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Title

Intracellular *Staphylococcus aureus* and host cell death pathways

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Take Away:

- *Staphlyococcus aureus* is capable of persistence within host cells.
- *S. aureus* is exposed to cell surveillance mechanisms and programmed cell death (PCD).
- This review focuses on how *S. aureus* disrupts the intracellular, interferes with PCD
- In the absence of effectors, *S. aureus* uses pathoadaptive mutants to persist in the face of PCDs

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Abstract

Staphylococcus aureus is a major opportunistic human pathogen that is globally prevalent. Although *S. aureus* and humans may have co-evolved to the point of commensalism, the bacterium is equipped with virulence factors causing devastating infections. The adoption of an intracellular lifestyle by *S. aureus* is an important facet of its pathogenesis. Occupying a privileged intracellular compartment permits evasion from the bactericidal actions of host immunity and antibiotics. However, this localization exposes *S. aureus* to cell-intrinsic processes comprising autophagy, metabolic challenges, and clearance mechanisms orchestrated by host programmed cell death pathways (PCDs), including apoptosis, pyroptosis and necroptosis. Mounting evidence suggest that *S. aureus* deploys pathoadaptive mechanisms that modulate the expression of its virulence factors to prevent elimination through PCD pathways. In this review, we critically analyze the current literature on the interplay between *S. aureus* virulence factors with the key, intertwined nodes of PCD. We discuss how *S. aureus* adaptation to the human host plays an essential role in the evasion of PCD, and we consider future directions to study *S. aureus*-PCD interactions.

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1. Introduction

Well-studied intracellular bacterial pathogens such as *Salmonella*, *Yersinia* and *Listeria* co-opt and subvert host processes to support their cytosolic residency, ranging from cytoskeletal scaffolding, vacuolar trafficking to catabolism (Mostowy and Shenoy, 2015; Omotade and Roy, 2019). Bacterial pathogens also increase their chances of survival by disrupting host programmed cell death (PCD), an intrinsic cellular response that eliminates cells under certain stressors (Baxt et al., 2013; Sanchez-Garrido et al., 2020). PCD pathways are fundamental for the maintenance of host cell homeostasis (Tang et al., 2019). In the context of infection, cell death is considered as a limiting mechanism hampering the dissemination of pathogens by exposing them to extracellular immune surveillance mechanisms. In the past decade, breakthroughs in PCD have been facilitated by advances in the understanding of bacterial manipulation of cell death. Bacteria exploiting intracellular spaces are endowed with repertoires of proteins called effectors that promote host cytoskeletal rearrangements, interact with cell-autonomous mechanisms and interfere with PCD (Gan et al., 2021; Gaudet et al., 2016; Mostowy and Shenoy, 2015). The in-depth characterization of bacterial effectors subverting key nodes of PCD pathways, such as those critical for inflammasome surveillance, has revealed mechanisms whereby pathogens avoid elimination and dictate the fate of their host cells (Demarco et al., 2020; Eng et al., 2020; Sanchez-Garrido et al., 2020).

S. aureus is a gram-positive human commensal bacterium, asymptomatically colonizing up to 30% of the human population in their nasal cavity (Sakr et al., 2018). Yet, *S. aureus* also causes life-threatening infections upon invasion of the bloodstream, skin, soft tissues and bones (Tong et al., 2015). The bacterial determinants promoting the transition of *S. aureus* from benign commensal to a life-threatening pathogen are not completely understood, and their expression

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may be subjected to different and selective pressures from the host and in response to the local microbiome (Boldock et al., 2018; Sakr et al., 2018; Young et al., 2017a).

Whilst considered a devasting extracellular organism, mounting evidence support *S. aureus* as a proficient intracellular pathogen. *S. aureus* is capable of entry, replication and persistence in host cells to evade bactericidal immunity insults (opsonization and circulating antibodies for example), antibiotic treatment, and detection by surface exposed pattern recognition receptors (PRR) (Casadevall and Fang, 2020; Flannagan et al., 2016; Kwiecinski and Horswill, 2020; Lowy, 2000). Yet, intracellular residency exposes *S. aureus* to cell-autonomous surveillance mechanisms (Randow et al., 2013). Many *S. aureus* virulence disrupt this privileged space, and those affecting PCD pathways are listed in Table 1. Moreover, cell wall components of intracellular *S. aureus* can be detected by the host as pathogen-associated molecular patterns (PAMPs) (those of interest with respect to PCD are listed in Table 2) (Fournier and Philpott, 2005), and can trigger innate immune responses (Davis et al., 2011).

The ability of *S. aureus* to switch from an indolent organism to a lethal pathogen is not well understood and challenges the roles of PCDs on *S. aureus* commensalism. However, current findings suggest that pathogenic *S. aureus* triggers a specific PCD over another, depending on its infection stage and the targeted host cells. It appears that the engagement of PCD pathways is also conditioned by bacterial sensing of environmental cues, such as host nutrient availability and degradative processes, to which pathogens respond by adjusting their metabolism or by modulating the expression of specific bacterial virulence factors, respectively (Bravo-Santano et al., 2018; Das et al., 2016; Wong Fok Lung and Prince, 2020). This review assesses the strategies employed by *S. aureus* to establish and maintain an intracellular lifestyle in the context of PCDs, grouped into *non-lytic* and *lytic* forms (Fig. 1A), the connectivity between

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PCD pathways (Fig. 1B) and explores the contributions of autophagy and host-pathogen metabolic interplay that are engaged during the bacterial intracellular lifestyle (Fig. 1A). We discuss avenues for future investigation to study PCDs and the pathogenesis of this highly adapted, yet genetically flexible pathogen.

2. Entering and disseminating in epithelia: a preambular dance with PCD

Crossing the epithelial barrier enables the transmigration of *S. aureus* into deeper sections of host tissues. The plethora of surface proteins decorating *S. aureus* are recognized by cognate host receptors and promote bacterial adhesion and entry into non-phagocytic cells (reviewed in (Foster et al., 2014; van Dalen et al., 2020)). Cell internalization protects *S. aureus* from antibiotic treatments and systemic immune insults (Strobel et al., 2016; Surewaard et al., 2016). Importantly, whilst this offers the bacterium a replicative niche, where it can thrive despite the lysosomal degradative processes (Lacoma et al., 2017; Moldovan and Fraunholz, 2019; Tranchemontagne et al., 2016), it also implies that *S. aureus* interferes with PCD to ensure the viability of its newfound shelter.

The breach of epithelial tissues is orchestrated by *S. aureus* alpha-toxin (Hla), instigating the invasion of keratinocytes (Inoshima et al., 2012). The cytolytic activity of Hla acts in concert with the enzymatic function of its host receptor, A-disintegrin-and-metalloprotease-10 (ADAM10) (Giebeler and Zigrino, 2016; Wilke and Wardenburg, 2010), which in turn enables dissemination of *S. aureus* to deeper epithelial sections (Inoshima et al., 2011; Kintarak et al., 2004).

Autophagy is an essential catabolic mechanism supporting cell survival by recycling cellular components and contributing to the degradation of invading pathogens into presentable

antigens (Deretic et al., 2013; Galluzzi et al., 2014; Mostowy, 2013). Autophagy is manipulated by intracellular *S. aureus* in multiple ways. While eluding autophagic elimination, *S. aureus* diverts the nutrients generated by this pathway to meet its metabolic needs (Bravo-Santano et al., 2018; O'Keeffe et al., 2015).

During the infection process, membranes damaged by pore forming toxins (PFTs) are recycled by autophagy, thereby preserving the integrity of epithelia. This repairing seems to also support *S. aureus* dissemination. Mice affected in autophagy (*ATG16L1*^{HM}) display upregulated expression levels of ADAM10 in epithelial and endothelial cells; increasing the availability of receptors to Hla and promoting *S. aureus* propagation deeper into skin tissue (Fig. 2A) (Maurer et al. 2015). Although *ATG16L1*^{HM} mice showed exacerbated symptoms as compared to infected WT mice, they do not present a higher bacterial burden (Maurer et al., 2015b). This observation challenges the pathogen-restrictive functions of autophagy, suggesting that *S. aureus* co-opted autophagic processes during its evolution with humans as it reduces the tissue damage during bacterial dissemination.

