Pore-Forming Toxins Trigger the Purge

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The intestinal epithelium responds to pathogens by coordinating microbial elimination with tissue repair, both required to survive an infection. In this issue of *Cell Host & Microbe*, Lee et al. (2016) discover a rapid and evolutionarily conserved response to pore-forming toxins in the gut, involving cytoplasm ejection and enterocyte regrowth.

To survive infection by pathogenic bacteria, the digestive tract needs to first eliminate the bacterial invader through immune responses and then to cope with the stress and damage inflicted by both the pathogen and the immune response (Ferrandon, 2013). Work in *Drosophila melanogaster* and mouse models have demonstrated conserved responses to infectious damage that involve the reprogramming of intestinal stem cells (ISCs) to repair the gut epithelium. Infection by diverse bacteria, including the human pathogen *Salmonella typhimurium* and the insect pathogens *Erwinia carotovora carotovora 15* (*Ecc15*) and *Pseudomonas entomophila* (*Pe*), triggers compensatory ISC proliferation in the gut (Buchon et al., 2009; Jiang et al., 2009; Karin and Clevers, 2016; Liu et al., 2010). Pioneering work in *Drosophila* has identified the molecular mechanisms that initiate this regenerative response. Upon infection, enterocytes produce cytokines that stimulate the secretion of growth factors, ultimately leading to increased ISC proliferation and differentiation, and replenishment of the damaged cells (Buchon et al., 2013). ISC-mediated tissue repair is, however, a long-term response to cell loss, and very little is known concerning the immediate response of enterocytes to bacteria-induced tissue damage.

Pore-forming toxins (PFTs) are the most common type of cytotoxic proteins produced by bacterial pathogens. PFTs are central to infection by a plethora of pathogenic bacteria, including human pathogens such as *Vibrio cholera* and *Staphylococcus aureus*, and the commonly used bioinsecticide *Bacillus thuringiensis* (*Bt*) (Melo et al., 2016; Dal Peraro and van der Goot, 2016). PFTs typically recognize a binding partner on cellular membranes and assemble into pores through progressive oligomerization and concomitant membrane insertion. Pore formation leads to the influx or efflux of small molecules such as ions and proteins from the target cell or cellular compartment, thus disrupting normal functions. Multiple responses are induced by cells to survive the stress inflicted by pore formation. The decrease in cellular potassium triggers activation of stress- and mitogen-activated protein kinases, as well as the release of calcium stores. In addition, membrane repair is initiated either by endocytosis of the pores or by shedding of vesicles containing PFTs, followed by endosomal sorting complex required for transport (ESCRT pathway) dependent membrane trafficking (Dal Peraro and van der Goot, 2016).

In this issue of *Cell Host & Microbe*, the study by Lee et al. (2016) expands our understanding of the complex interactions between the PFT secreting bacteria *Serratia marcescens Db11 (SmDb11)* and the gut epithelium. Lee and colleagues demonstrate that the *Drosophila* midgut epithelium undergoes remarkable alterations within a few hours after ingesting *SmDb11* (Lee et al., 2016), and that the *SmDb11* PFT, hemolysin, is both necessary and sufficient for this phenomenon. Contrary to previous models of infection, exposure to PFTs does not result in either enterocyte death or delamination, but instead promotes the accumulation of lipid droplets in enterocytes, followed by enlargement of the mitochondria and disorganization of endoplasmic reticulum. Subsequently, the apical contents of the enterocytes are extruded, which results in an apparent thinning of the epithelium without gut leakage or cell lysis. Finally, enterocytes regrow to their initial size within 12 hr (Figure 1). Monalysin, the PFT of *Pe*, triggers similar cytopathology in the midgut, suggesting that this represents a standard enterocyte reaction to PFT exposure. Accordingly, these effects are reminiscent of the general cellular response to PFTs and are likely consequences of cellular potassium depletion, alongside histone dephosphorylation and translational inhibition that occur upon PFT exposure. It has been proposed that such cellular changes are emblematic of a low-energy mode that may be required for survival (Dal Peraro and van der Goot, 2016).

The function of cytoplasmic extrusion remains uncertain, but it is possible that it assists in purging bacteria from the gut lumen. Accordingly, a hemolysin-deficient *S. marcescens* strain (*Sm21C4)* does not induce a cytoplasmic purge and shows increased virulence and reduced host survival compared to *SmDb11*. An alternative hypothesis is that cytoplasmic extrusion is a strategy for enterocytes to manage cellular stress. Upon ingestion of *SmDb11*, ISC proliferation is only marginally induced, and only in the late phase of infection. Surprisingly, infection with *Sm21C4* triggers higher compensatory ISC proliferation than infection with *SmDb11*, suggesting that PFT-induced cytoplasmic purge could lower epithelial stress. In such a model, cytoplasmic extrusion could be a response of the cell to either expel membrane-embedded PFTs or purge damaged organelles (Dal Peraro and van der Goot, 2016). A last possibility is that cytoplasmic extrusion represents a damage response mechanism in situations where the normal enterocyte response is prevented. Previous studies have shown that *Pe* induces strong ISC proliferation at low doses (Jiang et al., 2009), but at high doses PFT-dependent translational inhibition is triggered in enterocytes, leading to a loss of ISC function (Chakrabarti

