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REVIEWS

Spotlight

Staphylococcus aureus α-Toxin's Close Contacts Ensure the Kill

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The membrane pore-forming α-toxin is an important virulence factor of *Staphylococcus aureus*. Target cells can remove pores from their surface, but recent work shows that α-toxin may undermine this self-defense by clinging to epithelial cell junctions. The findings could lead to the development of novel remedies against *S. aureus* infections.

S. aureus α-toxin, also known as α-hemolysin or HLA, is the archetype of a membrane pore-forming toxin [1]. In models of staphylococcal skin disease and pneumonia, HLA has been shown to serve as an important virulence factor. The majority of *S. aureus* isolates secrete large amounts of toxin monomer, a water-soluble 33.2 kDa protein. Upon contact with susceptible target cells, HLA forms stable, heptameric pores which span the plasma membrane. The unregulated flux of ions and water through these channels triggers a multitude of cellular responses, some of which culminate in the initiation of cell-death programs, while others promote survival [2]. Intriguingly, some cell types are able to reverse the loss of cytosolic K^+ ions and ATP, and survive HLA attack by removing plasma membrane pore complexes via endocytosis. [3]; this rescue process is blocked by dynasore, a low-molecular-weight inhibitor of the large cellular GTPase dynamin. Cellular recovery from HLA attack is most efficient at moderate toxin concentrations, where binding depends on high-affinity sites. The transmembrane disintegrin- and metalloproteinase-containing protein ADAM10 is the putative high-affinity receptor for HLA [4].

In addition to ADAM10, functional screenings identified other host cell proteins which impact HLA-dependent cytotoxicity [5,6]. Conspicuously, several hits are involved in membrane traffic, or formation of cell–cell junctions. A puzzling finding was that knockout of one of these proteins, PLEKHA7 (pleckstrin homology domain-containing family A member 7), which is important for the integrity of adherens junctions (AJs), enabled HAP-1 cells (a near-haploid cell line originating from a chronic myeloid leukemia cell) to recover from HLA attack [5]. Moreover, PLEKHA7 knockout mice were more resistant to skin infection or pneumonia caused by HLA-producing *S. aureus*. The collective data suggested that AJs impair the recovery of perforated cells, but the underlying mechanism remained unclear. In a paper by Shah *et al.*, recently published in *Cell Reports*, new data were presented which provide plausible explanations [7]. The authors propose that ADAM10 and HLA pores cluster at AJs, where they become locked. Consequently, cell death would occur, in part because pores could not be efficiently removed from the plasma membrane. Shah *et al.* characterize the protein interactions involved in the proposed docking of toxin pores to, and locking at, AJs. A role for TspanC8 proteins (a subgroup of tetraspanins, proteins characterized by four transmembrane domains) in the traffic of ADAM10 has been documented previously, but the role of these proteins in the localization of ADAM10 to AJs, and in the toxicity of HLA, was not known at the beginning of this study. Using confocal microscopy and biochemical approaches, Shah *et al.* show that ADAM10 binds to tetraspanin Tsp33, which, in the presence of PDZD11 (PDZ domain containing 11, an ATPase-interacting PDZ protein), docks it to junctions by interacting with the WW (tryptophan/tryptophan)-domain of the zonula adhaerens protein PLEKHA7. Further, the authors show that the N terminus of afadin, which is essential for the organization of AJs, directly binds to the C terminus of ADAM10 and tethers it to the zonular actin belt. Consistent with this, a C-terminally truncated derivative of ADAM10 has been shown to confer reduced cytotoxicity in HAP-1 cells, as compared to full-length ADAM10 [8].

Although focusing on cell biological aspects, the findings of Shah *et al.* may also have important implications for the basic mechanism of pore formation by HLA: the proposed locking mechanism implies that pore complexes remain associated with the putative HLA receptor. This could not be anticipated, because the interaction of ADAM10 with HLA was originally discovered by using a mutant HLA/glutathione-*S*-transferase-fusion protein (which is presumably monomeric) as bait in pulldown experiments [4]. That ADAM10 remains associated with HLA after pore formation invites investigations into the stoichiometry of the ADAM10/HLA pore complex that accumulates at cell junctions. Is a single ADAM10 molecule sufficient to nucleate formation of a pore? That pores, not just toxin monomers or pre-pore stages, are arrested at junctions was concluded on the basis of the persistence of stable toxin oligomers and the concomitant failure of cells to recover from toxin attack. Whether pore formation impacts junctional clustering of oligomers remains to be investigated; this could be done by using HLA mutants which can oligomerize but which do not form channels, or with inhibitors that clog the HLA pore.

As is usually the case with important new findings, they bring forth more questions. Does clustering of HLA pores at AJs impact ADAM10-dependent cleavage

