Staphylococcus aureus toxins

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Abstract

Staphylococcus aureus is a dangerous pathogen that causes a variety of severe diseases. The virulence of *S. aureus* is defined by a large repertoire of virulence factors, among which secreted toxins play a preeminent role. Many *S. aureus* toxins damage biological membranes, leading to cell death. In particular, *S. aureus* produces potent hemolysins and leukotoxins. Among the latter, some were recently identified to lyse neutrophils after ingestion, representing an especially powerful weapon against bacterial elimination by innate host defense. Furthermore, *S. aureus* secretes many factors that inhibit the complement cascade or prevent recognition by host defenses. Several further toxins add to this multi-faceted program of *S. aureus* to evade elimination in the host. This review will give an overview over *S. aureus* toxins focusing on recent advances in our understanding of how leukotoxins work in receptor-mediated or receptor-independent fashions.

Introduction

Staphylococcus aureus is a dangerous and versatile pathogen that can cause a multitude of different diseases. Most frequently, it causes skin infections and infections of the respiratory tract. Skin infections are usually community-acquired, whereas infections of the lung dominate among nosocomial *S. aureus* infections. Among nosocomial pathogens, *S. aureus* is the most common and associated with high morbidity and mortality. *S. aureus* pneumonia often develops in hospitalized patients with underlying conditions, such as in patients suffering from immune deficiencies or viral infections. However, *S. aureus* may also cause a variety of other sometimes very severe and life-threatening diseases, such as infective endocarditis, toxic shock syndrome, scalded skin syndrome, or osteomyelitis, to name but a few. Even necrotizing fasciitis and necrotizing pneumonia were reported with *S. aureus* as the causative agent [1,2].

The versatility of *S. aureus* as a pathogen stems from the fact that *S. aureus* strains possess a varying repertoire of virulence factors, many of which are encoded on mobile genetic elements (MGEs), such as plasmids or prophages, and can be transferred between strains by horizontal gene transfer (HGT). HGT in *S. aureus* may happen via phage transduction, conjugation, or – as recently found – by direct uptake of "naked" DNA by genetic competence [3].

Many *S. aureus* virulence factors can be described as toxins. Toxins are usually defined as poisonous substances. Thus, the distinction from other virulence factors – molecules that

increase the potential of a pathogen to cause disease in a broader sense – is that they are secreted by the producing organism and interfere directly with the host. Toxins thus do not include molecules that, for example, combat mechanism of host defense in the intracellular space of the bacteria, such as intracellular reactive oxygen scavenging mechanisms. Also, *S. aureus* produces a large set of secreted, surface-bound proteins that enable the pathogen to attach to host tissue. Although this is an important mechanism of the *S. aureus* pathogenesis program, these surface-located proteins will not be regarded as toxins for the purpose of this review and the reader is referred to other reviews dealing specifically with those proteins [4]. Furthermore, molecules that are secreted but serve the defense from host immunity in a passive way, such as exopolymers with a function in providing resistance to antimicrobial peptides or leukocyte phagocytosis, will not be included here. Rather, this review will cover secreted *S. aureus* molecules that in some way or another directly harm the host. These are grouped in three categories: 1) membrane-damaging toxins, which may work in a receptor-mediated or receptor-independent fashion, 2) toxins that interfere with receptor function but are not membrane-damaging, and 3) secreted enzymes, such as those that degrade host molecules or affect important host defense mechanisms.

Membrane-damaging toxins

The cytoplasmic membrane is the target of a large series of bacterial toxins, including several that are produced by *S. aureus*. These toxins cause pore formation in the membrane, leading to the efflux of vital molecules and metabolites, and therefore are cytolytic. Two subgroups can be distinguished: those for which subsequent lysis is dependent on initial receptor interaction, and which thus show high target cell specificity, and those that interfere with membranes in a less specific fashion without receptor interaction (Fig. 1).

Receptor-mediated

S. aureus produces a variety of cytolytic toxins. Most are infamous for lysing red and/or white blood cells. Those that lyse red blood cells are called hemolysins, while those that target white blood cells are leukotoxins. Many cytolytic toxins of *S. aureus* have only recently been shown to require receptor interaction for their lytic function.

