Interactions Between Microbes and the Gut Epithelium

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Abstract: Gut microbes interact with the epithelium through cell surface components, fermentation products, and extracellular secreted proteins. Host-microbial interactions primarily involve TLRs (toll-like receptors) and NLR (nucleotide-binding oligomerization domain and leucine-rich repeat containing proteins). In a strain and dose-dependent manner, several probiotic strains directly alter tight junction protein expression and/or localization in gut epithelial cells through the release of secreted compounds. Interactions between gut microbes and intestinal epithelial and immune cells are necessary for the development and maintenance of intestinal homeostasis.

Key Words: epithelium, toll-like receptors, tight junctions

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Gut microbes interact with the host through various mechanisms, including structural components [lipopolysaccharide (LPS), S-proteins, DNA, peptidoglycan (PGN)], fermentation products (butyrate, acetate, propionate), and extracellular secreted proteins.[1] Host cells that respond to these mediators include intestinal epithelial cells, goblet cells, paneth cells, macrophages, dendritic cells, and T and B cells.[2] Gut microbes can also respond to signals from other microbes[3] and mediators from the host.[4]

BACTERIAL STRUCTURAL COMPONENTS

LPS is found in the outer membrane of gram-negative bacteria and consists of a lipid and polysaccharide joined by a covalent bond. LPS acts as an endotoxin, and interacts with the CD14/TLR4/MD2 receptor complex to elicit strong immune responses in epithelial and immune cells. LPS activates monocytes/macrophages to produce inflammatory cytokines, including tumor necrosis factor-α, interleukin (IL)-1, and IL-6, which contribute to septic shock.[5] Both gram-positive and gram-negative microbes have PGN within their cell walls. PGN consists of alternating residues of β-(1,4) linked N-acetylglycosamine and N-acetylmuramic acid attached to peptide chains. Bacterial PGNs are recognized by the host's innate immune system primarily through the nucleotide-binding oligomerization domain (NOD)/caspase recruitment domain (CARD) family, which induces inflammation by activating nuclear factor (NF)-κB. The cell wall structure, including the amino acid sequence of lipoteichoic acid (LTA) plays a critical role in determining both the character and magnitude of immune response elicited from host immune and epithelial cells.[6] The importance of LTA in the effects of particular probiotic strains was demonstrated by Mohamadzadeh et al[7] who showed that *Lactobacillus acidophilus* deficient in LTA was able to ameliorate chemically induced colitis, whereas *L. acidophilus* with LTA had no effect.

In 2004, Cobb et al[8] showed that a specific zwitterionic polysaccharide called polysaccharide A (PSA) found on the cell surface of *Bacteroides fragilis* was processed by antigen presenting cells and presented to $CD4^+$ T cells via major histocompatibility complex class II proteins. Further studies demonstrated that PSA was able to induce immunoregulatory and protective responses in the colons of normal mice, which were associated with increased levels of Foxp3+ regulatory cells.[9,10] These effects were dependent on the expression of PSA, as *B. fragilis* lacking PSA did not have the same beneficial effects.[9,10]

Numerous protein or glycoprotein subunits, ranging from 25 to 71 kd, are found in the outermost region of the cell envelope of several strains of bacteria. These surface layers (S-layers) are arranged in a monomolecular crystalline array and have been shown to have numerous functional properties, including acting as virulence factors on pathogens such as *Campylobacter fetus* spp. *fetus* and *Aeromonas salmonicida*[11]; being a depository for surface-exposed enzymes (eg, *Bacillus stearothermophilus*); determining shape in such organisms as *Thermoproteus tenax*[12]; and mediating adhesion on epithelial cells and collagens/laminin (eg, *Lactobacillus helveticus*.[13–15] The S-layer appears to have multiple functions; given the variability in structure and their presence throughout the microbial world, many questions remain as to the antigenic potential of this structure.

