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Staphylococcus aureus Secreted Toxins & Extracellular Enzymes

Kayan Tam and **Victor J. Torres**

Department of Microbiology, New York University School of Medicine, 430 East 29th Street, Alexandria Center for Life Science | Room 311, New York, New York 10016, U.S.A.

Abstract

S. aureus is a formidable pathogen capable of causing infections in different sites of the body in a variety of vertebrate animals, including humans and livestock. A major contribution to the success of *S. aureus* as a pathogen is the plethora of virulence factors that manipulate the host's innate and adaptive immune responses. Many of these immune modulating virulence factors are secreted toxins, cofactors for activating host zymogens, and exoenzymes. Secreted toxins, such as pore-forming toxins and superantigens are highly inflammatory and can cause leukocyte cell death by cytolysis and clonal deletion, respectively. Coagulases and staphylokinases are cofactors that hijack the host's coagulation system. Exoenzymes, including nucleases and proteases, cleave and inactivate various immune defense and surveillance molecules, such as complement factors, antimicrobial peptides, and surface receptors important for leukocyte chemotaxis. Additionally, some of these secreted toxins and exoenzymes can cause disruption of endothelial and epithelial barriers through cell lysis and cleavage of junction proteins. A unique feature when examining the repertoire of *S. aureus* secreted virulence factors is the apparent functional redundancy exhibited by the majority of the toxins and exoenzymes. However, closer examination of each virulence factor revealed that each has unique properties that have important functional consequences. This chapter will provide a brief overview of the current understanding on the major secreted virulence factors critical for *S. aureus* pathogenesis.

Section I: Exotoxins

Introduction

Staphylococcus aureus is a highly successful pathogen that colonizes ~30% of the population asymptomatically, but it is also capable of causing infections ranging from mild skin and soft tissue infections to invasive infections, such as sepsis and pneumonia (1). When *S. aureus* infects the host, it produces many different virulence factors that promote the manipulation of the host's immune responses while ensuring bacterial survival. These virulence factors include secreted toxins (exotoxins), which represent approximately 10% of the total secretome (2). While there are over 40 known exotoxins produced by these bacteria, many of them have similar functions and have high structural similarities. Closer examination of these seemingly redundant exotoxins revealed that each has unique properties. Exotoxins fall into three broad groups based on their known functions: cytotoxins, superantigens, and cytotoxic enzymes (Table 1). Cytotoxins act on the host cell membranes, resulting in lysis of target cells and inflammation. Superantigens mediate

massive cytokine production and trigger T and B cell proliferation. Secreted cytotoxic enzymes damage mammalian cells. Collectively, these exotoxins modulate the host immune system and are critical for *S. aureus* infections.

A) Cytotoxins

β-barrel pore-forming toxins

α-toxin, the prototypic pore forming toxin: α-toxin (also known as α-hemolysin or Hla), is encoded by the gene *hla* as part of a monocistronic operon in the core genome of *S. aureus*. Like all conventionally secreted proteins, α-toxin is synthesized with a N-terminal signal peptide. Water-soluble, α-toxin monomers form heptameric β-barrel pores in target cell membrane, resulting in cell lysis (Figure 1a) (3). The α-toxin heptamer resembles a mushroom and has 3 major domains: an extracellular cap domain, a stem domain that forms the β-barrel pore, and a rim domain that confers receptor specificity (Figures 2a–c) (4). The β-barrel pore is formed from a prepore by a conformational change in a toxin substructure known as the amino latch (5). The critical role of the amino latch in the β-barrel pore formation is exemplified by a single amino acid mutation at His-35, which disrupts interprotomer stabilization, thus preventing pore formation and inactivating the toxin (6–8).

The role of α-toxin in disease has been studied extensively. α-toxin causes lysis of many different cell types: erythrocytes, platelets, endothelial cells, epithelial cells, and certain leukocytes (9–12). For many years, α-toxin was thought to mediate cytolysis through nonspecific binding to the lipid bilayer of cells. However, this model did not explain the species specificity exhibited by the toxin (i.e. lysis of rabbit but not human erythrocytes). In 2010, Wilke *et al.* identified the protein ADAM-10 (a disintegrin and metalloprotease 10), as the cellular receptor for α-toxin receptor, thus providing an explanation for the observed species and cell type specificities (Figure 3) (13). Recently, the mammalian junction protein, PLEKHA7 (plekstrin-homology domain containing protein A7), was also demonstrated to be involved in α-toxin cytotoxicity and is thought to contribute to α-toxin-mediated tissue injuries in murine skin infection and pneumonia models (14).

α-toxin is not only lethal, but can also modulate cellular responses at sublytic concentrations, including the release of nitric oxide from endothelial and epithelial cells, extracellular Ca^{2+} influx, production of proinflammatory cytokines, and pyroptosis of monocytes through the activation of caspase-1 and the production of NLRP3-inflammasomes (10, 15–19). Additionally, sublytic levels of α-toxin upregulate the expression of ADAM10 and activate the ADAM10 protease to cleave the junction protein E-cadherin, resulting in disruption of the epithelial barrier (11). Nanogram to microgram amounts of α-toxin can cause severe dermonecrosis when administered subcutaneously in rabbits and mice (20, 21). Moreover, intravenous administration of this toxin also results in rapid lethality of the animals (20, 21). *S. aureus* Δ*hla* strains are severely attenuated in several infection models, resulting in enhanced host survival, decreased bacterial burden, inflammation, and tissue injuries (22–27).

The bicomponent pore-forming toxins—The bicomponent pore-forming toxins (PFTs) are distant relatives to α-toxin (Figure 4), share structural homology with α-toxin,

and have a similar pore formation mechanism (Figures 1–2). However, in contrast to α-toxin, the bicomponent PFTs require two subunits: the fast-eluting subunit, F-subunit, and the slow-eluting subunit, S-subunit, named on the basis of their liquid chromatography behavior (28, 29). The current model for leukocidin pore formation suggests that the S-subunit recognizes and binds to a surface receptor on the target cell, then recruits the F-subunit for dimerization (30–32). This is followed by oligomerization with 3 additional dimers to form an octameric pre-pore on the target cell membrane (33). Next, the stem domains of the prepore extend in the center of the structure, forming a β-barrel pore that inserts into the target cell membrane, resulting in cell lysis (Figure 1b) (33, 34). Similar to the α-toxin heptamer, the bicomponent PFT octamer also resembles a mushroom, consisting of the cap, the rim, and the stem domains (Figure 2).

The bicomponent PFTs primarily target leukocytes, thus they are also known as leukocidins (Luk). Currently, 5 of the leukocidins are known to be associated with human infections: LukSF-PV (originally known as Panton-Valentine leukocidin, PVL), γ-hemolysins AB and CB (HlgAB, HlgCB), LukED, and LukAB (also known as LukHG) (30–32). Two other bicomponent PFTs, LukMF' and LukPQ, are associated with animal infections (35–37).

