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Probiotics: progress toward novel therapies for intestinal diseases

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Abstract

Purpose of review—As the beneficial effects of probiotics on health and disease prevention and treatment have been well recognized, the demand for probiotics in clinical applications and as functional foods has significantly increased in spite of limited understanding of the mechanisms. This review focuses on the most recent advances in probiotic research from genetics to biological consequences regulated by probiotics and probiotic-derived factors.

Recent findings—Genomic and proteomic studies reveal genes and proteins involved in probiotic adaptation in the host and while exerting their beneficial effects. Recent studies in cell culture and in animal models emphasize probiotic functions in intestinal development, nutrition, host microbial balance, cytoprotection, barrier function, innate immunity, and inflammation. Most importantly, several novel and known probiotic-derived factors have been characterized, which regulate host-signaling pathways and mediate probiotic function.

Summary—Progress in understanding probiotic mechanisms of action will increase our basic understanding of biological crosstalk and provide the rationale to support the development of new hypothesis-driven studies to define the clinical efficacy of probiotics for intestinal disorders.

Keywords

homeostasis; immune response; inflammation; intestine epithelium; microbiota; probiotics

Introduction

The gastrointestinal tract of humans and other animals harbors a diverse, complex, and dynamic community of microbial flora, called the intestinal microbiota. The continuous contact between the gastrointestinal epithelial cell monolayer and the intestinal microbiota forms a functional relationship that profoundly contributes to host intestinal development, nutrition, immunity, and intestinal epithelial homeostasis. Interruption of the normal

microbial–host interactions has been linked to various pathological conditions, including inflammatory bowel diseases (IBDs) and irritable bowel syndrome (IBS). Thus, manipulation of the intestinal microbiota is emerging as a potential alternative therapy for disease prevention and treatment. Probiotics were first described as selective nonpathogenic living microorganisms, including some present as commensal bacterial flora, which have beneficial effects on host health and disease prevention and/or treatment by Lilly and Stillwell [1]. Given the substantial increase in probiotic research in laboratory and clinical studies, probiotics are currently defined as ‘live microorganisms which, when consumed in adequate amounts as part of food, confer a health benefit on the host’. Examples of probiotics that have been studied extensively in humans and animals include *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces*.

The purpose of this review is to address the most recent probiotic advances (published after January 2008) in clinical applications and mechanisms of action. Two significant areas advancing our understanding of the molecular basis for probiotic health-promoting activities are highlighted, genomic analysis of probiotics and functional studies of probiotic-derived factors.

Genomic and proteomic studies of probiotics

As in other fields, genomics has proven a valuable tool to accelerate probiotic research. Analysis of current available probiotic genome sequences, including eight *Lactobacillus* and six *Bifidobacterium* strains, have revealed genes involved in adaptation to the human gut and exerting their beneficial effects (reviewed in [2•]). Annotation of *Bifidobacterium* genomes show genes that encode enzymes required for the breakdown of complex sugars, which generate ecological niches in the human gastrointestinal tract for bacterial adaptation [3]. Genomic analysis of *Lactobacillus rhamnosus* GG (LGG) reveals that a mucus-binding pilus on the surface of LGG is a key factor for adhesion of LGG to the host intestinal mucus [4••]. Another recently reported LGG gene cluster encodes the enzymes, transporters, and regulatory proteins involved in the biosynthesis of long, galactose-rich extracellular polymeric substance molecules. These proteins mediate adherence to mucus and gut epithelial cells and biofilm formation by LGG [5].

Proteomics plays a pivotal role in linking the genome and the transcriptome to potential biological functions. A study [6•] using two-dimensional differential gel electrophoresis and mass spectrometry shows that protein production, including purine, fatty acid biosynthesis, galactose metabolism, translation, and stress response, is differentially regulated between laboratory and industrial-type growth media. Interestingly, expression of several LGG proteolytic enzymes is growth medium-dependent [6•]. As all of these proteins are responsible for LGG’s survival and function in the host, it is likely necessary that these processes should be considered when extrapolating in-vitro effects to in-vivo conditions in the host for designing probiotic-based clinical trials.

