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COMMENTARY

Microbial Invasion Across the Intestinal Epithelial Barrier

Commentary on the article by Burns *et al.* on page 30

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The intestine forms a barrier that separates two opposing compartments. A continuous monolayer of columnar epithelial cells sealed together by circumferential tight junctions insulates a surface area of nearly 200 m^2 from passive diffusion of small and large solutes, particles, microorganisms and cells. Such barrier function is required for efficient vectorial transport of water and solutes and for host defense (1).

In the adult human, the intestine must function as an effective barrier against invasion of 10^8 to 10^{10} bacteria that normally colonize the intestinal lumen. Certain bacterial pathogens, however, such as *Salmonella*, *Shigella*, and *Yersinia*, opportunistically invade the healthy intestine. These pathogens first breech the epithelial barrier by exploiting the transepithelial transport activity of M cells located in the so-called dome epithelium that covers organized lymphoid follicles of the intestine (2).

This may not be the only route of bacterial invasion across mucosal surfaces. Available evidence indicates that certain microorganisms may also breech the intestinal barrier at sites separate from the dome epithelium. For example, *Salmonella* entry into the mucosa *via* M cells requires the coordinate action of specific bacterial virulence factors, but certain *Salmonella typhimurium* strains deficient in such virulence factors can still invade the host intestine, spread to the liver and spleen and cause lethal disease. In a recent paper, Vazquez-Torres et. al. suggested that these invasion-deficient *S. typhimurium* may breech the intestinal mucosa by sequestering themselves first within CD18 positive mononuclear cells that shuttle the microbe across the villus epithelium into the systemic circulation (3).

In this issue of *Pediatric Research*, Burns *et. al.* provide evidence for another route of bacterial invasion. This study indicates that certain microbial pathogens, in this case *Escherichia coli* K1, may breech the epithelial barrier by direct transit through the absorptive villus enterocyte. *E. coli* K1 accounts for nearly 40% of septicemia and 80% of meningitis caused by *E. coli* infection in the newborn period, and the intestinal tract is considered the primary site of colonization and presumably invasion.

To demonstrate invasion *via* this pathway, Burns *et al.* applied pathogenic and nonpathogenic strains of *E. coli* to apical surfaces of polarized intestinal T84 and Caco2 cells in culture. When grown on permeable supports, both cell lines form confluent polarized epithelial monolayers that exhibit tight intercellular junctions with high transepithelial resistance. These intercellular junctions represent the rate-limiting barrier against passive flux of small and large solutes. Bacteria cannot penetrate such junctional complexes unless the junctions are disassembled. The data show that pathogenic *E. coli* K1 strains crossed intact T84 and Caco2 cell monolayers from apical to basolateral reservoir nearly 100-fold more efficiently than nonpathogenic strains. Intercellular tight junctions were not disrupted, and such transport cannot be due to passive diffusion around cells.

This is an important observation. In general, solutes and insoluble particles such as microbes may cross the epithelial barrier by one of two routes. Some solutes and water can cross the intestine by passive diffusion or convection around cells through tight junctions. This is termed the paracellular pathway. Some solutes can cross the mucosa by transit through the cell, the transcellular pathway. The paracellular pathway exhibits charge and size selectivity, and few, if any, macromolecules can cross epithelial barriers by passive diffusion. Microbes, of much larger size and stokes radius, are more severely restricted. Thus, these observations, that pathogenic strains of *E.coli* K1 cross intact T84 or Caco2 monolayers and nonpathogenic strains do not, provide evidence that certain microbes exhibit the ability to enter and traverse the absorptive epithelial cell itself. Previous studies indicate that pathogenic *Neisseria* (4), the HIV (5), group B streptococci (6), and possibly *Salmonella* (7) can do the same.

The mechanism for such transepithelial transport of *E. coli*, however, remains unknown. The authors indicate that invasion of T84 monolayers by *E. coli* K1 may be saturable and suggest a receptor mediated mechanism, but these data are not convincing.

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on the other hand, transepithelial transport across Caco2 monolayers seems to be dependent on cell differentiation. These data indicate that factors endogenous to the host cell are likely involved. This is a plausible argument. While intestinal function depends on the integrity of the epithelial barrier, the resistance to macromolecular and cellular transport across the general crypt-villus epithelium is not absolute. Macromolecules, such as sIgA and IgG, are normally transported intact across intestinal epithelial cells and, in certain disease states, bacterial toxins co-opt these transcellular pathways to invade the host (8–10). We also now know that certain microbes can induce their own entry into host epithelial cells by co-opting the machinery for phagocytosis and some pathogens can sequester themselves within intracellular vesicles of unique pathogen-elicited protein and lipid composition (11, 12). Thus, the idea that certain microbes, such as *E. coli* K1, *Salmonella*, *Shigella*, and *Yersinia*, may utilize endocytic vesicles for transport across the epithelial barrier by transcytosis is both plausible and attractive. Future studies on the pathogenesis of these evolutionarily refined microbes undoubtedly will lead us to understand the fundamental mechanisms that regulate vesicular and macromolecular traffic in the host epithelial cell. The results of this effort could set the stage for the development of new approaches to prevent and treat microbial infections that depend on invasion across mucosal surfaces.

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