INTRODUCTION

The origin of fermented foods and cultured milk products goes so far back that it predates recorded history. However, it was not until the beginning of the 20th century that Metchnikoff made observations that human health and longevity are associated with the consumption of sour milk containing *Lactobacillus bulgaricus*. The probiotic organism, a secreted substance, or parts of it, such as DNA, can produce benefits when ingested. Probiotics have an antimicrobial effect through modifying the microflora, secreting antibacterial substances, competing with pathogens to prevent their adhesion to the intestine, competing for nutrients necessary for pathogen survival, and producing an antitoxin effect. Probiotics are also capable of modulating the immune system, regulating allergic response of the body and reducing proliferation in cancer. It is also interesting to note that the effects of the probiotics go beyond the gastrointestinal tract to distant areas, such as the urogenital and respiratory mucosa.

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Probiotics are live microbial feed, which provide benefits to the host. The significance of probiotics was recognized at the turn of the century when health benefits were associated with the consumption of sour milk containing *Lactobacillus bulgaricus*. The probiotic organism, a secreted substance, or parts of it, such as DNA, can produce benefits when ingested. Probiotics have an antimicrobial effect through modifying the microflora, secreting antibacterial substances, competing with pathogens to prevent their adhesion to the intestine, competing for nutrients necessary for pathogen survival, and producing an antitoxin effect. Probiotics are also capable of modulating the immune system, regulating allergic response of the body and reducing proliferation in cancer. It is also interesting to note that the effects of the probiotics go beyond the gastrointestinal tract to distant areas, such as the urogenital and respiratory mucosa.

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The Mechanism of Action of Probiotics

There is considerable evidence to support that components of the microflora contribute to the development, not only to gastrointestinal disorders such as inflammatory bowel disease (3,4), colon cancer (5–7), and irritable bowel syndrome (8,9), but also systemic disorders such as atopic dermatitis (10).

Interestingly, it may not be necessary to administer the intact probiotic organism to achieve benefit. Secreted proteins and DNA from probiotics (VSL#3) can block inflammation and stop the death of epithelial cells (11,12). In another study, DNA from the same probiotic preparation as well as specific E-coli strains suppressed experimental colitis in several animal models (13). Probiotic bacteria can also be genetically modified for use as carriers for antigen delivery into diseased sites in the intestine (14).

It is recognized that considerable differences exist in the biological activities, doses and composition between the different probiotic preparations. Further studies are necessary to increase our understanding of how the probiotic agents produce a beneficiary effect on the host as different strains of probiotic bacteria may work by distinctly different mechanisms. It is also important to recognize that in vitro effects of a probiotic may display opposite behavior in vivo (15).

The mechanism of action of probiotics can be classified based on specific effects of the bacteria on the microbial milieu, intestinal epithelium, immune response, allergic diseases, distant mucosal sites, and cancer (Figure 1).

**ANTIMICROBIAL EFFECTS**

**Effect on Microflora**

Modification of microflora has long been considered as the mechanism of action of probiotics. Several studies suggest that ingestion of certain lactobacilli and bifidobacteria species decrease the fecal concentrations of clostridia, Bacteroides, and E-coli, and can increase the endogenous levels of lactobacilli and bifidobacteria, but more importantly affect the metabolic activities of the flora by decreasing the production of carcinogenic substances such as fecal azoreductase, nitroreductase and β-glucoronidase (16). Whether colonization is critical for probiotics to have their effect remains unresolved (17). In a infant study by Agarwal, et al, colonization with Lactobacillus GG occurred in 21% of infants who weighed less than 1500 grams versus 47% of larger infants. The use of antibiotics interfered with the colonization ability of the probiotic. Therefore, the neonatal response to probiotic preparations is dependent on gestational age, weight, postnatal age and prior antibiotic exposure (18).

