Pathologic and physiologic interactions of bacteria with the gastrointestinal epithelium

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ABSTRACT Communication between microorganisms and the gastrointestinal epithelium, ie, bacterial-epithelial “crosstalk,” is examined. Because most basic research on the molecular interaction of bacteria with the gut epithelium relates to pathogen-epithelial cell interaction, crosstalk with pathologic bacterial is considered in detail. Through their interactions with the intestinal epithelium, pathogens can modify epithelial function to enhance their penetration across the epithelial barrier and to exploit mucosal host defenses for their own benefit. Three representative pathogens are used to illustrate the various adaptive techniques used to colonize and penetrate the mucosal barrier. Salmonella enterica typhimurium interacts with the physiologic receptor for enteropathogenic bacteria, and resident flora with the intestine is the focus of this review. A particular emphasis will be the signaling pathways used in this “crosstalk” between enteric pathogens and their intestinal host.

Bacterial pathogens possess highly specialized adaptive processes that enable them to co-opt epithelial cell functions to augment their penetration of the host intestinal epithelium to cause disease. A necessary step in the successful colonization and ultimate production of disease is the ability of bacterial pathogens to adhere to host surfaces, which is an important determinant of virulence. Generally, binding to intestinal host cells is essential for the bacteria to resist the fluid flow of the luminal contents and the peristalsis of intestinal contraction. Once bound to the epithelial surface, bacteria may colonize and establish a permanent residence in the gut. Bacterial adhesion to host cells or surfaces is often an essential first stage in the disease process because pathogens have become localized to an appropriate target site. A wide range of mammalian cell surface constituents, including glycoproteins and glycolipids, can serve as receptors for bacterial attachment (3). The host cell is often an active participant in the adhesion process and does not function simply as an inert surface for attachment. Many pathogens possess a specific set of virulence factors that have evolved to affect the host. On the other hand, the host in turn has specialized strategies to resist such infections, often in response to these virulence factors or the damage caused by them. This interaction defines the disease process or lack thereof (4).

MECHANISMS OF BACTERIAL-EPITHELIAL CROSSTALK

Many microbial pathogens attach to and invade host cells that are not phagocytic. The process involves the interaction of...
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Microbial surface determinants with host receptors capable of transmitting signals that can disrupt the cytoskeleton and trigger entry of the pathogen (5). These pathogens share common mechanisms of interaction with the host, but each species has also evolved a repertoire of unique approaches to exploit host processes. For example, the entry of the enteric pathogen Salmonella enterica typhimurium into cultured epithelial cells is coincident with tyrosine phosphorylation of the receptor for epidermal growth factor (EGF) (6). Also, both S. typhimurium and EGF induce patterns of host tyrosine phosphorylations that are remarkably similar (6). In other words, in this case, the bacterial pathogen enhances its own attachment by co-opting the host cell’s receptor phosphorylation to a signal transduction pathway that favors the pathogen.

More dramatically still is the sequence of events involved in the adhesion of enteropathogenic Escherichia coli (EPEC), pathogens that cause diarrhea in young children. EPEC adhere to intestinal epithelial cell surfaces by destroying host microvilli and rearranging the actin cytoskeleton to form a pedestal on the host cell surface (7). The results of recent studies suggest that EPEC can translocate their own protein receptor termed Tir (for translocated intimin receptor) into the host cell. Tir has ≥ 3 functions: to bind intimin, to cause cytoskeleton rearrangements induced by EPEC, and, once attachment has occurred (8) in conjunction with intimin, to cause additional host signaling events.

Many enteric pathogens have developed a specialized secretion system, called type III secretion, to mediate the direct transfer of proteins into the host cell membrane. Through this mechanism, extracellular bacteria that are in close contact with eukaryotic cells can deliver bacterial proteins into the cytosol of these cells (9). Many effectors of type III secretion that appear to act directly on intracellular host factors (10, 11) have been identified in Salmonella spp., Shigella spp., EPEC and enterohemorrhagic E. coli, and Yersinia spp. Once secreted into host cells, these soluble molecules activate epithelial cells by tyrosine kinase phosphorylation, Ca²⁺ mobilization from intracellular stores, or activation of small GTP-binding proteins that belong to the Ras superfamily, eg, Rac, Rho, and CDC42. This process eventually leads to an actin-cytoskeleton rearrangement and entry of the bacteria into the cell (12).