Alpha-toxin is probably the best-known toxin of *S. aureus* [5] and the first identified example of the beta-barrel forming toxins, which predominantly consist of beta sheets. It is lytic to red blood cells and a series of leukocytes, but not neutrophils [6]. It is 293 amino acids in length and forms a heptameric pore that leads to the efflux of mono- and, at higher concentration, divalent ions. At higher concentrations, pore formation may be receptor-independent, but pore formation at lower concentrations has recently been shown to be dependent on the interaction with the ADAM10 receptor [7,8]. Binding of alpha-toxin to ADAM10, a disintegrin and metalloproteinase, ultimately leads to the disruption of focal adhesions. In particular, cleavage of E-cadherin in epithelial cells leads to loss of epithelial barrier function. Independently, alpha-toxin also causes apoptosis in human monocytes, T and B cells [9].

S. aureus also produces are series of bi-component toxins that are structurally similar to alpha-toxin and belong to the beta-barrel pore-forming toxin family: the Panton-Valentine leukocidin (PVL, consisting of the LukS and LukF proteins), the leukocidins LukDE and LukAB (LukGH), and gamma-toxin (gamma-hemolysin, HlgA, HlgB, HlgC). Intensified research has recently been prompted by the association of PVL with infections by community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains [10], although the involvement of PVL in CA-MRSA disease remains controversial [11]. Initially often believed to function in a receptor-independent fashion, the discovery of the LukDE receptor (CCR5) [12], and the subsequent discoveries of the PVL and LukGH (LukAB) receptors

(C5aR, C5L2 and CD11b, respectively) [13,14], clearly showed that these toxins require receptor interaction for cytolytic activity. Notably, these findings explained the often-noted species and target cell specificity of the bi-component *S. aureus* toxins [15].

Non-receptor mediated

In 2007, it was discovered that the long-known *S. aureus* delta-toxin (delta-hemolysin) is but one member of a family of secreted peptides called the phenol-soluble modulins (PSMs), which have multiple function in staphylococcal pathogenesis [16]. Importantly, some PSMs have pronounced, non-specific cytolytic activity. While members of the PSM family also occur in other, less pathogenic staphylococci [17], *S. aureus* produces high amounts of strongly cytolytic PSMs, in particular the PSMα peptides PSMα1 – α4 encoded in the *psmα* locus, of which PSMα3 is by far the most active. PSMs trigger inflammatory responses by interaction with the FPR2 receptor, but their cytolytic activity is FPR2-independent [18]. They are small, amphipathic peptides with detergent-like properties. Accordingly, pores formed by delta-toxin are short-lived [19] and it can be assumed that other PSMs work similarly. According to a recent study that performed an alanine exchange peptide library screen with PSMα3, pro-inflammatory, cytolytic, and other properties of PSMs can be attributed to specific amino acid positions and different parts in the peptide [20]. Notably, similar to alpha-toxin and in contrast to many bi-component leukocidins, PSMs are produced by most *S. aureus* strains [16,21]. Only strains dysfunctional in the global virulence regulator Agr, which regulates most *S. aureus* toxins and exoenzymes, lack PSM production. Recently, PSMα peptides of *S. aureus* were identified as the toxins that contribute to neutrophil lysis after phagocytosis, a pathogenesis mechanism of immense importance for the high toxicity found in strongly aggressive *S. aureus* strains [22–24] (Fig. 2). Notably, lysis after phagocytosis might explain at least in part why the development of vaccines for *S. aureus* that work by enhancement of opsonophagocytosis failed so far [25]. Among the other leukocidins, only LukAB (LukGH) also lyses neutrophils after uptake [26,27]. Finally, recent work indicates that S. aureus δ-toxin contributes to the allergic skin disease atopic dermatitis by inducing mast cell degranulation [28]. Interestingly, only δ-toxin but not PSMα peptides contributed to that phenotype, exemplifying that PSM peptides have undergone divergent evolution to fulfill different functions in pathogenesis.

