Epithelial Cells and Their Neighbors.

IV. Bacterial contributions to intestinal epithelial barrier integrity

Anisa S. Ismail and Lora V. Hooper

Center for Immunology, University of Texas Southwestern Medical Center, Dallas, Texas

MAMMALS have coevolved with vast populations of commensal bacteria. The majority of these microbes are found in the intestine, where they are in constant contact with gut epithelial surfaces. The gut’s bacterial consortia colonize starting at birth, eventually reaching population levels as high as $10^{12}$ organisms. Given the size of this population and its intimate contact with intestinal surfaces, it is likely that these bacteria profoundly influence many aspects of intestinal physiology.

For the most part, we share a mutually beneficial relationship with our prokaryotic counterparts. Humans and other mammals depend on intestinal microbes to extract maximum nutritional benefit from their diets (24). Gut bacterial societies are metabolically active, degrading dietary substances that otherwise would be indigestible by the host (23). The microbes derive benefit from these associations as well, as they are given a protected, nutrient-rich habitat in which to multiply. In an environment where nutrients are in short supply, natural selection would likely favor such host-microbial associations, which may explain why these interactions evolved and have been maintained.

Although indigenous bacterial populations provide important metabolic benefits to their hosts, such host-bacterial relationships remain “friendly” only as long as these microbes are effectively coralled in the gut lumen. However, the magnitude of the intestinal surface area renders the underlying tissues highly vulnerable to microbial incursions that can lead to inflammation or sepsis. The intestine is thus faced with challenges that are unique relative to other organs in the body. On the one hand, the gut must accommodate large luminal bacterial populations without mounting an overzealous inflammatory response that could cause collateral damage to host tissues. On the other hand, the gut must be poised to trigger such responses if luminal microbes invade the epithelial barrier.

As a result of these challenges, intestinal surface epithelia have evolved several key functions that facilitate a peaceful coexistence with luminal microbial populations while maintaining immunological vigilance against invading microbes. These functions include downregulation of proinflammatory signaling pathways, expression of key antimicrobial proteins that actively defend epithelial surfaces, and initiation of epithelial repair after mucosal injury. This review focuses on accumulating evidence indicating that resident bacteria play an important role in shaping these functions. These findings underscore the central role of commensal microbes in the development and maintenance of epithelial barrier integrity and suggest that disrupting these host-bacterial associations with broad-spectrum antibiotics may lead to disease.

CELLULAR MAKEUP OF THE INTESTINAL EPITHELIAL BARRIER

The intestine’s internal tissues are separated from the microbe-filled lumen by a single epithelial layer that is only ~20 μm thick. Far from being a homogeneous cell population, however, gut epithelial surfaces are composed of several distinct cell types, each of which contributes in a unique way to mucosal defense and the maintenance of barrier integrity.

The enterocyte is the most abundant cell type at both small and large intestinal epithelial surfaces (Fig. 1). Enterocyte membranes, as well as the tight junctions that are formed between these cells, form an important physical barrier to microbial penetration. However, enterocytes also assume a more active role in defending epithelial surfaces by secreting a variety of antimicrobial proteins. These include two families of small, highly conserved antimicrobial peptides: β-defensins and cathelicidins. Members of both families exhibit broad-spectrum antimicrobial activity against bacteria, fungi, and protozoa by disrupting microbial membranes (25).

Gut surfaces harbor other less-abundant epithelial cell lineages that also help to protect mucosal surfaces from bacterial encroachments. Goblet cells, found in both the small and large intestines, secrete large quantities of mucin, which is composed of highly glycosylated proteins that form a protective layer of gel-like mucus over the surface epithelium. In the small intestine, Paneth cells are the key effectors of antimicrobial defense. These specialized epithelial cells are situated at the base of small intestinal crypts (Fig. 1) and harbor secretory granules containing several microbialidal proteins including α-defensins, lysozyme, and phospholipase A₂. Paneth cells sense bacterial proximity by an unknown mechanism and react by discharging their microbicidal granule contents into the gut lumen (1).
BACTERIA-SENSING MECHANISMS

Activation of epithelial defensive mechanisms frequently requires sensing of bacterial proximity by intestinal surface cells. Recognition of bacteria by epithelial cells is mediated by host-encoded receptors that bind to conserved molecular patterns unique to prokaryotes. These molecular patterns include bacterial cell wall components such as lipopolysaccharide (LPS) and peptidoglycan or protein components of specialized bacterial structures such as flagella. Ligand binding to pattern recognition receptors activates signaling cascades that control transcription of defensive or proinflammatory genes.