HOST RECEPTORS TOLL-LIKE RECEPTORS

Host-microbial interactions primarily involve TLRs (toll-like receptors) and NLR (NOD and leucine-rich repeat containing proteins). The TLR family is a group of pattern-recognition receptors, which recognize conserved motifs referred to as microbe-associated molecular patterns.[16] TLRs detect numerous microbe-associated molecular patterns, including LPS by TLR4; lipoproteins and LTAs by TLR2; flagellin by TLR5; CpG DNA by TLR9; dsRNA by TLR3; and ssRNA by TLR7. Activation of TLRs results in the stimulation of cellular pathways to preserve cellular integrity as well as pathways aimed at defense against microbes. These responses can include the induction of tissue repair and cell survival pathways, cytokine and antimicrobial peptide production and secretion, and maturation of antigen presenting cells.[17] In addition to ligand specificity, individual TLRs differ in their expression patterns and the signal transduction pathways they activate

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appear to be cell-type dependent. TLR signaling in epithelial cells is controlled through numerous mechanisms, including the expression of inhibitory molecules such as toll-interacting protein (TOLLIP), single immunoglobulin IL-1 – related receptor (SIGIRR), IL-1 R-associated kinase 3 (IRAK3), and A20.[18] Intestinal homeostasis and repair is dependent upon the sensing of the intestinal microflora by TLRs.[19] This was clearly demonstrated by Rakoff-Nahoum et al[20], who showed that mice deficient in MyD88 had significant defects in the gut mucosa and deficient repair of the intestinal barrier after radiation or chemically induced colitis.

TLR9 signaling in the gut seems to play a major role in maintaining gut homeostasis. In epithelial cells, TLR9 is expressed on both the apical and basolateral surfaces,[21] and its expression can be up-regulated by pathogenic DNA.[22] In contrast, in immune cells, TLR9 is sequestered within the endoplasmic reticulum, and interacts with endocytosed DNA within endosomes.[23] Epithelial cells can clearly differentiate between DNA isolated from different bacterial species, and respond in a differential fashion.[24] Although probiotic bacterial DNA inhibits NF-κB activation, pathogenic bacterial DNA up-regulates expression of TLR9 on the apical surface of epithelial cells [22] and enhances the release of IL-8 from epithelial cells through activation of NF-κB.[24] Signaling through apical or basolateral TLR9 receptors also results in a differential response by epithelial cells.[21] Apical TLR9 signaling induces the secretion of type 1 interferons and the production of ligands of the Wnt pathway that act to modulate antibacterial peptide production, resulting in the induction of tolerance.[21] In contrast, basolateral signaling of TLR9 induces a classical pro-inflammatory response.[21] TLR9 signaling also has been shown to modulate the equilibrium between T regulatory and T effector cells.[25]

HOST RECEPTORS NLR RECEPTORS

Within the NOD/CARD family of proteins are NOD1 (Card4) and NOD2 (Card15). These 2 intracellular proteins respond to distinct bacterial PGN substructures: NOD2 detects muramyl dipeptide, a molecular motif common to gram-negative and gram-positive bacteria, whereas NOD1 detects PGNs containing meso-diaminopimelic acid, which is more commonly found in gram-negative bacteria.[26,27] Activation of NOD receptors triggers inflammation by the activation of NF-κB and mitogen-activated protein kinase (MAPK) pathways.[28] Other intracellular NLRs include NLRP (NLR family, pyrin domain-containing protein) proteins, NLRC4 (NLR family, CARD-containing protein 4; or IPAF), and NAIP (NLF family, apoptosis inhibitory protein). These NLRs act to recruit and activate inflammatory caspases into complexes called inflammasomes.[29] Caspase-1 processes pro-IL-1β and pro-IL-18 into their biologically active forms and induces cell death.[30] IL-18 appears to exert a dual role in gut homeostasis, with low expression required for wound healing and repair, while excessive production is linked with inflammation.[31] Other receptors involved in the recognition of extracellular proteins secreted by microbes include the ICE protease-activating factor (IPAF)[32–34] and the C-type lectin receptor DC-specific ICAM 3-grabbing non-integrin (DC-SIGN).[35]

