Probiotic microbes: do they need to be alive to be beneficial?

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An essential symbiotic relationship exists between intestinal cells and commensal bacteria within the human gastrointestinal tract. Alteration or absence of this interaction may play a role in the development of human disease. Use of probiotic organisms has yielded improvement of certain medical conditions, such as inflammatory and infectious gastrointestinal disease, although the mechanisms of benefit remain poorly defined. The administration of live organisms is not without risk, both potential and realized, particularly in certain populations. Therefore, it is of considerable interest to determine if the health benefits of probiotics can be attained without the risks associated with administration of a live organism. Reviewed here is the evidence that heat-killed, ultraviolet-inactivated, and even components of these agents may be just as effective and considerably safer for the host.

INTRODUCTION

The human gastrointestinal (GI) tract is home to a complex ecosystem that is composed of 10–100 trillion microorganisms consisting of approximately 2000 microbial genes that number an order of magnitude greater than somatic cells. This suggests that this symbiotic dyad comprising a super-organism is only 10% human.1–3 As such, it is not surprising that the intestinal microbiota contributes greatly to the regulation of intestinal physiology, the development and functions of the intestinal epithelium, and to the regulation of inflammatory conditions.4 The GI epithelium serves as a defense system for its internal environment by distinguishing between commensal microorganisms that reside in the GI tract and pathogenic microorganisms that attempt to breach this barrier.5 Recent studies suggest that the interaction of the GI microflora and its components with intestinal epithelial cells plays a key role in the development of mucosal and systemic immunity as well as in the prevention and treatment of inflammatory bowel diseases, periodontal disease, rheumatoid arthritis, atherosclerosis, allergy, multi-organ failure, and colon cancer.6 It has been suggested that lack of formation of a diverse microbiota during childhood may result in imbalances in effector and regulatory immune responses, which in turn predispose to various diseases such as type 1 diabetes, allergy, asthma, and multiple sclerosis.7,8 The benefits of maintenance of normal endogenous microbiota along with the observation that probiotics may provide health benefits have led to the development of a large industry producing probiotics for popular use.

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Probiotics have demonstrated effectiveness in the prevention and treatment of various causes of diarrhea as well as inflammatory diseases of the GI tract.9 There is accumulating evidence that probiotics are also useful in the treatment and prevention of allergic diseases as well as in stimulation of the immune system.10

Accompanying these encouraging findings are concerns about potentially detrimental effects of these agents. This is due to the relative paucity of information about their mechanisms of action or if there are potentially safer alternatives that may provide similar benefits. Whether probiotic bacteria need to be “alive” and able to proliferate and survive for prolonged periods in order to exert their beneficial effect is a question that is being addressed in several studies, some of which will be summarized here. We will also discuss some of the common

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Key words: inactivated, inflammation, intestine, microbes, probiotics

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mechanisms of action of “dead” probiotics or their components that may result in beneficial effects.

CONCERNS ABOUT ADMINISTRATION OF LIVE MICROBES

Despite the large body of evidence supporting the health benefits of probiotics, several concerns have been raised about the possibility of unwanted side-effects, including the following: higher risk of problems in sick patients or very young individuals; virulence factors in probiotic bacterial strains; spread of undesired resistance genes in intestinal bacterial populations; ignition of inflammatory response; translocation to the locally draining tissues and blood; possible detrimental effects in patients with atopic dermatitis; possible increased risk for sepsis with use in very premature infants; possible formation of a persistent colony that prevents normal colonization of other microflora. This concern is especially valid in sick patients or very young individuals in whom manipulation may result in adverse effects. Another key issue concerns the safety aspects of bacteria added to particular products marketed for improvement of general health or treatment of (post) infectious symptoms. In some cases, virulence factors have been detected in probiotic bacterial strains, and the implications of these traits for safety assessments need to be considered in that horizontal transfer can result in the acquisition of virulence genes or antimicrobial resistance in probiotic bacteria. Antimicrobial resistance in these probiotic bacteria can potentially aid the spread of undesired resistance in intestinal bacterial populations. The relative risk of such gene transfers needs to be considered in any recommendations for routine or widespread use.

One of the known mechanisms of probiotics is the capability to modulate an over-aggressive inflammatory response, but in some cases probiotics may actually incite an inflammatory response of their own in highly susceptible individuals. There is current concern that these live bacteria may translocate to the locally draining tissues and blood, thereby causing bacteremia, especially in immunocompromised individuals. Contrary to some of the initial studies that demonstrated positive benefits of probiotics in patients with atopic dermatitis, considerable controversy is rising as studies are beginning to show there may be detrimental effects. For example, supplementation with *Lactobacillus rhamnosus* GG (LGG) during pregnancy and early infancy neither reduced the incidence of atopic dermatitis nor altered the severity of atopic dermatitis in affected children, but it was associated with an increased rate of recurrent episodes of wheezing bronchitis.

Despite considerable enthusiasm for the use of probiotics in premature infants for the prevention of necrotizing enterocolitis, based on randomized controlled trials, data from one of the most recent multicenter trials suggests the smallest group of these infants may be at increased risk for sepsis when given prophylactic probiotics. There is also concern that the prophylactic probiotics may form a persistent colony that prevents normal colonization of other microflora in the GI tract with subsequent alteration of normal immune system development. In addition concern exists that manipulation in newborns prior to the establishment of a normal core microbiome may incur risks.

