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Review

Defying death: Cellular survival strategies following plasmalemmal injury by bacterial toxins

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ABSTRACT

The perforation of the plasmalemma by pore-forming toxins causes an influx of Ca^{2+} and an efflux of cytoplasmic constituents. In order to ensure survival, the cell needs to identify, plug and remove lesions from its membrane. Quarantined by membrane folds and isolated by membrane fusion, the pores are removed from the plasmalemma and expelled into the extracellular space. Outward vesiculation and microparticle shedding seem to be the strategies of choice to eliminate toxin-perforated membrane regions from the plasmalemma of host cells. Depending on the cell type and the nature of injury, the membrane lesion can also be taken up by endocytosis and degraded internally. Host cells make excellent use of an initial, moderate rise in intracellular [Ca^{2+}], which triggers containment of the toxin-inflicted damage and resealing of the damaged plasmalemma. Additional Ca^{2+}-dependent defensive cellular actions range from the release of effector molecules in order to warn neighbouring cells, to the activation of caspases for the initiation of apoptosis in order to eliminate heavily damaged, dysregulated cells. Injury to the plasmalemma by bacterial toxins can be prevented by the early sequestration of bacterial toxins. Artificial liposomes can act as a decoy system preferentially binding and neutralizing bacterial toxins.

Contents

1. Introduction

Plasmalemmal integrity is critical for the division of the intracellular from the extracellular milieu. Since a breach in the plasmalemmal continuum leads to a potentially fatal imbalance in cellular homeostasis, damage control mechanisms are a necessity for the survival of eukaryotic cells. Both the immediate containment and repair of membrane damage as well as the alert of neighbouring cells to impending danger require an efficient intracellular diagnostic and therapeutic team.

The localization of a cell within the organism is critical for determining its likelihood of damage: by mechanical disruption: at skin or mucosa, by cyclic strain: in muscle cells or by chemical interference: perforation through pore-forming proteins in

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epithelia, immune cells and vascular endothelium. Plasmalemmal repair mechanisms and the necessary machinery are therefore adapted to the needs of the individual cell and its potential risk scenario (rev. by [1]).

Mechanical injuries of irregular size and shape are preferentially patched by lysosomal fusion and exocytotic membrane transport rev. by [2,3]. Also, contraction-associated injuries are taken care of by repair proteins, specialized for sarcolemmal lesions [4,5]. In contrast, the perforation of the plasmalemma by bacterial toxins requires a "surgical" removal of the lesions, which are subsequently expelled in the form of vesicles or taken up and degraded internally [6,7]. And in many cases, a combination of different repair mechanisms can be observed, e.g. when lysosomal exocytosis is coupled to endocytotic uptake [8] or when the initial perforation by a protein-lined toxin pore gives rise to a secondary mechanical lesion set off by Ca^{2+}-induced acto-myosin (over)contraction [9].

2. Plasmalemmal damage by bacterial toxins

Bacterial toxins are essential virulence factors for a large number of pathogens. The largest family (~30% of all bacterial toxins) are the pore-forming toxins. Bacterial pore-forming toxins generate either small (0.5–5 nm) or large (20–100 nm) protein-lined pores [10–12]. Characterized by their large pore size are the cholesterol dependent cytolysins (CDCs), which constitute a family of more than 20 members originating from 24 different Gram positive bacterial species, such as *Clostridium*, *Streptococcus*, *Listeria* and *Bacillus* [13]. Prevalent pathogens producing CDCs are *Streptococcus pyogenes* (streptolysin O, SLO) and *S. pneumoniae* (pneumolysin, PLY). Secreted as soluble monomers, these toxins bind to the plasmalemma, oligomerize and are assembled into pre-pores rev. by [11]. Conformational changes lead to the insertion of β-hairpins into the membrane and the creation of transmembrane toxin pores of ~300 Å in diameter [14]. Depending on the cell type and the degree of injury, a great variation in cellular reactions have been described (Table 1) ranging from cytokine release to cell lysis.

Other members of the pore-forming toxin family form heptameric units thus creating small pores (e.g. *S. aureus* α-toxin, leukocidins). They contribute to the exchange of ions, thereby constituting key virulence factors [11,37–39].

In addition, host cells have to protect themselves against a multitude of other toxins, which widely differ in lethal properties. Toxins conveying enzymatic activity such as phospholipases or sphingomyelinases have been demonstrated for *S. aureus*, *Clostridium perfringens* or *Pseudomonas aeruginosa* [40]. By cleaving the phospholipid PIP2 (by phospholipase C) or by hydrolyzing sphingomyelin to ceramide and phosphocholine (by sphingomyelinases) they prompt a multitude of signalling pathways, which eventually lead to apoptosis.

Yet, luckily for the organism, even sustained attacks by bacterial toxins are successfully countered by numerous cellular mechanisms for membrane repair and damage prevention.