Importance of bacterial-epithelial crosstalk

The interfaces between a mammalian host and its microflora in the intestinal lumen consist of the mucous layer, the underlying cell coat (or glycocalyx), and glycoconjugates on the apical surface of the enterocyte (3). The first challenge to potential luminal pathogens is to successfully attach to the intestinal surface. The indigenous intestinal microflora is known to interfere with colonization by pathogens, and competition for binding sites is likely to play a role in this protection. It is now apparent that the presence of indigenous intestinal microflora can also influence the expression of glycoconjugates by host epithelial cells. Recent studies suggest that the host epithelial cell can express specific glycoconjugates in response to the presence of bacteria (D Dai, unpublished observations, 2000). These bacteria can utilize the sugar moiety that makes up the oligosaccharide unit of these surface macromolecules. These oligosaccharides can serve as receptors for the attachment of microorganisms (3).

It seems that pathogenic microorganisms have evolved to exploit the otherwise mutually beneficial crosstalk between indigenous microflora and the host by attaching to these same glycoconjugates (Figure 1). This observation has implications for the use of model systems to study disease pathogenesis and for strategies to prevent or to treat gastrointestinal infectious disease.

Membranous epithelial cells (M cells) of the gut, in addition to sampling and transporting luminal antigens, are also a potential pathway for pathogen invasion. M cells reside throughout the gut as follicle-associated epithelium that overlays lymphoid follicles, eg, Peyer’s patches, and belong to functional units that constitute inductive sites for the mucosal immune response (13). M cells have a poorly organized brush border, an active endocytic capacity, and lymphocytes juxtaposed to their basolateral surface. The lack of mucus and glycocalyx facilitates contact between molecules and particles and the apical surface of the M cells. Several microorganisms, particularly bacteria, take advantage of the transcytotic function of M cells and use them to cross the otherwise impermeable epithelial lining of the gut. In experimental animal models, several gram-negative bacteria that cause disease by colonizing or invading the intestinal mucosa were noted to bind either preferentially or exclusively to M cells. This binding is the case for E. coli, S. typhimurium, Shigella flexneri, and other pathogens (14). These interactions of pathogens with M cells may lead to changes in the host cell cytoskeleton that result in bacterial entry into the cell. In addition, microbial entry into epithelial cells results in the production and release of proinflammatory cytokines (15). These mucosal surfaces of the gut serve as an interface between the internal microenvironment of the body and the milieu of the gut lumen, which varies widely and frequently contains microbes and other potentially injurious agents. Accordingly, epithelial cells must be able to mount an acute host defense response to contain these agents. Thus, in response to bacterial invasion, intestinal epithelial cells can activate intracellular signals that lead to the initiation and amplification of an acute mucosal inflammatory response.

To maintain the integrity of these vulnerable cellular barrier functions, mucosal surfaces are equipped with specialized innate and adaptive defense mechanisms, many of which have an immune component, eg, the mucosa-associated lymphoid tissue (16). Besides the need for lymphoepithelial interaction, the

FIGURE 1. Crosstalk between intestinal bacteria and the host epithelium. Colonization by indigenous microflora induces the expression of fucosylated glycoconjugates on the host intestinal epithelium. The expression of the glycoconjugates provides lectin-like receptors for the attachment of luminal pathogens and eventually confers susceptibility to pathogen colonization and disease. Adapted from reference 3.
development of Peyer’s patches and M cells seems to require an interaction with the intestinal microflora (17). Germfree mice have a reduced number of Peyer’s patches even though they are fully immunocompetent, but lymphoid follicles and M cells increase in number after transfer of germfree mice to a conventionalized environment. Repopulating germfree mice with a single strain of nonpathogenic bacteria or with a single pathogen is sufficient to restore the normal number of Peyer’s patches with follicular-associated epithelium or M cells (17).

