Eukaryotic-Microbiota Crosstalk: Potential Mechanisms for Health Benefits of Prebiotics and Probiotics

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Abstract

The ability to link dietary consumption of prebiotic food ingredients and probiotic microorganisms to health benefits rests, in part, on our ability to identify both the extent to which these factors alter human microbiome activity and/or structure and the ability to engage eukaryotic cells necessary to transduce signals originating from the microbiome. The human microbiome consists of bacterial, archaeal, and fungal components that reside in mucosal surfaces of the gut, the airways, and the urogenital tract. Characterization of the symbiotic nature of the relationship between eukaryotic cells and the bacterial and archaeal components of the microbiota has revealed significant contributions in energy balance, bowel function, immunologic function, sensory perception, glycemic control, and blood pressure regulation. Elucidating the complex interactions between the microbiota and their associated epithelial, immune, and neural cells may provide mechanistic insights and a rational basis for our belief that dietary consumption of probiotic microorganisms and prebiotics produces health benefits.

Contents

INTRODUCTION

The attribution of efficacy to the consumption of probiotic microorganisms for health benefits has occurred in reverse order of the available scientific evidence. Awarded the Nobel Prize in Medicine in 1908 for his cellular (phagocytic) theory of immunity, pioneering immunologist Elie Metchnikoff has inspired generations of scientists and food product developers with his proposal to transform the "toxic" flora of the large intestine into a host-friendly colony of *Bacillus bulgaricus* (53). A century later, we still lack sufficient systematic evidence for broad public health recommendations for the consumption of prebiotic dietary substances and probiotic microorganisms in the prevention and treatment of specific diseases and health conditions.

Probiotic bacteria are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (16). To be characterized as "probiotic," microorganisms must be demonstrated to exert a beneficial effect on the consumer, be nonpathogenic, nontoxic, and free of significant adverse side effects; retain stability during the intended shelf life of the product; contain an adequate number of viable cells to confer the health benefit; be compatible with product format to maintain desired sensory properties; and be labeled in a truthful and informative manner to the consumer (72). **Table 1** contains the recommendations of an expert panel convened by the Council for Agricultural Science and Technology to provide guidance for research and policy developments and recommendations for future improvements in the field of probiotics (72).

Prebiotics are dietary ingredients that alter the composition, or metabolism, of the gut

Table 1 Recommendations made by an expert committee of food scientists, microbiologists, and nutritionists convened by the Council for Agricultural Science and Technology to enhance the validity of labeling and health claims of probiotic-containing food products

Establish a standard of identity for the term "probiotic" based on the FAO/WHO definition.
Regulate probiotics based on their intended use, but expand regulatory conceptualization of health benefit claims.
Adopt the use of third-party verification of label claims.
Use probiotics selectively in clinical conditions.
Consider multiple factors when evaluating probiotics.
Focus research on the important role of human native microbiota in health.
Use a science-based assessment of the benefits and risks of genetically engineered probiotic microbes.
Provide better information to consumers.

microbiota in a beneficial manner (49). The genera typically affected by prebiotic consumption include *Lactobacillus* and *Bifidobacterium*. Prebiotics consist of fructan-based oligosaccharides and fructooligosaccharides that exist naturally in foods or that are added in processing. Nonfructan food sources include various forms of resistant starches. Conceptually, prebiotics may exert health benefits in ways similar to probiotics, but they do so more economically because they are associated with decreased risk of bacterial translocation and may be easier to incorporate into the diet than are probiotics. The reader is referred to recent reviews that have discussed a growing list of physiologic targets, including enhanced mineral absorption affecting bone health and amelioration of symptoms due to inflammatory bowel diseases associated with consumption of prebiotic dietary substances (49, 64, 69).

HEALTH BENEFITS OF PREBIOTICS AND PROBIOTICS: THE NEED FOR SYSTEMATIC, EVIDENCE-BASED REVIEW

The potential to regulate the composition of the microbiota by prebiotic dietary substances and probiotic microorganisms is, without question, an exciting but underdeveloped approach to the prevention and treatment of some diseases. Preliminary evidence exists for the clinical effectiveness of specific prebiotics and probiotics for numerous conditions. For example, specific probiotics have been used to treat gastrointestinal (GI) conditions such as lactose intolerance, acute diarrhea, and antibiotic-associated GI side effects (12). Similarly, probiotics administration has been associated with decreased risk of systemic conditions such as allergy, asthma, cancer, and infections of the ear, urinary tract, and vagina (41). It is noteworthy that even in the rare instances where professional medical societies make evidence-based recommendations to consume, for example, probiotics during pregnancy and nursing for the prevention of atopic dermatitis, no evidence-based guidance is provided concerning consumption levels, frequency, duration, or specific probiotic microorganisms to consume (29). The provision of evidence-based guidance to consume prebiotics and probiotics for specific health benefits will benefit from using systematic methodologies (i.e., *American Dietetic Association Evidence Analysis Manual*). This type of systematic peer review of available studies is considered more rigorous than narrative reviews (11) and enhances the veracity of recommendations as it occurs independent of the food industry sponsors of research in these areas.