Toxins that interfere with receptor function (other than membrane-damaging)

Enterotoxins are secreted toxins of ~ 20 to 30 kD that interfere with intestine function and typically cause emesis and diarrhea [29]. They are superantigens, molecules that trigger T cell activation and proliferation without the need for antigen processing by allowing non-specific interaction of the class II major histocompatibility complex MHC II with T cell receptors. *S. aureus* strains can produce a wide array (~ 20) of enterotoxins and enterotoxin-like toxins. Enterotoxins, also produced by some other bacteria, share a common structure comprising a two-domain fold, a long central alpha-helix, the characteristic N-terminal "oligosaccharide/oligonucleotide fold" with beta-barrel structure and a C-terminal "beta grasp" motif. The mechanisms by which staphylococcal enterotoxins work are not well known, but may include the activation of cytokine release, ultimately causing cell death by apoptosis [30]. Staphylococcal enterotoxin B (SEB) is considered a biological warfare weapon [31]. Staphylococcal enterotoxin C (SEC) has been shown to promote infective endocarditis, sepsis, and kidney injury in the CA-MRSA strain MW2 [32].

The most famous *S. aureus* superantigen, the 22-kD toxic shock syndrome toxin (TSST), causes toxic shock syndrome (TSS) by stimulating release of IL-1, IL-2, TNF-α, and other cytokines. TSS is a severe and potentially fatal disease mostly known for the outbreak

associated with tampon use in the 1980s. In contrast to the enterotoxin superantigens, TSST does not cause emesis. With the exception of SelX, a recently described core genome encoded enterotoxin produced by 95 % of isolates, all enterotoxins and TSST are present in only a small number of *S. aureus* strains [33].

S. aureus produces a series of secreted proteins that interfere with leukocyte receptors to evade recognition and prevent subsequent activation of the immune system. CHIPS (chemotaxis inhibitory protein of *S. aureus*) binds specifically to the C5aR and FPR receptors, thereby impairing the recognition of bacterial formylated peptides by FPR and blocking activation of leukocytes via C5a, a terminal effector of the complement system [34]. FLIPr (FPR-like 1 inhibitory protein) and its homologue FLIPr-like also block recognition of formylated peptides by the FPR receptor, with FLIPr-like having ~ 100 times more potency [35,36]. Additionally, FLIPr is an efficient antagonist of FPR2 (formerly named FPRL1), which is the receptor recognizing *S. aureus* PSM peptides. Finally, both FLIPr and FLIPr-like have recently been demonstrated to competitively block IgG-ligand binding by FcγR receptors, inhibiting neutrophil phagocytosis and subsequent killing of *S. aureus* [37]. CHIPS, FLIPr and FLIPR-like are encoded within pathogenicity islands, but show relatively high frequency among *S. aureus* isolates.

Enzymes

Many secreted *S. aureus* enzymes degrade host molecules or interfere with host metabolic or signaling cascades. Several of those are proteases. Relatively non-specific proteases degrade host proteins in a broad fashion, leading to tissue destruction, but may also have a more specific impact. The protease aureolysin (*S. aureus* neutral proteinase) cleaves many proteins including insulin B, with a preference of cleaving after hydrophobic residues. Furthermore, aureolysin inactivates PSMs, thus having a major impact on the pathogenesis of osteomyelitis [38]. It also leads to maturation of another non-specific *S. aureus* exoprotease, the glutamyl endopeptidase SspA, which cleaves after glutamate residues. Aureolysin, glutamyl endopeptidase, and the cysteine proteases staphopain A and B all interfere with complement factors, leading to evasion of complement-mediated bacterial killing [39]. The biological function of further *S. aureus* proteases, a series of serine proteases, is not well understood, except for the exfoliative toxin serine proteases. The exfoliative toxins specifically cleave desmosomal cadherins of the superficial skin layers [40], leading to staphylococcal scalded skin syndrome (SSSS), a severe skin disease presenting with rash, blisters, and severe lesional damage of the skin. Finally, *S. aureus* may produce a protease that degrades collagen, called collagenase.

