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Probiotics and immune health

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Abstract

Purpose of review—The beneficial effects of probiotics have been demonstrated in many diseases. One of the major mechanisms of probiotic action is through the regulation of host immune response. This review highlights the recent scientific research findings that advance our understanding of probiotic regulation of the host immune response with potential application for disease prevention and treatment.

Recent findings—Probiotic genomic and proteomic studies have identified several genes and specific compounds derived from probiotics, which mediate immunoregulatory effects. Studies regarding the biological consequences of probiotics in host immunity suggested that they regulate the functions of systemic and mucosal immune cells and intestinal epithelial cells. Thus, probiotics showed therapeutic potential for diseases, including several immune response-related diseases, such as allergy, eczema, viral infection, and potentiating vaccination responses.

Summary—Probiotics may provide novel approaches for both disease prevention and treatment. However, the results of clinical studies regarding probiotic application are preliminary and require further confirmation.

Keywords

allergy; immune response; intestinal epithelium; microbiota; probiotics

Introduction

The immune response is initiated by innate immunity following exposure to foreign substances or tissue injury. Innate immunity exerts protective roles in host homeostasis in part by priming adaptive immune responses against persisting insults and inducing

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Conflicts of interest

None declared.

inflammation. However, the unbalanced immune response leads to severe inflammation and uncontrolled tissue damage and disease. Sensing of the intestinal microbiota by the host mucosal immune system plays significant roles in maintaining intestinal homeostasis and inducing systemic protective responses. Thus, manipulation of the intestinal microbiota is a potential alternative approach for maintaining health and preventing and/or treating diseases. Probiotics were defined as 'live microorganisms which, when consumed in adequate amounts as part of food, confer a health benefit on the host'. *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces* are three extensively studied and commonly used probiotics in humans and animals.

Several beneficial effects of probiotics on the host intestinal mucosal defenses system have been identified. These include blocking pathogenic bacterial effects by producing bacteriocidal substances and competing with pathogens and toxins for adherence to the intestinal epithelium. For intestinal epithelial homeostasis, probiotics promote intestinal epithelial cell survival, enhance barrier function, and stimulate protective responses from intestinal epithelial cells. Most importantly, modulation of the immune system is one of the most plausible mechanisms underlying the beneficial effects of probiotics on human health. Probiotics have been found to enhance the innate immunity and modulate pathogen-induced inflammation via toll-like receptor-regulated signaling pathways [1].

The purpose of this review is to address the most recent findings regarding probiotic regulation of immune health (published after January 2010). Clinical applications and mechanisms of action are highlighted, which include probiotic genes and probiotic-derived factors involved in the regulation of host immunity, molecular targets of probiotic action responsible for the host immune responses, and roles and mechanisms of probiotics in vaccination and prevention and treatment of diseases, such as allergy, eczema, and viral infections.

Probiotic genes involved in the regulation of host immune responses

Metagenomic analysis has expanded our understanding of the probiotic genes which are involved in the regulation of the host immune responses. Forty-two *Lactobacillus plantarum* strains isolated from diverse environmental and human sources were evaluated for their capacity to stimulate interleukin 10 (IL-10) and IL-12 produced by peripheral blood mononuclear cells. By comparison of the strain-specific cytokine responses and comparative genome hybridization profiles obtained using *L. plantarum* WCFS1 DNA microarrays, six candidate genes with immunomodulatory capacities were identified. These genes are involved in encoding an *N*-acetyl-glucosamine/galactosamine phosphotransferase system, the LamBDCA quorum-sensing system, components of bacteriocin biosynthesis and transport pathway. Deletion of these genes in *L. plantarum* WCFS1 resulted in abolishing the capacity to stimulate cytokine production [2]. Furthermore, the same bacteria and the methods were applied to study gene loci that regulate IL-10 and IL-12 production by dendritic cells. Several different genes from those involved in the regulation of cytokine production by peripheral blood mononuclear cells were identified, which include six genes involved in bacteriocin production or secretion, one encoded a bile salt hydrolase and

another encoded a transcription regulator [3^{*}]. Thus, these results suggest that regulation of responses by different immune cells is likewise probiotic gene specific.