All the leukocidins share structural homology and sequence identity, ranging from 40–90% within each S-subunit and F-subunit family (Figure 4) (38). The only exception is LukAB, which shares only ~30% sequence similarity with the others (38). LukA has a ~33-amino acid sequence at the N-terminus and a 10-amino acid C-terminal tail that are absent from other S-subunits, contributing to its divergence. Nevertheless, the structure of LukAB remains homologous to the other bicomponent PFTs (39).

In additional to mediating cell lysis, many of the leukocidins have sublytic effects, causing extracellular Ca^{2+} influx on host cells (40–42) and production of proinflammatory cytokines (40, 43–47). Several of the leukocidins, PVL, HlgAB, and LukAB, stimulate K^+ efflux, production of the NLRP3-inflammasome, and activation of caspase-1, resulting in a form of inflammatory cell death, known as pyroptosis (43, 45–47).

Although the bicomponent PFTs exhibit many similarities, there are subtle differences that confer unique properties on each of them, which are briefly summarized below (see references (30–32) for in depth summaries).

LukSF-PV (Panton-Valentine leukocidin, PVL): The *pvl* locus is encoded within the genomes of at least six different prophages (48–51). Although less than 40% of clinical isolates from the United States carry a *pvl*-encoding prophage, over 90% of strains associated with severe necrotizing pneumonia and community-acquired infections carry one (1, 52). The relationship between *pvl*-encoding *S. aureus* and severe infections in humans is supported by strong epidemiological data and recent data using animal models of infection (53–57).

PVL exhibits species specificity, killing only rabbit and human leukocytes. The species specificity is due to the targeting of the human and rabbit G-protein-coupled receptors (GPCRs), C5aR1 and C5aR2, but not the murine counterparts (Figure 3) (58, 59).

Consequently, regular mice are inappropriate for the study of this toxin; the availability of rabbit models and human *ex vivo* models have provided insight into the complexity of PVL-mediated pathology. PVL is a critical factor in invasive diseases such as osteomyelitis and pneumonia in rabbits. Deletion of *pvl* results in lower inflammation, reduces tissue injuries and bacterial burden, and promotes host survival (57, 60). Remarkably, sublytic levels of PVL can enhance phagocytosis and killing of the bacteria by primary human neutrophils (61). In contrast, the role of PVL during skin and soft tissue infections is unclear. While one study demonstrated that PVL did not contribute to lesion sizes and bacterial burden (62), another study showed that infections caused by Δ*pvl* strains have smaller lesions compared to the wildtype and complemented controls (63). These studies seem to suggest that the pathogenic effect of PVL could be dependent on the site of infection, but more work must be done to test this hypothesis.

γ-hemolysin (HlgAB, HlgCB): The γ-hemolysin locus is part of the core *S. aureus* genome, present in ~99% of sequenced *S. aureus* genomes (64, 65). This locus is comprised of 3 genes: the *hlgA* gene, transcribed by its own promoter, followed by an operon containing *hlgC and hlgB*, transcribed by a different promoter (66). HlgA and HlgC are S-subunits that share the same F-subunit, HlgB, to form two leukocidins – HlgAB and HlgCB, each possessing its own unique properties.

HlgAB binds to the human receptors CXCR1, CXCR2, CCR2, and the Duffy antigen receptor for chemokine (DARC), lysing human erythrocytes, neutrophils, monocytes, and macrophages (Figure 3) (44, 67). HlgAB can also target murine monocytes and macrophages but cannot target murine neutrophils as it cannot bind to murine CXCR2 (44). In contrast, HlgCB is a human specific toxin that targets cells expressing the receptors C5aR1 and C5aR2 (the same receptors targeted by PVL) (Figure 3) (44).

γ-hemolysins cause acute tissue injury and inflammation and contribute to *S. aureus* disease in different animal models. Retroorbital administration of microgram amounts of HlgAB is lethal to mice (68). Intravitreal injection of γ-hemolysins in rabbits is highly toxic, resulting in destruction of the eye and tissue injury in surrounding areas (69). The tissue damage could be the result of a combination of toxin-mediated cell lysis and pyroptosis caused by sublytic concentration of the toxins (47). The contribution of HlgAB to disease has been further demonstrated in several infection models with strains that do not produce HlgAB. With such strains, there is reduced neutrophil lysis, less inflammation, reduced bacterial burden, and enhanced host survival (44, 70–72).

LukED: The *lukED* locus is in the νSaβ gene cluster. The *lukED* locus is present in ~70% of *S. aureus* isolates and is conserved in a lineage specific manner (64, 65). The two genes *lukE and lukD* in the locus are co-transcribed during the late exponential phase (73).

Early studies on LukED demonstrated the lytic activity of the toxin in rabbit and human erythrocytes and neutrophils (74). Subsequently, LukED was demonstrated to also mediate lysis of many different human and murine bone-marrow derived cells (75, 76). LukED targets the GPCRs – CXCR1, CXCR2, CCR5, and DARC on neutrophils, monocytes, macrophages, dendritic cells, NK cells, T-cells and red blood cells, conferring on the toxin

broad leukocidal activity (Figure 3) (67, 76, 77). Moreover, the pathogenic effects of LukED are receptor-dependent (75–77).

LukED is an important contributor to the virulence of *S. aureus*. Initial studies on LukED demonstrated toxin-induced dermonecrosis of rabbit skin (73). Retroorbital administration of microgram amounts of the toxin leads to acute lethality in mice (68). Corroborating the intoxication studies, Δ*lukED* strains are severely attenuated, resulting in lowered inflammatory responses, reduced bacterial burden, and enhanced host survival in murine systemic infections (75–77).

LukAB (also known as LukHG): The *lukAB* locus is part of the core *S. aureus* genome found in 99% of *S. aureus*. The toxin is found in abundance in the secreted proteome during the late exponential growth phase, which led to its discovery (78, 79). The C-terminal region of LukA is critical for toxin activity, as its deletion or mutation within this region, (i.e. the E323A mutation) renders the toxin inactive (80). Unlike the other leukocidins, which are secreted as monomers, LukAB is secreted as heterodimers (80).

LukAB exhibits sharp species specificity – with its greatest potency being for human & primate cells, followed by rabbit, and it is ~1000-fold less active in mice (81). The selectivity of LukAB was explained when it was discovered that LukAB mediates cytotoxicity through targeting the I-domain of the CD11b receptor present on leukocytes, including neutrophils, monocytes, macrophages, dendritic cells, and NK cells (Figure 3) (80, 82).

Since LukAB is only weakly active toward murine leukocytes and mildly active with rabbit cells, the role of LukAB during infection remains to be fully elucidated. Although the field currently lacks robust *in vivo* models to directly assess the pathogenicity of the toxin, *in vitro* and *ex vivo* studies have provided insights into the role of LukAB in disease, revealing that *lukAB*-defective mutants are greatly attenuated for virulence. For example, primary human neutrophils infected with Δ*lukAB* strains exhibit enhanced survival compared to wildtype (78). Additionally, LukAB promotes the escape of phagocytosed *S. aureus* from neutrophils and monocytes (46, 83). Anti-LukAB antibodies were observed in the serum of patients with invasive *S. aureus* disease, thus demonstrating production of the toxin during infection (84, 85).