Toll-like receptors (TLRs) are a key group of pattern recognition receptors in mammals. To date, 11 mouse and 10 human TLRs have been identified. Each family member recognizes a distinct set of molecules derived from viruses, bacteria, or fungi. At least four TLRs are specific for bacterial patterns. TLR2 and TLR4 recognize the bacterial cell wall components lipoteichoic acid and LPS, respectively. TLR5 detects flagellin, a major protein component of gram-negative flagella. TLR9 binds to unmethylated CpG DNA, which is found in bacteria but not eukaryotic cells. Although TLR9 is localized intracellularly (13), the remaining TLRs are expressed predominantly on the cell surface, where they likely detect extracellular bacteria. Upon ligand binding, each of the TLRs initiates a signaling cascade that triggers the activation and nuclear translocation of the transcription factor NF-κB, which directs expression of proinflammatory genes such as IL-8 and tumor necrosis factor.

The members of the TLR family are expressed variably throughout the intestine. It is still not entirely clear which TLRs are expressed in the gut in vivo and which cell types support their expression. TLR4, for example, is expressed on several intestinal epithelial cell lines (3) and is present in intestinal crypt epithelial cells in vivo (19). However, its expression in gut epithelia is restricted to the cytoplasm (9), suggesting that gut epithelial TLR4 is not stimulated by extracellular LPS. Indeed, it seems reasonable to expect that epithelial cells would be minimally responsive to extracellular LPS, as healthy gut epithelia are not inflamed despite being in constant contact with large bacterial populations and their products. Consistent with this idea, TLR5 is localized to the basolateral surfaces of epithelial cells and promotes an inflammatory response only when bacteria penetrate the tight junctions between epithelial cells (6). Thus compartmentalization of TLRs in the gut is likely important for preventing luminal bacterial populations from triggering uncontrolled inflammation.

A second major group of pattern recognition receptors is the nucleotide-binding oligomerization domain (NOD) family, a group of cytoplasmic proteins. NODs are thought to recognize intracellular microbial components, likely derived from invading bacteria. The best-characterized members of this family are NOD1 and 2, which bind to muramyl peptide, a constituent of peptidoglycan. Whereas NOD1 recognizes muramyl tripeptides from gram-negative bacteria only (7), NOD2 binds a specific muramyl dipeptide common to both types of bacteria (10). Like TLRs, NOD ligand binding activates cytoplasmic signaling cascades leading to NF-κB activation and proinflammatory gene transcription (17). NF-κB is thus a nexus of proinflammatory signaling, receiving and coordinating inputs from multiple pattern recognition receptors.
The continuous and intimate contact between gut bacteria and intestinal mucosal surfaces suggests that indigenous microbes profoundly influence neighboring host cell functions. Consistent with this prediction, a growing body of experimental evidence reveals that luminal bacteria drive key epithelial cell functions that help to maintain barrier integrity. These interactions are likely important for keeping inflammatory processes in check, thus preserving a mutually beneficial relationship with the gut’s indigenous microbial populations.

**Attenuation of proinflammatory responses.** As discussed above, the gut avoids mounting an inflammatory response against its prokaryotic residents despite the proximity of these large bacterial populations to host intestinal tissues. This is underscored by the fact that neutrophil infiltrates, which are hallmarks of clinically significant inflammation, are virtually absent in healthy intestine. As discussed above, such tolerance can be explained in part by the compartmentalization of pattern recognition receptors such that they are activated only by invading bacteria. Additionally, two key studies suggest that certain nonpathogenic gut bacteria actively attenuate the transcription of inflammatory cytokines in surface epithelial cells. Interestingly, the mechanisms revealed by both studies involve interference with the master proinflammatory transcription factor NF-κB.