The interaction of Hla with ADAM10 has consequences for PCD, as Hla activates the acid sphingomyelinase (ASM) (Becker et al., 2017), resulting in lysosomal leakage of cathepsin D (CTSD) and B (CTSB), activators of the inflammasome (Fig. 2B) (Ma et al., 2017). However, evidence suggest that intracellular *S. aureus* develops mutations in the *agr* regulon (reviewed in (Bronesky et al., 2016)) that negatively impact the production of Hla, and modulate inflammasome activation, also through autophagy. *S. aureus* infected keratinocytes chemically stimulated to induce autophagy displayed lower inflammasome activation and reduced mortality, while inhibition of autophagy resulted in increased cell death levels (Soong et al., 2015). This raises the possibility that host-adapted *S. aureus* avoids cell detection through the

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activity of unknown Agr-dependent factors, redirecting inflammasome components towards autophagy, thus silencing signaling leading to PCD (Schnaith et al., 2007). The subversion of autophagy to impede PCD has also been observed in primary polymorphonuclear cells, wherein *S. aureus* surviving in autophagosomes simultaneously inhibit apoptosis (Mulcahy et al., 2020). Such a strategy suggests that intracellular *S. aureus* may promote the survival of circulating cell reservoirs supporting bacterial dissemination and the formation of secondary infection sites.

3. *S. aureus* interferes with apoptosis, a non-lytic PCD.

Apoptosis is triggered either through the intrinsic pathway, *via* mitochondrial insults, or *via* the extrinsic pathway in response to cell surface receptor engagement; with both leading to the activation of initiator caspases, such as caspase-8 (Fig. 1B). The two pathways converge by activating the executioner caspases 3, 6, and 7, which in turn activate substrates leading to the physical manifestations of apoptosis (for review see (Singh et al., 2019)).

Although staphyloccocal toxins lead to a wide range of biological consequences resulting in apoptosis (Table 1) (Zhang et al., 2017), *S. aureus* also has factors that are specifically targeting this PCD. These can either inhibit apoptosis to enable the survival of infected host cells (Korea et al., 2014), or promote it to neutralize cells operating efferocytosis, a mechanism whereby dying cells are eliminated by patrolling immune cells (Lawrence et al., 2020; Winstel et al., 2019). The non-lytic and non-inflammatory nature of apoptosis is advantageous to intracellular pathogens, as this PCD likely prevents their clearance by the immune system upon their release from dying cells (Demarco et al., 2020).

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The diversity of *S. aureus* PFTs represents as many opportunities to trigger apoptosis. For instance, the toxin Hla, in isolation or secreted during bacterial infection can form pores into mitochondria, which triggers intrinsic apoptosis (Fig. 1B) (Bantel et al., 2001). Exposure of monocytes and epithelial cells to Hla and staphylococcal enterotoxin B (SEB) induce the extrinsic apoptosis (Haslinger et al., 2003; Liang and Ji, 2007; Yu et al., 2013; Zhang et al., 2017). Luk-S, a component of the Panton-Valentine leukocidin (PVL), stimulates intrinsic apoptosis in polymorphonuclear neutrophils, as observed in the lung sections from patients succumbing to *S. aureus* necrotizing pneumonia (Genestier et al., 2005) and is characterized by elevated levels of BAX, caspase-3 and -9 in toxin-treated THP1 cells (Bu et al., 2013; Shan et al., 2015). In addition to stimulating apoptosis in human keratinocytes, PVL is also required for the bacterial escape from the endosomal space, highlighting the importance of PFTs to the intracellular lifestyle of *S. aureus* (Chi et al., 2014).

Conversely, pathoadaptive mutations in master regulator genes controlling PFT production could be regarded as a mechanism to preclude apoptosis (Laabei et al., 2015). Reports of upregulated levels of the anti-apoptotic factors BCL-2 and MCL-1, in persistently infected phagocytes, suggested that *S. aureus* may promote a pro-survival cell program (Koziel et al., 2013, 2009; Kubica et al., 2008). The genome sequencing of strains used in these studies revealed the presence of *agrA* and *saeR* mutations (Sause et al., 2017). Reduced PFTs levels and attenuated cytotoxicity are typical phenotypes for these mutants (Moldovan and Fraunholz, 2019; Sause et al., 2017). These observations further support the existence of unidentified effectors affecting apoptotic processes controlled by the *agr* regulon to promote intracellular persistence, as observed in keratinocytes (Soong et al., 2015) .

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In a balancing act to PFT-driven apoptosis, the *S. aureus* Type Seven Secretion System (T7SS, also referred to as ESAT-6-like Secretion System) can directly manipulate this PCD (Unnikrishnan et al., 2017; Warne et al., 2016). The T7SS effector EsxA was shown to prevent apoptosis during epithelial cell infection, even under staurosporin treatment, an inducer of apoptosis (Fig. 2C) (Korea et al., 2014). This activity was confirmed in human dendritic cells, wherein T7SS effectors dampened the production of pro-inflammatory cytokines (Cruciani et al., 2017), and stimulated an anti-inflammatory response in infected mice (Ohr et al., 2017). However, the identity of apoptotic pathway nodes affected by T7SS effectors remain undefined. Interestingly, T7SS effectors also mediate interbacterial competition under both laboratory conditions and during host infection (Ohr et al., 2017; Ulhuq et al., 2020); sharing similarities with the trans-kingdom activities displayed by some Type VI Secretion System effectors (Hachani et al., 2016). Whether the *S. aureus* T7SS effectors mediating bacterial antagonism are also affecting apoptosis is to date unknown.

S. aureus kills neutrophils and induces apoptosis to blunt efferocytosis

Neutrophils are the most abundant and bactericidal circulating cells, constituting an instrumental arm of the innate immunity to control staphylococcal infections (McDonald et al., 2012; András N. Spaan et al., 2013). Neutrophils engulf and capture bacteria using NETosis, a mechanism whereby they project neutrophil extracellular traps (NETs) containing condensed chromatin and antimicrobial peptides (Brinkmann, 2004). They also secrete inflammatory cytokines that recruit phagocytes performing efferocytosis; a process clearing apoptotic and dying cells from infection sites to maintain tissue homeostasis and resolve inflammatory damages (reviewed in (Doran et al., 2020)). While during pneumonia, *S. aureus* Hla directly interferes with efferocytosis by alveolar macrophages (Cohen et al., 2016; Greenlee-Wacker et al., 2014), bacteria can also indirectly impact this process by targeting NETs. Indeed, using

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two secreted enzymes, the adenosine synthase A (AdsA) and Nuc, *S. aureus* counteracts the NETs microbicidal activity by converting their deoxyadenosine monophosphate (dAMP) content into deoxyadenosine (dAdo) (Thammavongsa et al., 2013). Macrophages recruited to clear neutralized neutrophils undergo caspase-3-mediated apoptosis following dAdo exposure (Thammavongsa et al., 2013) (Fig. 2C and Table 1). Conversely, caspase-3-deficient macrophages are insensitive to dAdo toxicity, suggesting that individuals bearing loss-of-function *CASP3* polymorphisms may be less susceptible to *S. aureus* infection (Winstel et al., 2019).

Overall, *S. aureus* appears to dynamically manipulate apoptosis to prevent the escalation of immune responses subsequent to acute staphylococcal infections. Yet, the pathogen present numerous PAMPs at its surface which can signal its extracellular presence to the host immune system.