Mechanisms of probiotics and probiotic-derived factors regulating host homeostasis

To aid in the development of hypothesis-driven studies testing the efficacy of probiotics, promising basic research has revealed several general mechanisms of probiotic action (reviewed in [7•]). These mechanisms include increasing enzyme production, enhancing digestion and nutrient uptake, maintaining the host microbial balance in the intestinal tract through producing bactericidal substances that compete with pathogens and toxins for adherence to the intestinal epithelium, promoting intestinal epithelial cell survival, barrier function, and protective responses, and regulating immune responses by enhancing the

innate immunity and preventing pathogen-induced inflammation. These responses are mediated via regulation of signaling pathways, including nuclear factor kappa B (NF- κ B), phosphatidylinositol-3'-kinase (PI3K)/Akt, and mitogen-activated protein kinase (MAPK) in intestinal epithelial and immune cells to facilitate probiotic action. Interestingly, some of these mechanisms of action appear to be mediated by probiotic-derived soluble factors.

Intestinal development

Gnotobiotic studies provide much of our current understanding regarding the role of intestinal microbiota in the development of normal gut. In the absence of microbes, there are profound deficiencies in intestinal epithelial and mucosal immunological development and function, including the inability to generate proper immune responses to protect against infection and inflammation (reviewed in [8]). The impaired development and maturation of isolated lymphoid follicles in germ-free mice is reversed following the introduction of gut bacteria [9]. Furthermore, a surface carbohydrate molecule of *Bacteroides fragilis*, polysaccharide A, appears to modulate the maturation of the intestinal immune system [10].

Recent findings show that probiotics may exert protective effects for developing the healthy intestinal system. The administration of a probiotic bacterium, *Lactobacillus casei* DN-114001 in fermented milk to nursing mice or their offspring improves the gut immune response in mothers and their offspring through the stimulation of the immunoglobulin A⁺ cells, macrophages, and dendritic cells [11]. Furthermore, LGG decreases chemically induced apoptosis and increases expression of genes primarily involved in cytoprotective responses in the developing mouse small intestine [12].

Nutrition

Studies have repeatedly demonstrated that the intestinal microbiota promotes human and other host nutritional status, including promotion of polysaccharide digestion and uptake of nutrients by intestinal cells (reviewed in [13]). Studies of lean and obese mice suggest that the gut microbiota regulates energy balance by influencing the efficiency of calorie harvest from the diet, as well as the usage and storage of this harvested energy [14,15]. More recent studies indicate that there may be a core gut microbiome, which exists at the level of shared genes, including an important component involved in various metabolic functions. In fact, it has been hypothesized that deviations from this core are associated with aberrant physiological states such as obesity [16••].

Probiotic bacteria are widely used as nutritional supplements to improve the digestibility and uptake of some dietary nutrients by host intestinal cells. For example, bacterial lactase is a well known enzyme produced by probiotic bacteria that degrades lactose in the intestine and stomach and prevents symptoms of lactose intolerance. A study [17] focusing on the role of probiotics in the function of intestinal electrolyte absorption reveals that *Lactobacillus acidophilus* secretes soluble molecule(s) that stimulate apical chloride/hydroxyl exchange activity via a PI3K-dependent mechanism.

The role of probiotics in malnutrition under disease states has also been tested. A double-blind randomized control trial [18] in Malawi was performed to evaluate clinical and nutritional efficacy of a probiotic and prebiotic functional food for the treatment of severe acute malnutrition in HIV-prevalent children. Although severe acute malnutrition outcomes are not improved, the observation of reduced outpatient mortality is interesting and suggests further studies of probiotics in malnutrition are warranted [18].

Intestinal epithelial homeostasis

In addition to nutritional functions, the intestinal epithelial cells are critical for maintaining normal intestinal homeostasis through several protective defense mechanisms, including barrier function formed by tight junctions, antibacterial substances synthesis, and active involvement in innate and adaptive immunity. Probiotics facilitate intestinal epithelial homeostasis through a number of biological responses, including promoting proliferation, migration, survival, barrier integrity, antimicrobial substance secretion, and competition for pathogen interaction with epithelial cells (reviewed in [7•,13]).

To enhance antibacterial and anti-inflammatory activities of the intestinal epithelium, probiotics stimulate cytoprotective protective protein synthesis and secretion, including heat shock protein, defensin, angiogenin, and mucin by intestinal epithelial cells (reviewed in [7•, 13]). Recent studies show probiotics prevent inflammation through regulating secretion of several factors from intestinal epithelial cells. For example, *L. casei* cell surface proteins inhibit tumor necrosis factor (TNF)-induced T-cell chemokine interferon (IFN)-inducible protein (IP-10) secretion via impairing vesicular pathways, followed by degradation of this proinflammatory chemokine [19].

