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EDITORIAL

Brush Border Destruction by Enterohemorrhagic *Escherichia coli* (EHEC): New Insights From Organoid Culture

The microvillus-rich brush border found at the apex of enterocytes and colonocytes serves as the gut's functional interface, playing roles in nutrient processing, solute transport, and host defense.[1] Infectious or inherited diseases that impact the function of this organelle have significant effects on gut homeostasis and can lead to death in susceptible populations. An important example is provided by disease-causing, gram-negative attaching/effacing (A/E) pathogens, which have evolved efficient mechanisms for destroying the brush border during efforts to promote their own survival.[2]

Enterohemorrhagic *Escherichia coli* serotype O157:H7 (EHEC) is one A/E pathogen that represents a major cause of food-borne enteric infection in North America, leading to hundreds of deaths per year.[3] Once in the gastrointestinal tract, EHEC preferentially colonizes the proximal colon. EHEC illness is at least partially caused by release of Shiga toxin, which poisons multiple organ systems, but there also are direct effects on the colonic epithelium. EHEC uses a type III secretion system to inject colonocytes with virulence factors that stimulate a massive remodeling of the apical actin cytoskeleton. This leads to formation of adherent pedestals that allow these bugs to remain tightly associated with the epithelium and establish microcolonies. Although Shiga toxins and pedestal formation have been the focus of intense study for years, how EHEC establishes initial contact with the epithelium and the subsequent dynamics of effacement remain poorly understood. This gap has persisted in part owing to a lack of model systems that faithfully recapitulate human colonocyte biology.

However, the advent of organoid culture in recent years has fundamentally changed how biologists think about cultured cell experiments.[4] Gut organoids are cultured either from stem cells or stem cell–containing crypts isolated from small intestine or colon, derived from mice or humans. These primary cultures are grown in a basement membrane substrate and can be induced to differentiate, building a crypt-villus axis similar to that observed in vivo. Although gut organoids have already proven their utility in studies of stem cell maintenance and human diseases,[5,6] a major limitation relates to their shape; organoids are cyst-like, consisting of a single layer of epithelial cells surrounding a hollow lumen. This closed system limits access to the apical surface of the epithelium and also presents major challenges for light microscopy.

In the current issue of *Cellular and Molecular Gastroenterology and Hepatology*, In et al[7] describe an approach for flattening human colonoid cultures into monolayers, to enable mechanistic studies of EHEC infection. After initial growth, human colonoids are coaxed into monolayers by seeding on filter substrates coated with collagen. The resulting cultures show biochemical and morphologic hallmarks of differentiated colonocytes, including assembly of a well-organized apical brush border and copious mucus secretion. Importantly, growth of organoids as monolayers allows unfettered access to the apical surface, and clearer microscopic imaging with cells limited to a single plane. Other groups also recently developed colonoid monolayers,[8] suggesting that this approach represents a robust, next-generation gut epithelial biology model system.

In et al[7] took advantage of human colonoid monolayers to examine the early stages of EHEC contact with monolayers and generate new insights on infection. The initial target of EHEC appears to be the Mucin-2 (MUC2)-enriched mucus layer, which could serve as both an energy source and substrate for initial attachment. Indeed, exposure to EHEC significantly reduced MUC2 levels on the surface of colonoid monolayers. Intriguingly, the authors also found that EHEC disrupts protocadherin-24 (PCDH24)-dependent intermicrovillar adhesion, which was recently discovered to be a critical driver of microvillar packing during brush-border assembly.[9] In et al[7] observed robust PCDH24 expression and apical localization in colonoid cultures, and showed that PCDH24 levels decrease significantly in response to infection. Using genetically modified variants of EHEC, the authors also established that the secreted serine protease, EspP, is the culprit enzyme responsible for loss of PCDH24. Without EspP, EHEC is unable to induce perturbations to colonoid brush borders. Finally, the authors showed that addition of purified EspP to the surface of colonoid monolayers is sufficient for PCDH24 degradation. Together these results suggest that a key step in EHEC-induced microvillar effacement is loss of intermicrovillar adhesion, caused by EspP-mediated degradation of PCDH24.

These results are fascinating for several reasons. This report shows that intermicrovillar adhesion molecules are targeted by EHEC's pathogenic mechanism. This connection makes biological sense given that perturbations in brush-border morphology induced by loss of intermicrovillar adhesion complexes[9] phenocopy those induced by EHEC infection. These data further suggest that adhesion between microvilli provides a physical barrier to infection. Eliminating this barrier and opening gaps between adjacent microvilli might allow EHEC to make closer and more continuous contact with the apical membrane, ultimately promoting A/E lesion formation. This would be reminiscent of previous work showing that splaying of brush-border microvilli is a key step in the uptake of commensal microbes.[10] Because initial studies on intermicrovillar adhesion also established a potential link between PCDH24

expression and stabilization of microvillar actin bundles,[9] degradation of PCDH24 also might represent the first step in remodeling the apical actin network into A/E lesions. Future studies along these lines will need to focus on clarifying the molecular relationship between EHEC-induced PCDH24 degradation, type III secretion system function, and A/E lesion formation.

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