These concerns prompt consideration of alternative agents such as prebiotics, which are usually indigestible oligosaccharides that prompt growth of resident (hopefully beneficial) microorganisms. Postbiotics, i.e., products of microbial fermentation such as the short-chain fatty acids acetate, propionate, and butyrate, may also provide beneficial effects. Accumulating evidence suggests that other specific components of microorganisms (usually those acting on Toll-like and other signal transduction receptors in the intestinal epithelium, dendritic cells, and other immunoreactive intestinal cells) may confer the same benefits as probiotics without incurring the risks associated with a live organism.

**BENEFICIAL DEAD PROBIOTICS AND THEIR COMPONENTS: EVIDENCE FOR BENEFIT?**

Previous studies have evaluated the probiotic *Lactobacillus rhamnosus* GG (LGG) and its effects on inflammation in human intestinal epithelial cells, specifically the Caco-2 cell line. One study demonstrated that pretreatment with both live and heat-killed LGG downregulated the production of IL-8 when an inflammatory response is generated in this cell line following cell stimulation by tumor necrosis factor-α (TNF-α). However, when an inflammatory response was not stimulated by any type of pathogenic ligand, high doses of live LGG significantly increased the production of inflammatory markers in intestinal epithelial cells, whereas heat-killed LGG only caused a slight increase. These data suggest that although pretreatment with both forms of LGG were effective in downregulating the TNF-α inflammatory response, high doses of the live agent without pre-existing inflammatory mediator stimulation actually caused a large increase in IL-8 production, whereas this was minimal with the heat-killed form. Thus, one might speculate that under certain conditions, the heat-killed form may be a safer alternative. The effects of live and ultraviolet (UV)-inactivated LGG on flagellin-induced IL-8 production in Caco-2 cells has also been evaluated. That study showed a brisk induction of IL-8 production in the Caco-2 cells stimulated with flagellin that was blunted with pretreatment with both live and UV-inactivated LGG. Similar effects have been demon-
strated in an animal model wherein pretreatment with heat-killed LGG diminished the LPS-stimulated production of cytokine-induced neutrophil chemoattractant. In a study designed to compare relative inhibition of attachment of pathogenic bacteria to Caco-2 cells, live versus heat-inactivated Lactobacillus acidophilus displayed similar benefits.

MECHANISMS OF HOMEOSTATIC REGULATION

The intestinal mucosa represents a huge surface area populated by several different cell types exposed to a myriad of microbes and other antigenic substances. A comprehensive review of the interactions between the mucosa and the microbes is beyond the scope of this review. Here, we will focus on the epithelium and some of the pertinent signaling processes. Since intestinal epithelial cells represent unique populations that exist in direct contact with a biomass of bacteria, there is a huge potential for unregulated inflammation. Prolonged and excessive activation of receptors that signal the inflammatory response can lead to uncontrolled inflammation detrimental to the host. Inflammatory signaling is regulated by several factors including the collective effects of several negative regulators that include interleukin-1 receptor-associated kinase-M, Toll-interacting protein, single negative regulators that include interleukin-1 receptor-associated factor 6 (Figure 1). Evidence suggests there is regulation of cytokine production via an intracellular mechanism that renders cells relatively resistant to these stimuli after repeated exposures.

Activation of intracellular proteins by pro-inflammatory stimuli such as commensal or pathogenic microorganisms triggers a series of enzymatic reactions that will alter inhibitor kB, including phosphorylation, ubiquitination, and degradation. Similarly, phosphoguanine nucleotide, such as flagellin, causes a series of intracellular signaling events to occur; these eventually lead to the dissociation of the transcription factor nuclear-factor kB (NF-kB) from inhibitor kB and translocation of NF-kB into the nucleus, thereby stimulating transcription of inflammatory mediators (Figure 1). Evidence suggests there is regulation of cytokine production via an intracellular mechanism that renders cells relatively resistant to these stimuli after repeated exposures.

Receptors for various microbial components expressed on epithelial cell surfaces appear to play a role in the transduction of signals arising from microbes in the intestinal lumen, which result in the regulation of the production of inflammatory mediators. Of interest is that several of the receptors appear to act through common pathways. Thus, LPS, LTA, cytosine phosphoguanine nucleotides, and flagellin may result in similar responses.

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Conclusion

Although literature to date has shown that live agents are effective in significantly downregulating the intestinal inflammatory response, there is still concern that these live agents may be detrimental to the host. However,
agents that are heat-killed or UV-inactivated, and even components of these agents, may be just as effective and considerably safer for the host. Our data as well as data from other studies provide evidence that these Toll agonists may provide the cells with a certain degree of tolerance, thereby decreasing the inflammatory response that would have otherwise been exacerbated in their absence. Although current literature shows that the NF-kB pathway is affected in this process, it is not clear whether it is the only pathway affected or simply one outcome of many. Further dissection of these intracellular signaling pathways and the conditions under which they perform these regulatory functions will certainly lead to important therapeutic and preventative strategies.

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