Adherence to and uptake of microorganisms by M cells appear to allow an efficient sampling of foreign antigens by inductive sites of the mucosal immune system, resulting in secretion of protective polymeric immunoglobulin A (pIgA) antibodies to prevent uptake of antigens, thus limiting the intensity and duration of the disease (15). However, as stated above, some pathogenic microorganisms exploit the M cell transport process to gain access to the underlying intestinal mucosa and have developed diverse strategies to escape the host’s protective immune responses (13).

When a pathogen comes into contact with the host, a struggle between the pathogen and the local innate host defense systems ensues. The resolution of this encounter is a critical determinant of whether the interaction leads to infection and overt disease. Localized host defenses are only the first line of defense and are in close communication with the humoral immune system (11, 18, 19). Thus, microorganisms that survive the initial interaction with local host defenses usually activate other protective immune responses that attempt to contain the infection locally (19). Because of this selective defense, successful bacterial pathogens must develop strategies to avoid or circumvent established host defense mechanisms. Several pathogens can directly alter components of the host’s immunophysiologic response and modulate this activity to their own advantage. For instance, infection of monolayers of human colon epithelial cell lines with *Shigella*

results in the coordinate expression and production of a specific array of proinflammatory cytokines, such as interleukin 8, monocyte chemoattractant protein 1, granulocyte-macrophage colony-stimulating factor, and tumor necrosis factor α. Presumably, this response is geared to protect the host by evoking inflammation, but may also be used by the pathogen to destroy the epithelial barrier and gain greater access to the intestinal milieu (20).

Pathologic bacterial–epithelial crosstalk

Recent advances in the fields of molecular microbiology and immunology have provided insights into the interactions between some highly adapted enteric pathogens and their hosts. A recent development in this field is the identification of a highly specialized protein secretion system by several gram-negative organisms. This process, which is termed type III secretion, has evolved to deliver proteins from bacteria to the host cell. These bacterial proteins can then stimulate or interfere with host cellular processes, thereby dictating the terms of the bacterial-host interaction. In this review, this crosstalk between pathologic bacteria and the epithelium will be illustrated by considering the representative enteric pathogens EPEC, *Salmonella* spp., and *Shigella* spp.

**EPEC**

EPEC are one of a class of pathogens that cause attachment and effacement lesions on intestinal cells, characterized by localized destruction of brush border microvilli, intimate bacterial adhesion, and gross cytoskeletal rearrangement leading to the formation of actin-rich pedestals (7). EPEC require intimate attachment to host cells for maximum virulence to occur. This involves several bacterial factors, including EPEC-secreted proteins (EspS), type III secretion, and the expression of the outer membrane protein intimin. Release of extracellular proteins via a type III secretion apparatus is necessary for the formation of attaching and effacing lesions by EPEC (21, 22). The intimate attachment of the bacteria with the host cells is via intimin binding to a 90-kDa tyrosine phosphorylated protein in the host membrane (23). This receptor, Tir, originally thought to be a host protein, was recently found to be of bacterial origin. Tir is translocated from the bacterial cell into the host membrane, where it becomes phosphorylated on tyrosine residues and binds to intimin (24). The tight association between intimin and Tir necessitates steps leading to the formation of an actin-rich pedestal (8).

The host cell undergoes several changes during infection by EPEC (Figure 2). The most striking change in the cellular structure is the actin cytoskeletal rearrangement that leads to the formation of an actin-rich pedestal (25). Several signal transduction pathways appear to be stimulated in epithelial cells after infection with EPEC. Shifts in intracellular calcium concentrations seem to play a role in the pathogenesis of EPEC (26). Furthermore, an increase in intracellular calcium results in the depolymerization of actin by villin and a breakdown of the host cytoskeleton similar to that seen in EPEC-infected cells. Elevated inositol triphosphate concentrations and fluxes have also been observed in EPEC-infected cells.