Functional genomic analysis was performed on three very closely related *Escherichia coli* strains, strains 83972 and Nissle 1917, which are probiotic strains of urinary tract and fecal origin, respectively, and strain CFT073, a uropathogen. Transcriptomic profiling revealed that the active genomic profiles of these three strains are closely related. This study also showed that *E. coli* Nissle 1917 grew in urine and formed biofilm, which required three genes, *yhaK*, *yhcN*, and *ybiJ* [4]. This evidence indicates that similar functional gene profiles are present in both probiotics and pathogens. It will be important to understand how these bacteria with similar transcriptions function quite differently in cellular or other context-dependent manners.

Host factors have also been shown to exert effects on regulation of the transcription of probiotic genes. Genes associated with stress and adhesion in *Lactobacillus acidophilus* NCFM were studied in an in-vitro gastrointestinal tract model. Expression of the genes encoding the stress-related proteins, GroEL, DnaK, and ClpP, were upregulated in *L. acidophilus* NCFM preincubated with acidified milk during gastric digestion and declined upon subsequent duodenal digestion. Whereas genes encoding mucin-binding and fibronectin-binding proteins were not influenced by saliva or gastric juice, they were significantly increased during incubation in duodenal juice and bile. These results provide elegant examples of the complexity and functionality of probiotics during passage through the gastrointestinal tract [5].

Probiotic components which regulate immune responses in the host

To define effectors of probiotic action, active components of probiotics have recently been studied. Two-dimensional gel coupled with matrix-assisted laser desorption ionization time-of-flight mass spectrometry analysis of *Bifidobacterium animalis* subsp. *lactis* BB-12 secreted proteins revealed 74 distinct proteins. Thirty-one proteins are predicted to carry out their physiological role either outside the cell or on its surface, including solute-binding proteins for oligosaccharides, amino acids and manganese, and cell wall-metabolizing proteins. Eighteen proteins mediate interaction with human host epithelial cells or extracellular matrix proteins. The potential functions include binding of plasminogen, formation of fimbriae, adhesion to collagen, attachment to mucin and intestinal cells as well as induction of immunomodulative responses. These findings suggest a role of bacterial proteins in colonization of the gastrointestinal tract, adhesion to host tissues, or immunomodulation of the host immune system [6^{*}].

Recently, a *Lactobacillus rhamnosus* GG-derived soluble protein, p40, was shown to prevent and treat dextran sulfate sodium-induced intestinal injury and acute colitis and oxazolone-induced colitis. p40 treatment reduced intestinal epithelial apoptosis and disruption of barrier function in the colon epithelium in an epidermal growth factor receptor-dependent manner in mouse models of colitis. Furthermore, p40 reduced tumor necrosis factor (TNF), IL-6, keratinocyte chemoattractant, and interferon (IFN)- γ production, but not IL-1 β , IL-10, or IL-17 expression in dextran sulfate sodium-treated mice, nor did it affect

IL-13 production in oxazolone-treated mice. These findings suggest that p40 plays a role in the regulation of innate immunity and the Th1 immune response [7**]. In another report, two active compounds produced by *Lactobacillus reuteri* RC-14, cyclic dipeptides cyclo (L-Tyr-LPro) and cyclo (L-Phe-L-Pro), were shown to inhibit the staphylococcal quorum-sensing system agr and decrease the expression of toxic shock syndrome toxin-1 in *Staphylococcus aureus* MN8, a pathogen in menstrual toxic shock syndrome [8]. These reported probiotic-derived compounds may therefore be candidates for clinical applications in disease prevention and treatment.

