Mechanisms of Action of Probiotics

W. Allan Walker
Division of Nutrition and Department of Pediatrics, Harvard Medical School, Boston, and Mucosal Immunology Laboratory, Massachusetts General Hospital for Children, Charlestown, Massachusetts

At birth, the newborn leaves the germ-free intrauterine environment and enters a highly contaminated extraterine world, which requires potent host defenses to prevent disease. Intestinal defenses develop during gestation and have the capacity to respond but first must be exposed to colonizing bacteria. I review the importance of bacterial colonization for the appearance of normal mucosal immune function and the clinical consequences of inadequate colonization with regard to development of disease. For example, we now know that an imbalance in T-helper (Th) cells (e.g., Th2 levels greater than Th1 levels) can predispose to autoimmune disease and gut inflammation or disease, such as necrotizing enterocolitis. As we determine the role of bacterial colonization in the gut (bacterial-epithelial “cross talk”), we should have more-appropriate ways to modulate the gut immune responses—for example, by use of probiotics to prevent the expression of these gastrointestinal diseases.

If we define probiotics as “live microorganisms that when ingested have a positive effect on health” [1], we can begin to include as probiotics certain commensal bacteria found in the human gut. During the past decade, microbiologists, immunologists, and gastroenterologists have actively studied the mechanism by which commensal bacteria improve mucosal defenses of the gastrointestinal tract. This article will review selected aspects of these observations.

BACTERIAL-EPITHELIAL “CROSS TALK”

It has been known that colonizing bacteria that interact with the gastrointestinal mucosa can communicate with the underlying epithelial and mucosal lymphoid elements and that such interaction stimulates host defenses in the gut [2]. However, it was not until recently that investigators began to understand the so-called bacterial-epithelial cross talk at the cellular level. With the discovery of toll-like receptors (TLRs) on eukaryotic epithelial, endothelial, and lymphoid cells, which could interact with molecular patterns on both pathogens and commensal bacteria [3], a molecular and cellular basis for communication could be appreciated. The TLR that has been studied the most is TLR-4, whose primary ligand is gram-negative lipopolysaccharide (LPS) (i.e., endotoxin). This receptor interacts with LPS as an LPS-binding protein complex after being anchored to the cell surface by a surface molecule, CD14. With this interaction, a series of signaling molecules is activated in the cell to release the transcription factor nuclear factor κB (NFκB) into the nucleus, which in turn transcribes inflammatory cytokines—for example, IL-8 and IL-6—to provide the basis for an acute innate inflammatory response to an invading pathogen. Since the initial discovery of TLR-4, ten additional TLRs have been cloned, and a variety of microbial molecular patterns have been identified as ligands, including patterns on commensal bacteria [4]. The appreciation of microbial patterns that interact with pattern-recognition receptors on eukaryotic cells has been the basis of our understanding of bacterial-epithelial cross talk and its role in both innate and adaptive mucosal immunity.

DEVELOPMENT OF HOST DEFENSE

A vital practical application of bacterial-epithelial cross talk is the communication between colonizing bacteria and the germ-free neonatal gastrointestinal tract im-
The nature and composition of these colonizing bacteria are important contributors to the development of gastrointestinal host defenses against infection and allergic reaction. Table 1 lists several important components of host defenses affected by this initial colonization. At birth, the full-term neonate has the developmental capacity to mount an adequate host defense. However, for the efferent (i.e., active) component of that defense to occur, the gut lymphoid tissue must first be stimulated by colonizing bacteria [5]. This includes expression of follicular epithelial (i.e., microfold) cells over Peyer’s patches in the ileum and colon. These cells facilitate interaction between microbes and lymphoid cells [6]. In addition, intraepithelial and lamina propria sites are activated to produce protective cytokines [7], and mesenteric lymph nodes secrete polymeric IgA (pIgA) [8]. Recently, it has been demonstrated that dendritic cells in the lamina propria can extend their appendices between epithelial cells, and, via TLR-2 and TLR-4 on their surface, they can sample commensal-bacterial molecular patterns (figure 1) [9]. The interaction leads to maturation of the dendritic cells and to the release of cytokines, which orchestrate the conversion of naive T-helper cells (Th0) into a mature, balanced response of T-helper cells (Th1, Th2, and Th3/Tr1), an important component in the prevention of disease. Furthermore, commensal bacteria can cross microfold cells and interact with antigen-presenting cells in mesenteric lymph nodes to activate naive plasma cells into becoming pIgA-producing B cells [10]. pIgA, in turn, coats the mucosal surface to control subsequent microbial and antigen penetration.