Recent studies provide significant evidence that probiotics and probiotic-derived soluble factor prevent cytokine and chemical-induced epithelial apoptosis and disruption of barrier function. Two novel proteins present in LGG culture supernatant, p40 and p75, show protective roles in cytokine-induced intestinal epithelial cell apoptosis through activating antiapoptotic PI3K/Akt signaling *in vitro* and in colon organ culture [20]. p40 and p75 also prevent hydrogen peroxide-induced insult through enhancing membrane translocation of tight junctional complex proteins, including ZO-1, occludin, protein kinase C (PKC) β 1, and PKC ϵ , in an extracellular signal-regulated kinase 1/2 MAPK-dependent manner [21]. Another study [22] reports that *Bifidobacterium infantis* conditioned medium enhances intestinal epithelial barrier function in experimental colitis and prevents cytokine-induced disruption of tight junctions through regulation MAPK activation and tight junction protein expression. These findings suggest that it may be possible to identify specific probiotic bacterial products for prevention, treatment of gastrointestinal diseases, or both.

Intestinal epithelial cells play important immunomodulatory roles through complex interactions with immune cells to induce appropriate innate or adaptive immunity. For example, *Lactobacillus helveticus* R0011 protects against enterohemorrhagic *Escherichia coli* O157:H7-induced inhibition of innate immune responses through preventing pathogen-mediated IFN γ -induced signal transducer and activator of transcription 1 signaling in intestinal epithelial cells [23].

NF- κ B signaling is a critical mediator of intestinal epithelial cell crosstalk with immune cells. Optimal NF- κ B activity plays a significant role in maintaining normal intestinal homeostasis and injury repair responses. However, hyperactivation of NF- κ B results in chronic intestinal inflammatory disorders (reviewed in [24]). One mechanism of probiotic effects is through the suppression of NF- κ B signaling to limit excessive inflammation. Soluble factors released by *Bifidobacterium breve* C50 in the culture supernatant reduce TNF-induced cytokine production through inhibition of NF- κ B and activator protein 1-dependent transcription in intestinal epithelial cells. These soluble factors and soluble factors-conditioned dendritic cells prevent trinitrobenzene sulfonic acid-induced colitis in mice. However, these effects were not found in response to *B. breve* ATCC 15698, *L. rhamnosus* ATCC 10863, and *Eubacterium rectale* L15 [25]. However, factors present in conditioned media of *Lactobacillus plantarum*, but not *L. acidophilus*, *L. paracasei*, *B. fragilis*, *B. breve*, *E. coli* F18 or enteropathogenic *E. coli*, inhibit TNF-induced NF- κ B-binding capacity, I κ B degradation, and proteasome activity in intestinal epithelial cells,

macrophages, and dendritic cells [26]. These studies indicate probiotic strain-specific secretion of factors regulate NF- κ B activation. However, current studies lack information regarding further characterization of these probiotic-derived soluble factors. Interestingly, hydrogen peroxide produced by *Lactobacillus crispatus* M247 has been shown to act as a signal-transducing molecule to suppress NF- κ B activity in colon epithelial cells, whereas other *Lactobacillus* strains producing little hydrogen peroxide fail to block NF- κ B activity [27•].

Probiotic immunoregulatory effects independent of NF- κ B have also been reported. *E. coli* Nissle 1917 expresses a direct anti-inflammatory activity on human epithelial cells via a secreted factor, which suppresses TNF-induced interleukin (IL)-8 transactivation, which occurs in the absence of NF- κ B inhibition [28].

Intestinal immune responses

Both in-vitro and in-vivo studies have shown effects of probiotics on defining and maintaining the delicate balance between necessary and excessive defense mechanisms, including innate and adaptive immune responses: upregulation of immune function may improve the ability to fight infections; downregulation may prevent the onset of intestinal inflammation and autoimmunity. Previous studies have demonstrated that probiotics perform these immunoregulatory effects through enhancing innate immunity, promoting anti-inflammatory, and inhibiting proinflammatory cytokine production. Activation of Toll-like receptor-regulated signaling is one of the known mechanisms for probiotic regulation of immune functions (reviewed in [7•]).