The extensive list of health conditions and diseases for which either prebiotics or probiotic microorganisms are under investigation may prompt the skeptical reader to liken these unproven health claims to the practices of past and current health charlatans. However, given the sheer numbers of microbiota, their extensive interactions with mucosal surfaces of the body and

the dynamic nature of epithelial, immune, and neuroendocrine intercourse renders these investigations highly promising. Clearly, we are witnessing an exciting, albeit nascent, stage of biomedical discovery.

HEALTH BENEFITS OF PREBIOTIC AND PROBIOTICS: PROVIDING PHYSIOLOGIC CONTEXT

Our current inability to make scientific consensus-based public health recommendations for the consumption of prebiotics (i.e., source, type, amount, and duration) or the consumption of specific genus, species, and strain of probiotic microorganism is scientifically untenable. There is a critical need to delineate structure-function relationships between prebiotics, probiotics, and their metabolic products and the risk of specific health conditions. Why? First, clinical studies have revealed heterogeneity of biological responses to different genus, species, and strains of probiotic microorganisms. Heterogeneous results in efficacy trials for probiotics in clinical studies may be due to lack of appropriate biological activity of the microorganisms used, improper strain choice, or inadequate dose (51, 57, 77). Knowledge of the structural determinants of these organisms and the effects of their metabolic products will help clarify the physiologic routes through which their phenotypic effects are observed. Second, probiotics are not innocuous life forms. Like acetylsalicylic acid or aspirin, prebiotics and probiotics were consumed long before potential mechanisms were proposed. As with the ascription of cyclooxygenase inhibition as the mechanism of action for aspirin, we must move beyond general statements like "enhances immune health" in order to justify health benefits for prebiotics and probiotics. Just as aspirin can produce GI disorders due to cyclooxygenase inhibition, probiotic microorganisms may have negative effects in specific physiologic contexts. Results from a recent clinical trial of probiotic administration to decrease infectious complications due to acute necrotizing pancreatitis showed increased mortality, apparently due to bowel ischemia (5). Although exceedingly rare, these negative effects associated with probiotic administration should raise our vigilance to the possibility of biologically plausible phenotypes that may be harmful. Third, the approval of health claims on food product labels on products containing prebiotics and probiotics will likely become more arduous with the necessity for support from systematic, evidence-based reviews. Even so, a limited ability to demonstrate clinical efficacy of dietary interventions for disease treatment and prevention has seldom stood in the way of commercial food product viability. As such, commercial sales growth of products containing prebiotics and probiotics is occurring in Japan, Europe, and United States.

Attribution of health benefits to phenotypic effects caused by consumption of prebiotics and probiotics will require knowledge of the bidirectional interactions between these components of the microbiota and the epithelial, immune, neurologic, and endocrine physiologic processes initiated by these compounds and organisms. Fortunately, new paradigms are being established detailing the absolute physiologic interdependence of human microbiota and eukaryotic cells, which will enrich our vocabulary to help describe the mechanisms by which prebiotics and probiotics function (43). Emerging data link the metabolic effects of the microbiota beyond the long-appreciated physiologic roles in digestion, including the anaerobic fermentation of carbohydrates and proteins, and the synthesis of biotin, folate, and vitamin K (74). Emerging evidence supports biologically plausible mechanisms for microbiota-exerted regulatory control over metabolism of food and xenobiotics, development and maintenance of local and systemic immune responses, as well as neural pathways involved in glycemic control, pain perception, blood pressure control, and mental state. This review provides an overview of the eukaryotic cellular components on which prebiotics and probiotics in the GI tract have been demonstrated to play roles in the transfer of information from the microbiota. As technologies are applied for the identification and

quantification of all members of the microbiota, it will be possible to characterize genotype and phylotype of the GI ecosystem, which is a necessary first step in elucidating its role in health and disease (14).

Microbiota as a Dynamic Organ

The human body supports unique ecological niches in the skin, GI tract, genitourinary tract, and the ducts of exocrine glands for ~100 trillion bacteria, archaea, fungi, and viruses (45, 54). Though one-thousandth the size of the human genome and up to 2% of body mass, the microbial genomes, by virtue of the large number of their species, contribute over 100 million more genes and >99% of the total cellular composition than do their eukaryotic host (25). The intimate contact between these microbes, the epithelial layers lining these ecological niches, and the associated immune and neural systems supporting these tissues is suggestive of dynamic interplay that serves as the biological basis for the symbiotic relationship between microbiota and their eukaryotic dependents.

