themes

Microbes and Microbial Toxins: Paradigms for Microbial-Mucosal Interactions

VII. Enteropathogenic *Escherichia coli*: physiological alterations from an extracellular position

GAIL HECHT
Department of Medicine, Section of Digestive and Liver Diseases, University of Illinois at Chicago, West Side Department of Veterans Affairs Medical Center, Chicago, Illinois 60612

Hecht, Gail. Microbes and Microbial Toxins: Paradigms for Microbial-Mucosal Interactions. VII. Enteropathogenic *Escherichia coli*: physiological alterations from an extracellular position. *Am J Physiol Gastrointest Liver Physiol* 281: G1–G7, 2001.—Enteropathogenic *Escherichia coli* (EPEC) is primarily associated with infantile diarrhea in developing countries. This intriguing pathogen exerts numerous physiological effects on its host target tissue, the intestinal epithelium, all from an extracellular location. Expression of a type III secretory apparatus allows this organism to transfer bacterial effector molecules directly into host cells. As a result of EPEC attachment to and/or translocation of proteins into intestinal epithelial cells, many signaling cascades are activated. Ultimately, host functions are perturbed, including alteration of ion transport, disruption of the tight junction barrier, and activation of the inflammatory response.

In 1945, Bray (1) identified a unique strain of *Escherichia coli* that caused life-threatening diarrhea in infants. What differentiated this particular strain of pathogenic *E. coli* from others was that its virulence mechanisms could not be attributed to an enterotoxin. Hence, the term enteropathogenic *E. coli* (EPEC) was coined by Neter (24) to refer to specific serotypes of *E. coli* found responsible for these outbreaks of infantile diarrhea that occurred in the 1940s and 1950s.

Intriguingly, after more than 50 years, the pathogenic mechanisms of this important microbe still remain elusive. Unlike prototypic enteric bacterial pathogens, EPEC is essentially noninvasive and produces no toxins. It was observed, however, that attachment of EPEC to host intestinal epithelial cells caused a unique morphological change called an attaching and effacing (A/E) lesion (Fig. 1). A/E lesions are characterized by elevation of the host cell membrane up to 10 μM above the cell, a central invagination wherein the bacterial microcolony is situated in intimate association with its host, and accumulation of cytoskeletal proteins beneath the adherent microcolony. These sites of cup and pedestal formation are accompanied by the degeneration of surrounding microvilli, hence the description of this lesion as effacing. This latter finding led to the assumption that the dissolution of surface hydrolases and overall loss of absorptive surface area would result in malabsorption. Although malabsorption may well contribute to EPEC-associated diarrhea, it cannot fully account for the enhanced fluid and electrolyte loss. This statement is supported by the fact that diarrhea can manifest as early as 3 h after ingestion of this pathogen (5).

Over the past decade, major advances toward defining the pathogenesis of EPEC have been made. These forward strides have resulted from diverse investigative approaches including genetics, cell biology, biochemistry, and host intestinal physiology. Unfortunately, space constraints do not allow an in-depth review here of the large body of literature that now exists concerning EPEC. The reader is therefore referred to recent extensive reviews of this pathogen (13, 34) for additional information. Although this themes article includes a general overview of the pathogen, it is written with a focus on the host intestinal epithelium and its myriad of responses to this most interesting organism.

**EPEC PATHOGENICITY**

As it became apparent that the mechanisms whereby EPEC induces disease were not readily identifiable, new investigative tactics were used; one tactic was aimed at dissecting the genetic complex pathogenicity, another was aimed at exploring the basis of interaction between pathogen and host, and, last, one tactic was
directed at examining the impact of infection on host cell events, signaling as well as function. One of the major advances toward unraveling the pathogenesis of EPEC was the sequencing of the 35-kb pathogenicity island, called the locus of enterocyte effacement (LEE) (21). The LEE houses 41 open reading frames divided into 5 operons. Contained within three of these operons are genes that encode a type III secretory apparatus (see below). Another houses genes involved in the intimate attachment of EPEC to host cells, and the last contains genes that encode proteins secreted by type III secretion, called Esps (E. coli-secreted proteins).