Modification of the bacterial genome has been shown as an approach for facilitating the regulatory effects of probiotics. *L. acidophilus* NCFM, with the deletion of the phosphoglycerol transferase gene, which mediates biosynthesis of lipoteichoic acid, downregulated IL-12 and TNF, but enhanced IL-10 production in dendritic cells and controlled the costimulatory functions of dendritic cells, resulting in their inability to induce CD4⁺ T-cell activation. In addition, treatment of mice with these mutant bacteria significantly decreased dextran sulfate sodium and CD4⁺CD45RB^{high}T cell-induced colitis. Upregulation of IL-10 and CD4⁺FoxP3⁺ T regulatory cells by these mutant bacteria was correlated with decreased mucosal inflammation [9*]. Thus, further understanding of probiotic structure–functional relationship with intestinal cells will enhance targeted effects by probiotics.

Host immune responses regulated by probiotics

Probiotics play a role in defining and maintaining the delicate balance between necessary and excessive defense mechanisms including innate and adaptive immune responses. Points of interaction with the immune regulation for probiotics include bacteria direct interaction with intestinal epithelial cells, or following internalization by M cells through interaction with dendritic cells and follicle-associated epithelial cells, initiating responses mediated by macrophages and T and B lymphocytes. Regulation of gene expression and signaling pathways in the host cells are two major mechanisms underlying probiotic action leading to immunomodulation.

Host genes

Genetic variability of the host contributes to diversity of response to identical stimuli and this plays out in probiotic effects too. A double-blind, placebo-controlled study was performed in healthy volunteers to determine mucosal responses to *L. acidophilus* Lafti L10, *Lactobacillus casei* CRL-431, and *L. rhamnosus* GG. Transcriptomes clustered per person, not per intervention, which suggests that person-to-person variation in gene expression was the largest determinant of differences between transcriptomes. In addition, these three probiotic bacteria induced differential gene-regulatory networks and pathways in the human proximal small intestinal mucosa. *L. acidophilus* regulated genes mediating immune response, hormonal regulation of tissue growth and development, and ion homeostasis. For example, *L. acidophilus* modulated transcriptional regulation of the mucosal inflammatory bowel disease-associated IL-23 signaling pathway. Wound healing, IFN response, and ion homeostasis were associated with *L. rhamnosus*. The major altered transcriptional networks and pathways regulated by *L. rhamnosus* involved cellular growth, proliferation, and

development, with major roles in JUN, JAK2 and STAT4, and IGF1. Mucosal responses to *L. casei* involved proliferation, Th1–Th2 balance, and hormonal regulation of blood pressure. *L. casei* promoted a shift of a Th1/Th2 balance to a Th2 type and/or Th17 type, with upregulation of IL-17D and IL-21, which enhance the development of natural killer cells. Thus, these comprehensive analyses revealed that probiotic regulation of mucosal immunity at the gene expression levels in humans is both host genetics-dependent and strain specific [10**].

Another study showing whole genome microarray analysis revealed that *L. acidophilus* NCFM upregulated genes related to viral defense in murine bone-marrow-derived dendritic cells, including IFN- β , IL-12, and IL-10. In addition, *L. acidophilus* NCFM-triggered expression of viral defense genes in dendritic cells depended on TLR-2 [11]. These effects were not seen for *Bifidobacterium bifidum* Z9 and *E. coli* Nissle 1917.

Saccharomyces cerevisiae strain CNCM I-3856, a noncommensal and nonpathogenic yeast used as a probiotic in gastrointestinal diseases, has been shown to regulate inflammatory gene expression in porcine intestinal epithelial IPEC-1 cells. Viable *S. cerevisiae* inhibited the enterotoxigenic *E. coli* (ETEC)-induced expression of proinflammatory cytokines and chemokines at both transcriptional and protein expression levels, including IL-6, IL-8, CCL20, CXCL2, and CXCL10. This inhibition was associated with a decrease of ERK1/2 and p38 MAPK phosphorylation, an agglutination of ETEC and an increase of the anti-inflammatory PPAR- γ nuclear receptor mRNA level. However, *S. cerevisiae* failed to maintain the barrier integrity in monolayer exposed to ETEC suggesting that this yeast does not directly inhibit ETEC enterotoxin activity [12].