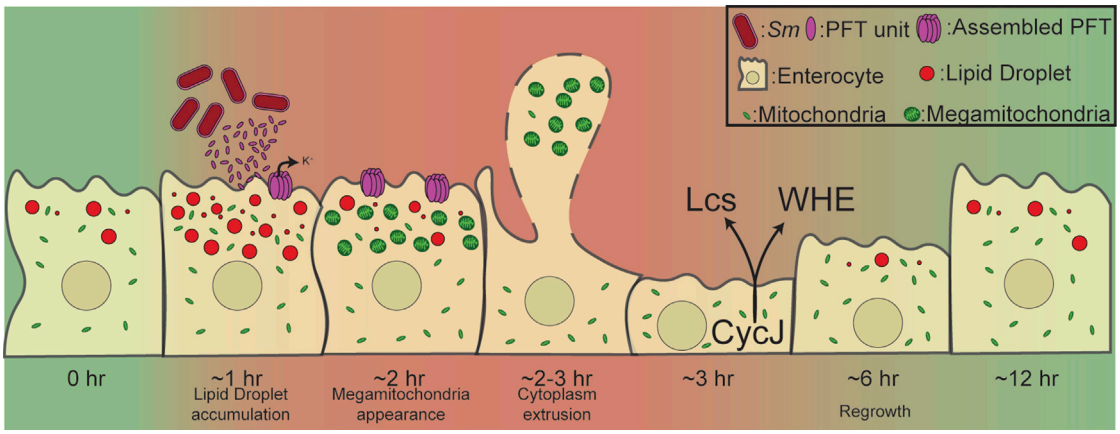

Figure 1. The Pore-Forming Toxin, Hemolysin, Induces Cytoplasmic Purge followed by Regrowth in the Gut of *Drosophila*
In the early phase of infection, *SmDb11* releases PFTs that trigger pore formation on enterocyte cell membranes. As a response, enterocytes accumulate lipid droplets (red dots) after ~1 hr from infection. After ~2 hr, these lipid droplets reabsorb while mega-mitochondria (green globules) accumulate in enterocytes. At ~3 hr post ingestion, enterocytes expel the apical fraction of their cytoplasm into the lumen, which decreases infection-associated stress and helps to maintain barrier integrity against *SmDb11*. Finally, CycJ in enterocytes leads to the expression of the short secreted proteins la costa (Lcs) and what else class of genes (WHE), allowing enterocytes to regrow to their initial size.

et al., 2012). Such translational blockage has also been described for *Sm* (Dal Peraro and van der Goot, 2016). It is therefore possible that cytoplasmic extrusion evolved as a general response to PFTs that does not require protein synthesis and allows for timely buffering of infection-induced stress in a low-energy mode (Dal Peraro and van der Goot, 2016). However, this must be an immediate response since enterocyte death and compensatory ISC proliferation occur during the late stages of infection.

Lee et al. (2016) also began to uncover the molecular mechanisms underlying the regrowth of enterocytes after cytoplasm extrusion. *CycJ* is a highly conserved cyclin of unknown function that is upregulated upon *SmDb11* infection. A *CycJ* mutation resulted in the inability of midgut epithelium to restore its thickness and was associated with increased host mortality. Surprisingly, clonal analysis revealed that the role of *CycJ* is non-cell-autonomous. Transcriptomic data revealed that *CycJ* acts as a transcriptional regulator that induces expression of a group of secreted proteins (e.g., Lcs, WHE) required for epithelial regrowth upon *SmDb11* infection. Overexpression of these secreted proteins rescued the ability of *CycJ* mutant epithelium to recover. Importantly, cytoplasmic extrusion and recovery are seemingly conserved from insects to mammals. Ingestion of *SmDb11* by the honeybee or injection of hemolysin-expressing *E. coli* into mouse intestines both lead to very similar cytopathologies. However, while this mechanism is clearly conserved, it is probable that different effector proteins allow epithelial regrowth in different host systems. In support of this, the small peptides induced by CycJ upon *SmDb11* infection are present only in *Drosophila melanogaster*, suggesting that additional effectors mediate a similar response in mammals or other insects.

Overall, the manuscript by Lee et al. (2016) describes a novel, conserved resilience mechanism that allows the intestinal epithelium to cope with infection-induced stress. Future work should clarify whether cytoplasmic extrusion is limited to the gut epithelium or if it is a more general cell response to PFTs, and whether a similar response occurs in the respiratory tract due to PFT exposure or infection with *Staphylococcus aureus*. The paradoxically increased pathogenicity of bacterial strains lacking hemolysin demonstrates that the in vivo consequences of well-characterized virulence factors can be surprising, and that the attack by PFTs can act as a danger signal that benefits the host. It will be interesting to determine whether other PFTs or virulence factors also act as danger signals for the cell. In addition, this paradox demonstrates once more the importance of in vivo models of infection to capture the complexity of bacterial pathogenesis. Without a doubt, *Drosophila* will continue to serve as a powerful model to dissect the molecular mechanisms underlying complex host-pathogen interactions and to pave the way for studies relevant to human health.

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