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Review

Apoptosis induced by *Staphylococcus aureus* toxins

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ABSTRACT

Apoptosis stimulated by bacterial toxins is common during infection and is now considered important in disease processes. As a major human pathogen, *Staphylococcus aureus* also causes apoptosis during infection. In some diseases such as atopic dermatitis and sepsis, the apoptosis induced by *S. aureus* influences the severity and outcome of diseases. *S. aureus* has various toxins, many of which have reportedly triggered apoptosis. In this review, we focused on the apoptosis-inducing toxins secreted by *S. aureus,* and their underlying mechanisms. Novel therapies for cancer that utilized the reconstructed *S. aureus* toxins were also discussed.

1. Introduction

Staphylococcus aureus is one of the most infamous human pathogens, causing diseases from mild skin and wound infections to fatal sepsis or multiple organ failure (Yang et al., 2017). During infection, *S. aureus* can induce cell apoptosis through various pathways (Grassm et al., 2001). Apoptosis is a programmed cell death procedure that relies on an active cascade of cysteine endopeptidases called caspases (Fink and Cookson, 2005). Accumulated research data showed that apoptosis is pivotal in certain diseases caused by *S. aureus*, such as atopic dermatitis (AD) and sepsis (Ayala et al., 2007; Xu and Mccormick, 2012; Aziz et al., 2014). Cell apoptosis of the host immune system may conceivably facilitate *S. aureus* infection, and apoptosis of tissue cells can also trim the immune response by influencing cytokine production and T cell differentiation (Torchinsky et al., 2010). Thus, apoptosis can significantly affect *S. aureus* pathogenesis.

A remarkable feature of *S. aureus* is its vast arsenal of virulence factors, including toxins and other molecules that increase the potential of diseases. Toxins are secreted poisonous substances that directly interfere with the host; toxins have three categories, namely, membrane-damaging toxins, toxins that interfere with receptors, and secreted enzymes (Otto, 2014). Many *S. aureus* toxins, such as staphylococcal enterotoxins (SEs) and alpha-toxin (α-toxin), show proapoptotic activities (Ulett and Adderson, 2006). However, the exact apoptotic cell types induced by *S. aureus* toxins and the underlying mechanisms are still obscure. In this review, we focus on the apoptosis brought by *S. aureus* toxins and the mechanisms by which they induce apoptosis. However, other virulence factors, including surface-located proteins, such as staphylococcal protein A (SPA), also potentially trigger apoptosis (Das et al., 2002). In line with the abovementioned definition of toxins, these virulence factors will not be discussed in this review.

2. Membrane-damaging toxins

Membrane-damaging toxins can be further divided into two, namely, those that lyse cells dependently on initial receptor interaction and those that interfere with membranes without receptor interaction (Otto, 2014). Although *S. aureus* possesses many membrane-damaging toxins, including hemolysins, bi-component leukocidins, and phenol-soluble modulins, only α-toxin (α-hemolysin) and Panton-Valentine leukocidin (PVL) were capable of promoting apoptosis. However, α-toxin and PVL have different apoptosis-inducing abilities.

2.1. Alpha-toxin

As a major virulence factor of *S. aureus*, the *hla* gene on the chromosome encodes the α-toxin, which manifests as a secreted 33.3-kDa water-soluble monomer. Upon cell-binding, α-toxin oligomerizes to heptameric pores in the host cell plasma membrane (Iacovache et al., 2010). Although a high concentration of lipid bilayer could allow the absorption of α-toxin at low specificity, a membrane metalloproteinase called ADAM10 was recently reported as the α-toxin receptor (Inoshima et al., 2011). Besides pore formation, α-toxin reportedly interfered with cell membrane integrin and Caveolin-1 (Vijayvargia et al., 2004; Liang and Ji, 2006).