Staphylokinase activates plasminogen to plasmin, which degrades fibrin clots. The biological significance of this activity is to diminish the function of the fibrin meshwork in keeping a staphylococcal infection localized. It also cleaves the complement factor C3b [41], adding to the broad attack of other staphylococcal proteases and further molecules, such as the fibrinogen-binding protein Efb and SCIN (staphylococcal complement inhibitor) [42,43], on complement function. While staphylokinase facilitates bacterial penetration through the skin barrier, it decreases the severity of skin infections by leading to drainage [44].

S. aureus produces two coagulases, staphylocoagulase and von Willebrand factor (vWF), which contribute to the formation of fibrin clots after binding to prothrombin (forming a complex called staphylothrombin) and several other plasma proteins, thereby triggering the conversion of fibrinogen to fibrin [45]. This leads to fibrin clots on the surface of *S. aureus* cells, inhibiting phagocytosis, causing abscess formation [46] and adhesion of *S. aureus* to catheters during biofilm-associated infection [47].

S. aureus beta-toxin is a sphingomyelinase of type C that degrades the sphingomyelin present on the surface of a variety of host cells, leading to cell lysis. In many virulent *S. aureus* strains, the gene encoding beta-toxin (*hlb*) is disrupted by a pathogenicity island [48]. Beta-toxin is thus not considered a virulence factor contributing significantly to the pathogenicity of virulent *S. aureus*.

Finally, *S. aureus* produces lipases and nucleases, whose functions in pathogenesis are poorly understood. Possibly, nucleases may decrease the antibacterial activity of neutrophil extracellular traps (NETs), which consist of DNA released from lysed neutrophils [49].

Other toxins

Some *S. aureus* secreted host-damaging factors cannot be classified in the categories used in this review. These include the abovementioned Efb and SCIN, which are potent inhibitors of the function of convertase C3, a crucial enzyme in the complement pathway.

Conclusions

Main developments in recent *S. aureus* toxin research include the discovery of (i) the PSMs, (ii) a large series of complement-inhibiting factors, (iii) molecules that block recognition by host immune cells, and the finding that leukotoxins and alpha-toxin bind to specific receptors. These findings and discoveries will prompt further research in those areas, aimed to investigate for example the exact mechanisms by which PSMα peptides and specific leukotoxins cause cell death and phagosomal escape.

Acknowledgments

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• of special interest

•• of outstanding interest

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Highlights

- *S. aureus* produces a wide variety of toxins mostly aimed to evade elimination by host defenses.

- Many *S. aureus* toxins are encoded on mobile genetic elements, leading to a strongly varying repertoire of toxins in different isolates.

- Some toxins, such as alpha-toxin and PSMs, are produced by virtually all strains, representing an *S. aureus* toxin "core set".

- Classical leukotoxins of *S. aureus* work in a receptor-mediated, while PSMs lyse in a receptor-independent fashion.

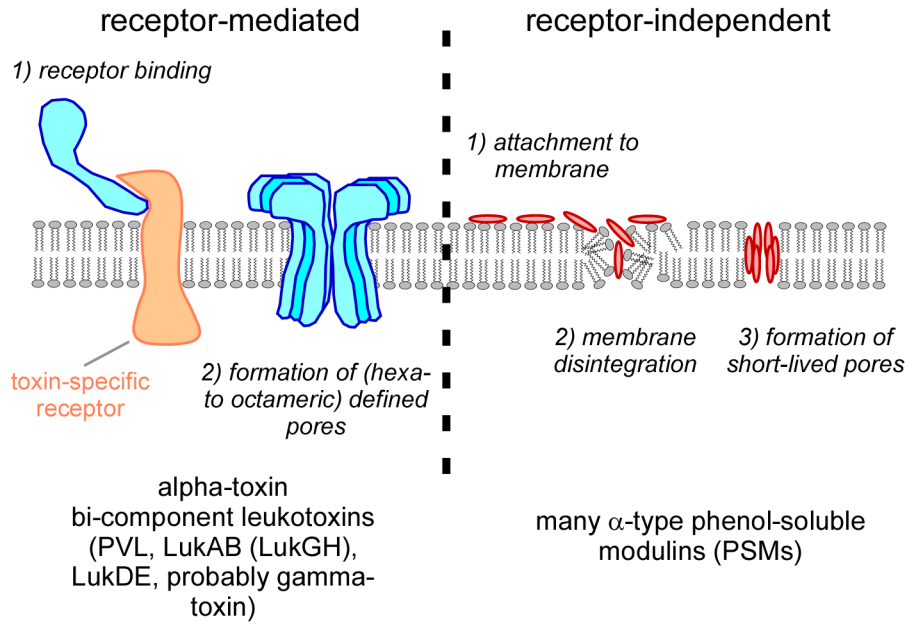- The leukotoxin LukAB (LukGH) and PSMs can lyse after phagocytosis.