An important component of mucosal host defense in the gut is the capacity of epithelial and lymphoid cells, after an acute innate immune response, to turn off the inflammatory response in the absence of a self-limited manner. In the absence of a self-limited and innate inflammatory response, chronic clinical conditions such as inflammatory bowel disease and necrotizing enterocolitis (NEC) can occur. Accordingly, several mechanisms have evolved to control chronic inflammation of the gut. These include the interaction between CpG DNA of bacteria and the intracellular receptor TLR-9, to which the bacteria bind and thus activate T regulatory cells by the production of anti-inflammatory cytokines (e.g., IL-10) (figure 2) [11]. Commensal bacteria can also secrete small molecules that can enter intestinal epithelial cells to inhibit activation of NFκB [12]. In addition, it has been reported recently that prolonged exposure to molecular patterns (of peptidoglycans and LPS) that interact with TLR-2 and TLR-4 can activate cell surface and intracellular negative regulators, respectively, which, in turn, can turn off transcription factors, leading to a reduction in the production of inflammatory cytokines and chemokines [13]. These cellular mechanisms are critical to both intestinal host defense and the prevention of chronic disease in the gastrointestinal tract. With an inadequate initial bacterial coloni-
zation, probiotics can be used to accomplish the same balanced immune response [14].

OTHER PROTECTIVE EFFECTS OF PROBIOTICS

Probiotics have been demonstrated to have an adjuvant effect on immunologic responses. As mentioned above, their interaction with mesenteric lymph nodes can result in an up-regulation of IgA against intestinal pathogens and food antigens [10]. In addition, Saccharomyces boulardii, a probiotic fungal organism, has been demonstrated to enhance the specific IgG and IgA antibody response to Clostridium difficile toxin A after Clostridium infection [15]. Infants with rotaviral gastroenteritis who were given Lactobacillus rhamnosus strain GG had an increased IgA response to the virus [16]. Finally, a pilot study involving healthy adults about to receive typhoid vaccine for foreign travel showed that those given Lactobacillus GG for 10 days before vaccination had significantly higher levels of antityphoid antibodies than did those who received a placebo [17]. Furthermore, the effect of Lactobacillus GG was specific because levels of antibodies to antigens in other vaccines received previously were not increased.

The use of probiotics has also been effective in enhancing the mucosal barrier to pathogens and antigen presentation. A recent study reported that known probiotics, Lactobacillus strains, could stimulate up-regulation of mucous genes in intestinal goblet cells [18]. Furthermore, the effect of these probiotics on the activation and secretion of mucus in the intestine was directly correlated with the inhibition of pathogenic Escherichia coli attachment and of damage to the intestinal tract.