Intestinal NF-κB is a heterodimer composed of two related proteins, p50 and RelA. In its resting, unactivated state, the NF-κB heterodimer is complexed with IκB (Fig. 2). This interaction blocks nuclear translocation of NF-κB, inhibiting its ability to upregulate proinflammatory gene transcription. Proinflammatory stimuli (such as binding of bacterial ligands to TLR or NOD receptors) elicit a cascade of phosphorylation events culminating in IκB phosphorylation. This, in turn, triggers IκB ubiquitination, targeting the protein for degradation by the 26S proteasome and releasing NF-κB for translo-
cation into the nucleus, where it modulates target gene transcription.

Studies in model epithelia have revealed that nonpathogenic Salmonella strains suppress inflammatory responses by interfering with the dissociation of the NF-κB/IκB complex (16). The mechanism involves inhibition of IκB ubiquitination, which blocks its degradation. The net result is that NF-κB is not released from the inhibitory complex, preventing its translocation into the nucleus to activate proinflammatory gene transcription. Interestingly, pathogenic Salmonella are unable to inhibit IκB degradation and subsequent nuclear transport of NF-κB. Furthermore, if epithelial cells are first exposed to nonvirulent Salmonella, they become refractory to subsequent delivery of proinflammatory stimuli by pathogenic bacteria (16).

A second anti-inflammatory mechanism has been elucidated in the case of Bacteroides thetaiotaomicron. Although the mechanism also involves inhibition of NF-κB-mediated gene transcription, this prevalent member of the human gut flora utilizes a mechanism of interference with NF-κB function that is distinct from nonpathogenic Salmonella. Instead of preventing nuclear import of NF-κB, B. thetaiotaomicron promotes nuclear export of the RelA subunit of NF-κB (Fig. 2), thus interfering with transcription of NF-κB-dependent genes (11). The nuclear hormone receptor peroxisome proliferator-activated receptor-γ is stimulated (through an as-yet-unknown mechanism) to complex with RelA and shuttle it from the nucleus into the cytoplasm. As with Salmonella, this anti-inflammatory effect is bacterial species specific, as the related strain Bacteroides vulgatus does not attenuate proinflammatory signaling by this mechanism (11).

Together, these studies suggest that certain commensal species can directly influence the inflammatory status of gut epithelial cells. However, a number of questions remain. First, what specific bacterial cues attenuate epithelial inflammatory responses? Second, although inflammatory pathways may need to be dampened under normal circumstances, they must be activated when pathogens penetrate the epithelial barrier. How is inflammatory attenuation overridden when invading bacteria are detected? Third, it is not yet clear whether these anti-inflammatory pathways operate in vivo. Nevertheless, these findings strongly point to the idea that commensal bacteria engage in active cross talk with epithelial cells, profoundly affecting epithelial integrity and mucosal health.

**Bacterial modulation of gut antimicrobial defense.** Although downmodulation of proinflammatory signaling undoubtedly contributes to mucosal tolerance to commensal bacteria, other compelling evidence suggests that under normal, healthy conditions, the systemic immune system is largely ignorant of noninvasive luminal bacteria. Mucosal secretions such as secretory IgA and antimicrobial proteins play a critical role in preventing luminal bacteria from crossing the epithelial barrier, where they can initiate adaptive immune responses and inflammation (12, 15). Recent evidence suggests that indigenous gut bacteria collaborate with the host to maintain this state of immunological ignorance by inducing expression of at least one component of the intestinal antimicrobial protein arsenal.

Angiogenin 4 (Ang4) is a protein found in abundant quantities in Paneth cell secretory granules (Fig. 1). Although it is a member of the angiogenin family, which includes proteins with proposed angiogenic functions, Ang4 and its related family members exhibit potent bactericidal activity (8). Its microbicidal activity, its localization in Paneth cell granules, and the fact that it is discharged into the lumen in response to bacterial signals indicate that Ang4 plays a role in the defense of mucosal surfaces. By preferentially targeting gram-positive organisms (8), Ang4 may also help to establish and maintain the predominantly gram-negative bacterial populations found in adults.