Immune cells

Probiotics regulate host innate and adaptive immune responses by modulating the functions of dendritic cells, macrophages, and T and B lymphocytes [1,13]. One of the mechanisms of probiotics regulating immunomodulatory functions is through the activation of toll-like receptors.

A recent study demonstrated how probiotics activated innate immunity to prime the adaptive immune responses. A probiotics mixture consisting of *L. acidophilus*, *L. casei*, *L. reuteri*, *B. bifidum*, and *Streptococcus thermophilus* stimulated regulatory dendritic cells that express high levels of IL-10, TGF- β , COX-2, and indoleamine 2,3-dioxygenase, which in turn promoted the generation of CD4⁺Foxp3⁺ regulatory T cells (Tregs) from the CD4⁺CD25[−] population and increased the suppressor activity of naturally occurring CD4⁺CD25⁺Tregs. In addition, this probiotic mixture induced both T-cell and B-cell hyporesponsiveness and downregulated T helper (Th) 1, Th2, and Th17 cytokines without inducing apoptosis. In-vivo studies revealed that this mixture suppressed 2,4,6-trinitrobenzenesulfonic acid-induced intestinal inflammation, which was associated with enrichment of CD4⁺Foxp3⁺ Tregs in the inflamed regions. Thus, probiotics that enhance the generation of regulatory dendritic cells to induce Tregs represent a potential therapeutic approach for inflammatory disorders [14**]. Another study of the effects of probiotics on dendritic cells showed that higher treatment ‘doses’ of *L. rhamnosus* Lcr35 (multiplicity of infection, MOI 100 compared to MOI 0.01), induced a large-scale change in gene expression, mainly involving immune responses in

human monocyte-derived immature dendritic cells, and induced a strong dose-dependent increase of the production of the pro-Th1/Th17 cytokines, such as TNF, IL-1 β , IL-12p70, IL-12p40, and IL-23, but only a slight increase of IL-10. *L. rhamnosus* Lcr35 also stimulated a dose-dependent maturation of the dendritic cell membrane phenotype with an upregulation of the membrane expression of CD86, CD83, HLA-DR, and TLR4, and a downregulation of DC-SIGN, MR, and CD14. Thus, *L. rhamnosus* Lcr35 induces a dose-dependent immunomodulation of human dendritic cells leading to the semimaturational of these cells and a strong proinflammatory effect [15].

Induction of Foxp3⁺ Tregs by *Bifidobacterium breve* AH1205, *B. longum* AH1206, and *Lactobacillus salivarius* AH102 *in vivo* was shown to be strain-specific and induction of Foxp3⁺ Tregs was associated with protection from ovalbumin respiratory allergy and the ovalbumin-cholera toxin dietary allergy. *B. longum* AH1206 increased the numbers of Foxp3 Tregs in infant, adult, and germ-free animals and protected against airway inflammation in these two models of allergy. However, *B. breve* AH1205 induced Foxp3⁺ Tregs expansion only in infant mice, whereas neither *L. salivarius* AH102 nor *B. breve* AH1205 altered the number of Tregs or provided protection in either animal model [16]. Interestingly, another report found that *L. acidophilus* NCFM and *L. salivarius* Ls-33 treatment completely protected from colitis in SCID mice with low numbers of Tregs in addition to the disease-inducing T cells. Gene expression patterns of rectum samples of protected mice that received either of the probiotics showed a closer resemblance to naïve SCID mice than did the patterns of the control group. Thus, one mechanism of action of probiotics appears to be an indirect effect by inducing a Tregs-favorable environment rather than a direct effect on the Tregs [17].