LukMF': The *lukMF'* locus is encoded by temperate phage ΦSa1 (37). While *lukMF'* is infrequently found among human isolates, it is commonly found among animal isolates (86). LukMF' exhibits high cytolytic activity towards bovine neutrophils and macrophages through targeting the surface receptor bovine CCR1, CCR2, and CCR5 (Figure 3) (87). LukMF' can be isolated from bovine mastitis tissue samples, indicating a role of this toxin in disease progression (36). However, unlike the other leukocidins, purified LukMF' does not elicit a strong proinflammatory response when incubated with primary bovine macrophages (88).

LukPQ: LukPQ is encoded by the temperate phage ΦSaeq1 (35). Like other bicomponent PFTs, LukPQ demonstrates species and cell type specificities. Equine neutrophils are most

sensitive to the lytic effect of LukPQ, followed by bovine neutrophils; human neutrophils are relatively insensitive to the toxin (Figure 3) (35). LukPQ targets the equine GPCRs, CXCRA and CXCR2, to initiate cytolysis (35). At high concentration, the toxin also targets equine CCR5. However, toxin binding can be inhibited by cytokines binding to these receptors, suggesting LukP and the receptor ligands may be sharing a common binding site (35). Uniquely, the F-subunit, LukQ, is responsible for conferring species specificity of LukPQ, whereas species specificity is conferred by the S-subunit in the other leukocidins (35).

Other cytotoxins

Phenol Soluble Modulins: Phenol soluble modulins (PSMs) belong to a family of amphipathic peptides uniquely found in staphylococci. In *S. aureus*, PSMs are encoded in 3 loci in the core genome: 1) the *psmα* operon, encoding PSMα1–4, 2) the *psmβ* operon, encoding PSMβ1–2, and 3) *hld* encoding δ-toxin (89, 90). *hld* is also part of the coding sequence of RNAIII, the master regulatory RNA in staphylococci. Additionally, certain methicillin resistant staphylococci carry PSM-mec, encoded by *psm-mec* in the staphylococcal cassette chromosome mec (SSCmec) (91). Like *hld, psm-mec* is encoded within a regulatory RNA (92).

Unlike the other cytotoxins described thus far, PSM peptides are often secreted without a signal peptide (90). Therefore, most PSM peptides isolated from staphylococci contain a N-terminal formylmethionine. However, some PSM peptides lack this N-formylmethoinine due to the cytoplasmic enzyme N-deformylase (93). PSMs are secreted by the ABC transporter, phenol-soluble modulin transporter (Pmt). Absence of Pmt causes accumulation of PSM in the cytosol, resulting in cell death (94).

PSMs are classified based on length (95). The α-type PSMs are typically 20–25 amino acids long, most having neutral or positive net charge. PSMα and δ-toxin are α-type PSMs, the entire protein forming one α-helix (96, 97). In contrast, the β-type PSMs are longer, typically 43–45 amino acids in length, most having negative net charge. The structure of the β-type PSM – PSMβ2, consists of 3 α-helices that fold to a “v”-like shape (97).

PSMs have multiple roles in *S. aureus* pathogenesis, including cell lysis, biofilm formation, and immune modulation. α-type PSM peptides have high potency in lysing eukaryotic cells in a receptor independent manner through targeting the cell membranes (90, 98–100). However, lipoproteins present in the serum can inhibit the cytolytic activity of PSMs (101). Therefore, the role of PSMs in extracellular cytolysis *in vivo* is unclear. In contrast, phagocytosed *S. aureus* produces PSMs to lyse neutrophils and osteoblasts intracellularly (99, 100). As such, the role of PSMs could be to mediate intracellular escape of *S. aureus*.

Sublytic concentration of PSMs have immune modulatory effects on host cells. In humans, PSMs are detected by the pattern recognition receptor formyl peptide receptor 2 (FPR2) (102). FPR2 is a member of the G-protein-coupled receptor family that specializes in recognizing pathogen-associated molecular patterns (PAMPs) produced by bacteria. FPR2 is predominately expressed on innate immune cell types, including neutrophils, monocytes, macrophages, and immature dendritic cells. Upon activation by PSMs, FPR2 induces a

series of proinflammatory responses, including cytokine production, neutrophil chemotaxis, and leukocyte activation (102).

PSMs can shape biofilms by forming channels needed for nutrient delivery and dissemination (103). Additionally, the α-type can cause a leaky *S. aureus* membrane, resulting in the release of cytoplasmic proteins (104).

ε-toxin—Merriman *et al.* identified ε-toxin in 2015. The gene encoding ε-toxin, *cytE*, is conserved in the core genome of *S. aureus* (105). Since this is a recently identified toxin, the regulation of *ε*-toxin expression and the mode of action of the toxin are unknown. However, ε-toxin lyses rabbit erythrocytes and human keratinocytes. Lytic concentration of ε-toxin in keratinocytes promotes the secretion proinflammatory cytokine, IL-8 (105). In contrast, sublytic concentration of ε-toxin slows the rate of keratinocyte proliferation, suggesting a role for the toxin in impairing normal wound healing (105). Microgram amount of ε-toxin can result in neutrophil recruitment to the injection site when administered subcutaneously in rabbits (105). Moreover, the same dosage of ε-toxin can cause rabbits to develop fever after intravenous administration of the toxin (105). The role of this toxin in *S. aureus* infections remains to be fully elucidated.

B) Staphylococcal superantigens

T cell superantigens—T cell superantigens (SAgs) represent the largest family of exotoxins produced by *S. aureus*. Their molecular weights range from 19–30kDa. SAgs are unique because they are resistant to heat, proteolysis, and desiccation (106). Due to their extreme stability and high toxicity in humans, some of them are classified as select agents for bioterrorism (i.e. SEB).

The genes encoding SAgs are found in various components of the *S. aureus* genome. The recently discovered *selX* is part of the core genome (107). The other SAgs are encoded in different mobile genetic elements, such as bacteriophages, plasmids, or pathogenicity islands (108–111). However, the distribution of SAgs is highly variable in the same mobile genetic element found between different strains (112).

SAgs share structural homology to another family of closely related proteins, the superantigen-like proteins (SSLs). The *ssl* are encoded in the gene cluster νSaα (113) This family of proteins was originally called SETs for staphylococcal enterotoxin-like proteins (113). However, they were renamed by the International Nomenclature Committee for Staphylococcal Superantigens in 2004 to reflect their lack of emetic and mitogenic properties (114). The primary role of these proteins seems to be immune evasion (See reference (111) for a review on the SSLs).

SAgs exhibit tremendous sequence diversity (Figure 5), but their overall structures are similar. SAgs have two primary domains: a N-terminal oligosaccharide/oligonucleotide binding (O/B) fold that is shaped like a β-barrel and a C-terminal β-grasp domain comprised of anti-parallel β-sheets. The two domains are connected by an α-helix (115). Additionally, all SAgs have a dodecapeptide binding site, a Vβ T cell receptor (TCR) binding site, and up to two major histocompatibility complex (MHC) binding sites (106).