**Production of Antimicrobial Factors**

Probiotics are capable of producing short chain fatty acids, which lower the colonic pH, favoring the growth of less pathogenic organisms (19). Bacteriocins, which are antimicrobial proteins elaborated by probiotic organisms, are especially effective against gram-positive organisms (20). Lactobacilli also produce substances that inactivate viral particles. Soluble substances produced by Lactobacillus rhamnosus GR-1 and L. fermentum RC-14 can inactivate adenovirus and the vesicular stomatitis virus within minutes (21). Lactobacillus GG produces compounds that inhibit the growth of several gram-positive and gram-negative bacteria by producing antimicrobial substances such as lactic acid, hydrogen peroxide, and pyroglutamate (22,23). In addition, Lactobacillus acidophilus strain LA1 produces a non-bacteriocin and non-lactic acid antimicrobial substance against a variety of gram-negative and gram-positive bacteria (24). Moreover, specific microflora isolated from an infant, were found to be bactericidal against Salmonella typhimurium (25).
**The Mechanism of Action of Probiotics**

**PROBIOTICS: THE HOPE, THE HYPE, AND THE REALITY, SERIES #2**

**Competition for Adhesion**

Binding to intestinal epithelium is one of the determinants in establishing the efficacy of a probiotic (26). Colonization resistance occurs through this binding, competitively inhibiting adhesion of pathogenic bacteria (27–29). For example, *Lactobacillus* GG and *Lactobacillus plantarum* 299V competitively inhibit the attachment of enterohemorrhagic *Escherichia coli* 0157H7 to HT-29 cells (30). Other lactobacillus strains have been shown to compete with enteropathic *E. coli* for attachment to mucus in pig ileum (31). *Saccharomyces boulardii* inhibits the attachment of *Entamoeba histolytica* trophozoites to erythrocytes in vitro (32). Furthermore, certain strains of lactobacilli are capable of blocking receptor sites preventing the invasion of pathogens (33).

**Competition for Nutrients**

Probiotics may also compete for nutrients otherwise consumed by pathogenic organisms. For example, consumption of monosaccharides by a probiotic may reduce the growth of *Clostridium difficile*, which is dependent on monosaccharides for growth (34).

**Antitoxin Effect**

Probiotics may also modify toxin receptors through an enzymatic mechanism, which has been seen with *S. boulardii* through its effect on the *C. difficile* toxin A receptor (35). The effect of *S. boulardii* on *C. difficile* toxins was suspected when investigators observed clinical improvement without a change in the concentration of *C. difficile* in the stools. Similar effects have been postulated for the cholera toxin receptor. In animal studies, gut commensals offer host resistance against pathogens, where the host is able to withstand lethal doses of pathogens such as *Salmonella enteritidis* (36) (Table 1).

**EFFECTS ON INTESTINAL EPITHELIUM, MUCUS PRODUCTION, AND BARRIER FUNCTION**

**Effects on Barrier Function**

Probiotic bacteria can enhance barrier function by different mechanisms. First, probiotic bacteria such as *Streptococcus thermophilus* and *Lactobacillus acidophilus* enhance activation of tight junction proteins avoiding the development of a leaky intestine (37). Second, other probiotic bacteria such as *Lactobacillus rhamnosus* GG can prevent inflammation and programmed cell death of the lining intestinal epithelial cells (12). Finally, an effect on barrier function with a lactobacillus strain has been demonstrated by decreased mucosal permeability to mannitol in germ-free rats (38).

**Down-regulation of the Secretory and Motility Defenses**

Mucins produced by the host constitute one of the defense mechanisms against pathogens, and *MUC2* and *MUC3* mRNA expression is increased in response to lactobacilli, protecting cells against the adhesion of pathogenic bacterial (30). Moreover, *Lactobacillus plantarum* 299v was found to decrease the damaging effect of a specific type of *E. coli* on intestinal epithelial cells (39,40).

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Trophic Action of Probiotics

Several studies suggest a trophic intestinal effect secondary to the ingestion of *S. boulardii* by increasing the brush border enzymes in jejunal mucosa (41). Also, the same probiotic, through its production of polyamines, may enhance intestinal enzyme expression (42) (Table 2).