EFFECTS OF SECRETED MOLECULES ON EPITHELIAL BARRIER

In a strain and dose-dependent manner, several probiotic strains have been shown to directly alter tight junction protein expression and/or localization in gut epithelial cells through the release of secreted compounds.[36–46] *Lactobacillus rhamnosus* and *Bifidobacterium lactis* were shown to increase epithelial resistance in conjunction with increased phosphorylation of ZO-1 and occludin.[36] Conditioned media from bacterial strains found in the probiotic mixture, VSL#3, (*Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium breve*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus plantarum*, *Streptococcus salivarius* subsp. *thermophilus*) increased resistance of T84 cells, with *B. infantis* exerting the largest effect.[47] This increase in resistance was accompanied by decreased claudin-2 protein expression and increased protein expression of ZO-1 and occludin.[38] *B. infantis*-conditioned medium increased levels of phospho-ERK1 and 2 and decreased phospho-p38, suggesting a role for the MAPK pathway in the effects on epithelial barrier function.[38] *Lactobacillus* GG and LGG-derived soluble proteins (p40 and p75) maintained epithelial barrier function in the presence of hydrogen peroxide-induced disruption by increasing membrane translocation of ZO-1, occludin, PKCβ1, and PKC€ in an extracellular signal-related kinase (ERK1/2) and MAPK-dependent manner.[48]

Probiotics have also been shown to enhance epithelial gut barrier function via increased production of cytoprotective molecules such as heat shock proteins.[49] Heat shock proteins are constitutively expressed in epithelial cells and are induced in stressed cells to help maintain homeostasis.[49] Soluble factors released from *Lactobacillus* GG induced cytoprotective heat shock protein synthesis in intestinal epithelial cells in a p38-dependent and JNK/MAPK-dependent manner.[50] Quorum-sensing molecules secreted by *Bacillus subtilis* also induced epithelial expression of cytoprotective heat shock proteins.[51]

In addition to their effects on tight junction proteins, probiotic strains are also able to prevent cytokine-induced and oxidant-induced epithelial damage by promoting cell survival. *Lactobacillus* GG and soluble factors (p75 and p40) released from LGG prevented epithelial cell apoptosis through activating anti-apoptotic Akt in a phosphatidylinositol-3′-kinase (PI3K)-dependent manner and inhibiting pro-apoptotic p38/MAPK activation.[52,53] This reduction in apoptosis would help to maintain epithelial barrier integrity and increase resistance to pathogens by reducing breaks in the mucosal barrier (Fig. 1).

CONCLUSION

Current understanding of interactions between gut microbes and intestinal epithelial and immune cells suggests that these interactions are necessary for the development and maintenance of intestinal homeostasis. Immune and epithelial responses to microbes depend on the species under study, the concentration of microbes used and the type of cell studied. A barrier between luminal microorganisms and the host immune system is maintained through intestinal epithelial cells, mucus and antimicrobial production, and IgA secretion. It is clear that host-microbial interactions at the gut mucosal surface are critical for