By using microarray analysis, a randomized double-blind placebo-controlled study addressed the in-vivo responses to *L. plantarum* in healthy humans and identified mucosal gene expression patterns and cellular pathways that correlated with the establishment of immune tolerance. These altered gene profiles focused on NF- κ B-dependent pathways [29•]. For the probiotic mixture, VSL#3 (a combination of eight strains of *Bifidobacterium*, *Lactobacillus*, and *Streptococcus*) supplementation alters the distribution of the dendritic cell subsets within the intestinal mucosa in mice, which may be important in the alteration of mucosal immunity seen in humans using this therapy [30].

Studies focusing on immunoregulatory effects of probiotics on colitis show that the mixture of *L. acidophilus* and *Bifidobacterium longum* prevents experimental colitis through expansion of intestinal intraepithelial $\gamma\delta$ T cells and regulatory T cells (Treg), as well as downregulation of proinflammatory cytokines, TNF, and monocyte chemotactic protein 1 and upregulation of the anti-inflammatory cytokine IL-10 [31]. In another in-vivo study [32•], *B. infantis* drives the generation and function of Treg cells to suppress lipopolysaccharide (LPS)-induced NF- κ B activation and *Salmonella typhimurium* infection.

Furthermore, several probiotic-derived factors mediate probiotic function in immunity. For example, *Lactobacillus reuteri* secreted factors promote TNF-induced apoptosis and antiapoptotic protein production (Bcl-2 and Bcl-xL) in human myeloid cells by inhibiting NF- κ B activation and enhancing MAPK signaling [33]. Two identified factors of probiotics have been reported. S layer protein A of *L. acidophilus* NCFM promotes dendritic cell maturation and function to stimulate T helper cell 2 T-cell polarization [34•]. Polysaccharide A, made by *B. fragilis*, protects against experimental colitis through inducing IL-10 production [35••].

Clinical application of probiotics

Results from clinical trials have suggested potential applications of probiotics for prevention and/or treatment of a large number of gastrointestinal disorders, including IBD, antibiotic-associated diarrhea, IBS, neonatal necrotizing enterocolitis (NEC), enteropathy in HIV infection, gluten intolerance, gastroenteritis, *Helicobacter pylori* infection, and colon cancer. Other diseases that have been reportedly treated with probiotics include allergy and infections in the urogenital tract and the respiratory system (reviewed in [7•,36,37]). The following section reviews recent progress toward probiotic therapies in IBD, IBS, NEC, and HIV infection.

Inflammatory bowel diseases

IBD, including Crohn's disease and ulcerative colitis, is a chronic inflammatory condition of the gastrointestinal tract. Primary or secondary alteration of the intestinal microbiota is thought to play a central role in the pathogenesis of IBD and thus investigators have pursued clinical studies to manipulate intestinal microflora using probiotics and prebiotics. Probiotics, including VSL#3, LGG, and *E. coli* Nissle 1917, show potential for therapeutic application in ulcerative colitis by induction of remission, decreasing clinical activity index scores, and preventing onset of pouchitis, a common complication of ileal pouch–anal anastomosis surgery for ulcerative colitis (reviewed in [7•]). Recent clinical studies show that VSL#3 treatment significantly increases the number of mucosal regulatory T cells in patients with ileal pouch–anal anastomosis, indicating a potential beneficial immunoregulatory mechanism of probiotic action in this disease [38].

However, to date, the potential for use of probiotics in prevention or treatment of Crohn's disease remains controversial. A meta-analysis [39] including eight randomized placebo-controlled clinical trials using *Lactobacillus johnsonii*, LGG, *E. coli* Nissle 1917, or *Saccharomyces boulardii* fail to show efficacy of probiotics in maintaining remission and preventing clinical and endoscopic recurrence in Crohn's disease. The failure for Crohn's disease benefits may be reflective of both the lack of a mechanistic understanding of probiotics in trial design and the current state of knowledge regarding the pathogenesis of this disease.