**Salmonella**

*S. typhimurium* is a species of gram-negative bacteria that causes various diseases from gastroenteritis to typhoid fever. *S. typhimurium* infections are contracted by oral ingestion of the pathogen and penetration of the pathogen across the intestinal epithelium before the induction of systemic disease. Before
invasion, bacteria must encounter and attach to one or more enterocyte cell types found in the intestinal tract (Figure 3). *S. typhimurium* also induces transepithelial migration of neutrophils in an in vitro assay system (27). Once in close contact with the epithelium, salmonellae induce degeneration of the enterocyte’s microvilli, followed by profound membrane “ruffling” localized to the area of bacteria–host cell attachment. Membrane ruffling is accompanied by extensive endocytosis and internalization of the bacteria into host cells. The mechanisms by which salmonellae stimulate nonphagocytic cells to internalize bacteria are clearly complex and include components of a type III secretion apparatus, enterocyte regulatory proteins, and secreted effector proteins and their chaperones (28). Type III secretion is activated on host–bacteria contact and results in the export of virulent determinants directly into the host cell, where they influence steps in the bacteria’s uptake (29).

Invasive *S. typhimurium* induces a dramatic actin rearrangement on the luminal surface of mammalian cells as part of its pathogenic mechanism. This process requires many host factors. For example, the Rho subfamily member Cdc42 is needed to mediate bacterial uptake through membrane ruffling (reviewed in references 15, 28, 30, 31). Membrane ruffling similar to that induced by *S. typhimurium* also results after exposure to physiologic amounts of growth factors. These changes closely resemble the membrane changes that occur when a growth factor binds to its receptor and it was suggested that the EGF receptor on enterocytes plays an important part in salmonella invasion in vitro (32). Other experiments showed that infection of cultured epithelial cells with wild-type *S. typhimurium* is accompanied by a tyrosine phosphorylation of the EGF receptor, whereas infection with a noninvasive *S. typhimurium* invA mutant did not have that effect. Furthermore, the administration of EGF to cultured cells allowed for invasion by *S. typhimurium* ΔinvA, but not by an adherent, noninvasive *E. coli* strain (6). These collective observations implicate tyrosine phosphorylation of the EGF receptor as being centrally involved in bacterial-induced host signal transduction and communication with the cytoskeleton.

**Shigella**

*S. flexneri* is an invasive pathogen that causes both secretory and bloody diarrhea (bacillary dysentery). *S. flexneri* directs its own uptake into the colonic mucosa through membrane ruffling in a manner similar to salmonella uptake, namely, via a plasmid-encoded Mxi/Spa apparatus (a gene cluster whose products are responsible for invasion) and secreted effector proteins that induce endocytosis of shigellae by colonic M cells (14). Shigellae use the M cell for their initial entry. Once across the luminal surface, the bacteria may invade adjacent intestinal epithelial cells (Figure 4). By co-opting cytoskeletal components of the host, the bacteria can obtain greater intracellular movement (12). Once the microbes have escaped their vacuole, they quickly become coated with a filamentous actin and ultimately form an actin tail. This actin polymerization provides a propeller for the bacteria to cross the cytoplasm. When the pathogen reaches the plasma membrane of the cell, it forms a long protrusion into the neighboring cell, which subsequently internalizes the microbe (12). This process leads to lateral translocation through the epithelial basolateral surface, invasion of epithelial cells, and stimulation of an intense inflammatory reaction that eventually causes tissue destruction (33).

**Summary**

Bacterial pathogens have evolved several mechanisms to utilize the host’s cell signaling machinery and disrupt the cytoskeleton. EPEC mediate their effects on the host cell from the cellular surface, secreting their own receptor (Tir) into the host and then binding intimately to the host by its intimin. Salmonellae, on the other hand, actively invade intestinal epithelial cells by inducing membrane ruffling and endocytosis. Salmonellae may directly bind to an EGF receptor or may indirectly activate this signal transduction pathway. Shigellae are also an invasive pathogen that lyse their endocytic vacuole and co-opt host mobile proteins to initiate intracellular actin-based locomotion to spread from cell to cell via the cytoplasm. Despite the outward differences between each mode of pathogenesis, EPEC, salmonellae, and shigellae have effectively managed to subvert the host cytokinet machinery for their own purposes and cause substantial diarrheal disease (reviewed in 11, 12, 15, and 31) via bacterial-epithelial crosstalk.