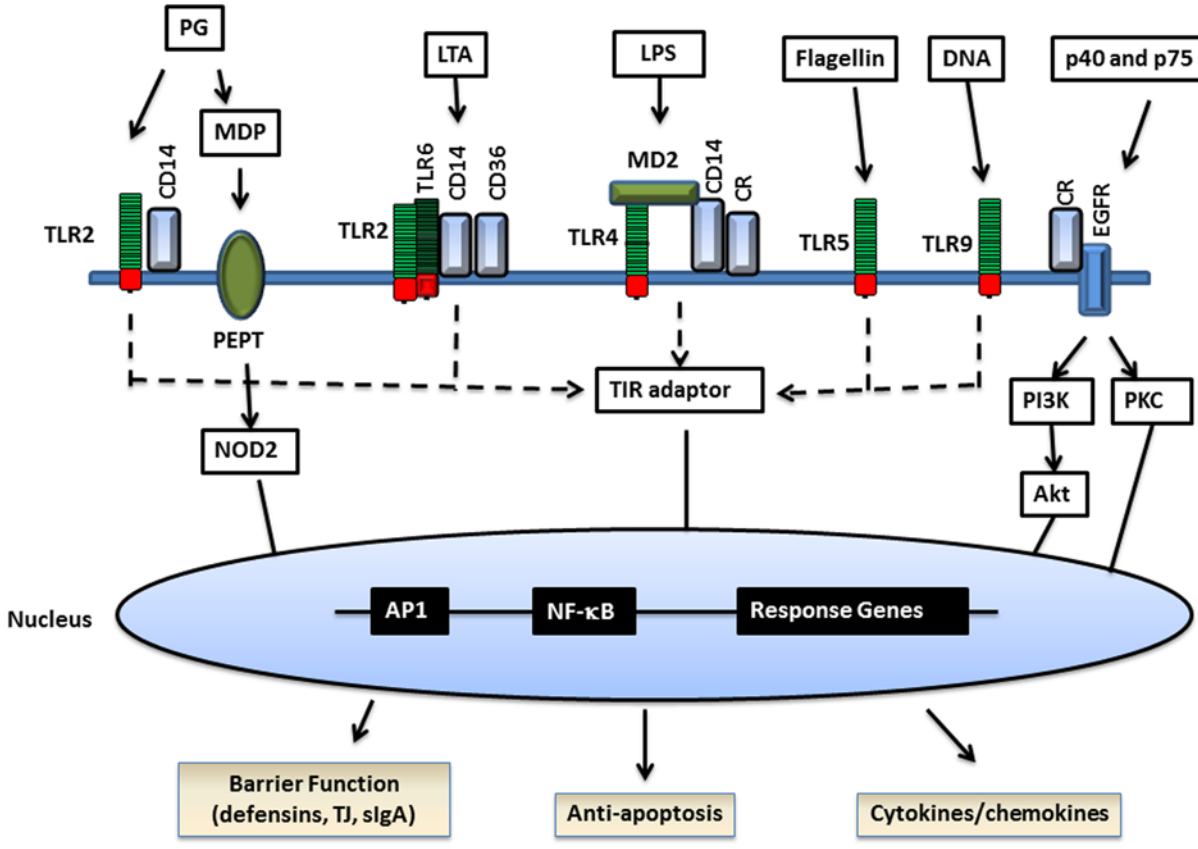

FIGURE 1. Molecules either secreted from or shed from the cellular surface of probiotic bacteria interact with intestinal epithelial cells through numerous receptors. Peptidoglycan (PGN) and lipoteichoic acid (LTA) interact with TLR2. Muramyl dipeptide (MDP) is taken up by the apical peptide transporter PEPT1 and then interacts with NOD2. Lipopolysaccharide (LPS) interacts through TLR4; flagellin through TLR5; and bacterial DNA through TLR9. Proteins released from *Lactobacillus rhamnosus* GG (p40 and p75) can interact with the epidermal growth factor receptor (EGFR). Co-receptors (CR) associated with toll-like receptors in lipid rafts can modulate TLR signaling. Responses to probiotics include the induction of human β-defensin 2; enhancement of barrier function through effects on tight junction (TJ) proteins and sIGA; anti-apoptotic mechanisms; and modulation of cytokines and chemokine secretion. NF-κB indicates nuclear factor-κB; PI3K, phosphoinositol 3-kinase; PKC, protein kinase C; TIR, Toll/interleukin-1 receptor. Adapted with permission from Lebeer et al.[54]

health and overall homeostasis and further, that probiotics may be harnessed to enhance barrier and immune function to maintain health and protect against disease.

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