3. Identification of a lesion: the critical role of Ca^{2+}

The process of plasma membrane resealing consists of sensing, detection and repair of the injuries. We have previously shown that protein-lined toxin pores generated by SLO or PLY lead to an influx of Ca^{2+} and an efflux of cytoplasmic contents [7,9,17,41]. The initial surge in Ca^{2+} through the perforated plasma membrane is countered by Ca^{2+} storage in cellular organelles such as the endoplasmic reticulum (Fig. 1a). The inability to restore the homeostatic ion balance leads to irreversible permeabilization of the plasma membrane with complete loss of cytoplasmic

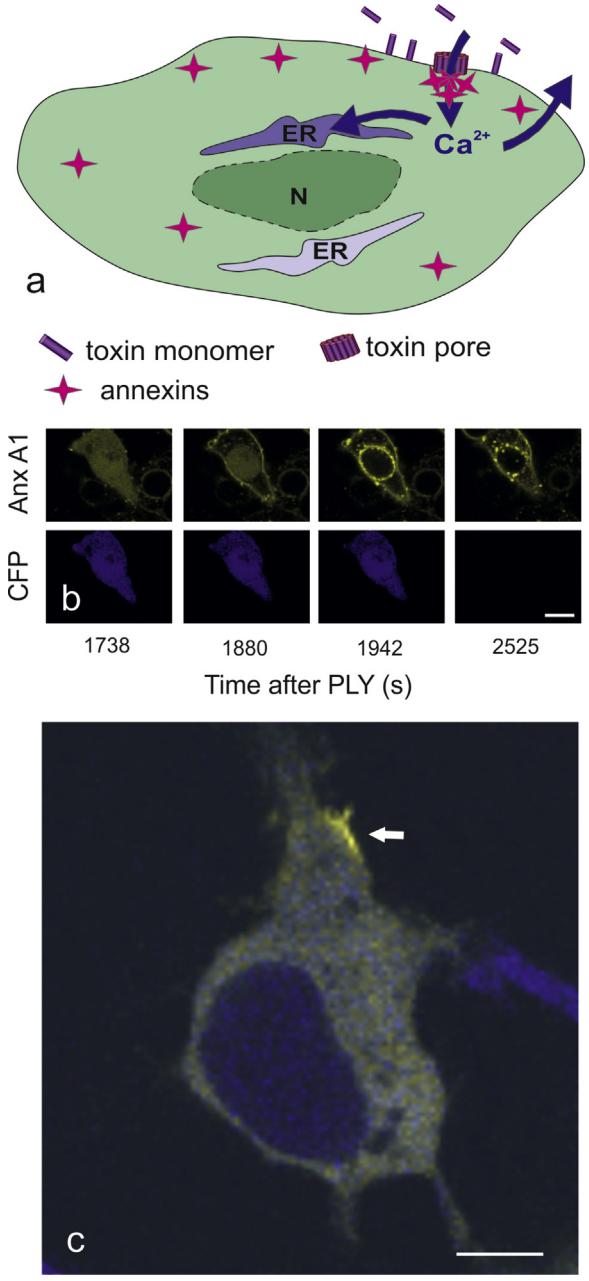

Fig. 1. Toxin-mediated Ca^{2+} influx leads to the translocation of annexins. (a) Secreted monomers of pore-forming toxins oligomerize and perforate the plasma membrane. Ca^{2+} influx leads to the filling of endoplasmic reticulum (ER) and the translocation of annexins. N = nucleus. (b) Fluorescently labelled annexins serve as a read-out for plasmalemmal permeabilization and repair of PLY pores. HEK293 cells expressing annexin A1 (Anx A1-YFP, yellow) and cytoplasmic CFP (CFP, blue) were treated with pneumolysin (PLY). Anx A1-YFP remains membrane-bound even after a complete loss of cytoplasmic CFP. (c) PLY pore dynamics in individual perforated cells. Confocal micrographs of HEK293 cells expressing Anx A1-YFP (yellow) and cytoplasmic CFP (blue) were challenged with PLY. Annexin A1 binding occurs at distinct plasmalemmal sites of PLY perforation due to the influx of extracellular Ca^{2+}. Scale bars = 10 μm.
Adapted from [17].

constituents (Fig. 1b). The cell's management of $[Ca^{2+}]_i$ is critical in the reaction to plasmalemmal damage: the extent of $[Ca^{2+}]_i$ elevation in perforated cells is defined by the balance between pore-induced Ca^{2+} entry and the activity of intracellular Ca^{2+} sequestrating mechanisms. The elevation in $[Ca^{2+}]_i$ plays a dual role in plasmalemmal repair: an increase in $[Ca^{2+}]_i$ due to Ca^{2+} entry from the extracellular milieu activates the plasmalemmal

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