4. The roles of lytic PCDs during *S. aureus* intracellular infection

The extracellular detection of *S. aureus* is an important arm of host defenses to mount a cellular immune response against this pathogen (Fournier, 2013). In particular, the specific recognition of staphyloccocal PAMPs (listed in Table 2) by Toll-like receptor 2 (TLR2) present at the cell surface, is necessary to initiate a signaling cascade leading to NF-kB induction and pro-inflammatory cytokine production (Fig. 1B) (Dziarski and Gupta, 2005; Fournier, 2013; Iwaki et al., 2002; Schwandner et al., 1999). TLR2 signaling contributes to control *S. aureus* infection, as infected TLR2 knockout mice display exacerbated symptoms (Takeuchi et al., 2000; Yimin et al., 2013), and human TLR2 polymorphism is associated with increased susceptibility to staphylococcal infections (Stappers et al., 2014). *S. aureus* possesses multiple strategies to disrupt TLR2-mediated signaling, including superantigen-like proteins to impair heterodimer TLR2-TLR6 association (Bardoel et al., 2012; Koymans et al., 2015; Yokoyama

et al., 2012), TIR domain-containing proteins to reduce TLR signaling and NF-kb activation by structural mimicry (Patot et al., 2017), the surfactant activities of phenol-soluble modulin (PSM) peptides that modulate shedding of bacterial lipopeptide TLR2 agonists (Hanzelmann et al., 2016), and a lipase with glycerol ester hydrolase (Geh) activity also targeting surface-exposed *S. aureus* lipoproteins (Chen and Alonzo, 2019).

While the adoption of an intracellular niche can occlude *S. aureus* from extracellular detection, it exposes the bacteria to inflammasome cytosolic surveillance, a key element in lytic PCD (Vince and Silke, 2016). The inflammasome is a conserved microbial surveillance platform consisting of pattern recognition receptors (PRRs) bearing NLR-pyrin domains (such as NLRP3) recognizing staphylococcal PAMPs (Fig. 1B) (Fournier and Philpott, 2005; Martinon et al., 2002) that include peptidoglycan, lipoteichoic acid, lipoproteins, or internalized bacterial vesicles (Fig. 2D) (Wang et al., 2020). PAMP detection by the inflammasome activates a priming signal (Shimada et al., 2010; Wang et al., 2020), leading to the transcription of NF-kB, and the upregulation of NLRPs subunits and pro-inflammatory cytokines genes, with the second signal promoting the proteolysis and maturation of caspase-1, instrumental in the orchestration of pyroptosis, a destructive form of PCD (Hara et al., 2018)

Pyroptosis and S. aureus

During infection by intracellular pathogens, pyroptosis - a highly inflammatory PCD - culminates in cell membrane perforation, ion efflux and the release of pro-inflammatory cytokines (Fig. 1B) (Hara et al., 2018; Miao et al., 2010). Staphyloccocal PAMPs detection, ROS generation or lysosomal damage caused by the escape of *S. aureus* to the cytosol, are cues contributing to the activation and engagement of caspase-1, pro-inflammatory interleukins and gasdermin D (GSDMD) (Fig. 1B). Caspase-1 is critical in the maturation of pro-IL-1β and pro-IL-18, and the cleavage of GSDMD into GSDMD-N (also performed by caspase-4/5 in humans

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and caspase-11 in mice) (Shi et al., 2015). Subunits of GSDMD-N oligomerize as a pore into the plasma membrane (Kayagaki et al., 2015; Liu et al., 2016), allowing the release of leaderless IL-1 and IL-18 into the extracellular space, to induce a strong inflammatory response (Broz and Dixit, 2016; Kayagaki et al., 2015; Liu et al., 2016). Interestingly, GSDMD pore formation is controlled by the endosomal sorting complexes required for transport (ESCRT), a membrane repair machinery, to mitigate the progression of cell lysis (Rühl et al., 2018). However, such repair systems cannot amend the damage caused by *S. aureus* PFTs (Bouillot et al., 2018).

The degree of inflammasome activation by *S. aureus* will depend on the cell type targeted by *S. aureus*. Contrary to the trajectory of infection within macrophages where the intracellular sensing of peptidoglycan is required to initiate IL-1β production, keratinocytes present a readily activated inflammasome as they constitutively express pro-IL-1β (Shimada et al., 2010; Soong et al., 2012). Considering the deleterious activities of its PFT toxins, *S. aureus* inevitably activates the inflammasome (Melehani and Duncan, 2016), and consequently inflammation (Miller et al., 2007; Muñoz-Planillo et al., 2009; Soong et al., 2012). These observations were collected from animal studies, so they should carefully be considered in light of the species tropism of *S. aureus*. Infection of human-induced pluripotent stem cell- derived macrophages and their cognate PFT receptor knock-outs showed that *S. aureus* inflammasome activation results in different outcomes depending on the studied PFT (Chow et al., 2020).

As a bactericidal process, pyroptosis should be examined in the context of *S. aureus* adaptation to cytosolic environments during both colonization and chronic infection (Young et al., 2017b). The recognition of bacterial cardiolipin by cell-autonomous surveillance has recently emerged as a conserved antimicrobial strategy (Liu and Lieberman, 2017). For instance, by targeting the

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cardiolipin rich domains of bacterial membranes, GSDMD-N itself is a potent anti-microbial peptide against *S. aureus* (Liu et al., 2016). These domains are also targeted by septins that are components of the cell cytoskeleton recognizing dividing bacterial pathogens to restrict them into "septin cages" and direct them towards autophagy (Krokowski et al., 2018; Krokowski and Mostowy, 2016). In view of its manipulation of autophagy, it is possible that septin recognition of cardiolipin benefits directly *S. aureus* by eluding the bactericidal activity of pyroptosis during chronic infections.

Given that intracellular *S. aureus* redirect inflammasome components towards autophagy, it maybe that the bacteria are evading elimination by preventing pyroptotic cell death and thus limiting exposure to extracellular immunity (Shi et al., 2012; Soong et al., 2015). Genomic analyses of host-adapted *S. aureus* clinical isolates have highlighted mutations in master regulators controlling production of key toxins (Jenul and Horswill, 2018), that reduce toxicity and favor persistence in the host (Das et al., 2016). These observations support evasion of inflammasome activation and pyroptosis as essential for *S. aureus* host-adaptation. However, as these mutations are phase variable and reversible (Gor et al., 2019), *S. aureus* could resume "pathogenic behavior" triggering pyroptosis later during host colonization and provoke an inflammatory relapse. Pyroptosis intersects with apoptosis via caspase-1, t-Bid and caspase-3 (Tsuchiya et al., 2019). This suggests that in the absence of GSDMD and canonical pyroptosis, PCD might still be engaged via apoptosis under certain conditions during *S. aureus* infection.

It is notable that pyroptosis is also important for NETosis, as the generation of NETs depends on GSDMD (Sollberger et al., 2018). Whether *S. aureus* contribute directly to the processes governing NETosis remains to be investigated. Due to its highly inflammatory nature, pyroptosis could be preferentially engaged during acute *S. aureus* infections. This also suggests

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that conversely, lytic PCD occurring during chronic intracellular *S. aureus* infections may be associated with necroptosis.

Necroptosis: backup lytic PCD during S. aureus chronic infection?

Necroptosis, a caspase-independent PCD, can be initiated through the engagement of death-domain containing receptors, such as the TNF-α stimulation of the receptor TNFR1 (Wilson et al., 2009) and/or detection of PAMPs by TLRs. (He et al., 2011) (Fig. 1B). These signaling cascades leads to the formation of the necrosome and activate the necroptosis executioner, the Mixed Lineage Kinase Domain Like pseudokinase (MLKL). Oligomerization of MLKL at the cell membrane forms cation-selective pores (Xia et al., 2016) and subsequent cell death (Murphy et al., 2013) (Fig. 1B).