An SAg exerts its mitogenic property by crosslinking the Vβ TCR on a T cell with the MHC class II molecule (MHCII) on an antigen presenting cell (APC), resulting in polyclonal T cell proliferation (Figure 6) (116). SAgs are highly effective T cell mitogens that can stimulate up to 50% of T cells (106). SAg-induced T cell proliferation is followed by a state of T cell anergy, where activated T cells failed to proliferate and/or undergo apoptosis. SAgs are one of the many ways *S. aureus* manipulates the host immune system to prevent the generation of functional adaptive immunity.

SAgs can be broadly divided into three groups, staphylococcal enterotoxins (SEs), staphylococcal enterotoxin-like (SE-*l*) superantigens, and toxic shock syndrome toxin-1 (TSST-1). Each group of SAgs will be briefly summarized below (see reference (106)).

Staphylococcal enterotoxins: There are 7 distinct SEs, SEA to SEE, and SEG. Additionally, several variants of SEB and SEC have been identified. They were originally named for their ability to induce emesis, a key characteristic of staphylococcal food poisoning (114). Ingestion of SEs causes vomiting and diarrhea. However, the disease is usually self-limiting. The emetic activity of SEs is correlated with the presence of a 9–19 amino acid-long disulfide loop in the protein.

SEs can have up to two MHC-binding sites. SEBs and SECs contain only one MHC-binding site, while the other SEs have two MHC-binding sites (106, 117, 118). The low-affinity binding site targeting the MHCII α-chain is common to all SAgs. The second site is a Zn^{2+}-dependent high-affinity binding site that targets the β-chain of MHCII (119). SAgs that have two MHC-binding sites are 10–1000-fold more potent compared to SAgs that have only one. However, they are produced at a much lower abundance compared to some other SAgs that contain 1 MHC-binding site, such as TSST-1 and SEB (106).

Staphylococcal enterotoxin-like superantigens (SE-*l*s): The other 15 SAgs are SE-*l*H to SE-*l*X (106, 114). SE-*l*s include all newly identified SAgs that are T cell mitogens but have unproven emetic activity (114). However, this group also contains SE-*l*s that lack the emesis-associated disulfide loop and are proven to not induce emesis (106). SE-*l*s can have up to two MHC binding sites and a 15-amino acid extension for specific TCR interactions (106, 120).

Toxic shock syndrome toxin-1: The gene *tst* is encoded in several pathogenicity islands, including SaPI1, SaPI2, and SaPIbov1 (121). TSST-1 has only one low-affinity MHC-binding site targeting the MHCII α-chain, a Vβ TCR binding site, and a dodecapeptide binding site (122). This dodecapeptide binding site is proposed to be important for the interaction of TSST-1 with epithelial cells and the immune stimulatory molecules, CD40 and CD28 (123–125).

TSST-1 originally was known as SEF. In 1984, it was renamed to reflect the lack of emetic activity and its association to toxic shock syndrome (TSS). TSS is an acute systemic illness characterized by hypotension, fever, rash, and desquamation 1–2 weeks after onset. As defined by the Center for Disease Control, TSS involves at least 3 different organ systems – gastrointestinal, muscular, mucous membrane, renal, hepatic, hematologic, or central

nervous system (126). TSS can be further classified as menstrual and non-menstrual TSS. Menstrual TSS is usually associated with vaginal/cervical mucosae colonization of TSST-1-producing *S. aureus* and tampon use (127, 128). Approximately 50% of non-menstrual TSS are caused by TSST-1-producing strains and the remaining are caused by strains producing SEB or SEC (129).

B cell superantigen—Staphylococcal protein A (SpA) is the only known B cell superantigen produced by *S. aureus*. A majority of clinical isolates contain *spa* in the core genome (130). The SpA precursor has a N-terminal signal peptide that is cleaved prior to the secretion of the mature protein. Mature SpA has 4–5 highly conserved Ig-binding domains connected by short linkers at the N-terminus (131). This is followed by a hypervariable region called Region X, comprised of subregions Xr and Xc (132). The highly variable and repetitive octapeptide in Xr is the basis of SpA-typing, a high throughput method of grouping *S. aureus* isolates (130). Region X is followed by the C-terminal LPXTG motif for covalent anchoring of the protein to the cell wall (131). However, SpA proteins can be released from the cell wall by the cell wall hydrolase, LytM (133).

The Ig-binding domains confer upon SpA the ability to bind Fcγ portion of immunoglobulins (Ig) to prevent opsonization (134). These Ig-binding domains also mediate SpA binding to B cells by crosslinking V_H3-expresssing B cell receptor (BCR), which results in B cell activation; however, activation without costimulatory signals results in death and subsequent clonal deletion of B cells (135–137).

Conventional antigen recognition by BCR requires antigen recognition at the complementarity-determining region (CDR). In contrast, SpA exerts its mitogenic activity by binding to the variable region of the heavy chain, away from the CDR, thus bypassing the antigen specificity requirement for B cell activation (Figure 6) (138, 139). SpA-mediated clonal deletion of V_H3-expressing B cells can lead to the impairment of the B cell repertoire important for mounting effective antimicrobial defenses against the pathogen (140, 141).

During intravenous infection, SpA prevents opsonophagocytosis of the bacteria by binding to immunoglobulins (Ig) and impedes the development of specific anti-*S. aureus* antibodies (142). In contrast, isogenic strains that lack *spa* or express variants that cannot bind to Ig exhibited reduced kidney abscess formation and elicited specific anti-*S. aureus* antibodies (142). Mice immunized with the Ig-binding deficient SpA variant, SpA_{KKAA}, acquired protective immunity and could mount a more effective humoral response against *S. aureus* antigens (143).

C) Cytotoxic enzymes

β-toxin (also known as β-hemolysin)—The β-toxin encoding gene, *hlb*, is part of the core *S. aureus* genome. However, due to the presence of the *hlb*-converting prophage (i.e. ΦSa3, Φ13), which disrupts the gene, only a limited number of human clinical isolates produce β-toxin (144, 145). The prophage carries the immune evasion gene cluster encoding for immune evasion factors, such as the staphylococcal complement inhibitor proteins (SCIN), chemotaxis-inhibitory proteins (CHIPS), and staphylokinase (145). These virulence factors are thought to be involved in *S. aureus* immune evasion and survival in the human

host. The *hlb*-converting prophage is prevalent in strains associated with human infections (~90%), but it is less frequently found in animal isolates (~30%) (146). However, chronic infections or environmental pressures (i.e. oxidative stress, antibiotics, temperature) can promote the excision of the phage and the production of β-toxin (147–150). Thus, the contribution of β-toxin during human infection is unclear.

β-toxin is a Mg^{2+}-dependent neutral sphingomyelinase (SMase), a phospholipase that specifically cleaves sphingomyelin to produce ceramide and phosphocholine (151). This toxin was first identified in 1935 by Glenny and Stevens based on several unique observations: hemolysis of erythrocytes in the presence of α-toxin neutralizing serum, lysis of sheep but not rabbit erythrocytes, and enhanced hemolysis caused by temperature shifting from 37°C to a lower temperature (152). As such, β-toxin is also known as a hot-cold hemolysin. This unique phenomenon is the result of ceramide hydrolysis products at 37°C being held together by cohesive forces in the membrane. When temperature decreases (i.e. 4°C), this causes a phase separation that condenses ceramide into pools and collapses the lipid bilayer, resulting in the invaginations observed on erythrocyte membranes by electron microscopy (153).