α-Toxin could stimulate the apoptosis of T cells that were purified from peripheral blood and cultured human umbilical vein endothelial cells even at sub-lytic concentrations (Jonas et al., 1994; Menzies and Kourteva, 2000). α-Toxin also triggered Jurkat T cell apoptosis through the intrinsic pathway, thereby releasing cytochrome C and upregulating

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the activities of caspases-3 and −9. Bcl-2 overexpression could reduce the α-toxin-induced apoptosis in Jurkat T cells, whereas Fas could not (Bantel et al., 2001). However, the authors argued 2 years later that α-toxin-induced cell death was more necrotic than apoptotic because the pan caspase inhibitor z-VAD-fmk could not inhibit the cell death process in Jurkat T cells, and the dead cells released lactate dehydrogenase (LDH) and HMGB-1, the molecular markers of necrotic cells. However, overexpression of Bcl-2 still inhibited the DNA fragmentation activated by α-toxin (Essmann et al., 2003). Another team came up with a conflicting result in the same year; either z-IETD-fmk (caspase-8 inhibitor) or z-LEHD-fmk (caspase-9 inhibitor) inhibited Jurkat T cell apoptosis brought by α-toxin, whereas tumor necrosis factor α (TNFα) did the opposite (Haslinger et al., 2003). Srivastava et al. (2009) partially explained this controversy by constructing an α-toxin H35N oligomerization-deficient mutant and found H35N as a promoter of apoptosis in A431 (derived from a human epidermal carcinoma of the vulva) and HeLa cells in the intrinsic pathway, thereby differentiating the proapoptotic abilities of H35N and the wild type α-toxin. The authors explained that α-toxins could be manifested in three forms, which were cell-bound monomer, the non-lytic pre-pore, and the lytic pore, upon binding to the target cell. Therefore, the cell death process was a combination of all three forms because only a fraction of the bound α-toxin underwent all conformational changes to form the lytic pore when the natural α-toxins were tested.

The α5β1-integrin interacted with α-toxin and affected α-toxin-induced apoptosis in A549 lung epithelial cell line where TNFα had a major role (Liang and Ji, 2007). Imre et al. (2012) demonstrated that the initial caspase of α-toxin-promoted apoptosis in HeLa cells was caspase-2, followed by caspases-8 and −9. Potassium efflux elicited by α-toxin regulated the activation of caspase-2, and another oligomerization-deficient mutant of α-toxin, D152C, could not induce apoptosis in HeLa cells (Imre et al., 2012). α-Toxin could induce apoptosis in ECV304 endothelial cells (ECV304 is now argued as a bladder cancer cell line) through the extrinsic pathway via TNFα (Yu et al., 2013). These two studies broadened the mechanistic information of α-toxin-induced apoptosis besides the intrinsic pathway.

Thus, α-toxin-induced apoptosis has various pathways in different cell types, including intrinsic pathway, extrinsic pathway, and even caspase-2-initiated pathway. However, TNFα is the only cytokine that has demonstrated importance in α-toxin-triggered apoptosis. Given its propensity to oligomerization, α-toxin could exert a more profound influence on cell death than apoptosis. As demonstrated by Srivastava et al. (2009), H35N mutant showed a different cell death-inducing ability from wild type α-toxin, where the process caused by the former could be a combined phenomenon of different cell death types and stages. Hence, simple utilization of caspase inhibitors could reduce but not block α-toxin-induced cell death. Even though α-toxin stimulated the release of LDH and HMGB-1 in Jurkat T cells, the cell death process could not simply attribute to α-toxin-induced necrosis because secondary necrosis usually occurred following apoptosis (Silva, 2010). As a major toxin of *S. aureus,* α-toxin has multiple functions aside from pore-formation, but its exact proapoptotic role needs further investigation. Apparently, the apoptosis-inducing abilities of α-toxin in different cell types and the underlying mechanisms remain complex, and more cell types should be selected to determine the apoptotic roles of α-toxin.

2.2. PVL

PVL is the most infamous representative of *S. aureus* bi-component pore-forming toxins, and reportedly lyses human polymorphonuclear neutrophils (PMNs), monocytes, and macrophages, but not erythrocytes (Hu et al., 2015). Epidemiological studies have revealed that PVL is mainly carried by community-acquired methicillin-resistant *S. aureus* and is closely related to skin and soft-tissue infections (Shallcross et al., 2013). As a bi-component toxin, the pore formation requires the presence of both LukS-PV and LukF-PV, which are secreted before assembling into a pore-forming octamer. Recently, C5a receptor (C5aR, CD88) was also determined as a PVL receptor (Spaan et al., 2013). LukS-PV initiates C5aR-binding and subsequently dimerizes with LukF-PV, followed by alternate serial binding of LukF-PV and LukS-PV until the octamer assembly is complete (Kaneko and Kamio, 2004).