Studies comparing germ-free mice with those harboring a diverse microbial flora have disclosed that Ang4 expression is triggered by commensal bacteria (Fig. 1) (8). In mice that have normal gut flora, Ang4 expression increases dramatically during weaning (when young mice switch from mother’s milk to a regular diet) and quickly reaches adult levels. By contrast, germ-free mice never achieve high Ang4 expression levels, indicating that full expression of Ang4 in Paneth cells requires the presence of commensal bacteria. However, this deficiency is reversible. By exposing adult germ-free mice to commensal bacteria, Ang4 message levels rise to match those found in conventionally-colonized mice. Furthermore, the gut commensal species B. thetaiotaomicron is sufficient to stimulate conventional adult Ang4 expression levels (8).

The bacterial signals that lead to Ang4 induction remain to be defined. It will also be important to determine whether Paneth cells are direct recipients of bacterial signals or whether they receive signals relayed from surrounding host cells. Consistent with the direct interaction model, Paneth cells express at least one pattern recognition receptor, NOD2 (18). Furthermore, NOD2 has been linked to Paneth cell expression of at least two members of the α-defensin family of antimicrobial peptides (12).

Ang4’s postnatal expression pattern suggests that commensal bacterial interactions with Paneth cells help to shape the intestinal antimicrobial arsenal during weaning, a key developmental transition in the gut. One view of this interaction is that it represents an active host effort to maintain epithelial barrier integrity and limit bacterial penetration of mucosal surfaces despite the withdrawal of maternal antibodies during this period. In addition, microbe-stimulated antimicrobial proteins such as Ang4 may contribute to shaping the composition of the gut’s microbial community, which undergoes a dramatic shift in species composition during weaning. Although it is not yet known whether commensal bacteria stimulate expression of other Paneth cell antimicrobial proteins in addition to Ang4, bacteria-regulated antimicrobial defenses could help to promote intestinal colonization by beneficial microbes while minimizing penetration of mucosal barriers by these bacteria.

**Bacterial stimulation of intestinal epithelial repair.** The vast surface area of the gut epithelium is constantly exposed to ingested foreign substances as well as to luminal microbes. It is thus susceptible to damage by a variety of factors, including environmental toxins and pathogenic bacteria. The presence of large indigenous microbial populations means that gut epithelial damage can quickly lead to bacterial penetration, inflammation, and sepsis. The intestinal mucosal surface must therefore be able to recognize and repair damage rapidly and efficiently. Interestingly, two recent studies reveal that luminal gut bacteria actively trigger mucosal repair through a mechanism involving TLR signaling.

Numerous analyses of the effects of gut mucosal damage have relied on the dextran sulfate sodium (DSS)-induced...
model of epithelial injury. In this model, direct colonic epithelial injury is initiated in mice within a few days after ad libitum administration of DSS in drinking water. Epithelial damage is apparent through the appearance of focal colonic lesions, is accompanied by increasing mucosal permeability, and can be detected well in advance of an ensuing inflammatory response. Removal of DSS from drinking water initiates a complex tissue repair pathway that results in a vigorous enterocyte proliferative response and restoration of intact epithelium (4).

Recent work has revealed that efficient colonic epithelial repair requires the presence of resident gut bacteria. Using the DSS-induced injury model, Rakhoff-Nahoum et al. (21) found that mice lacking most of their gut microflora due to broad-spectrum antibiotic treatment are more susceptible to DSS-induced epithelial injury than fully colonized mice. However, recolonization of antibiotic-treated mice with commensal bacteria restores their ability to repair damaged mucosa.

Mice deficient in TLR signaling are unable to fully heal epithelial damage even in the presence of commensal bacteria. MyD88 is an intracellular protein required for signaling through all TLRs. Mice lacking this protein show profound defects in their ability to repair DSS-induced mucosal damage. Bacterial activation of TLRs in damaged mucosa moreover induces expression of several factors known to contribute to cellular protection, including IL-6, KC-1, and heat shock proteins (21). Together, these results suggest that bacterial activation of TLR signaling pathways plays a critical role in directing colonic tissue repair processes.