Type III secretion systems, expressed exclusively by gram-negative pathogens, are comprised of ~20 proteins that collectively form a bridge between the bacterial pathogen and the host cell. Specifically, these proteins span the inner membrane, periplasmic space, and outer membrane of the microbe and, through filaments, composed of EspA for EPEC, bacterial effectors are delivered directly into host cells (Fig. 2). The contribution of specific EPEC proteins to the composition of the type III secretory machinery has been defined in part. EspA, mentioned above, is delivered across the EPEC membrane to form filaments through which other translocated proteins are delivered (17). EspB and EspD are believed to form pores within the host cell membrane, because transmembrane domains are predicted for both of these proteins and their association with host cell membranes has been reported (35). That EspB may also act as an effector molecule is suggested by its presence in the cytosol of infected host cells and by the finding that deletion of the gene espB ablates EPEC-associated activation of host cell signaling cascades (6). Furthermore, transfection of espB into HeLa cells alters actin stress fibers and cell morphology, suggesting a role for this protein in pathogenesis (33).

Recently, EspF has been identified as an effector molecule (23). The unique nature of EspF is highlighted by the fact that, unlike the other Esps whose expression is required for the formation of A/E lesions, deletion of the gene espF has no impact on the quantity or character of A/E lesions, actin nucleation, or tyrosine phosphorylation of the translocated intimin receptor (Tir, see below) (22). EspF, which is not a structural component of the type III secretory machinery, is translocated by type III secretion into host cells, as demonstrated by both confocal microscopy and a Bordetella pertussis adenylate cyclase reporter system, making it a candidate effector. Although initial morphological and biochemical studies were unable to identify a given phenotype for the espF mutant strain, the coupling of prokaryotic genetic engineering with host physiological investigations unveiled an important finding. EspF expression is required for the full impact of EPEC on intestinal epithelial tight junctions (TJs). This finding opens the door to new speculations concerning EPEC pathogenesis. The contribution of A/E lesion formation itself to EPEC-associated disease has not been clarified. Now that the espF deletion mutant has been demonstrated to form A/E lesions but not to exert full functional effects on host tissues, it is tempting to view EPEC intimate attachment and pedestal formation as a maneuver to anchor itself to the host cell in the face of increased fluid movement through the intestine, thus ensuring the delivery of effector molecules into those cells. Others have offered the suggestion that this altered host morphology may in some way prevent internalization into host cells and...
subsequent antigen presentation. In fact, EPEC even escapes phagocytosis by macrophages (12), thus supporting this contention. Nonetheless, the induction of this characteristic morphological change appears to be insufficient, in and of itself, to effect physiological alterations, such as on barrier function (23). Instead, the delivery of key effector molecules, such as EspF and possibly others, is crucial. EspF is an interesting protein in that it contains proline-rich domains that confer the capacity for interacting with host cell proteins (22). The specific mechanism by which EspF exerts effects on the host tissue barrier is not known, but a dose-dependent correlation between EspF expression, disruption of the TJ barrier, and redistribution of the transmembrane TJ protein occludin has been demonstrated (Ref. 23; see below).

Perhaps one of the most intriguing aspects of EPEC infection is that through type III secretion, this organism inserts its own receptor into host cell membranes. The Tir is injected into host cells, where it is then modified by host cell enzymes (4, 16). Specifically, Tir is phosphorylated on tyrosine, and possibly serine and threonine, residues and then inserted in the host cell membrane. In this location, Tir is available to interact with its EPEC outer membrane ligand, intimin. Interestingly, intimin also possesses a binding site for β1-integrin (7). Although the interaction between intimin and β1-integrin appears unnecessary for the induction of A/E lesion formation, it is possible that it contributes to EPEC-initiated signaling and physiological effects. Disruption of intestinal epithelial TJs allows molecules normally restricted to either the apical or basolateral domain of the cell to redistribute to the other membrane. Opening of TJs by either calcium chelation or neutrophil transmigration has been shown to allow the migration of β1-integrin from its normal basolateral location to the apical membrane, where it would be available to interact with luminal organisms. Yersinia has been shown to exploit β1-integrin in this way (20). Because EPEC perturbs TJs, it is possible that the same scenario applies to this pathogen. Within the past few years, exploitation of host cell integrin molecules seems to have been an emerging paradigm for microbial pathogenesis.