Crystal structure of β-toxin reveals structural homology to members of the DNase I superfamily (154). β-toxin is a single domain protein consisting of 4 layers: 2 layers of β-sheets at the center and 2 outer layers comprised of α-helices and β-strands (154). Based on the structural homology to the DNase I superfamily, a secondary function for β-toxin was hypothesized. Later, β-toxin was shown to enhance biofilm formation through catalyzing the formation of nucleoprotein matrix in biofilms, therefore β-toxin is also a biofilm ligase (155).

β-toxin exhibits species-dependent hemolytic activity that correlates with the amount of sphingomyelin content in erythrocytes: sheep, cow, and goat erythrocytes are highly sensitive to the toxin, rabbits and human exhibit intermediate sensitivity, while murine and canine erythrocytes are resistant (156). The SMase activity of β-toxin also causes the lysis of human keratinocytes, monocytes, T cells, and bovine epithelial cells (154, 157–159). β-toxin stimulates the production of proinflammatory cytokines in human monocytes (158), but suppresses IL8 production and cell adhesion molecules expressions in human endothelial cell, therefore the toxin can prevent leukocyte migration across the endothelium (160).

Infection with β-toxin producing *S. aureus* results in larger lesions in the organs without affecting the overall bacterial burden in the rabbit endocarditis and pneumonia models (147). The presence of β-toxin enhances *S. aureus* colonization of the skin (159) and induces injuries to the scleral epithelial cells during ocular keratitis in mice (27). In an infective endocarditis model, rabbits infected with *S. aureus* producing β-toxin mutants that lack SMase activity have enhanced survival and smaller lesions in the heart, but there were no differences in bacterial burden when compared to the isogenic β-toxin-producing strain (161). Intranasal administration of β-toxin induces the shedding of syndecan-1, a major heparan sulfate proteoglycan molecule on lung epithelial cells and causes neutrophil infiltration into the lungs in mice (162). The shedding of sydecan-1 is caused by the SMase

activity of β-toxin, as intranasal intoxication with SMase mutants have reduced shedding of this protein *in vivo*, resulting in reduced lung pathology (162).

Exfoliative toxins—Exfoliative toxins (ETs) are also known as epidermolytic toxins. There are 4 antigenically distinct forms found in *S. aureus*: ETA, ETB, ETC, and ETD. Each ET is encoded on a different mobile genetic element: *eta* is encoded in the genome of a temperate phage that has been shown to convert *eta*-negative strains to toxin producers (163, 164). *etb* is found on the plasmid pETB (165) and *etd* is encoded as part of a pathogenicity island (166). ETC was purified from a *S. aureus* isolate associated horse infection, however, the genetic locus of ETC has not been described (167).

Most of what is currently known about the ETs is based on ETA and ETB. ETs are the causative agents for staphylococcal scalded skin syndrome (SSSS), including Ritter's disease, toxic epidermal necrosis, bullous impetigo, and certain erythema cases. SSSS predominantly affects neonates, infants, and immunocompromised adult patients (168). Symptoms of SSSS are characterized by formation of blisters and superficial desquamation, involving only the skin layer (168). Although SSSS was initially described in 1878, its association with *S. aureus* infections was first suggested in 1967 and the contribution of ETs to the blistering symptoms was not identified until in the 1970s (163, 169–171). This delay was in part contributed by the ETs' unique mode of action. The lesions characteristic of SSSS are often sterile, because the ETs can be distributed through the bloodstream from a distant site to cause symptoms (171).

ETs are glutamate-specific serine proteases of the chymotrypsin family. The catalytic triad (histidine, aspartate, serine) is conserved in all ETs (168). ETA and ETB are similar in structure and share homology with other staphylococcal serine proteases – SspA and the serine protease-like proteins (Figure 7) (172–174). The N-terminal α-helical extension is required for enzyme activity (172, 174). The crystal structures of both ETA and ETB show that the key residues in Loop D occupy the oxyanion hole required for enzymatic activity of all serine proteases, thus the crystal structures represent the inactive forms of the enzymes (172, 174). These findings suggest that the protease activity of ET may require a specific cellular target and occur under specific condition.

In the early 2000's, ETs were shown to interact with human and mouse desmoglein 1 (Dsg1), causing blistering of the superficial skin (175, 176). ET recognition of Dsg1 is conformational, requiring the presence of Ca^{2+}. Lack of Ca^{2+} results in the unfolding of Dsg1, thus the inability of ETs to cleave the protein (177). Through domain swapping and site-mutagenesis studies, it was determined that five amino acids (Q271, Y274, T275, I276, E277) belonging to the extracellular domain 3 of Dsg1 are critical for ETA to exert its protease activity (178).

With the identification of Dsg1 as the substrate for ETs, the pathophysiology of the superficial skin blistering in SSSS was explained. In humans, there are 4 isoforms of desmogleins, Dsg1–4 (179). Desmogleins are cadherins required for desmosome cell-to-cell adhesion to maintain the integrity of the epidermis. ETs target only Dsg1, which is expressed throughout the human epidermis. Cleavage of Dsg1 disrupts the cell-to-cell

adhesion of the epidermis, resulting in blistering and desquamation of the superficial skin. The other strata of the epidermis are unaffected because of the presence of Dsg2–4, which are not targeted by ETs, and thus compensate for the destruction of Dsg1 (175, 180).

Section II: Exoenzymes

Introduction

S. aureus devotes a significant amount of its resources to produce virulence factors to evade the host immune system and to acquire necessary nutrients for its own survival. In the previous section, the mechanisms of toxin-mediated host immune evasion and their roles in *S. aureus* virulence were discussed. In addition to the toxins, *S. aureus* also produces a large number of virulence factors that have enzymatic properties. They can be broadly categorized into two groups: cofactors that activate host zymogens and enzymes for degradation of tissue components (Table 2). While these cofactors and secreted enzymes (exoenzymes) have different substrates and mechanisms of action, they function to break down bacterial and host molecules for nutrient acquisition, bacterial survival, and dissemination.

A) Cofactors for host enzyme activation

Coagulase (Coa), von Willebrand factor binding protein (vWbp), and staphylokinase (Sak) are cofactors produced by *S. aureus* that have no enzymatic activities by themselves, but they can activate host zymogens. These three proteins hijack different aspects of the host coagulation system, thereby manipulating the host innate defenses to promote bacterial survival and dissemination.

Staphylococcal coagulases: Coagulase & von Willebrand factor binding protein—The ability to induce coagulation is one of the key criteria used in modern medical microbiology for species classification in the genus Staphylococcus – separating "coagulase-positive" and "coagulase-negative" species. A majority of staphylococci are coagulase-negative, but few are coagulase-positive species, including *S. aureus* and *S. intermedius*; however, *S. schleiferi* has both coagulase-positive and coagulase-negative subspecies (181).