The DSS injury model has also yielded important clues about which intestinal cell populations drive microbe-regulated mucosal repair. Pull et al. (20) found that bacteria are required for the proliferation of epithelial progenitor cells that fuel the replacement of damaged epithelium with new cells. As in the Rakhoff-Nahoum study, TLR signaling plays an essential role in this damage response, as MyD88-deficient mice exhibit profound deficiencies in epithelial progenitor proliferation. Moreover, studies in mice lacking various immune cell populations reveal that macrophages are required for the colonic epithelial proliferative response. After DSS-induced injury, colonic macrophages are recruited to sites of active epithelial proliferation where they become juxtaposed to epithelial progenitor cells and express factors involved in stimulating cellular proliferation (20).

These results suggest a model in which epithelial repair is driven by bacterial activation of mobile subepithelial cell populations. The intact mucosal barrier likely prevents detection of bacteria by TLR-bearing cells such as macrophages. However, epithelial damage and subsequent bacterial penetration may be detected by engagement of macrophage TLRs. Bacterial penetration of mucosal barriers could thus drive a damage response program that induces macrophage migration to injured areas and expression of mitogenic factors that stimulate epithelial cell proliferation.

**IMPLICATIONS FOR HUMAN HEALTH**

Until recently, host-microbial associations have been viewed predominantly as threats to human health. This view has spurred the development of numerous antimicrobial therapies, such that many diseases that were life threatening in the past are now treatable by the simple administration of antibiotics. However, there is increasing concern that the widespread use of antibiotics may lead to a different set of problems, including an increasing occurrence of opportunistic infections and the emergence of antibiotic-resistant pathogens (14).

The role of indigenous gut bacteria in shaping mucosal barrier function suggests that antibiotic use may also severely compromise the maintenance of epithelial health. By disrupting the composition and stability of indigenous microbial populations, antibiotics likely interfere with the beneficial cross talk required to develop and maintain a robust epithelial barrier. This could facilitate both pathogenic and commensal insults to mucosal surfaces and lead to inflammation and sepsis.

Understanding the role that indigenous bacteria play in promoting development of healthy mucosal barrier function may also shed light on the fundamental causes of inflammatory bowel disease (IBD). IBD denotes a group of disorders characterized by chronic gut inflammation and includes both Crohn’s disease and ulcerative colitis. IBD is frequently characterized by an abrogation of tolerance toward luminal bacterial antigens, resulting in an excessive inflammatory response (22). A number of studies now implicate dysregulated epithelial barrier functions in IBD pathogenesis (12). This makes sense, as strict confinement of commensal bacteria to the luminal side of the mucosal barrier is likely essential for keeping inflammation in check. Interestingly, the rising incidence of IBD in the United States has been linked to increased antibiotic use (2) and excessive hygiene (5), suggesting that factors that disrupt normal host-bacterial cross talk may compromise barrier integrity and lead to increased invasion of indigenous bacteria with ensuing inflammation.

**SUMMARY AND FUTURE PROSPECTS**

In this review we have highlighted several ways in which commensal gut bacteria shape mucosal barrier function and epithelial barrier integrity. Indigenous microbes actively attenuate epithelial inflammatory responses, stimulate antimicrobial defenses, and promote gut epithelial repair. These contributions point to a profound intertwining of microbial and host biology in the intestine. However, a number of challenges remain, including gaining a better understanding of the composition of the intestine’s microbial populations and determining the molecular nature of the microbial signals that promote fundamental changes in the properties of the epithelial barrier. Meeting these challenges will undoubtedly yield new insights about the consequences of broad-spectrum antibiotic use and its impact on the development and maintenance of the mucosal barrier. By fully elucidating microbial contributions to intestinal physiology, we will be better able to harness the power of our bacterial allies to promote human health.

**ACKNOWLEDGMENTS**

Thanks to Heather Cash for comments and helpful discussions during preparation of the manuscript.

**GRANTS**

Work in the authors’ laboratory is supported by the Crohn’s and Colitis Foundation of America and by a Burroughs Wellcome Foundation Career Award in the Biomedical Sciences (to L. V. Hooper).
REFERENCES