PVL promoted PMN apoptosis at sub-lytic concentrations through the intrinsic pathway, destroying the mitochondrial membrane and releasing cytochrome C (Genestier et al., 2005). PVL also triggered apoptosis in human alveolar macrophages and mouse clonal MC3T3-E1 pre-osteoblastic cell line (Wu et al., 2010; Jin et al., 2013). Being different from other secreted toxins, PVL could facilitate the escape of intracellular *S. aureus* from the endosome and stimulate the apoptosis in RHEK-1 human keratinocyte cell line, which could be blocked by z-VAD-fmk (Chi et al., 2014). Moreover, Bu et al. (2013) and Shan et al. (2015) demonstrated that the binding component, LukS-PV, could even inhibit cell proliferation and activated apoptosis in human acute myeloid leukemia cell lines, THP-1 and HL-60.

To date, PVL-induced apoptosis falls under the intrinsic pathway. However, very limited studies have tested the proapoptotic ability of this toxin. More cell types aside from leukocytes could be further tested for PVL-induced apoptosis. As mentioned above, the binding component, LukS-PV, could only promote apoptosis in THP-1 and HL-60 cell lines, where the underlying mechanisms of this process are of interest. Because C5aR is the receptor for LukS-PV, and the interaction of C5aR with its native ligand C5a triggers apoptosis in thymocytes, neurocytes, and endothelial cells (Guo et al., 2000; Jacob et al., 2010; Mahajan et al., 2016), the possibility of whether PVL and C5a could stimulate apoptosis via similar mechanism is worthy of further exploration.

3. Enterotoxin superfamily

The only proapoptotic toxins in the second category belong to the enterotoxin superfamily, which consists of SEs, the staphylococcal enterotoxin-like (SELs) proteins, and toxic shock syndrome toxin-1 (TSST-1). SEs were originally named based on their abilities to cause food poisoning and included at least 10 members, namely SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SER, and SET. SEs are sometimes referred to as staphylococcal super antigens (SAgs) because of their abilities to activate T cells and to stimulate hyper-inflammatory responses (TSST-1 was terminated SEF before and also acted as a superantigen). SEL proteins, including SELJ, SELK, SELL, SELM, SELN, SELO, SELP, SELQ, SELS, SELU, SELV, and SELX, are homologous and are structurally similar to the SEs. However, SEL proteins can neither induce emesis nor activate T cells unlike SEs (Xu and Mccormick, 2012).

The common structure of this protein superfamily consists of an N-terminal β-barrel motif similar to an OB-fold, which can bind to the Vβ region of the T cell receptor (TCR), and a C-terminal β-grasp motif that can ligate with type II major histocompatibility complex (MHCII) molecules. Proteins from the enterotoxin superfamily can be classified into five groups based on their structure and homology. Group I only has TSST-1. Given its truncated C-terminal, TSST-1 ligates to MHCII at a low affinity and does not induce emesis. However, TSST-1 can still bind to TCR and triggers toxic shock syndrome. SEB and SEC belong to group II and have a low-affinity MHCII α-chain binding domain in the C-terminal. SEA, SED, SEE, and SEH fall under group III and contain a high-affinity and zinc-dependent MHC II β-chain binding domain in addition to the low-affinity α-chain binding domain, so group III toxins can crosslink MHC II molecules. Both Group II and III toxins contain a unique cysteine-loop structure that is important for emetic activity. All Group IV toxins are streptococcal SAgs and are therefore beyond the scope of this review. Group V toxins contain mostly staphylococcal SAgs that do not cause emesis (Pinchuk et al., 2010; Spaulding et al., 2013). SEs showed diverse apoptosis-activating abilities among family members. Even a given SE can yield a different apoptosis-inducing effect in different cell types.