S. aureus induced coagulation of human plasma was initially documented in 1903 (182). The causative agents, coagulase (Coa) and von Willebrand factor binding protein (vWbp), are highly active in coagulating human and rabbit plasma (183).

Both *coa* and *vwb* are chromosomally encoded. There are 12 different isoforms of *coa* that have been identified thus far; the majority of the variability is attributed to the high sequence variability (>50%) of the N-terminus coding region between different strains (184, 185). In contrast, *vwb*, encoding vWbp, is relatively conserved with only 2 different alleles known (184). However, a recent report identified several *vwb* paralogues carried by SaPIs that produce vWbps that coagulate ruminant and equine plasma (186).

Coa and vWbp share ~30% protein sequence homology at the N-terminus (187). They both have a D1D2 domain for prothrombin binding (188, 189). However, they differ significantly at the C-terminus. The C-terminus of Coa has a 188-residue linker region followed by a

repeat region comprised of tandem repeats of 27 residues responsible for fibrinogen binding (188, 189). In contrast, the C-terminus of vWbp has a von Willebrand factor (vWF) domain and a fibrinogen binding domain (188, 190).

Coa or vWbp binds to prothrombin at a ratio of 1:1 to form staphylothrombin. Insertion of the N-terminus of Coa into the Ile16 pocket of prothrombin causes a conformational shift resulting in the activation of the zymogen (189, 191). Staphylothrombin is highly efficient in converting fibrinogen to fibrin (Figure 8).

The activity of staphylothrombin cannot be inhibited by common anti-coagulants (i.e. EDTA, heparin) or thrombin inhibitors, such as hirudin and bivalirudin (189, 191–193). However, two recently discovered small molecules, argatroband and dabigatran, can inhibit the activity of staphylothrombin (194, 195).

Δ*coa*Δ*vwb* strain is less virulent compared to its wildtype parent, thus demonstrating a role of the coagulases during infection (187, 196, 197). However, coagulases must be present concurrently with the infecting strain to promote virulence. Ekstedt *et al* demostrated that while intracerebral co-injection of purified coagulase with coagulase-negative *S. aureus* enhances the virulence of coagulase-negative *S. aureus*, pre-injection of purified coagulase before infection has no effect (198). Additionally, Coa is suggested to have a role in the formation of device associated biofilm formation (199).

In an abscess, the coagulases generate a fibrin shield to protect *S. aureus* from immune cell infiltration. Coa is required for the formation of pseudocapsule immediately surrounding the abscess and both vWbp and Coa are required for fibrin formation around the pseudocapsule (187, 200).

Staphylokinase—Staphylokinase (Sak) is a cofactor that hijacks host plasmin to activate plasminogen for the breakdown of fibrin clots and promotes bacterial dissemination (Figure 8). Sak is produced by lysogenic strains of staphylococci; the prophage encoding Sak typically carries other genes encoding virulence factors such as enterotoxin A and chemotaxis inhibitory proteins (201, 202). There are three different groups of phages that carry the *sak* gene (203). Serotype B phages (i.e. ΦC) cause positive conversion of Sak without disrupting other genes (204, 205). Positive conversion of *sak* can also be mediated by some serotype F phages (i.e. ΦSa3, Φ42D), but the phage integration disrupts the *hlb* gene (144, 202, 206). The phage carrying *sak* has also been reported to disrupt the coding sequences of N-acetylmuramyl-L-alanine amidase and peptidoglycan hydrolase (207, 208).

Sak is a single domain protein consisting of a central α-helix, a 5-strand β-sheet and 2 shorter β-strands (Figure 9) (209). Sak forms a 1:1 complex with plasmin in the serum to form Sak-plasmin (210, 211). This complex is highly efficient in converting plasminogen to plasmin. Sak can also bind to plasminogen, however, this complex is inactive and must be converted to Sak-plasmin to have enzymatic activity (212). In an active Sak-plasmin complex, the first 10 residues at the N-terminus of mature Sak are removed to expose the charged residue - Lys11 (213). Deletion of Lys11 inactivates Sak (214). The binding of Sak to plasmin directs the active site of plasmin to favor cleavage of the activation loop in

plasminogen and promotes the conversion of plasminogen to plasmin by enhancing substrate presentation to plasmin. (Figure 9) (215).

Circulating Sak-plasmin complexes are sensitive to dissociation by α_2-antiplasmin, but fibrin-bound complexes are protected from inactivation (216). The fibrin-bound complexes cleave IgG and human C3b, thus preventing opsonization of the bacteria by the complement system (217). Additionally, Sak-plasmin complexes can activate the matrix metalloprotease 1 (MMP-1), important for leukocyte migration and activation (218). Importantly, Sak neutralizes the bactericidal activities of α-defensins and LL-37, two major human anti-microbial peptides (AMPs) (219, 220).

Sak is highly species-specific. Sak is active for human, dog, goat, rabbit, and sheep plasminogen, but inactive for mouse, pig, cow, and buffalo plasminogen (221). Using transgenic mice that produce human plasminogen, studies demonstrated that Sak facilitates *S. aureus* invasion of the skin barrier to generate large and open lesions (222, 223). However, plasmin activation is known to promote wound healing and to reduce inflammation. Thus, during skin infection, Sak may functions as vanguard to establish the primary infection, but after the infection is established, Sak limits the severity of infections to promote dissemination (223).

Furthermore, Sak reduces biofilm formation and facilitates the detachment of mature biofilm by activating plasminogen (221). Corroborating these observations, high Sak-producing strains are often associated with less biofilm formation *in vitro* and non-invasive infections in humans (221, 223).

B) Enzymes that degrade host tissue components

Nucleases—Staphylococcal nuclease, originally known as micrococcal DNase, was identified in the culture supernatants of *S. aureus* by Cunningham *et al.* in 1956 (224). Nuclease requires Ca^{2+} ions for activity, but not other divalent cations (224, 225). Staphylococcal nuclease is also known as thermonuclease, named after its resistance to heat inactivation (224, 225). Staphylococcal nuclease functions as both an endo- and exo-nuclease that break down DNA and RNA substrates through the cleavage of the 5'-phosphoryl ester bond (224, 225).

With the availability of whole genome sequencing in the late '90s, the sequence of *S. aureus* genome became available, which led to the identification of two different staphylococcal nuclease genes, *nuc* (SA0746) and *nuc2* (SA1160) (226, 227). The two genes are located at disparate regions in the genome, under the control of separate promoters. The two nucleases share 34% amino acid similarity overall and 42% similarity within the catalytic domain (228). Both nucleases are Ca^{2+} dependent, heat-resistant, and are able to use DNA & RNA as substrates (226, 228). A major difference between Nuc and Nuc2 is their cellular localization. Nuc is a secreted enzyme with two different isoforms, NucB and NucA (229, 230). In contrast, Nuc2 is surface-bound (228).

Much of what is currently known about nucleases is gathered from studies performed on Nuc. During infections, Nuc regulates biofilm formation and mediates bacterial escape from neutrophil extracellular traps (NETs).