EFFECTS ON HOST INTESTINAL EPITHELIAL FUNCTION

Ion transport. As a single layer of cells, the intestinal epithelium has three major physiological functions: the vectorial transport of ions and solutes, barrier function as provided by intercellular TJs, and surveillance of and response to the contents of the intestinal lumen. Although the pathogenesis of EPEC still remains undefined, infection by this microbe perturbs each of the basic intestinal epithelial functions. Most confusing, however, is the effect of EPEC on intestinal transport. Collington et al. (3) reported that infection of cultured human intestinal epithelial Caco-2 cells stimulated a rapid and transient increase in short-circuit current (Isc), which represents net ion transport activity. A portion of this response, but not all, was shown to be attributable to Cl− secretion. On the other hand, infection of a different cultured human intestinal epithelial cell line, T84, widely used to study the regulation of apical Cl− secretion, failed to demonstrate a similar response. In fact, no alteration in basal Isc, which is quite small, was seen. Instead, stimulation of EPEC-infected monolayers with the classic Ca2+- and cAMP-mediated secretagogues carbachol and forskolin yielded an attenuated response for which altered Cl− secretion was not responsible (14). Further investigation suggested that perturbation of bicarbonate-dependent transport processes accounted for this paradoxical response (14). The use of different cell lines, which clearly have varying ion transport properties, and different models of infection potentially explains these discrepant results.

Hypothetically, Caco-2 and T84 cells represent different epithelial cell types found in the intestine, absorptive villous and secretory crypt cells, respectively. The net impact of EPEC infection on the geographically and compositionally complex native intestinal tissue cannot be predicted or perfectly modeled in vitro. Furthermore, in vitro model systems examine only the direct impact of EPEC infection on the primary host tissue, the intestinal epithelium. In vivo, however, other cell populations and mediators released in response to this infection impact on ion transport activities. For example, after transepithelial migration, apically situated neutrophils release 5′-AMP, which is converted to the secretagogue adenosine. Also, expression of the heptaspanning receptor for galanin (galanin-1 receptor) is increased as a result of pathogen-induced nuclear factor (NF)-κB activation (15). Activation of this receptor by its ligand, galanin, stimulates Cl− secretion. Hence, the interplay of factors that influence intestinal ion transport in response to infection by enteric pathogens is very complex, and ultimately dissection of these events in an appropriate animal model will be required if a full understanding is to be gained.

The major problem concerning in vivo studies is that there is no small animal model of the human-specific pathogen EPEC. Although some human EPEC strains induce A/E lesion formation in other animals, none of these develops diarrhea. Alternatively, other A/E-inducing strains of E. coli pathogenic for other animal species, in particular rabbits (REPEC) and mice (Citrobacter rodentium), have been studied with the belief that the pathogenic mechanisms of these various strains are similar or identical to those of human EPEC. The few human volunteer trials that have been performed so far (5, 32) support the conclusions derived from in vitro studies with human EPEC strains and in vivo studies with other species-specific strains. However, to date, human trials have not focused on specific functional alterations. Therefore, studies of native human tissues infected with EPEC will be needed to dissect out the alterations in ion transport associated with this infection.
EPEC ALTERATION OF HOST INTESTINAL EPITHELIAL PHYSIOLOGY

Tight junction effects. One of the most exciting areas of investigation concerning EPEC has been its effects on TJs. Several groups have demonstrated that infection of monolayers of intestinal epithelial cells, both Caco-2 and T84, increases the permeability of these tissues, determined as a decrease in transepithelial electrical resistance (TER) (2, 30). Flux studies using the paracellular marker mannitol defined the permeability defect to be at the level of the TJ (30). It appears that EPEC may perturb TJs by various mechanisms. Contraction of the perijunctional cytoskeletal ring is one way in which the TJ barrier can be disrupted. The biochemical event regulating this process is phosphorylation of the 20-kDa light chain of myosin (MLC20).

