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UPEC Hemolysin: More Than Just for Making Holes

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Summary

During acute cystitis, uropathogenic *Escherichia coli* (UPEC) induce bladder epithelial cell exfoliation, which eliminates infected cells and promotes UPEC dissemination. Dhakal and Mulvey 2012 uncover the mechanism that induces this exfoliation and re-introduce the pore-forming toxin, hemolysin, as an effector that surprisingly targets multiple host pathways to facilitate infection.

Detailed models of epithelial infection using uropathogenic *Escherichia coli* (UPEC) have redefined intracellular pathogen lifestyles, bacterial suppression of host responses, and pathogen influences on cellular and developmental programs within epithelial tissues. Upon introduction into the urinary bladder, UPEC bind epithelial surfaces via an array of adhesive surface structures and gain access to the intracellular environment. Cell entry likely leverages the normal membrane-recycling activity of superficial bladder epithelial cells (BECs) that is required for accommodation of changing urine volume (Bishop et al., 2007). Studies by Mulvey's group and others have shown that Rho family GTPases and modulation of actin dynamics figure prominently in UPEC entry and may affect the downstream fate of internalized bacteria (Dhakal et al., 2008). A subset of these organisms then proliferate to form large cohesive colonies termed intracellular bacterial communities (IBCs). Numerous studies suggest that these IBCs represent a haven for pathogen multiplication, protected from the activity of neutrophils and macrophages that are the primary cellular defenders against mounting infection. The outcome of a complicated conversation between UPEC and the mammalian host, this UPEC developmental pathway is certainly driven by stepwise and concerted expression of a number of families of virulence determinants.

A consistent feature of acute cystitis, a urinary tract infection (UTI) most often caused by UPEC, in both mice and humans is the exfoliation of superficial BECs (Mulvey et al., 1998), accompanied by activation of host cell differentiation programs that normally run much more slowly (Mysorekar et al., 2009). On one hand, the discharge of infected BECs into the urine can certainly be viewed as an important host defense mechanism, a way for the infected organ to rid itself of perhaps 100,000 bacteria in a single event. However, this process exposes the underlying transitional cells to UPEC, which can subsequently establish

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nests of quiescent bacteria that resist antibiotic therapy, avoid detection by immune effectors, and seed recurrent UTI. Mulvey was first to report the exfoliation of BECs in response to infection (Mulvey et al., 1998), and now his group reports the first molecular details underlying exfoliation and the bacterial effector that drives this process.

UPEC liberates a number of toxins, including hemolysin and cytotoxic necrotizing factor 1, to optimize the host environment for propagating infection. Unlike some of its enteric relatives, UPEC foregoes the need for a dedicated contact-dependent secretion apparatus to inject manipulative proteins into the host cell. During cystitis, the simple residence of this pathogen within the host cell cytoplasm permits the delivery of an array of highly active molecules. *E. coli* hemolysin (encoded by the *hlyCABD* operon) is a prototypic repeat-in-toxin (RTX) molecule and an important model for acylation of virulence factors within the bacterial cytoplasm. The protoxin (HlyA) is modified by the acyltransferase HlyC in a step required for cytotoxic activity, and export is accomplished by the action of inner membrane components HlyB and HlyD along with the outer membrane protein TolC (reviewed in (Welch, 2005)). At high concentrations, the toxin forms multimeric pores in eukaryotic membranes, leading to cell lysis, while lower concentrations have appeared to interfere with host cell signaling pathways and to cause apoptotic cell death. The fresh approach of Dhakal and Mulvey illuminates these events, demonstrating that upon insertion into host membranes at sub-lytic levels, acylated HlyA triggers the degradation of paxillin, a cytoskeletal scaffold protein, and other host proteins important for cell-cell and cell-matrix interactions. The current work elegantly shows that HlyA activates the host serine protease mesotrypsin and other host proteases that unfetter BECs from these contacts to initiate exfoliation. Mulvey's group is now positioned to deliver further exciting insights into the mechanism by which HlyA activates this proteolytic cascade and the spectrum of its effects on the physiology of host cells.

The presence of hemolysin on the chromosome has been linked with disease severity (specifically increased tissue damage) during epithelial infection caused by several *E. coli* pathotypes, though *hly* genes can be found in UPEC isolates from both cystitis and pyelonephritis. In the context of updated paradigms in UTI pathogenesis, and in light of Mulvey's new experiments with sub-lytic concentrations of the toxin, one can now refine this view to speculate that it is not only the encoding of hemolysin but the spatiotemporal regulation of its expression that influences the pathogenic success of a given UPEC strain. For example, production of hemolysin by extracellular pathogens is thought to facilitate acquisition of host-sequestered essential nutrients, notably iron. However, these nutrients are directly available to pathogens residing in the host cell cytoplasm, and at this stage, formation of hemolysin pores in the membranes of superficial BECs might result in loss of these nutrient sources and provide arriving neutrophils access to the nascent IBC. Instead, moderation in toxin expression during this stage would favor survival of these intracellular bacteria, perhaps also providing much needed nutrition in the form of amino acids released via proteolysis to support rapid UPEC growth. In contrast, subsequent UPEC egress from an infected BEC might be facilitated through increased HlyA production, especially by phagocytosis-resistant filamentous organisms that propagate infection to naïve cells (Horvath et al., 2011; Justice et al., 2006). As aptly proposed by Dhakal and Mulvey, timely stimulation of exfoliation of infected BECs would also serve to promote spread of the pathogen to other hosts upon expulsion in the urine. A detailed study of the spatiotemporal expression of *hlyA* during cystitis promises to shed light on these suppositions.

The attenuation of soluble and cellular innate responses has also been a focus of many studies in UPEC cystitis (reviewed in (Hunstad and Justice, 2010)). Mulvey's findings implicate hemolysin as one of several effectors acting to suppress cytokine production by BECs. Moreover, activation of the proteolytic cascade observed upon BEC exposure to

HlyA was recapitulated in cultured macrophages, suggesting a possible means for UPEC to foil the function of host phagocytes. Finally, the data reported in this issue suggest a novel mechanism by which UPEC inhibits pro-inflammatory NF-κB signaling, one that involves β-catenin activation and may be independent of proteasomal IκBα degradation. The fertile ground to be explored on the heels of the current work will broaden our already burgeoning knowledge of innate immune regulation and the myriad ways in which diverse bacterial pathogens subvert these ancient host defense systems.

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