Irritable bowel syndrome

IBS is a functional gastrointestinal disorder with low-grade inflammation and immune responses. The evidence that patients with IBS show changes in fecal microflora supports the application of probiotics for IBS [40]. Probiotics such as *Lactobacillus* (LGG, *L. acidophilus*, *L. plantarum*, *L. salivarius*, and *L. reuteri*), *Bifidobacterium* (*B. infantis* and *B. animalis*), and *Saccharomyces* (*S. boulardii*) have shown varying degrees of efficacy in overall IBS symptoms, including decreasing flatulence, pain, and bloating (review in [41]). A randomized single-blind placebo-controlled clinical trial [42] of fermented milk containing *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *L. acidophilus*, and *B. longum* further indicates that small bowel permeability is decreased significantly in patients with diarrhea–predominant IBS, and that probiotics improve mucosal barrier function for IBS patients. Two recent systematic reviews [43,44] emphasize the effectiveness of probiotics. However, on the basis of their analyses, both suggest research on species and strain selection and optimal dose of probiotics for any future IBS treatment strategies.

Necrotizing enterocolitis

NEC is an inflammatory necrosis of the intestine leading to significant morbidity and mortality in premature infants. A number of recent clinical trials have shown efficacy for prevention of NEC. Probiotics that have shown beneficial effects on decreasing NEC

incidence include *Bifidobacteria* (*B. infantis*, *B. bifidum*, and *B. breve*), *Lactobacillia* (LGG, *L. acidophilus*), *S. thermophilus* and *S. boulardii*, and *E. coli* Nissle (reviewed in [45]). A systematic review [46] of randomized controlled trials estimated a lower risk of NEC and death in the probiotic-treated group than in controls. However, risk of sepsis did not differ significantly between these two groups [46]. Another meta-analysis [47] also showed that probiotics supplementation significantly reduced the incidence of severe NEC, but not nosocomial sepsis or days on total parenteral nutrition. No systemic infection with the supplemental probiotic organism was found [47]. However, caution should be noted for using probiotics in this high-risk population.

HIV infection

The gastrointestinal tract is a site of early HIV replication and CD4⁺ cell destruction, and HIV-associated enteropathy emerges as a critical outcome of the transition from HIV infection to AIDS. A clinical pilot project studied the potential application of probiotic containing yogurt in HIV enteropathy [48]. Improvement in both diarrhea and CD4 cell number occurred with *L. rhamnosus* GR-1 and *L. reuteri* RC-14 supplemented in yogurt in HIV/AIDS patients who had not received antiretroviral treatment [49]. In addition, antibiotics in combination with *L. rhamnosus* GR-1 and *L. reuteri* RC-14 increase the cure rate of bacterial vaginosis, which increases the risk of acquiring HIV infection as compared with that in the control group receiving antibiotic with a placebo [50]. Therefore, probiotics may provide benefit through both the management of HIV enteropathy and reduced transmission of disease; however, further investigation is needed including the safety of probiotic administration in this immunocompromised population.

Safety of using probiotics

Concerns for the safe use of probiotics are raised by the outcomes of several clinical trials. For example, a mixture of six probiotic bacteria (*L. acidophilus*, *L. casei*, *L. salivarius*, *L. lactis*, *B. bifidum*, and *B. infantis*) used to treat patients with severe acute pancreatitis increased their risk of mortality [51], although this bacterial mixture inhibited the growth of most pathogens that caused pancreatitis complications in the preclinical animal studies [52]. In addition, the occurrence of bacteremia and fungemia in ill patients and immunodeficient individuals has also been reported (reviewed in [53]). Another possible risk of using probiotics is transferring of antibiotic resistance genes to the host, as *L. reuteri* and *L. plantarum* have been found to carry such genes [54•]. Thus, future clinical trials using probiotics should be accompanied by safety monitoring.

Conclusion

Promising health benefits and efficacy of probiotics for preventing and treating a variety of diseases attract increasing attention to inclusion of these agents as functional foods and using probiotics and probiotic-derived factors for novel therapies. To unequivocally prove the clinical efficacy of probiotics, large and well controlled clinical trials, using mechanism-based hypothesis and protocol design, selection of appropriate study populations, and product characterization, are needed in the future.

It should also be noted that the efficacy might be probiotic specific. It is well known that probiotics can exert beneficial effects on the host through distinct cellular and molecular pathways. These mechanisms of action may vary from one probiotic to another for the same biological response and may be regulated by a combination of several events, thus making study of the underlying basis of probiotic action a challenging, complex, and fertile area for investigation. Another important concept is the 'bioavailability' of probiotics for intended therapeutic application. These are important details, as the correct combination and

concentration of probiotics may improve the efficacy of this approach for prevention and treatment of human diseases in the future.

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References and recommended reading

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 170–172).

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