Nuc disperses biofilm by breaking down extracellular DNA (eDNA). Biofilm formation is enhanced in strains that do not produce Nuc (230, 231). Expression of *nuc* is repressed during biofilm formation, providing evidence that *S. aureus* controls nuclease expression to regulate biofilm formation (230, 232). Furthermore, the *nuc* mutant has decreased fitness during intraperitoneal infection *in vivo* (232).

The second role of Nuc is to mediate bacterial escape from NETs. NET is an innate immune defense mechanism by which DNA released from dying neutrophils immobilizes and facilitates killing of invading pathogens (233). Nuc degrades NETs to allow *S. aureus* to escape (234).

Moreover, when Nuc degrades DNA in the abscess or NETs, the degradation products, monophosphate nucleotides, become substrate for another enzyme, adenosine synthase A (AdsA) (235). AdsA converts the degraded DNA to deoxyadenosine, which induces caspase-3 activation, leading to apoptosis of macrophages surrounding the abscess or the NET, thus promoting *S. aureus* survival (235).

The contribution of Nuc2 to *S. aureus* virulence is less clear due to its low expression level compared to Nuc (228). Purified Nuc2 has been demonstrated to disperse biofilms *in vitro* (228). A mutant expressing only Nuc2 but not Nuc showed that the nuclease is produced during intramuscular infections in mice, albeit at a much lower level (228). The identification of Nuc2 *in vivo* suggests that it may have a role in *S. aureus* virulence, possibly performing similar functions as the secreted Nuc but on the bacterial surface.

Proteases—Staphylococci encode 3 families of secreted proteases: metalloproteases, cysteine proteases, and serine proteases. Collectively, these proteases have roles in nutrient acquisition, bacterial dissemination, and immune evasion. Currently, *S. aureus* is known to produce 12 different proteases: one metalloprotease (aureolysin/Aur), two cysteine proteases (staphopain A (ScpA), staphopain B (SspB)), and nine different serine proteases. These serine proteases include V8 protease (SspA), serine protease-like proteins A-F (SplA-F), and exfoliative toxins A & B (ETA & ETB). Although Spls, ETA, and ETB are secreted as active enzymes, all the other proteases are secreted as zymogens, requiring proteolytic cleavage of the pro-peptide for activation (Figure 10). The roles of ETA and ETB in *S. aureus* virulence are described in the Exotoxin section.

In the following sections, we will discuss mode of action of each protease family and its proposed role in *S. aureus* virulence:

Metalloprotease – Aureolysin: The *S. aureus* metalloprotease, aureolysin (Aur), also known as Protease III, was identified in the culture supernatant of strain V8 by Arvidson *et al.* in 1972 (236, 237). The structure of Aur is comprised of two conserved domains common to bacterial metalloproteases of the thermolysin family: the N-terminal β-pleated domain and the C-terminal α-helical domain (238). The mechanisms of substrate binding and

protein catalysis are also common among the proteases in this family. However, unlike other bacterial metalloprotease in the thermolysin family, Aur does not have elastase activity (238, 239).

Aur self-activates by autoproteolysis through the cleavage of the N-terminal pro-peptide (Figure 10) (240). The active enzyme prefers to cleave peptide bonds at the N-terminal side of bulky hydrophobic residues, such as alanine, isoleucine, and tyrosine (241). The presence of Zn^{2+} is required for enzyme activity, but Co^{2+} can act as a substitute and increases enzyme activity (242). Additionally, binding to Ca^{2+} ions stabilize Aur; whereas chelating agents, such as EDTA, irreversibly denature the protein (236, 238).

The broad substrate specificity of Aur allows the metalloprotease to target a variety of substrates, including other *S. aureus* proteins that are important for virulence and host proteins that are important for immune defense. Aur activates SspA, the second protease in the staphylococcal protease activation cascade (Figure 10) (243). Additionally, Aur can degrade clumping factor B (ClfB) and the PSMα peptides (244, 245). Collectively, Aur can shape the secreted and surface proteome of *S. aureus* (246).

Aur contributes directly to *S. aureus* immune evasion and dissemination through the cleavage and inactivation of the antimicrobial peptide LL-37, thus promoting *S. aureus* survival (247). Aur can also degrade the human plasma protease inhibitors – α_1-proteinase inhibitor and α_1-antichymotrypsin present in the serum, albeit not as efficiently as SspA (248, 249). As such, Aur and SspA are proposed to work synergistically to achieve immune evasion.

Aur can affect complement activation by cleaving the complement protein C3 to C3a and C3b in serum (250). The anaphylatoxin, C3a, is further degraded by Aur, preventing leukocyte activation (250). The soluble C3b fragment is inhibited and degraded by Factor H and Factor I in the serum (250, 251). Degradation of C3 by Aur results in the depletion of C3 proteins, thus preventing the formation of the membrane attack complex (MAC) on the bacteria and promotes bacteria survival. Furthermore, Aur activates pro-thrombin and pro-urokinases and inactivates plasminogen inhibitors, thereby manipulating the host coagulation system (252, 253). The various roles of Aur in modulating *S. aureus* proteome and host innate defense molecules suggest Aur has an important role in promoting survival and dissemination of the bacteria *in vivo*. This is corroborated by the detection of Aur in phagocytosed *S. aureus*, suggesting the protease may have a role during intracellular infection (254).

Serine Proteases – SspA: The serine protease, SspA is also known as the V8 protease or GluV8. The gene encoding SspA (*sspA)* is part of the *staphylococcal serine protease* operon (*ssp*), consisting of 3 genes, *sspA, sspB, and sspC* (255). The functions of SspB and SspC will be discussed later in this section.

SspA was identified in the culture supernatants of strain V8 by Drapeau *et al.* in 1972 (256). Around the same time, Arvidson *et al.* identified Protease I that exhibited similar properties as the SspA identified by Drapeau *et al.*, however, whether these two reports describe the

same enzyme was difficult to decipher because there were differences in molecular weight and protease inhibitor sensitivity between the two reports (241, 257). Based on the report by Drapeau *et al.*, the enzymatic activity of SspA can be inhibited by the serine protease inhibitor disopropyl flurophosphate (DFP) (256).

SspA is a glutamyl endopeptidase, part of a small group of serine proteases that preferentially cleaves substrates at the C-terminal side of glutamate and aspartate (256). The preference for negatively charged residues as substrates at neutral pH is due to of the protein's positively charged N-terminus (258). Crystal structure of SspA showed the protein lacks the disulfide bonds commonly found in other proteins of this family (258). However, SspA shares high structural homology to the serine proteases – staphylococcal exfoliative toxins and bovine trypsin, despite having limited protein sequence similarity (258). The conserved trypsin-like serine protease catalytic triad, consisting of histidine, asparagine, and serine, is found in SspA. The C-terminal repeat domain consists of tandem repeats of Pro-Asp or Asn-Asn that ranges from 9–19 repeats (259–261), however, as this C-terminal repeat domain is not required for activity (262), its role in the function of the protein is unclear.