Fig. 1.
Membrane-damaging toxins. Alpha-toxin and the bi-component leukotoxins of *S. aureus* bind to specific receptors, upon which formation of a defined pore occurs. Receptors have been identified for alpha-toxin, PVL, LukAB (LukGH), and LukDE. Probably gamma-toxin also binds to a specific receptor. PSMs are believed to attach to the cytoplasmic membrane in a non-specific fashion and lead to membrane disintegration. Probably the phospholipid composition and membrane charge are important for cell susceptibility to PSMs. Pores formed by PSMs are likely short-lived, as shown for delta-toxin.
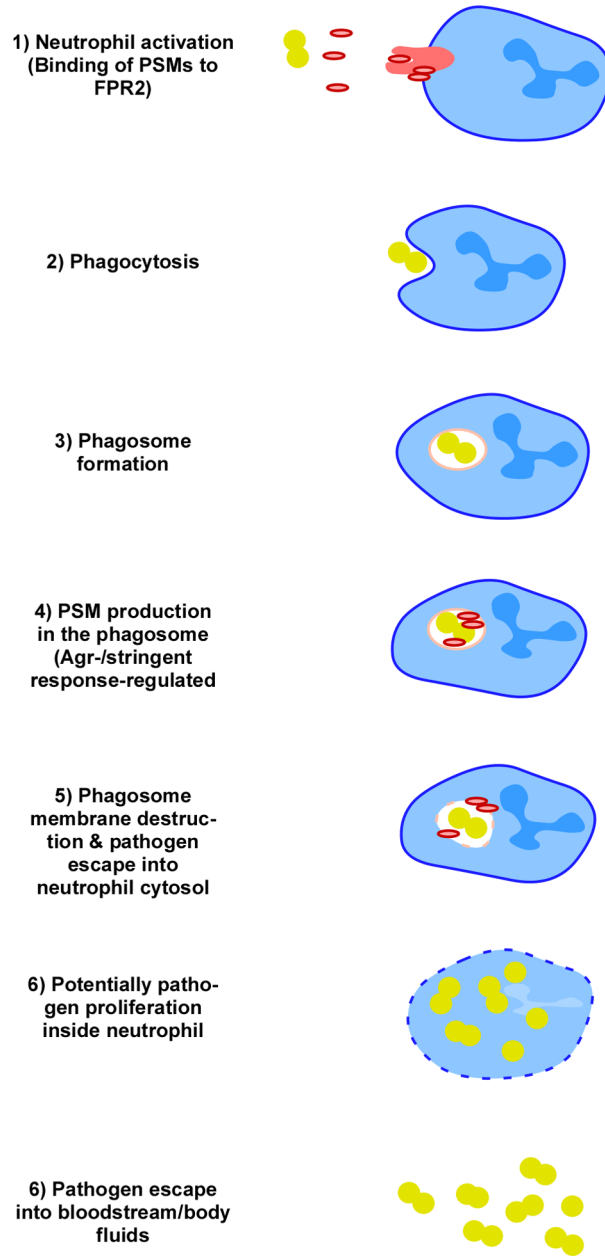
Fig. 2.
Neutrophil lysis after phagocytosis. Lysis after phagocytosis from within the neutrophil phagosome was demonstrated for *S. aureus* PSMα peptides and the leukotoxin LukAB (LukGH), illustrated here for PSMs. PSMs trigger neutrophil activation by interaction with the FPR2 receptor. After phagocytosis and phagosome formation, bacterial regulatory systems (Agr, stringent response) lead to PSM production and PSM-mediated phagosome lysis. Bacteria may further proliferate in the neutrophil. Upon ultimate neutrophil destruction the bacteria escape from the cell.