Years ago, Manjarrez-Hernandez et al. (19) reported that EPEC infection of eukaryotic cells stimulated the phosphorylation of a number of host proteins of which MLC20 was the most prominent. We found (36) that the functional consequence of this biochemical alteration was increased TJ permeability. The steps that lead to MLC20 phosphorylation are an initial increase in intracellular calcium and the formation of calcium-calmodulin complexes that then activate myosin light chain kinase (MLCK). Both sequestration of intracellular calcium and inhibition of MLCK diminished the EPEC-associated increase in TJ permeability (36).

Most recently, however, the structural basis of TJs has been identified. Tsukita and colleagues identified two TJ-associated transmembrane proteins, occludin (9) and a family of claudins (8), which consists of numerous isoforms. Both occludin and claudins possess four transmembrane domains that form two extracellular loops that project into the intercellular space and participate in formation of the barrier, likely by laterally polymerizing. The effect of EPEC, and other enteric pathogens, on TJ structure is just beginning to be explored. Studies from our laboratory (29) have demonstrated that EPEC infection of intestinal epithelial monolayers progressively perturbs the phosphorylation state and distribution of occludin. In other model systems, the importance of occludin phosphorylation in its localization to TJs has been shown. We found (29) that after infection with EPEC, occludin first assumed a beaded appearance in contrast to its normal uniform distribution at the TJ level of the membrane. These early morphological changes were not associated with functional perturbations. By 3 h after infection, however, a significant portion of occludin had dissociated from the TJ and redistributed to the cytoplasm (Fig. 3). Associated with this more dramatic morphological alteration was a significant drop in TER. Functional type III secretory machinery was found to be required for both the morphological and functional changes. In fact, progressively increased expression of EspF via infection with an isopropyl β-D-thiogalactopyranoside-inducible espF complemented strain revealed a dose-dependent perturbation in occludin localization and disruption of the TJ barrier (23). The prevention of both the morphological and functional changes by the serine/threonine phosphatase inhibitor calyculin A supports the contention that EPEC-induced dephosphorylation of occludin is at least in part responsible (29). The effect of EPEC on another TJ protein, ZO-1, has also been reported, but the events underlying these observations are only beginning to be explored.

Epithelial inflammatory response. One global response of the intestinal epithelium to infection by bacterial pathogens is initiation of the inflammatory response. Initially, it was demonstrated that invasive bacterial pathogens such as Salmonella stimulated this response. Using EPEC as a prototypic noninvasive pathogen, we found (26) that invasion is not necessary to trigger the cascade of signals that ultimately lead to inflammation, in this case defined as transepithelial migration of neutrophils. In fact, McCormick and co-workers have now demonstrated that Salmonella need not invade to stimulate neutrophil transmigration. Their recent report (18) shows that a secreted extracellular Salmonella product, SipA, is sufficient to stimulate a protein kinase C (PKC)-dependent signaling cascade that ultimately leads to neutrophil transmigration. Although a similar effector molecule of EPEC has not been identified, the future demonstration of a similar paradigm as defined for SipA of Salmonella would not be surprising. It should be mentioned, however, that a unique bacterial flagellin from select EPEC, enterohemorrhagic E. coli, and enteroadherent E. coli strains stimulates the secretion of interleukin (IL)-8 by host target intestinal epithelial cells (31). Interestingly, the basal, but not apical, exposure of intestinal epithelial monolayers to flagellin purified from Salmonella typhimurium activates the mucosal
inflammatory response (10). The underlying pathways by which these unique bacterial proteins induce this common host response deserve more intense investigation in the future.