The microbiota of the GI tract has received the most attention owing to its abundance, its roles in inflammation and inflammatory bowel diseases, its potential amenability to change by diet, and accessibility of the distal microbiota (feces). The 10^{13}–10^{14} bacteria and archaea associated with eukaryotic cells vary in number from ~10^{7-8} per milliliter (ml) vaginal fluid; along the GI tract, these bacteria and archaea increase from approximately 10^3 colony forming units (CFUs) per gram in the stomach to 10^7 CFU/g in the distal colon (31). Similarly, the identity of microbiota is a function of their location along and within the GI tract. Although adults harbor up to 1000 species of bacteria, most predominant are members of the *Bacteroidetes* and *Firmicutes* families of bacteria and one member of archaea, named *Methanobrevibacter smithii* (3, 14). Other prominent GI microbiota include the anaerobic genera *Bifidobacterium*, *Eubacterium*, *Fusobacterium*, *Clostridium*, and *Lactobacillus*. Aerobic genera include Gram-negative enteric bacteria (*Escherichia coli* and *Salmonella* spp.), the Gram-positive cocci (*Enterococcus*, *Staphylococcus*, and *Streptococcus*), and fungal species such as *Candida albicans* (54).

It has been noted that the unusually restricted microbial diversity of the GI microbiota is a function of strict adaptive mechanisms necessary for microbial survival in the gut (44). This limited number of autochthonous microorganisms that thrive in the GI niche may be due to the production of specific capsular polysaccharides by commensal bacteria (46). Commensal microorganisms are referred to as resident or autochthonous members of the microbiota, whereas probiotic microorganisms are generally considered noncolonizing or allochthonous members of the microbiota. The commensal bacterium *Bacteroides fragilis* employs several capsular polysaccharides in order to grow properly and successfully participate in symbiotic mutualism. Investigators employed *B. fragilis* deleted in the global regulator of polysaccharide expression and studied specific mutants with defects in capsule polysaccharide expression. Only restoration of expression of multiple capsular polysaccharides was able to completely restore the ability of *B. fragilis* mutants to compete for commensalism. These data support the observation that probiotic microorganisms, as allochthonous components of the microbiota, require frequent consumption in order to populate the microbiota. As such, it remains to be determined whether specific capsular polysaccharides that are necessary to compete for commensalism are expressed by probiotic microorganisms.

The study of the dynamic nature of symbiotic interactions between the microbiota and host has been pioneered by the elegant work of Dr. Jeffrey Gordon and collaborators (3). Emerging hypotheses from the work of Dr. Gordon and others have described new functions of microbiota. Largely inspired by this work, the National Human Genome Research Institute unveiled its plan for a Human Microbiome Project (80). The genomic diversity of entire microbial communities will be quantified through classical and metagenomic

DNA sequencing technologies. The microbial communities (GI tract, oral cavity, vagina, and skin) in people of different ages, origins, and health statuses will be compared to determine whether the community structure of microorganisms is associated with increased or decreased risk for certain diseases and whether it can be manipulated to improve human health. These studies will allow, for the first time, a rational framework in which to elucidate the effect of dietary probiotic bacteria and prebiotics on community structure and bacterial and archeal metabolism as well as potential health benefits.

EUKARYOTIC-MICROBIOTA MUTUALISM: ENERGY HARVEST AND WEIGHT HOMEOSTASIS AS ILLUSTRATIVE EXAMPLES

Microbial communities are characterized by unparalleled complexity. Our increasing technological ability to characterize this complexity will contribute to understanding the ecological processes that drive microbe-eukaryotic interactions. One of the most striking findings that helped to define this mutualistic relationship was the role of microbiota in energy harvest. Comparative studies of genetically obese mice, as well as those of obese human subjects, demonstrate an adiposity-associated increase in the *Firmicutes:Bacteroidetes* ratio compared with lean mice and humans, respectively (42, 45). This decrease in *Bactertoidetes* in obese people compared with lean people was reversible with weight loss on two types of low-calorie diet. In a similar vein, early differences in fecal microbiota in children have been associated with risk of overweight (33). Children of normal weight had higher bifidobacterial and lower *Staphylococcus aureus* concentrations than did children becoming overweight. These findings suggest that the microbiome exerts strong influences on weight homeostasis. These examples illustrate how microbiota provide us with metabolic attributes we have not been required to evolve on our own, including the ability to harvest otherwise inaccessible nutrients (3).

It is beyond the scope of this review to characterize the complex interactions and products resulting from interactions between components of the microbiome, including autochthonous and allochthonous microorganisms. It is acknowledged that products of these interactions, including production of short-chain fatty acids by anaerobic fermentation to provide substrates for oxidative metabolism in epithelial cells, the provision of protective factors against bacterial pathogens, and competition among components of the microbiota, are of central importance to eukaryotic-microbiota mutualism.