The combined activities of *S. aureus* toxins lead to MLKL pore-formation and result in necrotizing pneumonia during lung infection, however the mechanistic details supporting this process are obscured by the joint activities of the bacterium's many toxins (Kitur et al., 2015). Mice subjected to lung infection with *S. aureus* strain USA300 display necroptosis-induced inflammation, leading to the reduction of resident alveolar macrophage population and are associated with increased bacterial burden (Kitur et al., 2015). Increased bacterial loads were also observed in murine skin and sepsis infection models using *MLKL*$^{-/-}$ knock-out mice and chemical inhibition of RIPK1 and RIPK3, suggesting that necroptosis was required to restrict *S. aureus* infection by limiting local inflammation. *In vitro*, most of *S. aureus* secreted toxins played a role in the cytotoxicity of human derived THP1 monocytic cells, using either supernatants or directly toxin cognate mutants during cell infection (Kitur et al., 2016). Hla induced MLKL phosphorylation and pore formation, and specific inhibitors of RIPK1 (Takahashi et al., 2012) or MLKL (Sun et al., 2012) protected toxin-treated murine

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macrophages (González-Juarbe et al., 2015) and human airway epithelial cells from necroptosis (Gonzalez-Juarbe et al., 2018). PSM toxins can also contribute to necroptosis in neutrophils during pneumonia (Zhou et al., 2018). Although PVL is also a PFT, its role in necroptosis remained elusive in murine models (Kitur et al., 2015). This lack of evidence in mice may be attributed to the evolutionary adaptation of *S. aureus* to humans, as PVL toxin exhibit strong specificity with the human complement receptor C5aR (András N. Spaan et al., 2013). Indeed, using humanized mice showed that LukF-PV specifically interacted with the human receptor CD45 (Tromp et al., 2018). The fact that infected human polymorphonuclear leukocytes (PMN) underwent a RIPK3-dependent cell death, independently from TNF-α stimulation, MLKL and RIPK1, further underscored the important differences between murine and human models in necroptosis studies (Greenlee-Wacker et al., 2017).

Whilst necroptosis leads to cell death, it does not efficiently eradicate *S. aureus* during chronic infections, and a robust inflammatory response is still required to achieve bacterial clearance (Wong Fok Lung and Prince, 2020). Necroptosis is also critical for NETosis, as inhibition of RIPK1 activity and absence of MLKL prevented NETs formation and exacerbated *S. aureus* infection in mice (D'Cruz et al., 2018).

Metabolic interplay between host and S. aureus: consequences for necroptosis

During chronic infection, *S. aureus* adapts to its environment by switching to a persistent, slow-growing phenotype (the small colony variant, SCV) (Proctor et al., 2006; Tuchscherr et al., 2011). Phenotypically, SCVs may arise due to various mutations occurring in progenitor cells affecting auxotrophy and resistance to antibiotics (Lannergård et al., 2011). The mechanisms supporting this phenotypic switch do not necessarily result in stable genetic changes (Tuchscherr et al., 2020) and in some cases involve large structural chromosomal rearrangements (Gao et al., 2015; Guérillot et al., 2019). Despite their apparent reduced toxicity

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(Häffner et al., 2020), SCVs are associated with significant morbidity (Häffner et al., 2020; Vulin et al., 2018).

SCV metabolic adaptation to intracellular lifestyle also accounts for successful host colonization (Gabryszewski et al., 2019; Wong Fok Lung et al., 2020; Yang et al., 2018) . It was recently shown that *S. aureus* engages in metabolic competition for glucose in keratinocytes, with the mitochondrial ROS generated during this interplay leading to necroptosis. This PCD could be prevented with inhibitors of glycolysis and ROS scavengers, independently of the presence of toxins (Wong Fok Lung et al., 2020). Specifically, higher expression levels of *fumC*, coding for fumarate hydratase converting fumarate to malate, have been observed in clinical SCVs isolated from skin infection and lungs of cystic fibrosis patients (Acker et al., 2019; Gabryszewski et al., 2019). By controlling fumarate levels, itself a glycolytic inhibitor (Kornberg et al., 2018), *S. aureus* promotes glycolysis to favor its persistence during infection (Wickersham et al., 2017) (Fig. 2E). Furthermore, fumarate is also required for host-trained immunity, by inducing epigenetic changes in the promoters of proinflammatory cytokine genes (Arts et al., 2016). Thus, by fine-tuning its metabolism, *S. aureus* can control its persistence while tampering the immune response. This host-pathogen glycolytic interplay has also been reported during *S. aureus* bone infection, whereby metabolic competition with the host ensures durable bacterial colonization (Potter et al., 2020).

Ferroptosis: a newly described PCD potentially targeted by S. aureus

This iron-dependent form of cell death is characterized by the accumulation of ROS and unchecked lipid oxidation; and is initiated by interference with the glutathione-dependent antioxidant mechanism resulting in plasma membrane damage (Fig. 2F) (Conrad et al., 2018; Dixon et al., 2012). Modification of the glutathione levels, either through disruption of the

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cysteine antiporter (GPX4) or by specific inhibition using ferrostatin-1, increases the intracellular ROS levels. These oxygen species react with free intracellular iron, leading to the oxidative degradation of lipids in cell membranes and oxidative cell death (Feng and Stockwell, 2018). Interestingly, lipid peroxidation occurring during gram-negative sepsis drives caspase-11 activation and GSDMD pore formation in a murine model (Kang et al., 2018), suggesting that ferroptosis and pyroptosis are connected. Whether this occurs during *S. aureus* infection remains to be experimentally demonstrated. However, being adapted to live within (and without) acidic lysosomes/autophagosomes of infected cells endows *S. aureus* with the potential to interact with ferroptosis. *S. aureus* is well equipped to respond to metal availability and redox status in these environments, and to adjust toxin expression as a consequence of metal ion availability (Christmas et al., 2019; Torres et al., 2010). The glutathione peroxidase-4 (GPX4) recycling can potentially be interrupted by the release of CTSB occurring during *S. aureus* escape from the lysosomal compartment (Magtanong et al., 2016). The ROS levels generated during the metabolic crosstalk between staphylococci and keratinocytes (Wickersham et al., 2017; Wong Fok Lung et al., 2020) potentially supports activation of ferroptosis, possibly connecting it to necroptosis. It is equally conceivable that *S. aureus* could manipulate ferroptosis by competing with iron availability in host cells through some of its siderophores such as staphyloferrins (Courcol et al., 1997). Metal homeostasis can be modulated by metalloregulators controlled by the *fur* and *perR* regulons, which also control toxin production and heme metabolism, while contending with host oxidative stress (Friedman et al., 2006; Horsburgh et al., 2001; Torres et al., 2010). The sensitivity of *S. aureus* to host arachidonic acid and lipid peroxidation lend support to the involvement of ferroptosis during infection (Beavers et al., 2019; Bravo-Santano et al., 2019). *S. aureus* can incorporate some of these long chain fatty acids in its membrane (Parsons et al., 2014), or in the case of antimicrobial fatty acids, release them in the host cytosol using efflux pumps (Alnaseri et al.,

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2015; Beavers et al., 2019). Whilst the oxidation state of these lipids is unknown, this suggests that bacterial mechanisms may be at play to prevent lipid oxidation and consequently interrupt ferroptosis. In this vein, *M. tuberculosis* induces ferroptosis in macrophages, correlating with iron accumulation and lipid peroxidation, and this is prevented using ferrostatin-1 (Amaral et al., 2019). Whilst bacterial mechanisms interfering with ferroptosis are still underreported, it is notable that host-derived fatty acids can activate the *S. aureus* T7SS, also present in the mycobacterial genus (Lopez et al., 2017; Unnikrishnan et al., 2017). Studies of the oxidation of lipid species and their impact on the T7SS activation may reveal new insights on the link between this secretion system and ferroptosis during *S. aureus* infection.