Regarding EPEC-induced inflammation, the first studies confirmed that the interactions between EPEC and host intestinal epithelial cells were sufficient to recruit neutrophils across this layer and that IL-8 was in part responsible (27). Because IL-8 expression, like that of most inflammatory response genes, is in large part regulated by the transcription factor NF-κB, the ability of EPEC to activate this factor was explored and confirmed (25). Now the focus is on defining the proximal signaling pathways by which EPEC induces this response. Just as occurs in the classically defined cytokine-stimulated pathway, EPEC infection stimulates the phosphorylation and degradation of IκBα, the inhibitory molecule that maintains NF-κB in its inactive state in the cytoplasm. IκBα phosphorylation is an early event associated with EPEC infection, occurring within 15 min (28). Although the specific pathways leading to this response have yet to be defined, two important points have been demonstrated. First, a myriad of host cell signaling pathways, including mitogen-activated protein (MAP) kinases, PKCs, tyrosine kinases, and reactive oxygen intermediates, appear to contribute to the mucosal inflammatory response associated with EPEC infection. Second, enteric bacterial pathogens use different signals to elicit this common endpoint. For example, an initial increase in intracellular calcium concentration by S. typhimurium infection is key for NF-κB activation and IL-8 production (11). Although intracellular calcium concentration is likely increased after infection with EPEC (this point remains controversial), this molecule plays no role in the ensuing inflammation yet is required for perturbation of TJ permeability. Conversely, signals that effectuate the inflammatory response, such as MAP kinases, play no role in the permeability changes (28). Therefore, the many signals that are activated after EPEC attachment appear to diverge and impact separate physiological functions (Fig. 4).

**PERSPECTIVES**

Remarkable progress has been made in just the past decade toward understanding the pathogenesis of EPEC. One clear conclusion that has emerged from a multitude of studies is that neither one unifying mechanism of pathogenesis nor a single effector molecule is responsible for the symptoms that accompany EPEC infection. Instead, several EPEC virulence factors perturb many facets of host physiology that together culminate in diarrhea. Perturbations in intestinal ion transport, by both direct interactions between microbe and host and indirect effects that result from mediators released by other cell populations in response to infection, clearly contribute to the symptomatology. The maintenance of the intercellular TJ barrier is essential for the physiological functioning of the intestinal epithelium; hence, its disruption by EPEC infection has the potential to perturb many processes including transport, cell polarity, and protection of the underlying compartments from noxious contents of the intestinal lumen.

Seminal investigations into the genetics underlying EPEC pathogenesis have heralded a new approach to the study of EPEC. With the identification and sequencing of the pathogenicity island or LEE of EPEC, the union of genetic manipulation (single gene mutations) of the pathogen with the exploration of the impact on host function will serve to identify key effector proteins. It is this very approach that led to the finding that the secreted, but nonstructural, EPEC protein EspF is intimately involved in the disruption of TJ structure and barrier. The challenge now is to identify the host proteins that interact with EspF and other translocated EPEC effectors. The elucidation of the translocated EPEC receptor, Tir, affords the opportunity to determine the host proteins with which it interacts and the signaling pathways that are ultimately stimulated as a result.

Ultimately, the goal is to use in vitro models to identify candidate genes that deserve study in vivo. Several EPEC genes have been confirmed as virulence factors using this approach. The role of the outer mem-

---

**Fig. 4.** Host cell signaling pathways activated by EPEC infection diverge and alter separate physiological functions. MLCK, myosin light chain kinase; PKC, protein kinase C; MAP, mitogen-activated protein; IL, interleukin; IκB, nuclear factor-κB inhibitor; PMN, polymorphonuclear leukocyte; ROI, reactive oxygen intermediates; MLC-P, phosphorylated myosin light chain.
brane protein intimin, encoded by the gene eae, was highlighted in this way (5). Similarly, the importance of EspB in human infection was demonstrated (32). As functional assays are routinely used to screen newly created EPEC mutant strains, new insights into pathogenesis will be gained. This approach, which integrates genetics, both host and pathogen, cell biology, intestinal physiology, and immune response, will improve our understanding of this pathogen. With enhanced understanding comes opportunity for the development of targeted preventative and therapeutic strategies.

The author thanks her collaborators, Drs. James Kaper and Michael Donnenberg at the University of Maryland, Baltimore, MD, who have generously supplied her laboratory with many mutated strains of enteropathogenic E. coli for functional studies.

The author’s research is supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-50694 and DK-58964 and by the Department of Veterans Affairs through a Merit Review and Research Enhancement Awards Program.

REFERENCES


30. Steiner TS, Nataro JP, Poteet-Smith CE, Smith JA, and Guerrat RL. Enterocyte aggregating Escherichia coli expresses a