MECHANISMS OF ACTION: THE COMPLEX PHYSIOLOGY OF EUKARYOTIC-PROBIOTIC INTERACTIONS

Several general mechanisms have been proposed by which probiotic bacteria may exert their biological effects on the host, including: (*a*) preventing the colonization of host by pathogens by competing for nutrients and epithelial attachment site; (*b*) producing antimicrobial compounds and pH changes that make the environment for pathogens unfavorable; (*c*) recruiting immune cells and activating appropriate immune and/or inflammatory responses by altering cytokine and chemokine release; and (*d*) secreting antimicrobial peptides (48, 59). This review focuses on the third mechanism mentioned above, which involves the recognition of probiotics and their specific metabolites by cells of the GI tract, including mucosal immune cells, to influence local and systemic immune responses. It is noteworthy that this specific property of probiotic microorganisms has been suggested as an additional criterion for ascription of the functional term "probiotic" (20). The following discussion focuses on illustrations of the mechanistic basis for epithelial-immune cell interactions in the development of innate and adaptive immune responses. In addition, examples are provided to show

promising developments linking probiotic bacteria-mediated effects on the enteric nervous system.

THE GASTROINTESTINAL IMMUNE SYSTEM: SENSING AND REACTING TO LUMINAL CONTENTS

As the largest immune organ in the body, the GI tract contains approximately 65% of immune tissue overall and up to 80% of the immunoglobulin-producing tissues of the body (4, 6). Similarly, the GI tract possesses its own local nervous system, referred to as the enteric or intrinsic nervous system. Containing as many neurons as the spinal cord, the enteric nervous system along with the sympathetic and parasympathetic nervous systems constitute the autonomic nervous system (18). The devotion of such a large proportion of immunological and neurological resources for GI function is indicative of the central physiologic roles for the detection, acquisition, and processing of antigens and other molecules originating from the microbiota, the diet, and products of their interaction.

The ability of probiotic bacteria ingestion to alter the nasal and vaginal microbiota suggests crosstalk between the microbiota of disparate mucosa-associated lymphoid tissues (MALT) (26, 65). The following sections briefly describe the physiologic contexts responsible for interactions with microbiota in the GI lumen and other microbiota-exposed body surfaces. The primary anatomical platform for altering local and systemic immune responses by prebiotic dietary substances and probiotic microorganisms are those associated with MALT. MALT is composed of several compartments of lymphoid tissue including the gut-associated lymphoid tissue (GALT), tonsils, pharynx, nasal-associated lymphoid tissue (NALT) such as the adenoids, and bronchus-associated lymphoid tissue (BALT). GALT constitutes the major part of MALT and is composed of the Peyer's patches (specialized lymphoid follicles), the appendix, and numerous solitary lymphoid follicles especially in the large bowel (27). Since bacterial niches occupy microenvironments associated with MALT, NALT, and BALT, the acquisition of antigenic information from these disparate mucosal-associated microbiota may be a primary means of discourse in eukaryotic-microbiota communication.

Three types of epithelial cells, including intestinal epithelial cells, M cells, and enterochromaffin cells, as well as dendritic cells (DCs) and enteric neurons sense these luminal components and communicate information resident in bacterial antigens and metabolites to downstream components of the mucosal immune system, including B and T lymphocytes, and the central nervous system (19, 81, 82). This system also provides local defense mechanisms against environmental threats, including bacterial pathogens.

Antigen sampling by epithelial cells and DCs informs local protective effects such as secretory IgA (sIgA) production as well as systemic immune responses (60). Systemic responses can result from sampling of microbial antigens by DCs at the site of colonization or infection by microbial pathogens (usually under epithelial M cells). DCs can transport antigens to local lymph nodes, present antigens to naive immune cells, and activate disparate effector responses from B, T helper (T_h), and T regulatory (T_{reg}) cells. Bacterial challenges from commensal and pathogenic organisms can result in different patterns of dendritic cell cytokine production (56). T_h subsets that develop in response to dendritic cell priming release a distinct panel of cytokines that are capable of delivering activating and inhibitory feedback signals to effector immune cells. The activation of the appropriate T_h-cell subset is instrumental in the generation of a successful immune response, including memory response, to antigens. T_{reg} cell production of IL-10 may serve to dampen the tendency for inflammatory responses to gut microbiota (78). The ability to ascribe physiologic roles such as these to prebiotic and probiotic dietary components will inform efforts to establish public health recommendations for specific health conditions.

Components of the Microbiota in Communication with Eukaryotic Cells: Toll- and Nod-Like Receptors Ligands

Structural components of commensal and probiotic bacteria serve as ligands for Toll-like receptors (TLRs, also known as pattern recognition receptors or PRRs) and the nucleotide-binding domain, leucine-rich repeat-containing family of proteins (or NOD-like receptors, NLRs) that are two important families of membrane-associated and cytosolic molecules, respectively, required for microbial recognition, gut homeostasis, and induction and regulation of the innate and adaptive immune response (34, 63). Bacteria, yeast, fungi, viruses, protozoa, and molds contain structural components that serve as TLR ligands that stimulate mucosal immune responses. TLR ligand engagement by cells of the immune system is a primary means of antigen detection and discrimination (63). Thus, TLR signaling provides a portal for engagement of the microbiota to produce the low level of inflammation observed in the healthy GI tract. Studies in gnotobiotic animals demonstrate that this is the primary physiologic interaction required between luminal contents and mucosal eukaryotic cells to maintain cellular homeostasis in the GI tract (61).