5. Conclusions and future perspectives

While adapted to the point of commensalism with its human host, *S. aureus* can switch to lethal pathogen due to a formidable array of toxins, deployed during acute infection stages. The chronicity of some staphylococcal infections supports the notion that the species has also evolved intracellular phenotypes. Intracellular *S. aureus* will trigger PCDs that will eradicate infected cells. However, a growing body of evidence indicates *S. aureus* can circumvent these processes (Table 1). The multifaceted strategies employed by *S. aureus* whereby regulons of toxins are potentiated to preserve the integrity of the host by modulating processes such as autophagy and cellular metabolism. *S. aureus* in contrast to the classical and well-studied intracellular pathogens has a relatively smaller protein effector repertoire subverting host cell processes. Instead, *S. aureus* has evolved a strategy that seems to predominantly rely on sub-populations of bacteria with pathoadaptive mutations that favor persistence in the face of PCDs and metabolic challenges (Wong Fok Lung et al., 2020). These mutations frequently occur in regulatory genes, begging the question around the existence of potential “effectors” subverting cell-intrinsic mechanisms whose expression could be triggered in these regulatory mutants. So

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far, only the T7SS effector EsxA has been shown to prevent apoptosis, although EsxA molecular mechanisms of action are still unresolved. Examining the pathological context of staphylococcal infections using *bona fide* clinical *S. aureus* may shed light on other virulence mechanisms that cannot be identified using laboratory strains. For instance, a recent study interrogating the increased susceptibility of diabetic patients to specific staphylococcal infections highlighted the roles in virulence of *S. aureus* glucose transporter genes in host nutritional immunity (Thurlow et al., 2020). The limitations of some models used in *S. aureus*-host studies may also hamper the discovery of PCD mechanisms subverted during infection. The majority of PCD phenotypes described here (Table 1) relied on either cell lines, or mice models that may not reflect the complexity of staphylococcal infections. However, the emergence of humanized animal and cell models have the potential to improve our understanding of PCD in light of the *S. aureus* tropism for humans (Chow et al., 2020). The discovery of novel bacterial factors driving pathogenesis may be facilitated by exploiting the vast genomic data of *S. aureus* isolated from colonized and/or chronically infected patients. Applying comparative and functional genomic approaches on such isolates has the potential to uncover new staphylococcal genes and pathways instrumental in PCD (Allen et al., 2021). Finally, the connectivity between PCDs is increasingly recognized (Bedoui et al., 2020; Place et al., 2021), meaning that compensatory modalities of cell death may be engaged in response to bacterial cues arising during infection.

We have drawn attention to the underappreciated importance of *S. aureus* versatility in the context of PCDs and propose further avenues of investigation to understand the molecular interactions between PCDs and this pathogen.

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Conflicts of interest

The authors have no conflicts to declare

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Figure 1: Overview of PCDs in the context of Staphylococcus aureus infection.

(A) Concept map showing PCD pathways during S. aureusinfection. (B) Intertwined PCD Pathways. Apoptosis, necroptosis, and pyroptosis molecular determinants engaged during S. aureus infection are depicted as well as autophagy. In addition, TLR2-mediated NF-κB signalling through MyD88-IRAK-TRAF6 is shown. Although these pathways are initiated by distinct signals, their signalling cascades can be interconnected to lead to cell death during S. aureus infection. Red arrows: Interaction between apoptosis and pyroptosis. Blue arrows: Interaction between apoptosis and necroptosis. Brown arrows: Interaction between necroptosis and pyroptosis. Green arrows: Interaction between TLR2 signalling and PCD pathways, apoptosis, or necroptosis. Orange arrow: Interaction between apoptosis and autophagy. Bold Red arrows: canonical and non-canonical pyroptosis. Black dotted arrow: transport of pro-inflammatory cytokines out of the cell.

Figure 2: *S. aureus* determinants and PCD pathways.

(A) *The interaction between the Hla and its receptor ADAM10 promotes S. aureus dissemination through epithelia and exploits autophagy*. *The expression of Hla receptor, ADAM10, is higher in epithelial and endothelial cells in autophagy-deficient mice (ATG16L1^{HM}) as compared to WT mice. The upregulation of ADAM10 expression in absence of autophagy increases the susceptibility to S. aureus blood and lung infection. ATG16L1: Autophagy-related 16 like 1 gene.*

(B) The joint activities of S. aureus Hla and β- haemolysin mediate cathepsin B (CTSB) release from the lysosome and destabilize cell-cell adhesion. Hla binding to ADAM10 on epithelial and endothelial cells is followed by the activation of β-toxin, an acid sphingomyelinase promoting the breakdown of sphingomyelin to phosphorylcholine and ceramide. The accumulation of ceramide promotes the release of CTSB (right) from the lysosome (left), which activates the inflammasome and pyroptosis. The activity of β-toxin

contributes to the degradation of cadherins, thus disrupting tight junctions and tissue integrity, ultimately enabling bacterial dissemination in deeper tissue.

(C) *S. aureus* modulates apoptosis of efferocytic macrophages during NEtosis. *S. aureus* can directly induce apoptosis in macrophages, through its PVL toxins; or indirectly following interaction with neutrophils, whereby S. aureus neutralises the bactericidal activity of NETs using Nuc and AdsA. The activities of these enzymes generate dAdo, which triggers macrophage apoptosis. *S. aureus* can also directly inhibit apoptosis using the T7SS effector, EsxA. N: Neutrophil, MΦ: macrophages

(D) *S. aureus extracellular* vesicles containing leukocidins activate the inflammasome and lead to pyroptosis in macrophages. *S. aureus* vesicles decorated with leukocidins LukS-PV and Luk-AB are delivered to macrophages following binding to the toxins' cognate receptors, C5aR and CD11b, respectively. Upon internalization, these toxins promote the assembly of the NLRP3 inflammasome, the production of pro-inflammatory cytokines and trigger pyroptosis in macrophages. Pre-treatment of macrophages with potassium chloride inhibited IL-1β and IL-18 release, suggesting a potentiating role for leukocidins during pyroptosis.

(**E) *S. aureus* small colony variants (SCVs) promote necroptosis by potentiating host cell glycolysis.**

SCVs colonizing keratinocytes express higher levels of fumarate hydratase class II (*fumC*) as compared to WT *S. aureus*. FumC activity decreases the levels of fumarate which activates glycolysis in host cells, thus increasing ROS generation by mitochondria. These in turn induce MLKL phosphorylation and lead to necroptosis in keratinocytes.

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(F) Potential interventions of *S. aureus* on ferroptosis

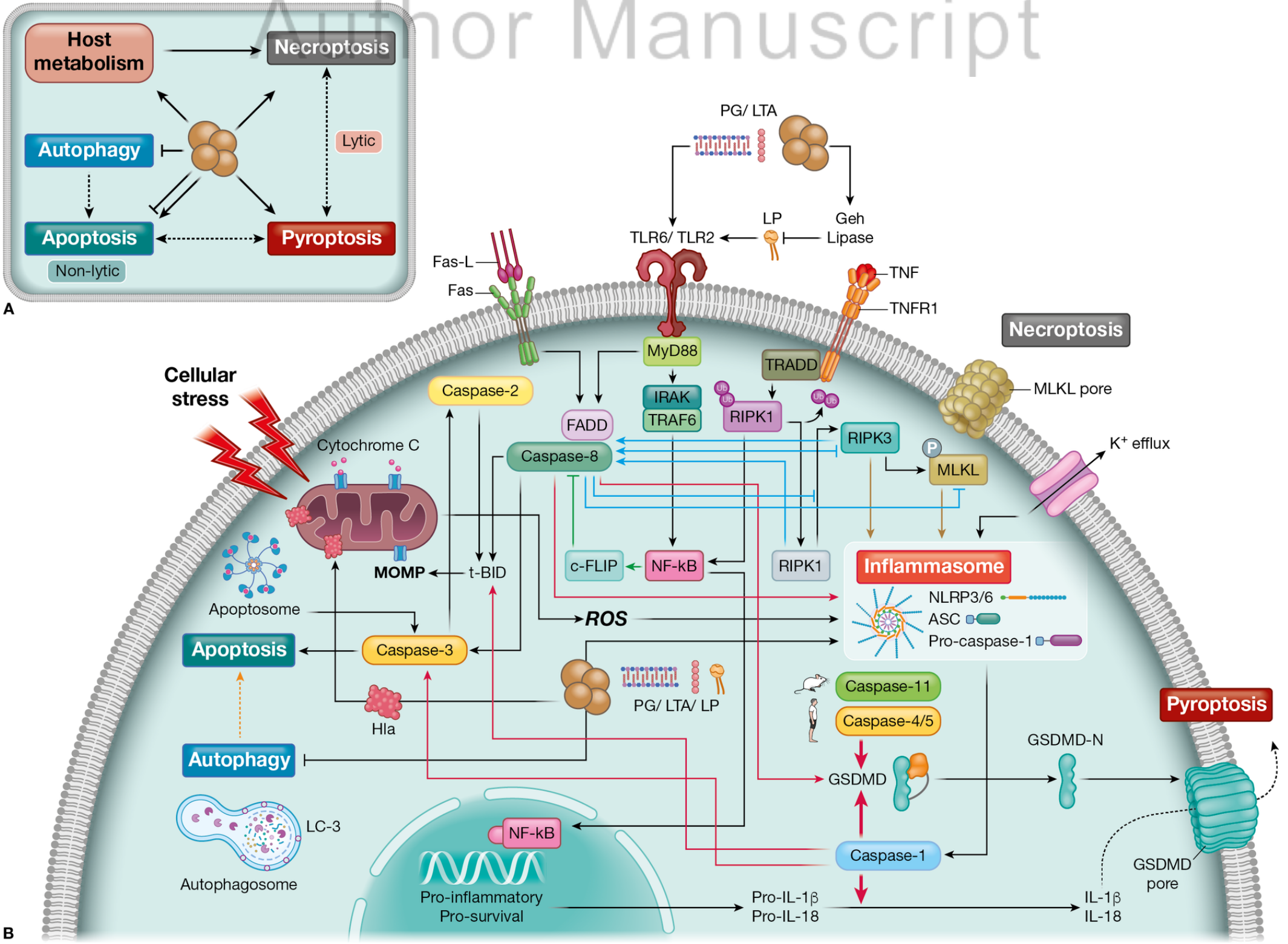
Ferroptosis is driven by the accumulation of mitochondrial-derived ROS, through interruption of GPX4 recycling and availability of free intracellular iron (Fe^{2+}) and/or associated with ferritin. By damaging lysosomes and releasing cathepsin B (CTSB), intracellular *S. aureus* could potentially interrupt the recycling of GPX4, thus interfering with ferroptosis. In addition, the siderophores staphyloferrin A and B may also sequester the intracellular pool of Fe^{2+}, thus affecting the Fenton reaction supporting ferroptosis.