**IMMUNE EFFECTS**

**Probiotics as Vehicles for Immune Modulation**

Probiotic agents such as lactobacilli can be genetically engineered to secrete substances that possess anti-inflammatory effect such as IL-10. When these genetically engineered probiotic agents are ingested by the host, anti-inflammatory cytokines can be released locally to the inflamed areas in the gastrointestinal tract (43).

**Effect on Molecular Signaling Inside the Cell**

The protective effects of probiotics may be mediated by their own DNA rather than by their metabolites or ability to colonize the colon. Toll-like receptor 9 (LR9) signaling is essential in mediating the anti-inflammatory effect of probiotics, and DNA derived from probiotic bacteria can be sufficient to attenuate experimental colitis (13). In a human and murine inflammatory model, VSL#3 DNA inhibited IL-8 secretion, reduced p38 mitogen-activated protein kinase activation, delayed nuclear factor kappaB activation, stabilized levels of IkappaB, and inhibited proteasome function (11). Similarly, *S. boulardii* prevented enterohemorrhagic *Escherichia coli* infection by interfering with the transduction pathways that control tight-junction structure as well as inhibiting NF-kappaB and MAPK signaling pathways leading to the production of IL-8 (44). A study by Petrof, et al demonstrated that probiotics inhibited the pro-inflammatory nuclear factor-kappaB pathway and triggered the expression of cell-protective heat shock proteins in the intestinal cells. Furthermore, the probiotic produced factors that inhibited the breakdown of the heat shock proteins, which would normally occur through intracellular protein destroyers known as proteasomes. Proteasome inhibition was an early event that began almost immediately after exposure of the colonic cells to the probiotic. The resulting inhibition of nuclear factor-kappaB and increased expression of heat shock proteins may account for the anti-inflammatory and cytoprotective effects reported for probiotics and may be a novel mechanism of microbial-epithelial interaction (45).

**Effect on Humoral Immunity**

Many studies demonstrate a strong and consistent capability of many probiotic agents in inducing specific antibody response. Viable *L. casei* strain GG stimulate rotavirus specific IgA antibody responses (46,47). Moreover, two probiotic strains; *Lactobacillus rhamnosus* GG or *Lactobacillus acidophilus* CRL431, induced an immunologic response towards poliovirus vaccine virus by affecting the production of virus neutralizing antibodies (48). Also, ingested *B. bifidum* significantly increased the number of immunoglobulin (IgM, IgG, and IgA) secreting cells mesenteric lymph nodes and spleen, in an animal model (49,50).

**Effect on Cytokine Release**

The effect of probiotics on cytokine release is a perfect model to highlight the differences in effect between similar probiotic bacteria, as well as differences in effect of the same probiotic bacteria when used at different doses. Not all probiotic strains have similar immune-modulating properties, as a matter of fact, they can exert opposite effects. Different species of lactobacilli exert very different dendritic cell activation patterns and, furthermore, at least one species may be capable of inhibiting activities of other species in the genus. Thus, the T(H)1, T(H)2, T(H)3-response of the dendritic cells in the intestine can be modulated according to composition of gut microflora, including ingested probiotics (51). Other mechanisms by which probiotics enhance cytokine production can be seen with the administration of VSL#3 in a murine model of colitis. The severity of recurrent colitis was reduced through increasing IL-10 production and increasing CD4(+) T cells bearing surface TGF-beta (52).

**Effect on Innate Immunity**

In a clinical trial involving forty-five healthy volunteers,
**L. casei** DN114001 consumption increased oxidative burst capacity of monocytes, as well as natural killer cells tumoricidal activity resulting in a positive effect in modulating the innate immune defense in healthy people (53). Moreover, probiotic bacteria appear to modulate the nonspecific immune response differently in healthy and hypersensitive subjects. This is seen as an immunostimulatory effect in healthy subjects, and as a down-regulation of immunoinflammatory response in milk-hypersensitive subjects (54). The probiotic *E. coli* Nissle 1917 bacteria have been shown to stimulate the intestinal innate immune system through up-regulation of antimicrobial peptides such as human beta defensin 2 (hBD-2) (55). *S. boulardii* was also found to activate complement and the reticuloendothelial system (56) (Table 3).