TLRs are a family of at least 10 membrane proteins that interact singly as homodimers or heterodimers or as heterodimers to recognize specific conserved components of microbes (58). For example, TLR-4 recognizes lipopolysaccharide (LPS), TLR-2 recognizes peptidoglycan (PGN) from Gram-positive bacteria, TLR-5 recognizes flagellin, and TLR-9 recognizes unmethylated CpG DNA (82). NOD1 (CARD4) and NOD2 (CARD15) are two members of the NOD family of NLRs (35). NOD1 and NOD2 are intracellular pattern-recognition molecules involved in the recognition of peptidoglycan (PGN); these two families of NLRs detect specific motifs within the PGN.

Little is known about how the specific presentation of TLR ligands from probiotic bacteria to mucosal immune cells may enhance local and systemic immune responses (82). However, tantalizing evidence from cell culture models and animal studies shows that ligands from probiotic bacteria may engage specific TLRs to result in decreased mucosal inflammatory tone. Elegant work has implicated DNA from probiotic bacteria as the TLR9 ligand necessary to observe the attenuation of experimental colitis (38, 40, 62). Although confirmation by other workers is necessary, these results have identified TLR9-induced type 1 interferon (IFN-ß) as the agent responsible for mediating the anti-inflammatory effects of probiotic DNA. Analogous to the commensal bacteria-mediated production of interleukin-6 (IL6) and keratinocyte-derived cytokine in mice (63), these findings underscore the diverse effects of indigenous microbial TLR ligands to stimulate epithelial cells to produce factors (such as IFN-ß) that participate in gut homeostasis.

Epithelial Cells: Toll-Like Receptor-Mediated Sensors of Luminal Contents

By far the most abundant cell type lining the small and large intestine, epithelial cells provide metabolic products that support luminal microbiota and provide a tight barrier between luminal contents and the underlying mucosa (9). Polarized epithelial cells also act as sensors to pathogen invasions that initiate defensive responses (39).

Because recognition of luminal components by epithelial cells is a primary influence on the development of immune responses, distinct mechanisms for TLR- and NLR-mediated effects may be exerted locally (i.e., inside the GI tract or GI mucosa) or systemically (i.e., amelioration of atopic conditions or alteration of nasal and vaginal mucosa microbiota) (32, 36). Although the roles of antigen sampling cells such as *M* cells and DCs are key to the development of systemic immune responses, the types of information contained in bacteria or their

metabolic products that result in vastly different genus-, species-, and strain-specific biological effects of probiotic bacteria are less well known. Current evidence points to a role for bacterial components to serve as ligands for TLRs and NLRs.

TLRs are expressed on various cell types of the GI tract, and each participates in host defense against pathogens in at least four ways: (*a*) recognition of molecular patterns on pathogens; (*b*) expression at the interface with the "environment" of the GI lumen; (*c*) secretion of proinflammatory or anti-inflammatory cytokines and chemokines that inform the adaptive immune response; and (*d*) induction of antimicrobial pathways (7, 8). In the healthy gut, TLR expression is functionally homeostatic, but with increased pathogenic threats, homeostasis could be altered to a disease state (55). In the normal, uninflamed intestine, there is a low level of expression of both TLR2 and TLR4; levels of TLR2 and TLR4 become increased during inflammatory conditions (1, 17). The following sections describe illustrative examples of the biological consequences of bacterial engagement of TLRs in the maintenance of gut homeostasis.

Intestinal epithelial cells play a primary role in stimulating sIgA production by mucosal plasma cells (61). sIgA has a required role in the mucosal defense against infectious microbes and reducing inflammatory tone in the gut. In human and mouse, IgA-producing plasma cells comprise approximately 20% of total plasma cells of peripheral lymphoid tissues, whereas more than 80% of plasma cells produce IgA in MALT. Luminal bacteria signal through TLRs to cause intestinal epithelial cells (IECs) to secrete a proliferation-inducing ligand (APRIL) (30). IEC-secreted APRIL activates DCs to process and present luminal antigens to naive B cells. In this manner, IECs may function as mucosal guardians orchestrating frontline IgG and IgA class switching through a TLR-inducible signaling program. Recent data show that probiotic bacteria can influence innate immune responses. *Lactobacillus casei* administration for seven days increased IgA+ cells and IL-6-producing cells (20). This association is critical because mucosal production of sIgA has been found to reduce the inflammatory response to commensal bacterium *Bacteroidetes thetaiotaomicron* in a gnotobiotic rodent model by reducing bacterial epitope exposure (61).