Intestinal epithelial cells

It is well known that the intestinal epithelium forms a physiological barrier against pathogenic microbes and detrimental substances present in the intestinal lumen. In fact, this monolayer is integral to both discrimination of pathogens and commensal bacteria and is actively involved in immune responses in the intestinal tract. Probiotics-regulated intestinal epithelial cellular responses have been recently reviewed [1,13], including restitution of damaged epithelial barrier, production of antibacterial substances and cell protective proteins, blockade of cytokine-induced intestinal epithelial cell apoptosis, and regulate intestinal epithelial immune function, such as cytokine production. Many of these responses result from probiotic stimulation of specific intracellular signaling pathways in the epithelial cells.

Regulation of intestinal epithelial immunological function by the probiotic *Lactobacillus johnsonii* N6.2 was shown using human Caco-2 cell monolayers. TLR7 and TLR9 expression levels were upregulated by *L. johnsonii* N6.2, followed by increased levels of IFN type 1 and IFN regulators Stat1 and IRF7, indicating that this probiotic bacterium stimulates TLR9 in the apical surface of intestinal epithelial cells resulting in a higher state of epithelial immunologic alertness [18]. These findings suggest that probiotics regulation of innate immunity in intestinal epithelial cells may serve as a mechanism for disease prevention and treatment.

The interaction between intestinal bacteria and the host epithelium lead to multiple consequences. Nonspecific secretory IgA (SIgA) enhanced probiotic adhesion to Caco-2 cell monolayer. *Lactobacillus* or *Bifidobacterium* alone or in complex with SIgA reinforced transepithelial electrical resistance, a phenomenon coupled with increased phosphorylation of tight junction proteins zonula occludens-1 and occludin. In contrast, association with SIgA resulted in both enhanced level of nuclear translocation of NF- κ B and production of epithelial polymeric Ig receptor as compared with bacteria alone. Moreover, thymic stromal lymphopoietin production was increased upon exposure to bacteria and further enhanced with SIgA-based complexes, whereas the level of proinflammatory epithelial cell mediators remained unaffected. Interestingly, SIgA-mediated potentiation of the Caco-2 cell responsiveness to the two probiotics tested involved Fab-independent interaction with the bacteria. These findings add to the multiple functions of SIgA and underscore a novel role of the antibody in interaction with intestinal bacteria [19].

Probiotics for immune disease prevention and treatment

Results of evidence-based analysis from human studies and animal models suggest that probiotics have potential for clinical effectiveness on intestinal diseases, including infectious diarrhea, antibiotic-associated diarrhea, atopic diseases, necrotizing enterocolitis, ulcerative colitis, and irritable bowel syndrome, and extraintestinal diseases, such as allergy.

Vaccination

Potentiating the effects of vaccination by probiotics has emerged as a benefit of probiotics. A randomized, double-blind, placebo-controlled pilot study showed that LGG treatment for 28 days after administration of live-attenuated influenza vaccine increased protection rates against the virus with no side-effects reported for the participants. Thus, LGG shows potential as an important adjuvant to improve influenza vaccine immunogenicity [20**]. *L. acidophilus* has been used as a live vehicle for oral immunization against chicken anemia virus. The AcmA-binding domains of *Lactococcus lactis* were used to display the VP1 protein of chicken anemia virus (CAV) on *L. acidophilus*. *L. acidophilus* carrying the CAV VP1 protein were used to immunize specific pathogen-free chickens through the oral route. The vaccinated groups showed a moderate level of neutralizing antibody to CAV in the serum, a VP1-specific proliferative response in splenocytes, and increased levels of Th1 cytokines, such as IL-2, IL-12, and IFN- γ . These studies suggest the exciting possibility that probiotics can be modified for delivery of vaccines [21].