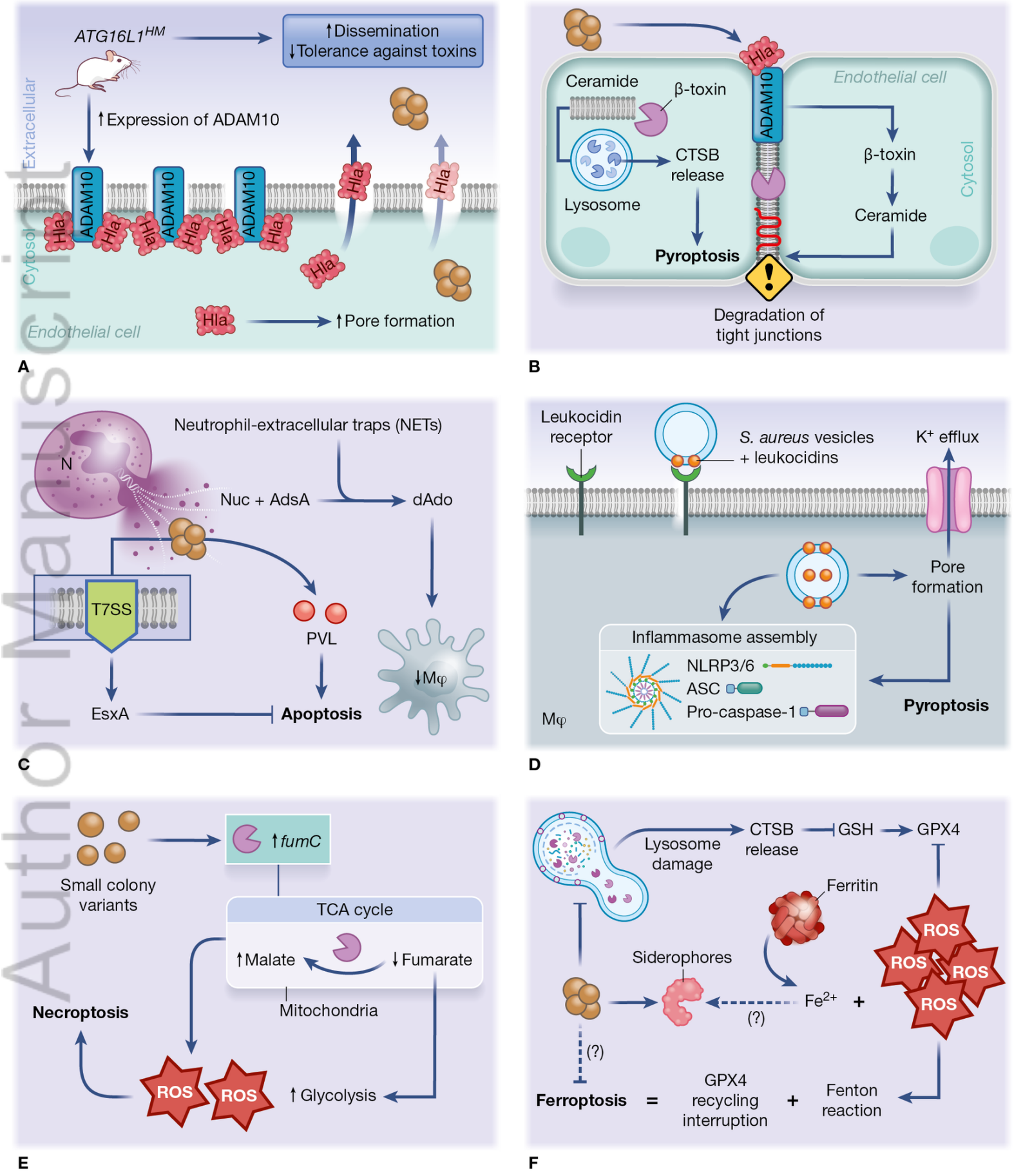
Table 1: *Staphylococcus aureus* Toxins, Enzymes, and Effectors involved in Programmed Cell Death Pathways.

Table 2: *Staphylococcus aureus* Pathogen-associated Molecular Patterns (PAMPs) involved in Programmed Cell Death Pathways.

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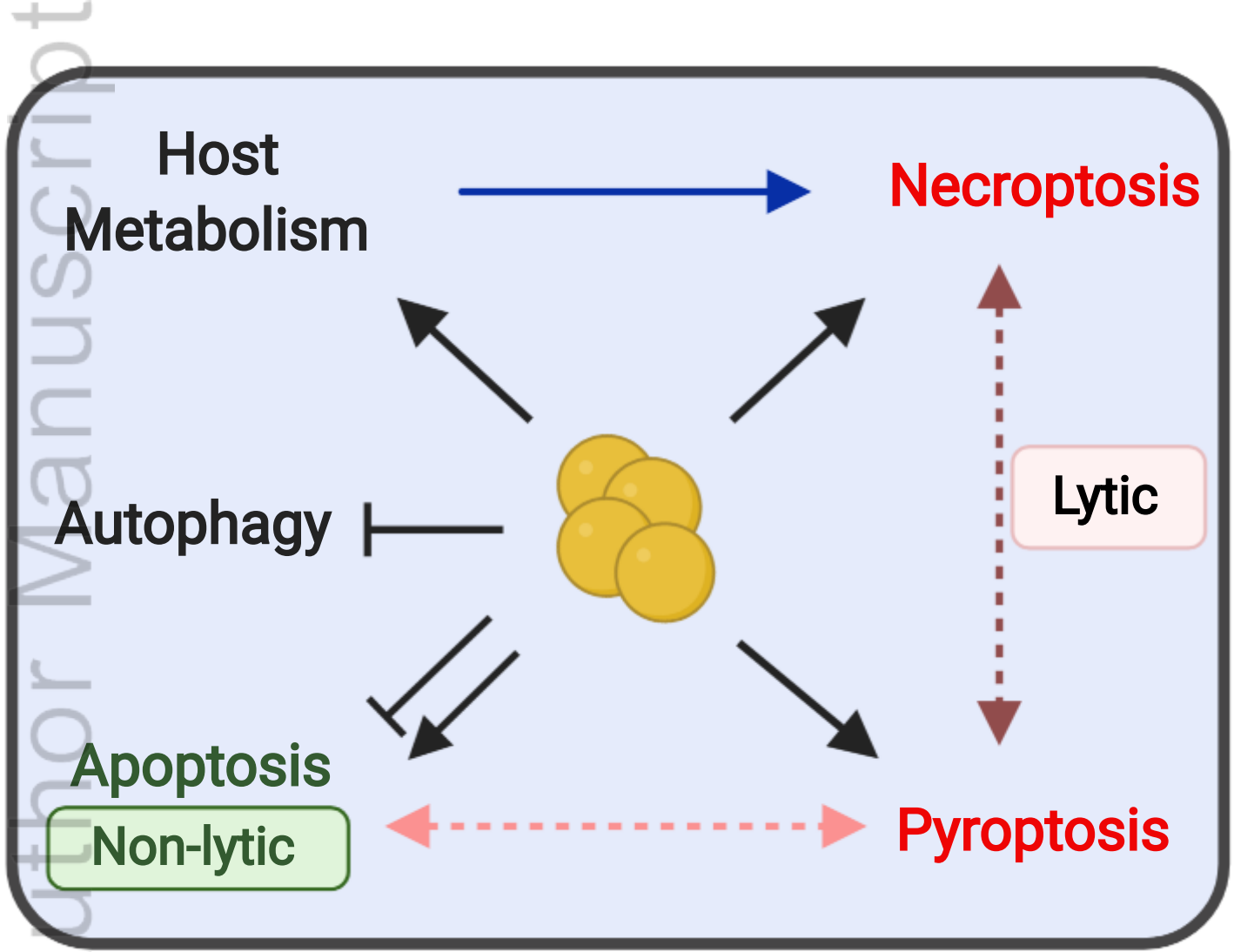
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The adoption of an intracellular lifestyle by *S. aureus* is an important facet of its pathogenesis. *S. aureus* exploits host cell processes to prevent its elimination from this niche by interfering with programmed cell death pathways including apoptosis, pyroptosis and necroptosis.