Dendritic Cells: Toll-Like Receptor-Mediated "First Responders" to Luminal Antigens

DCs are professional antigen-presenting cells that, together with intestinal epithelial cells and mucosal lymphocytes, control mucosal inflammatory tone. In addition to IEC, bacterial uptake from the gut lumen by DCs can be accomplished by extending dendrites between intestinal epithelial cells (66). DCs express receptors from the TLR family as well as C-type lectins such as dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) and mannose receptor, which recognize carbohydrate structures on pathogens and self-glycoproteins (68).

IECs discriminate between pathogenic and commensal bacteria by activating either of two distinct pathways leading to maintenance of a homeostatic, noninflammatory state or to the development of an inflammatory immune response. In the absence of inflammatory stimuli, resident immune cells maintain a noninflammatory phenotype and participate in the maintenance of gut homeostasis. Under these conditions, so-called IEC-conditioned DCs are blocked irreversibly in their ability to release proinflammatory IL-12 and to activate T helper 1 (Th1) cells and cannot produce inflammatory mediators even after an encounter with pathogenic bacteria such as *Salmonella* (67). Conversely, when invasive bacterial pathogens deliver their TLR ligands intracellularly, they initiate an inflammatory response in IECs, which release inflammatory cytokines and chemokines. These events result in the recruitment of naive phagocytes, including DCs, which are required to initiate anti-*Salmonella*-specific T-cell responses (71).

Interestingly, it has recently been shown that selective probiotic bacteria such as *Lactobacillus reuteri* and *Lactobacillus casei* can induce IL-10 production by T_{reg} cells by modulating DC function through DC-SIGN binding (75). Probiotic bacteria-induced IL-10 production would be expected to promote a tolerogenic phenotype in the gut via inhibition of the development of T_h1- and T_h2-type lymphocyte responses. Probiotic bacteria-exposed mononuclear cells *in vitro* have been shown to induce IL-10 production; however, no difference was observed between the *Lactobacillus rhamnosus GG* (LGG)-supplemented group and the placebo group in terms of the proliferative capacity of maternal or neonatal cord blood cells in response to IL-2, beta-lactoglobulin, or LGG (37).

The induction of T_{reg} cell-dependent IL-10 secretion by probiotic bacteria remains a viable intervention rationale because monocyte- and myeloid-derived DCs exposed to different probiotic bacteria, a process that causes DC maturation, lead to the production of different patterns of cytokines by bacteria-matured DCs (15). Blood-derived DCs responded to bacterial exposure with the production of IL-6 and tumor necrosis factor-alpha but elicited mainly IL-10 in monocyte-derived DC. In contrast, comparable IFN-gamma production patterns were found in both natural killer (NK) cells and T cells induced by all bacteria-matured DCs. A decrease in IFN-gamma production in bacteria-matured DCs was observed in all DC subtypes. The most potent responses were induced by monocyte-derived DCs, which could serve as a sensitive screening model for immunomodulatory species of probiotic bacteria.

EMERGING ROLE OF PROBIOTIC BACTERIA ON SIGNALING TO THE GUT-BRAIN AXIS

Recently published data have yielded insights into the role of the enteric nervous system in transducing information in gut luminal contents, including probiotic bacteria, to affect health conditions such as constipation and irritable bowel syndrome (IBS) (10, 81). This is, perhaps, more intuitive than it appears on first consideration. The gut is the only organ that can display reflexes and integrative neuronal activity when isolated from the central nervous system and can be triggered by luminal stimuli that are detected by nerves via epithelial intermediation (23). Alterations in the enteric nervous system can be manifested as altered intestinal motility and increased visceral sensitivity (50). These result from dysregulation of the bidirectional transfer of information between the gut with its enteric nervous system and the brain (the brain-gut axis), modulated by various psychosocial and environmental factors (e.g., infection, inflammation). Although numerous physiologic targets for probiotic bacteria exist, it is hypothetically possible that stress-induced changes in the microbiota play a primary role in IBS or secondary roles in the inflammatory bowel diseases. Restoration of the normal composition of the microbiota via consumption of prebiotics and probiotics may be the responsible mechanism (73).

Epithelial enterochromaffin cells (ECs) and vagal mucosal afferent nerves are two primary means of sensing, or sampling, luminal contents in the GI tract. ECs serve as sensory transducers of luminal contents that activate the mucosal processes of both intrinsic and extrinsic primary afferent neurons through their release of 5-hydroxytryptamine (5-HT) or serotonin (47). Serotonin activates these intrinsic and extrinsic primary afferent neurons to initiate peristaltic and secretory reflexes, respectively, and to transmit information to the central nervous system (24). Luminal contents are also sampled by vagal mucosal afferents within the lamina propria for substances absorbed across the epithelium or released from enteroendocrine cells (28).