**Physiologic bacterial-epithelial crosstalk**

The application of molecular techniques to the study of bacterial pathogenesis has resulted in remarkable discoveries and has changed the way we view bacterial-host interactions. Many critical reviews have discussed these mechanisms and the molecular basis of crosstalk between enteric pathogens and the intestinal epithelium. We summarized just a few of these interactions in the previous sections. However, as mentioned, a complex and dynamic microbial ecosystem inhabits the human intestine. The establishment of the intestinal microflora begins at birth and progresses throughout life. Normal gut flora inhabit the intestinal tract in large numbers and diversity and the interactions of these colonizing microbes with the host are complex. As the field of mucosal immunology has developed and the mechanisms of intestinal host defense have been better defined, we have gained a greater appreciation for the role of indigenous flora (probiotics) in this process. Studies of germfree animals have shed light on the interaction between the epithelium and resident microbes during normal development of the gut’s mucosal immune system, specifically, on the role of nonpathogenic bacteria in the development of the intestinal immune system and the prevention of pathogenic insults to the host. These studies have helped in our understanding of how probiotics alter host immune function for the host’s benefit.
Mechanisms of probiotic host defense

The results of several studies suggest that the resistance to colonization by enteric pathogens prompted by gut commensals results in preventing large inoculums of pathogens that could cause clinical symptoms and pathologic changes (34). These observations strongly suggest that the resistance to colonization conferred by the normal gut flora is a potent mediator of protection, most likely through several complex interacting mechanisms. These include competing more successfully for essential enteric nutrients or for epithelial attachment sites, producing antimicrobial compounds, and metabolizing nutrients into volatile fatty acids and chemically modified bile acids that in turn create a local environment that is generally unfavorable for the growth of many enteric pathogens. By inducing the recruitment of lamina propria immune cells, the normal gut flora also can activate appropriate inflammatory or immune mechanisms against chronic infection (35). In return for providing the host with nutritional benefits and enhanced defense against pathogens, the indigenous microflora gains access to a nutrient-enriched, stable environment, and thereby enters a symbiotic relation with the host’s intestinal tract (36).

Enhanced immunoresponsiveness

The fact that the normal flora substantially influences host defenses in the gastrointestinal tract was not well understood until germfree animals became available for study. For example, antibiotic disruption of the body flora of mice inhibits granulocyte formation in the bone marrow, suggesting that microbes or microbial products stimulate the host to generate this leukocyte lineage (37). In addition, when new bacterial species colonize the host at the time of weaning, there is an associated increase in the number of plasma cells throughout the intestine (38) that does not occur in germfree mice. The results of additional studies suggest that the normal colonization of the mammalian intestine with commensal microbes might contribute to the development of both the humoral and the cellular mucosal immune systems (39). These interactions between the mucosal immune system and enteric microbes maintain a physiologically controlled inflammation or activation of gut-associated lymphoid tissue throughout life (40). It has been known for decades that commensal microbes colonizing the neonatal mammalian gut can increase circulating specific and natural antimicrobial antibodies (2, 41). Also, the germinal center reactions of the Peyer’s patches, which preferentially generate IgA-committed, antigen-specific B cells, depend on chronic and novel gut mucosal stimulation by enteric bacterial antigens (2, 39). Stimulation of the intestinal immune system’s development by microflora and at the same time host tolerance of these same microflora, indicates a complex symbiotic mechanism that we are only beginning to explore at the mechanistic level. This mechanism is likely associated with bacterial-epithelial crosstalk.

Studies in germfree animals showed that in the absence of intestinal microflora the intestinal immune system is underdeveloped and intestinal morphology is disrupted (42). Altered immune profiles in germfree animals include underdeveloped Peyer’s patches, mesenteric lymph nodes lacking germinal centers and plasma cells (17), decreased macrophage chemotaxis, and a lower capacity for intracellular killing of pathogens compared with macrophages from conventionalized animals (37, 43). The functional capacity of the acquired immune system is generally similar in germfree and conventionalized animals, but the number of intestinal lymphocytes is greatly reduced in germfree animals (43). In most cases, germfree animals have a predominant production of IgM, little IgG, and no IgA. When germfree animals are transferred to a conventionalized facility or fed probiotics, their gut morphology and the mucosal immune system develop quickly and the animals begin to produce a greater diversity of antibody isotypes, including antibodies specific for resident intestinal bacteria (44).