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Table 1. *S. aureus* Toxins, Enzymes, and Effectors involved in Programmed Cell Death Pathways.

***S. aureus* factors**	**PCD-related pathways**	**Cells/Animal models**	**References**
Toxins			
α-hemolysin (Hla)	Apoptosis	Jurkat T lymphocytes (FADD- and caspase 8-deficient), human PBL and primary monocytes.	(Bantel et al., 2001)
		Jurkat T lymphocytes, PBMC.	(Haslinger et al., 2003)
		HeLa, primary human keratinocytes, MEF.	(Imre et al., 2012)
		A549, PC3.	(Liang & Ji, 2007)
		MAC-T.	(Wesson et al., 2000)
		ECV304.	(Yu et al., 2013)
	Necroptosis	A549.	(Gonzalez-Juarbe et al., 2018)
		MH-S, THP-1, BMDM (C57BL/6: WT, *Ripk3*$^{-/-}$, *Nlrp3*$^{-/-}$, *Myd88*$^{-/-}$, *Asc*$^{-/-}$).	(González-Juarbe et al., 2015)
		THP-1, primary human macrophages, human alveolar macrophages (BAL). *S. aureus* pneumonia infection model: NK, CD4^{+} T lymphocytes, DC, neutrophils, and macrophages (C57BL/6: BAL and lungs).	(Kitur et al., 2015)
	Pyroptosis	bEnd.3 and EOMA, murine bacteraemia model (C57BL/6H: WT and *Smpd1*$^{-/-}$).	(Becker et al., 2017)
		THP-1, BMDM and peritoneal macrophages (C57BL/6: WT, *Nlrp3*$^{-/-}$, *Asc*$^{-/-}$, and *Casp1*$^{-/-}$).	(Craven et al., 2009)
		Pulmonary and BAL macrophages (C57BL/6: *WT*, *Nlrp3*$^{-/-}$ and *Il1r*$^{-/-}$).	(Kebaier et al., 2012)
		BMDM (C57BL/6: WT and *Smpd1*$^{-/-}$).	(Ma et al., 2017)
		BMDM and peritoneal macrophages (C57BL/6N: WT, *Cias1*$^{-/-}$, *Asc*$^{-/-}$, *Nod2*$^{-/-}$, and *Ipaf*$^{-/-}$).	(Mariathasan et al., 2006)
		BMDM (C57BL/6: WT, *Asc*$^{-/-}$, *Casp-1*$^{-/-}$, *P2X7R*$^{-/-}$, *Myd88*$^{-/-}$, *Myd88*/*Trif*$^{+/-}$; and 129/C57BL/6: WT and *Nlrp3*$^{-/-}$).	(Muñoz-Planillo et al., 2009)
		HaCaT, 3D HuK.	(Soong et al., 2012)
		HaCaT, HEKn, 3D HuK, human skin grafts *in situ* - SCID mouse-human model.	(Soong et al., 2015)
	Autophagy	MEF, endothelial cells and peritoneal macrophages (C57BL/6: WT, *LC3B*$^{-/-}$, *Atg16L1*^{HM}, *Atg16L1*$^{fl/fl}$, *Atg16L1*$^{fl/fl}$-*Tie2Cre*, *Atg16L1*$^{fl/fl}$-*LyzCre*, and *Atg16L1*$^{fl/fl}$-*CD11cCre*, murine *S. aureus* pneumonia (intranasal) and sepsis (intravenous) infection.	(Maurer et al., 2015)

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		HaCaT, HEKn, 3D HuK, human skin grafts *in situ* - SCID mouse-human model.	(Soong et al., 2015)
β-hemolysin	Pyroptosis	bEnd.3 and EOMA cells, intravenous infection (C57BL/6H: WT, *Smpd1*$^{-/-}$*).*	(Becker et al., 2017)
		BMDM (C57BL/6: WT and *Smpd1*$^{-/-}$).	(Ma et al., 2017)
		BMDM and peritoneal macrophages (C57BL/6N: WT, *Cias1*$^{-/-}$, *Asc*$^{-/-}$, *Nod2*$^{-/-}$, and *Ipaf*$^{-/-}$).	(Mariathasan et al., 2006)
		BMDM (C57BL/6: WT, *Asc*$^{-/-}$, *Casp-1*$^{-/-}$, *P2X7R*$^{-/-}$, *Myd88*$^{-/-}$, *Myd88*/*Trif*$^{-/-}$; and 129/C57BL/6: WT and *Nlrp3*$^{-/-}$), murine subcutaneous infection.	(Muñoz-Planillo et al., 2009)
γ-hemolysin	Pyroptosis	BMDM and peritoneal macrophages (C57BL/6N: WT, *Cias1*$^{-/-}$, *Asc*$^{-/-}$, *Nod2*$^{-/-}$, and *Ipaf*$^{-/-}$).	(Mariathasan et al., 2006)
		BMDM (C57BL/6: WT, *Asc*$^{-/-}$, *Caspase-1*$^{-/-}$, *P2X7R*$^{-/-}$, *Myd88*$^{-/-}$, *Myd88*/*Trif*$^{-/-}$; 129/C57BL/6: WT and *Nlrp3*$^{-/-}$), murine subcutaneous infection.	(Muñoz-Planillo et al., 2009)
Enterotoxin A	Apoptosis	Human PBMC (depleted of CD8^{+} T cells, CD4^{+}/CD45RO^{+} T cells).	(Porichis et al., 2008)
Enterotoxin B	Apoptosis	Primary epithelial cells (human renal proximal tubule).	(Ionin et al., 2008)
		Hepatic mononuclear cells (T cells, NK cells, NKT cells, macrophages) and splenocytes (C57BL/6: WT, *CD44*$^{-/-}$, *CD44v7*$^{-/-}$).	(McKallip et al., 2005)
		Lymph node and peripheral T cells (C57BL/6: WT, congenic B6SmnC3H-gld – Fas-L deficient mice) and (C57BL/6.Tg93/lbm spf - T-cell Receptor Vβ8.2 transgenic mouse, backcrossed to BALB/c).	(Renno et al., 1996)
		PBMC, superantigen-induced CD80^{+} monocytes.	(Takahashi et al., 2001)
		ECV304.	(Yu et al., 2013)
		THP-1.	(Zhang et al., 2016)
Enterotoxin M	Apoptosis	MAC-T.	(Zhao et al., 2020)
Enterotoxin H	Apoptosis	Murine splenic lymphocytes, primary bovine mammary epithelial cells.	(Liu et al., 2014)
Leukocidins (PVL, LukAB)	Apoptosis	Primary human PMNs, CD4^{+} T cells, primary human monocytes-derived DC.	(Berends et al., 2019)
		THP-1.	(Bu et al., 2013)
		RHEK-1 AD12/SV40 immortalized keratinocyte cell line, rabbit intradermal lesions.	(Chi et al., 2014)
		Human neutrophils, PBL.	(Genestier et al., 2005)
		MC3T3-E1.	(Jin et al., 2013)