Allergy and eczema

Numerous studies have been performed to evaluate the effects of probiotics on allergy prevention and treatment. Results from animals and humans have shown promise for probiotics in the prevention and treatment of allergy. However, the contradictory results have been repeatedly reported. The heterogeneity of the research design, including probiotic strain dosage, administration time, and host genetic background, may contribute to the discrepant findings [22].

Recent studies showed that oral administration of VSL#3 to shrimp tropomyosin-sensitized mice significantly reduced symptom score and histamine release in the feces following allergen challenge, which was associated with the downregulation of IL-4, IL-5, and IL-13, and up-regulation of IL-10, TGF- β , and IFN- γ in the jejunum. The in-vitro studies on mouse spleen cells indicate that the VSL#3 preparation has the capacity to shift a polarized Th2 response to a Th1/T regulatory-type profile [23*]. By using a mouse model of polysensitization to birch and grass pollen allergens, mucosal application of *B. longum* NCC 3001 and *Lactobacillus paracasei* NCC 2461 at the time of sensitization and challenge led to significant suppression of airway inflammation and downregulated allergen-specific immune responses. In contrast, in the mice treated with probiotics prior to sensitization and challenge, only *B. longum* displayed protective effects. These findings suggest that both the specific probiotic and the timing of the application are crucial for tolerance induction [24].

In a randomized controlled trial of 250 pregnant women carrying infants at high risk of allergic disease, LGG failed to reduce the risk of eczema, or any change in cord blood immune markers, but was associated with decreased breast milk soluble CD14 and IgA levels. Thus, this study showed that prenatal LGG treatment was not sufficient to prevent eczema in infants. If probiotics are effective for preventing eczema, then a postnatal component to treatment or possibly an alternative probiotic strain may be necessary [25].

Virus infection

Protection from viral infection has also been shown as a benefit of probiotic action. Intranasal inoculation of wild-type mice with live or heat-inactivated *L. plantarum* or *L. reuteri* completely protected against the virulent rodent pathogen, pneumonia virus lethal infection and resulted in diminished granulocyte recruitment and expression of multiple proinflammatory cytokines and reduced virus recovery. Interestingly, these two probiotics also resulted in prolonged survival and protection against the lethal sequelae of pneumonia virus of mice infection in MyD88 gene-deleted mice, suggesting that these protective mechanisms may be TLR-independent [26*]. Furthermore, a randomized, parallel, double-blind, placebo-controlled study showed that consumption of *L. plantarum* HEAL 9 and *L. paracasei* 8700 : 2 for a 12-week period could reduce the risk of acquiring common cold infections in healthy individuals. For example, the incidence of acquiring one or more common cold episodes, the number of days with common cold symptoms, and pharyngeal symptoms were all reduced [27].

Conclusion

Current evidence shows promise for further developing health benefits and the efficacy of probiotics and probiotic-derived factors on the regulation of host homeostasis, including immune health. However, as probiotic research goes into the next stage, several questions have emerged that are to be answered to elucidate the mechanisms of probiotic action and to better apply probiotics for clinical uses. For example, what host factors need to be considered when designing studies and evaluating results? In addition, identifying biomarkers for evaluation of therapies, including probiotics in hosts is an emergent topic for translational and clinical research. As shown in human studies, there is person-to-person

variation in gene expression patterns upon probiotic administration [10**]. Given the potential demand for personalized medicine, future clinical trial study populations may be selected or characterized based on their baseline individual microflora and their individual genetic pattern responses to probiotic introduction. Thus, adding an intriguing and unexpected dimension to probiotic application in human disease prevention and treatment.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 588).

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Key points

- Regulation of host immune responses is probiotic gene specific, and the function of probiotic genes also depends on host microenvironment.
- Probiotic-derived factors mediate probiotic action in the regulation of host immune responses.
- Probiotics exert different levels of immune-regulatory effects in a host-dependent manner, including gene expression, protein synthesis, signaling pathways in immune cells and in intestinal epithelial cells.