PROBIOTIC MECHANISMS OF ACTION THAT ARE DISEASE SPECIFIC

Oral tolerance, allergy, and immunity. The capacity to develop oral tolerance is an important deterrent to the expression of allergy and autoimmune disease. Oral tolerance relates to commensal bacteria and innocuous antigens in the gut and prevents excessive immunologic responses to increased amounts of antigen stimulation of the gut. Oral tolerance occurs when innocuous antigens and commensal bacteria cross the mucosal surface and interact with mucosal regulatory T cells that release a cytokine (i.e., transforming growth factor β), which is thought to mediate the down-regulation of humeral and cellular immune responses to those antigens. A recent observation suggests that oral tolerance cannot occur in the absence of colonized bacteria. In a study that compared oral tolerance between germ-free and colonized animals exposed orally to ovalbumin and that measured specific IgE levels to quantify the humeral systemic response, it was noted that oral tolerance, seen as a down-regulation of the IgE anti-ova response, could not be achieved in the germ-free state [19]. In subsequent studies, both humeral and cellular systemic tolerance to oral antigens could be achieved in germ-free animals if they were conventionalized during the newborn period but not if conventionalized as adults, which suggests that early ex-
posure to colonizing bacteria is necessary to achieve oral tolerance [20]. Finally, recent reports have suggested that the expression of TLR-4 on enterocytes is necessary to achieve tolerance [21] and that extensive use of antibiotics would reduce levels of colonizing bacteria and thereby cause a loss of oral tolerance [22]. Finally, probiotics can be used to regain tolerance after extensive use of antibiotics [23].

NEC. A major condition that affects premature infants is NEC. It is thought that necrotic inflammation of the distal small intestine is due to an inadequate, immature, excessive inflammatory response to both pathogenic and commensal bacteria [24, 25]. A recent clinical study in Taiwan showed that the introduction of probiotics (Lactobacillus lactis and Bifidobacteria infantum) a few days after birth in premature babies with body weight <1500 g can prevent the expression of NEC [26]. A meta-analysis of different organisms used as probiotics in this situation has shown that results are generally positive [27]. We reported elsewhere that IxkB, the inhibitor of NFkB, is underdeveloped in premature enterocytes, which in part accounts for the NFkB-mediated, excessive, immature increase in IL-8 production and for the inflammation in NEC [28]. In subsequent studies using a known enterocyte-maturation factor, corticosteroids, we showed that pretreatment with this trophic factor in fetal enterocytes can reduce the IL-8 response to inflammatory stimuli by increased IxkB expression, suggesting that developmental reduction in the expression of genes that regulate the innate immune response may account for the expression of NEC in premature infants [29]. Most recently, using DNA microarrays of fetal enterocyte RNA and biopsy of intestinal specimens from older children, colleagues and I have shown that TLRs and their signaling-molecule genes are up-regulated, and the negative regulators of innate inflammatory response are down-regulated, again suggesting the developmental basis for the extensive inflammatory response (N. Nanthakumar and A. Walker, personal communication). We have also shown that culture media from the same probiotics shown to prevent clinical NEC (i.e., L. lactis and B. infantum [26]) can modify expression of genes of the innate immune response (e.g., TLRs and signaling molecules were decreased and negative regulators were increased) and, at the same time, can strikingly reduce the IL-8 response (N. Nanthakumar and A. Walker, personal communication), which further suggests a mechanism for protection against NEC.

CONCLUSIONS

In this review, I have suggested that, on the basis of the strict definition of probiotics, certain human commensal bacteria may qualify as probiotics. The demonstration of pattern-recognition receptors (e.g., TLRs) on eukaryotic cells, which interact with molecular patterns on pathogens and commensal bacteria, can provide the molecular basis for bacterial-epithelial cross talk, which results in both innate and acquired immune responses. Different probiotics can function differently in the development of intestinal host defenses, as an adjuvant of immune responses or to strengthen the mucosal barrier. Therefore, one needs to be careful when selecting a probiotic for a specific function in the gastrointestinal tract.

Acknowledgments

Financial support. National Institutes of Health (R37 HD12437, RO1 DK70260, P30 DK40561, and PO1 DK33506).

Supplement sponsorship. This article was published as part of a supplement entitled “Developing Probiotics as Foods and Drugs: Scientific and Regulatory Challenges,” sponsored by the Drug Information Association, the National Institutes of Health National Center for Complementary and Alternative Medicine (1R13AT003805-01 to Patricia L. Hibberd), the California Dairy Research Foundation, Chz. Hansen, the Dannon Company, General Mills, Institut Rosell, and Yakult International.

Potential conflicts of interest. W.A.W. is part of the Dannon/Yakult Scientific Advisory Board and is a consultant for the Dannon Company.

References

16. Isolauri E, Juntunen M, Rautanen T, et al. Lactobacillus strain (Luc-