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		HL-60, HL-60-SCID mouse xenograft model.	(Shan et al., 2015)
	Necroptosis	THP-1, primary human macrophages, human alveolar macrophages (BAL). Murine pneumonia model (BAL and lungs: NK, $CD4^+$ T lymphocytes, DCs, neutrophils and macrophages (C57BL/6: WT and *Rip3*$^{-/-}$).	(Kitur et al., 2015)
	Pyroptosis	Primary human PMNs, $CD4^+$ T cells, and DCs.	(Berends et al., 2019)
		THP-1, human-induced pluripotent stem cell-derived macrophages (*hC5aR1*$^{-/-}$), primary human monocytes derived macrophages, BMDM (C57BL/6: WT and humanized ($hC5aR^{+/+}$). Murine pulmonary infection.	(Chow et al., 2020)
		THP-1, primary human blood-derived monocytes, macrophages, neutrophils, lymphocytes and BMDM (C57BL/6).	(Holzinger et al., 2012)
		THP-1, primary human $CD14^+$ monocytes.	(Melehani et al., 2015)
		THP-1 (*Nlrp3*$^{-/-}$, *Casp-1*$^{-/-}$), J774A.1, human monocytes-derived macrophages. Intraperitoneal infection (C57BL/6H).	(Wang et al., 2020)
Phenol-soluble modulins (PSMs)	Necroptosis	HEK (transfected with TLR2, TLR1/2, TLR2/6); PMNs, PBMC and BMDM (C57BL/6 : WT and *TLR2*$^{-/-}$). Bacteraemia model (C57BL/6 *FPR2*$^{-/-}$) .	(Hanzelmann et al., 2016)
		THP-1, primary human macrophages, human alveolar macrophages (BAL). Murine NK cells, $CD4^+$ T lymphocytes, DCs, neutrophils and macrophages in BAL and lungs (C57BL/6: WT and *Rip3*$^{-/-}$). Murine Pneumonia model.	(Kitur et al., 2015)
		HaCaT, HEKn, 3D HuK, human skin grafts *in situ* – SCID mouse-human model.	(Soong et al., 2015)
		Primary human neutrophils. Murine pneumonia model: macrophages in peritoneal lavage fluid and BAL (BALB/c).	(Zhou et al., 2018)
Enzymes & Effectors			
T7SS/ EsxA	Apoptosis	Human $CD14^+$ monocytes-derived DCs, human $CD4^+$ T lymphocytes.	(Cruciani et al., 2017)
		A549.	(Korea et al., 2014)
Coagulase	Apoptosis	MC3T3-E1.	(Jin et al., 2013)
Nuclease (Nuc) and Adenosine synthase (AdsA)	Apoptosis & NETosis	BMDM (C57BL/6: WT and *Casp1*$^{-/-}$), U937, primary human neutrophils and monocytes, HL-60 neutrophils, THP-1. Bacteraemia model (BALB/c).	(Thammavongsa et al., 2013)
		U937 (WT, *Casp3*$^{-/-}$, *Slc29A1*$^{-/-}$), BMDM (C57BL/6: WT, *Casp3*$^{fl/fl}$, *Casp3*$^{fl/fl}$ Tie2-Cre^+). Murine renal abscess model.	(Winstel et al., 2019)

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Fumurate hydratase	Necroptosis	HEKn (*Tlr2* and *Tnfr1* knockdown), THP-1, PBMC. BMDM (C57BL/6: WT, *Mlkl*$^{-/-}$). Murine skin infection (C57BL/6: WT, *Rag2*$^{-/-}$, *Mlkl*$^{-/-}$, *Il1r1*$^{-/-}$, *Aim2*$^{-/-}$, and *Ripk1d* (*Ripk1*^{K45A})).	(Wong Fok Lung et al., 2020)
Glycerol ester hydrolase (Geh)	TLR2 signalling	BMDM (C57BL/6: WT, *Tlr2*$^{-/-}$, *Tlr4*$^{-/-}$, *Myd88*$^{-/-}$). Murine systemic infection model.	(Chen & Alonzo, 2019)
Protein A	Apoptosis	MC3T3-E1. Murine Ehrlich's ascites carcinoma model (Swiss albino mice), *in-vitro* co-culture of Ehrlich's ascites carcinoma with protein A-primed splenic cells. MC3T3-E1.	(Claro et al., 2011) (Das et al., 2002) (Jin et al., 2013)
Staphopain A & B	Apoptosis	Human PMNs and primary monocytes (adherent and non-adherent), human serum. HeLa, A549, 16HBE14o- human bronchial epithelial cell line, primary human tracheal epithelial cells. Murine pneumonia model (BALB/c).	(Smagur et al., 2009) (Stelzner et al., 2020)

Notes: A549: human alveolar epithelial cell line, BAL: Bronchoalveolar lavage, bEnd.3: Mouse endothelial cells, BMDM: murine bone-marrow derived macrophages, *Cias*: gene encoding cryopyrin, DCs: Dendritic cells, ECV304: human umbilical vein endothelial cell line, EOMA: hemangioendothelioma, HaCaT: human keratinocyte cell line, HeLa: human epithelial cell line, HEK: human embryonic kidney 293T cells, HEKn: primary human keratinocytes, 3D HuK: three-dimensional organotypic culture of human keratinocytes, HL-60: human leukemic cell line, J774A.1: murine macrophage cell line, MAC-T: bovine mammary epithelial cells, MC3T3-E1: mouse pre-osteoblastic cell line, MEF: mouse embryonic fibroblast, MH-S: murine alveolar macrophage cell line, NK: Natural-Killer cells, PBL: peripheral blood lymphocytes, PBMC: human peripheral blood mononuclear cells, PC3: human prostate carcinoma tumour cells, PMNs: polymorphonuclear leukocytes, SCID: severe combined immunodeficient, *Smpd1*: gene-encoding acid sphingomyelinase, THP-1: human monocytic cell line, U937: human macrophages cell line.

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Table 2. *S. aureus* Pathogen-associated Molecular Patterns (PAMPs) involved in Programmed Cell Death Pathways

PAMPs	Pattern Recognition Receptors (PRRs)	PCD pathways	References
Triacyl lipopeptides **Diacyl lipoproteins**	TLR1-TLR2 TLR2-TLR6	Pyroptosis	**PRRs:** (Stoll et al., 2005; Takeuchi et al., 2001), See Review: (Fournier, 2013; Oliveira-Nascimento et al., 2012) **TLR2 Interference** (Bardoel et al., 2012; Koymans et al., 2015; Patot et al., 2017; Yokoyama et al., 2012) **Pyroptosis:** (Muñoz-Planillo et al., 2009; X. Wang et al., 2020)
Lipoteichoic acid (LTA)	TLR2-TLR6	Apoptosis Pyroptosis	**PRRs**: See Review: (Fournier, 2013; Oliveira-Nascimento et al., 2012) **Apoptosis:** (Lotz et al., 2004; J. Wang et al., 2016) **Pyroptosis:** (Hara et al., 2018)
Peptidoglycan	TLR2 TLR2/NOD2	Apoptosis Pyroptosis	**PRRs**: (Dziarski & Gupta, 2005; Iwaki et al., 2002; Müller-Anstett et al., 2010; Schwandner et al., 1999), See Review: (Fournier, 2013; Oliveira-Nascimento et al., 2012) **Apoptosis:** (Towhid et al., 2012; Vázquez-Sánchez et al., 2014) **Pyroptosis:** (Müller et al., 2015; Shimada et al., 2010)**,** See Review: (Melehani & Duncan, 2016)