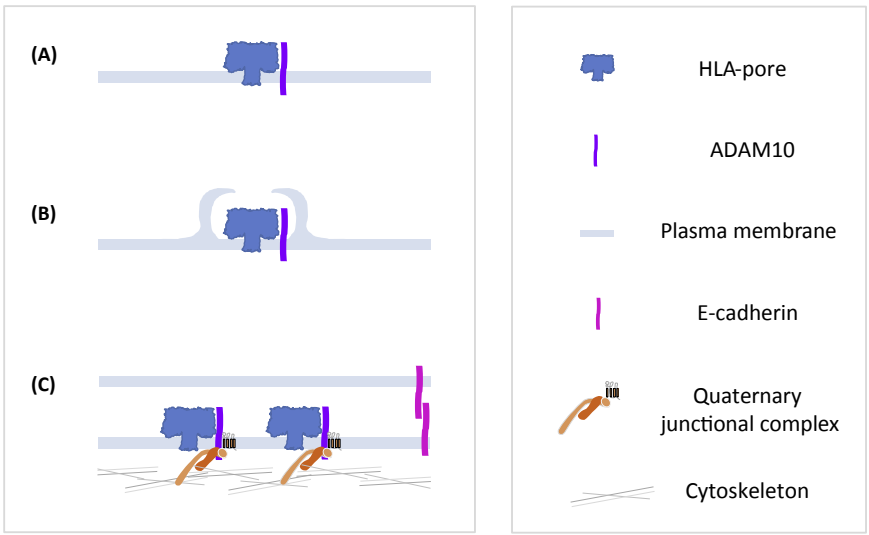

Figure 1. α-Hemolysin (HLA) Pores at Cell Junctions Escape Engulfment. (A) HLA binds to the plasma membrane via ADAM10, which leads to the formation of transmembrane pores. Uncontrolled flux of water and ions triggers multiple signaling pathways and may cause cell death. (B) Engulfment of pores by macropinocytosis, an inducible, high-capacity endocytic process, enables epithelial cells to survive an attack by HLA. (C) A quaternary junctional protein complex (comprising Tsp33, PLEKHA7, PDZD11, and afadin), organizes the clustering of ADAM10 at adherens junctions. HLA pores bound to junctional ADAM10 will escape engulfment, persist in the plasma membrane, and eventually kill the cell.

of substrates, or vice versa? Do similar interactions as those uncovered by Shah *et al.* play a role in other cell types? What is the fate of toxin monomers? Also, further details of HLA endocytosis deserve further analysis. Amiloride inhibited recovery, leading the authors to propose that uptake of HLA pores occurs via macropinocytosis. This would be in keeping with several previous observations, for example, the reduced endocytosis of HLA pores in the presence of dynasore [3], or inhibition of cellular recovery by cytochalasin D, an inhibitor of actin polymerization. Because macropinocytosis is regulated by the epidermal growth factor receptor, which is activated by HLA [9], and because it can internalize large patches of plasma membrane [10], it would seem possible that plasma membrane areas studded with HLA pores could be engulfed by this process. The concomitant intake of nutrients, a genuine function of macropinocytosis, could help cells to cope with amino acid starvation, a known consequence of membrane perforation by HLA and other pore-forming toxins [2]. Macropinocytosis could thus serve a dual function in target cells, namely, maintaining metabolic homeostasis and removing membrane pores. The paper by Shah *et al.* [7] and the foregoing work by Popov *et al.* [5] uncover how HLA targets a weak spot in cell autonomous defense (Figure 1), and they will certainly spur further research in this direction. In broader terms, their work exemplifies how investigations into host–pathogen interactions may elucidate basic cellular functions.

Acknowledgment

The authors would like to thank M. Wiedmann for critically reading the manuscript.

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https://doi.org/10.1016/j.tim.2018.11.010

References

1. Berube, B.J. and Bubeck Wardenburg, J. (2013) *Staphylococcus aureus* alpha-toxin: nearly a century of intrigue. *Toxins (Basel)* 5, 1140–1166
2. Bischofberger, M. *et al.* (2012) Pathogenic pore-forming proteins: function and host response. *Cell Host Microbe* 12, 266–275
3. Husmann, M. *et al.* (2009) Elimination of a bacterial pore-forming toxin by sequential endocytosis and exocytosis. *FEBS Lett.* 583, 337–344
4. Wilke, G.A. and Bubeck Wardenburg, J. (2010) Role of a disintegrin and metalloprotease 10 in *Staphylococcus aureus* alpha-hemolysin-mediated cellular injury. *Proc. Natl. Acad. Sci. U. S. A.* 107, 13473–13478
5. Popov, L.M. *et al.* (2015) The adherens junctions control susceptibility to *Staphylococcus aureus* alpha-toxin. *Proc. Natl. Acad. Sci. U. S. A.* 112, 14337–14342
6. Virreira Winter, S. *et al.* (2016) Genome-wide CRISPR screen reveals novel host factors required for *Staphylococcus aureus* alpha-hemolysin-mediated toxicity. *Sci. Rep.* 6, 24242
7. Shah, J. *et al.* (2018) A dock-and-lock mechanism clusters ADAM10 at cell–cell junctions to promote alpha-toxin cytotoxicity. *Cell Rep.* 25, 2132–2147 e7
8. von Hoven, G. *et al.* (2016) Dissecting the role of ADAM10 as a mediator of *Staphylococcus aureus* alpha-toxin action. *Biochem. J.* 473, 1929–1940
9. Haugwitz, U. *et al.* (2006) Pore-forming *Staphylococcus aureus* alpha-toxin triggers epidermal growth factor receptor-dependent proliferation. *Cell. Microbiol.* 8, 1591–1600
10. Watanabe, S. and Boucrot, E. (2017) Fast and ultrafast endocytosis. *Curr. Opin. Cell Biol.* 47, 64–71

Spotlight

Planting the Microbiome

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Plant-derived microRNAs stabilized by species-specific lipid nanoparticles mediate interkingdom communication through bacterial intermediates and impact consumer health. Ingested by distinct gut bacteria, these microRNA-containing particles alter bacterial gene expression to affect host immunity.