### Table 3

<table>
<thead>
<tr>
<th>Immune effects of probiotics</th>
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<tbody>
<tr>
<td>A. Probiotics as vehicles to deliver anti-inflammatory molecules to the intestine</td>
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<tr>
<td>B. Enhance signaling in host cells to reduce inflammatory response</td>
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<tr>
<td>C. Switch in immune response to reduce allergy</td>
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<tr>
<td>D. Induce antibody response to reduce infection</td>
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<td>E. Reduce the production of inflammatory substances</td>
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**ALLERGIC DISORDERS**

Experimental studies suggest that probiotics exert strain-specific anti-allergic effects at the level of the immune cells, the intestinal lumen, as well as the intestinal epithelial cells. Such effects include improved intestinal barrier function, allergic degrada-

tion, and down-regulation of immune responses at the local gastrointestinal site as well as distant mucosal sites (57). In a double blind, placebo-controlled study, *Lactobacillus* GG was found to alleviate atopic eczema in infants that are IgE-sensitized (58). Moreover, lactic acid bacteria can inhibit the secretion of T(H)2 cytokines (IL-4 and IL-5) in a manner dependant on antigen presenting cells, IL-12, and IFN-
gamma. This switch from a T(H)2 to a T(H)1 response is beneficial in allergic patients (59). In children with atopic dermatitis, lactobacilli may stabilize the intesti-
nal barrier function and decrease gastrointestinal symptoms (60). Probiotic bacteria reduce CD34+ hemopoietic precursor cells, which are increased in allergic subjects with systemic allergic inflammation (61). In infants with cow milk allergy, *Lactobacillus* GG can increase the secretion of IFN-gamma (62).

Specific strains of *Bifidobacterium* and *Lactobacillus* appear to be promising in the treatment and prevention of eczema and dermatitis in infants and children (63,64). It is interesting to note that in these studies, supplementation with the probiotic did not appear to alter bacterial microflora in the colon. This suggests that these results are related to altered immunity rather than altered colonization (65). For more details on the role of probiotics in allergic disorders, the reader is referred to an excellent review recently published by Kalliomaki (66).

### EFFECT OF PROBIOTICS ON DISTANT MUCOSAL SITES

*Lactobacillus* GG has been the most widely studied of probiotic agents. In addition to having been used with varying degrees of success for treating and preventing urinary tract infections, vulvo-vaginal candidiasis, otitis media (67), and bacterial vaginosis (68), *Lactobacillus* GG, in the form of a milk preparation, was recently reported as having some modest but consistent benefits in terms of preventing and reducing the severity of respiratory infections at day care centers (69).

### ANTIPOROLIFERATIVE EFFECT

Probiotics are able to modulate several intestinal functions such as detoxification, colonic fermentation, transit, and immune status, which may contribute to the development of colon cancer. The use of probiotics resulted in direct antiproliferative effects on tumor cells and immune cells (70). In rats, specific reduction of carcinogenic bacterial enzymes and modulation of gut and systemic immunity has been shown to have the potential to exert significant antiproliferative effects against colon cancer (71). Although the evidence is still mounting and more research is required, the data show promising evidence supporting the protective role of probiotics in colon cancer (72).

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SUMMARY

In summary, the areas of probiotics and intestinal microflora are becoming more and more fascinating as we realize their significance and the interesting mechanisms by which they exert benefit to the host. Much more research is needed to aid in the full understanding of how these organisms play a role in gastrointestinal and systemic conditions that afflict children and adults, how the immune system interacts with such bacteria, and what constitutes a healthy microbial milieu in individual gastrointestinal disorders.

References