Probiotic bacteria may influence the perception of GI pain by influencing signaling at the level of the epithelial cell receptor expression or by influencing stress hormones via the gut-brain axis. The following discussion highlights illustrative examples of probiotic bacteria on neural function in the gut. The last example

demonstrates a role for probiotic bacteria on vasomotor tone related to the maintenance of optimal blood pressure.

PERCEPTION OF PAIN AND STRESS

In colon epithelial cells in culture, the administration of *Lactobacillus acidophilus NCFM* (NCFM; Danisco, Madison, WI) induced the expression of μ-opioid and cannabinoid receptors (70). Increasing concentrations of NCFM in rats with butyric acid induced GI inflammation-mediated analgesic functions in the gut ostensibly through the expression of μ-opioid and cannabinoid receptors. These results suggest that composition of the luminal microbiota can influence our visceral perception of pain. If probiotic bacteria-induced enhancement of μ-opioid and cannabinoid receptors can be demonstrated in humans, these approaches may result in new treatments for abdominal pain and IBS. These results underscore the bidirectional nature of communication between the microbiota and the eukaryotic host as well as the specificity of the effect of probiotic strains on health outcomes.

Another type of stress that has been ameliorated by specific probiotic bacteria in an animal model is neonatal maternal separation (MS) of rat pups. MS of rat pups causes immediate and long-term changes in intestinal physiology due to the induction of stress. MS-induced gut dysfunction is manifested as increased hypothalamus-pituitary-adrenal (HPA) axis activity (indicated by corticosterone release), macromolecular permeability in the gut, enhanced electrical circuit activity, and bacterial adherence/penetration into the mucosa (22). Administration of two probiotic *Lactobacillus* species was shown to ameliorate MS-induced gut functional abnormalities and bacterial adhesion/penetration in rat pups and to reduce elevated corticosterone levels (21). The decrease in corticosterone release in the face of MS indicates that probiotic administration was associated with the normalization of HPA axis activity. The results indicate that altered enteric flora is responsible for MS-induced colonic pathophysiology. Similar observations have been made concerning the ability of *Lactobacillus paracasei* (NCC2461) therapy to ameliorate antibiotic-induced visceral hypersensitivity in mice (81). These data raise the prospect that the host physiological state can affect the microenvironment in which the microbiota reside and thereby alter the metabolic activity and composition of the microbiota. Probiotic therapy appears promising as a biologically plausible therapy to decrease symptoms of certain GI disorders by affecting the composition of the microbiota as well as enteric neural and immune function.

GLYCEMIC CONTROL

Preclinical observations in rodents demonstrate that oral administration of specific probiotic bacteria can exert hypoglycemic effects. Oral administration of *Lactobacillus johnsonii La 1* (LJLa1) in drinking water for two weeks also reduced the elevation of blood glucose and glucagon levels after an oral glucose load in streptozotocin-diabetic rats (83). These data suggest that LJLa1 might affect glucose metabolism by reducing autonomic nerve activity, thus decreasing glucagon-mediated glycogenolysis. The effect of intraduodenal administration of LJLa1 on adrenal sympathetic nerve activity (ASNA) in urethane-anesthetized rats was measured because the autonomic nervous system, including the adrenal sympathetic nerve, may directly affect blood glucose regulation. ASNA was suppressed by intraduodenal administration of LJLa1, which suggests that LJLa1 might improve glucose tolerance by reducing glucagon secretion via alteration of autonomic nerve activities including ASNA. These results warrant further investigation in humans under strict, metabolically controlled conditions.

BLOOD PRESSURE REGULATION

Two lines of evidence support a role for a hypotensive effect of specific peptides produced

by proteolytic activity of probiotic bacteria on milk-based proteins (79). Notably, milk fermented with lactic acid bacteria lowers blood pressure, a finding that suggests that metabolites or components of the bacteria have hypotensive action. Lactic acid bacteria possess strong proteinase activity that produce peptides with properties associated with both opioid and angiotensin-converting enzyme inhibitory activities (79). Angiotensin-converting enzyme normally functions to increase blood pressure by enzymatic conversion of angiotensin I to angiotensin II. Angiotensin II interacts with angiotensin receptors in the vasculature to promote systemic vasoconstriction.

Interestingly, the intraduodenal injection of *Lactobacillus johnsonii La1* (LJLa1) lowered blood pressure and reduced renal sympathetic nerve activity (RSNA) in urethane-anesthetized rats (76). Blocking the conduction of nerve impulses from histaminergic neurons with a histaminergic H3-receptor antagonist eliminated the effects of LJLa1 on RSNA and blood pressure. Creation of bilateral lesions in the hypothalamic suprachiasmatic nucleus abolished the suppression of RSNA and blood pressure caused by LJLa1. These findings suggest that LJLa1 or its metabolites might lower blood pressure by changing autonomic neurotransmission via the central histaminergic nerves and the suprachiasmatic nucleus in rats. These studies demonstrate strong biologic plausibility for the use of lactic acid bacteria or lactic acid bacteria-fermented milk proteins in the management of hypertension.

