in 8 of the 15 infants. There were significant differences in stool microbiota between the LGG-treated and control infants. In the LGG-treated infants, coliforms and lactobacilli were present in increased numbers as early as days 3–4 of life and, by 1 mo age, *Bifidobacterium* spp. had also increased. The newborn microbiota changes rapidly in the first weeks of life and at the time of weaning and is not thought to reflect adult patterns until 2 y age. Thus, the intestinal microbiota of infants may be more amenable to manipulation by probiotic supplementation than that of adults. However, Benno et al (64) have shown that probiotics can also alter the intestinal microbiota of adults. They administered LGG at a dose of 1.4×10^{10} CFU/d to 13 healthy adults for 4 wk. They found that the proportion of the fecal microflora represented by bifidobacteria rose from 16.9% before LGG administration to 36% after administration with the use of culture-based detection methods ($P < 0.05$). There was also an increase in lactobacilli and a decrease in the proportion of the fecal microflora represented by *Clostridium* spp. Tannock et al (65) used molecular methods to analyze stool bacterial populations and found less marked changes in the adult fecal microflora during supplementation with *L. rhamnosus* DR20 than reported by other workers. Molecular profiling methods can be more strain-specific than culture-based methods and may be less biased. However, either method of fecal profiling is limited to reflecting distal colonic luminal contents and provides little information regarding small intestinal colonization. Despite these limitations, these studies suggest that probiotics can affect the pattern of microbial colonic colonization.

Probiotics can also affect the intestinal microbiota in disease states. Some of the protective mechanisms through which they inhibit the actions of pathogenic microbes have been elucidated. For example, in disease states associated with increased intestinal mucosal permeability, it has been shown that the administration of *Lactobacillus* probiotics can decrease intestinal mucosal permeability (66). Probiotics produce bacteriocins, hydrogen peroxide, and biosurfactants to aid their survival in the gastrointestinal tract and can competitively inhibit the adherence of more pathogenic bacteria to the intestinal epithelium. Many probiotic species induce mucin production by intestinal epithelial cells in vitro and some also induce the production of defensin-$\beta 2$, an antimicrobial peptide (67). These appear to be important mechanisms through which some probiotic bacteria act in preventing the adherence of pathogens to the intestinal epithelium. Moreover, such antagonism of pathogenic bacteria appears to be most effective when probiotic strains themselves adhere to the intestinal epithelium (67). This supports the concept that probiotics need to colonize the intestine to exert a beneficial effect, and it is well established that some probiotic strains do colonize the intestine for >2 wk after administration (4). This transient colonization may be sufficient to protect the intestinal mucosa against colonization by more pathogenic microbes, stimulate local and systemic immune responses, and enhance mucosal barrier function. It may also transiently create the necessary microenvironment for other intestinal microbes to flourish, with these secondary microbes leading to clinical benefits. Whether colonization of the intestine is always necessary for probiotics to exert their beneficial effects is not certain.

Immunologic mechanisms

A range of probiotic immune effects have been described, but direct evidence for the immune mechanisms by which they achieve their beneficial effects is limited. Murine studies have defined some of the mechanisms through which the intestinal microbiota enhances intestinal epithelial barrier function, and this may also be an important function of probiotics. Hooper et al (68) discovered that intestinal commensals up-regulate mucin-encoding genes in the host intestinal epithelium, which stimulates the production of mucus to form a protective barrier. Other investigators have shown that Toll-like receptor (TLR) signaling by the commensal intestinal microbiota is essential for homeostasis of the intestinal epithelium and protection from epithelial injury. By recognizing pattern recognition molecules from commensal microorganisms, TLRs stimulate the production of epithelial repair factors. This is likely to be an important mechanism through which probiotics act (69). TLR activation by molecules such as lipopolysaccharide, flagellin, and lipoteichoic acid also generates the production of cytokines through intracellular signaling pathways, which activate transcription factors such as nuclear factor κB (NF-κB). Some nonpathogenic enteric bacteria have been shown to have an immunosuppressive effect on intestinal epithelial cells by directly inhibiting the NF-κB pathway (70). Others inhibit the same pathway by promoting the nuclear export of an NF-κB subunit, thus limiting the duration of NF-κB activation (71). These inhibitory effects on the proinflammatory NF-κB pathway may be an important mechanism by which microbes regulate intestinal inflammation.

Clinical studies have also shown some specific immunologic actions for particular probiotics. LGG increases mitogen-stimulated and circulating concentrations of the antiinflammatory cytokine IL-10 when administered to infants (72). LGG was also found to up-regulate markers of phagocyte activation in healthy persons while down-regulating the same markers in persons allergic to cow milk undergoing cow milk challenge (73). Specific probiotics have been shown to reduce intestinal inflammation and improve intestinal mucosal permeability in allergic disorders for which these markers are altered (74). In vitro studies have shown probiotic actions on dendritic cell function, which show considerable species-to-species variation (58). For example *Bifidobacterium bifidum, B. longum*, or *B. pseudocatenulatum* up-regulate cord blood dendritic cell IL-10 production in vitro, whereas *B. infantis* does not (59). More recently, specific probiotics have been engineered to produce IL-10 in the intestinal microenvironment, and future clinical studies of such strains will be of great interest (75). Thus, the immunologic effects of probiotics are likely to occur through both less specific TLR-mediated actions on intestinal epithelial homeostasis and strain-specific effects on particular immune functions. Further work is needed to elucidate these details for specific probiotics in specific disorders. The finding that a probiotic strain may have opposing effects in healthy persons and those with allergies is also noteworthy and warrants further evaluation.

CONCLUSIONS

Probiotics are increasingly being used by consumers for their health benefits and are advocated by many health care professionals. The evidence base for their use in specific clinical scenarios is strong, but they are commonly used in a much wider range of scenarios in which their efficacy is not well established. Herein we reviewed the safety of probiotics and highlighted deficiencies in our understanding of their appropriate administration and their mechanisms of action. We found that probiotics are safe for use in otherwise healthy persons, but should be used with caution in some persons because of the risk of sepsis. Newly developed probiotic strains should be thoroughly evaluated for safety before being marketed. Although much remains to be learned regarding the mechanisms of action and the appropriate administration of probiotic strains, it is clear that different strains can have very specific effects. Moreover their effects may vary in health and disease, in different disease states, and in different age groups. Thus, clinical trial results from one probiotic strain in one population cannot be automatically generalized to other strains or to different populations. Further studies are needed to explore mechanistic issues and probiotic interactions. In view of the increasing use of probiotics as health supplements and therapeutic agents, clinicians need to be aware of the risks and benefits of these treatments.

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