Other mechanisms

As stated, adhesion of pathogenic bacteria to the mucosal surface is considered to be the first step of intestinal infection. Furthermore, adhesion can be inhibited by physically blocking the receptor for specific adhesin analogues or by steric hindrance. Recent evidence suggests that colonization resistance to enteric pathogens is prompted by gut commensals, ie, a need for considerably larger doses of pathogens to cause clinical symptoms and pathologic changes (45, 46). Some probiotic bacteria with beneficial health effects have also been noted to adhere to the intestinal mucosa (46, 47), suggesting that adhesive probiotics
Bacterial-epithelial crosstalk can prevent the subsequent attachment of pathogens, a phenomenon known as competitive exclusion (Figure 5).

Probiotics are defined as live microbial food supplements that beneficially affect the host by improving its intestinal microbial balance. There are several reports of disease prevention or enhancement of immune function resulting from the oral administration of probiotics such as Lactobacillus and Bifidobacterium species. When ingested in large enough quantities (\(1 \times 10^{15}/L\)) and for long enough periods (\(>2\) wk), these bacteria can be incorporated into the resident gut flora (49). For example, certain Lactobacillus strains inhibit E. coli adhesion to porcine enterocytes and attachment to porcine mucus (50). Furthermore, metabolic end products of lactobacilli present in cell-free culture supernatant fluids inhibit adhesion or invasion by pathogenic bacteria (51). Probiotic Lactobacillus strains also inhibit mucosal adherence of EPEC and S. typhimurium in vitro (51).

In addition to preventing colonization by pathogens, probiotics may strengthen the epithelial barrier, thereby preventing pathogenic translocation of the epithelium (49, 52) by promoting accelerated epithelial repair. For example, oral administration of Lactobacillus acidophilus can ameliorate diarrhea in patients undergoing pelvic radiotherapy, perhaps by preventing radiation-induced damage to the intestinal epithelium (53). Furthermore, enhanced macromolecule intestinal permeability and translocation of intact proteins induced by cow milk was reversed in suckling rats fed milk supplemented with Lactobacillus casei GG (53).

These alterations in the function of the mucosal barrier induced by probiotics undoubtedly require a biochemical communication between the microorganisms and adherent enterocytes. For example, the probiotic organism may induce a signal transduction pathway that strengthens tight junctions between enterocytes and thereby reduces paracellular transport of antigens that cause inflammation. Additional studies of probiotic-epithelial crosstalk are necessary to better understand the role of commensal gut bacteria as immune modulators in health and disease.

### SUMMARY AND CONCLUSION

Mammalian epithelial surfaces are remarkable for their ability to provide critical physiologic functions in the face of frequent microbial challenges. In recent years, studies have focused on the molecular mechanisms of the interactions between gut bacteria, the intestinal epithelium, and immune cells. A better understanding of bacterial-enterocyte crosstalk has resulted in the identification of essential virulence gene products and the characterization of cellular components involved in pathogenicity. Our understanding of how intestinal pathogens interact with the intestinal mucosa to cause disease has advanced significantly during the past decade. Of importance is the integration of the host’s mucosal immune function and epithelial barriers, which plays a critical role in the outcome of infection. An understanding of this integration is necessary for a full understanding of pathogenesis. A new area of interest in human studies is the role of commensal flora and probiotics in protecting against bacterial disease. We must first understand the molecular mechanisms of probiotic-epithelial crosstalk to exploit the mechanisms of these bacteria to enhance mucosal immune responsiveness and inhibit pathogen colonization. A better understanding of probiotic-epithelial crosstalk can be used to devise new strategies to prevent and treat bacterial infections of the gut.

### REFERENCES