CONCLUSIONS

The Nobel Prize–winning immunologist Elie Metchnikoff ascribed all manner of pathophysiologic processes to the "putrefaction" of bacteria inside the human colon, including senility, atherosclerosis, and a less-than-optimal life span (52). He predicted that surgical removal of the colon would be routine in the future and serve as a preventive measure against putrefactive bacteria-driven illness. Having inspired the development of the scientific study of probiotic bacteria, Dr. Metchnikoff's now-antiquated ideas provide insight into the progress we have made and a preview of how we will view our progress 100 years hence.

Future research needs are described in **Table 2**. With few notable exceptions (13), information regarding the genus, species, and strain of probiotic bacteria or the type and quantity of prebiotic dietary carbohydrates necessary to effectively treat or reduce the risk of specific health conditions is lacking. Beyond the aforementioned need for evidence supporting efficacious consumption levels and duration of exposure, a broader limitation is the

Table 2 Future needs in biomedical research for probiotic bacteria

1. Identify and quantify the components of the human microbiome. Determine if specific patterns of composition and metabolic activity of the microbiome are related to the risk of specific health conditions.
2. Correlate the relationship between consumption of prebiotic dietary substances and probiotic microorganisms with changes in the community structure of the microbiota present at particular body sites and the risk of specific conditions and diseases.
3. Define the specific immune and endocrine parameters affected by the consumption of prebiotic dietary substances and probiotic microorganisms that are associated with a decreased risk of disease or the modulation of immune function.
4. Identify the structural components and metabolic end products of the microbiome components, including probiotic microorganisms, that influence eukaryotic development and homeostasis, including systemic changes in immunological and neural function.
5. Determine the effect of host inflammatory states and disease stage on the composition and metabolic activities of the microbiome, particularly in the gastrointestinal tract.
6. Utilize an evidence-based, systematic approach to the evaluation of the medical efficacy of prebiotics and probiotics to support public health recommendations for specific health conditions.
7. The support of public and private funding support will be required to encourage the development of multidisciplinary collaborative efforts and to address the broad nature of the research questions that will be generated.

lack of valid measures for the modulation of dietary factor–associated local or systemic immunological biomarkers associated with health risks (2). The relative dearth of supportive systematic medical evidence stands at odds with the tremendous commercial success of foods containing probiotics and prebiotics in Japan, Europe, and the United States.

Progress in understanding the mechanisms by which the microbiota interact with their eukaryotic hosts will provide a rational basis for health claims for dietary consumption of probiotics and prebiotics. The metabolomics-fueled unveiling of a host of metabolic capabilities of the microbiota will necessitate a broader definition of signal transduction with eukaryotic cells. Moreover, the tremendous scale of this discourse—within and between bacteria and among the cascade of signals generated by epithelial, immune, and neural cells—renders our current paradigms inadequate in describing the complexity of eukaryotic-microbiota communication. Multidisciplinary research in these areas will bolster our widely held belief that dietary consumption of prebiotics and probiotics results in health benefits.

SUMMARY POINTS

1. The human microbiota consist of ~100 trillion bacteria, archaea, fungi, and viruses that contribute over 100 million more genes and >99% of the total cellular composition than do their eukaryotic host.
2. In addition to participating in energy harvest and the synthesis of certain vitamins, the microbiota may support life by assisting the development and maintenance of immune system and enteric neural pathways involved in glycemic control, pain perception, and blood pressure control.
3. Information conveyed from components of the microbiota, including probiotic microorganisms and their metabolic products, is captured by intestinal epithelial cells, M cells, dendritic cells, macrophages, enterochromaffin cells, and enteric neurons.
4. Health effects from consumption of probiotic microorganisms may be produced when antigenic and endocrine information from innate immune cells and enteric neurons is communicated to mucosal immune cells and the central nervous system.
5. Evidence suggests that probiotic microorganisms act locally to modulate gut-associated immune function and systemically to alter mucosal microenvironments and risk of certain health conditions.
6. Not all probiotic bacteria produce health benefits when consumed; the genus, species, and strain of probiotic bacteria must be tested in human research to confirm how much must be consumed and for what duration to produce specific health benefits.
7. A century after Dr. Metchnikoff's conceptual establishment of the field of probiotics, we still lack sufficient systematic medical evidence for broad public health recommendations for the consumption of prebiotics and probiotics in the prevention and treatment of specific diseases and health conditions.
8. Our current inability to specify the genus, species, and strain of probiotic microorganism or type of prebiotics to consume for specific health effects is scientifically untenable. Growing commercial success of products containing probiotics and prebiotics continues to benefit more from belief in biologically plausible health benefits and limited clinical studies than from systematic evidence-based recommendations.

9. Progress in understanding the mechanisms by which the microbiota interact with their eukaryotic hosts will provide a rational basis for health claims for dietary consumption of probiotics and prebiotics.

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of this review.

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Errata

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