SspA is secreted by *S. aureus* as a zymogen. However, pro-SspA can undergo autoproteolysis to generate a shorter version of pro-SspA (243). Aur processing is required for both forms of pro-SspA to become active enzymes (Figure 10) (243).

SspA contributes to *S. aureus* immune evasion and dissemination by breaking down self and host proteins. SspA cleaves fibrinogen binding factors on *S. aureus* cell surface, thus reduces bacterial adhesion and enhances bacterial dissemination, and ultimately results in the breakdown of biofilms (263, 264). SspA can also degrade host proteins such as α_1-proteinase inhibitor (248), the IL-6 cytokine (265), and immunoglobulins (266, 267), thus SspA directly modulates immune activation and opsonization. SspA can cleave LL-37, but the cleavage does not inactivate the antimicrobial peptide (247).

SspA is produced upon *S. aureus* phagocytosis by neutrophils, suggesting its role in facilitating *S. aureus* intracellular escape, potentially through activating the cysteine protease, Staphopain B (254). SspA can disrupt epithelial barriers, compromising cell junction integrity (265, 268). Skin infection models of *sspA* mutants suggest a slight decrease in bacterial fitness *in vivo* (269). However, since the activity of SspA can be inhibited by α_2-macrogobulin present in the serum, its role as a soluble virulence factor in serum during *S. aureus* pathogenesis remains unclear (263).

Cysteine proteases – Staphopains: Staphopain A (ScpA) was the first cysteine protease identified in *S. aureus* by Arvidson *et al.* (described as Protease II) in 1973 (257). Subsequently, Staphopain B (SspB) was identified as ORFX in 1998 by Chan *et al.* (270). A third staphopain, Staphopain C has been described in avian-associated *S. aureus* isolates (271, 272). Most of what is known regarding staphopains is derived from studies on staphopains A & B.

The staphylococcal cysteine protease operon (*scp*), contains the genes *scpA* and *scpB*, encoding the proteins ScpA and its intracellular inhibitor staphostatin A, respectively (273).

The staphylococcal serine protease operon (*ssp*) contains the genes *sspA, sspB*, and *sspC*, encoding the V8 protease (SspA), staphopain B (SspB), and its intracellular inhibitor staphostatin B (SspC) (255).

ScpA and SspB are secreted as zymogens. Pro-SspB is processed by SspA as the last step of the proteolytic cascade that began with Aur (Figure 10) (255, 274). In contrast, processing of pro-ScpA is not mediated by Aur, SspA, or SspB (269). Instead, pro-ScpA undergoes rapid autoproteolysis, but this process also leads to rapid degradation of the protease (275).

Despite limited primary sequence identity, crystal structures of both staphopains demonstrated structural similarity to papain. Classical papain-like proteases contain 2 domains: the helical L-domain is comprised of the N-terminal part of the protein, containing the catalytic cysteine; and the R-domain is constituted by the C-terminal part of the protein, which folds into anti-parallel β-sheets forming a β-barrel-like structure, containing the catalytic histidine and aspartate (Figure 11) (276–278). The location of the catalytic triad is conserved in both staphopains (276, 277).

Although ScpA and SspB share high structural similarity, subtle differences between the proteases confer different substrate specificities. ScpA cleaves elastins found in connective tissues, pulmonary surfactant protein A in the lungs, and the chemokine receptor CXCR2 on leukocytes (239, 279–281). Additionally, ScpA promotes vascular leakage by activating the plasma kallikerin/kinin system, resulting in hypotension (281). The activity of ScpA in mediating vascular leakage is enhanced by SspB; however, SspB alone does not induce vascular leakage, demonstrating substrate specificity of the two proteases (281).

In contrast, SspB degrades antimicrobial peptide, LL-37, thereby promoting bacterial survival (282). SspB also cleaves CD11b and CD31, surface proteins important for the activation and survival of phagocytes, respectively (283, 284). Thus, SspB prevents *S. aureus* from phagocytosis while diminishing the leukocytes' abilities to detect pathogens. Paradoxically, SspB is also a potent activator of chimerin, a chemoattractant for dendritic cells and macrophages (285). *S. aureus* thrives intracellularly in macrophages and dendritic cells (286, 287). Therefore, SspB may function to promote the intracellular lifestyle of *S. aureus* for persistent infections. In fact, *S. aureus* has been demonstrated to produce SspB, SspA, and Aur after neutrophil phagocytosis (254).

Both ScpA and SspB are implicated in modulating biofilm formation (288, 289). The expression of both staphopains is repressed during biofilm formation and the production of staphopain result in the dispersal of biofilms (289).

In addition to promoting biofilm dispersal, staphopains have a direct effect on the host's connective tissue and coagulation systems. Staphopains inactivate a number of host proteins, including α_1-proteinase inhibitor, collagen, and fibrinogens; however, SspB has higher activity in cleaving fibrinogen and collagen compared to ScpA (248, 290).

The activities of staphopains are inhibited by the cysteine protease inhibitor E-64; heavy metals, such as Co^{2+}, Ag^{2+}, Hg^{+}, and Zn^{2+}; and host derived proteins, including α_2-macroglobulin in human plasma, and the epithelial serpin, SCCA1 (239, 257, 291).

Additionally, *S. aureus* produces inhibitors against the enzymes, known as staphostatins. Staphostatins are specific reversible inhibitors of staphopains. Staphostatin A can only inhibit staphopain A, but not staphopain B (273). Similarly, staphostatin B inhibits only staphopain B (273). Both staphostatins are similar in size and structure. These small proteins (~13 kDa) are each comprised of 8 β-strands forming a single mixed β-barrel domain (292). Staphostatin occupies the same binding site as substrate, thus they are competitive inhibitors (Figure 11) (276, 293). Staphostatins lack signal peptides, thus they are proposed to inhibit intracellular staphopain activities prior to secretion of the proenzyme (273, 294).

Serine proteases – the serine protease-like proteins: Serine protease-like proteins A-F (SplA-F) are the newest group of secreted staphylococcal serine proteases identified. SplC (named ORF-2 in the study) was the first Spl identified from a screen of *S. aureus* antigens reactive to serum antibodies from endocarditis patients (295). Soon after, *splC* was discovered as part of the *spl* operon, encoding *splA-F* (296). This operon is located in the gene cluster νSaβ, present in over 60% of *S. aureus* genomes (296, 297).

Spls share 40–60% protein sequence identity; except for SplD and SplF, which have 95% sequence similarity with each other (Figure 12) (296). The Spls are similar in size, ranging from 21–22 kDa. SplA-D have been characterized, and their structures were determined by X-ray crystallography (298–301). Crystal structures of the four Spls showed structural homology to the other staphylococcal serine proteases. Spls have a chymotrypsin-like fold, consisting of two β-barrel domains (Figure 12) (298–301). The catalytic triad typical of serine proteases (His, Asp, Ser) is conserved and is present in the center between the two domains (296, 298–301).

Based on the functional studies on the Spls, the precise cleavage of the signal peptide is critical for protease activity. An additional 2 amino acids (such as those resulting from a thrombin cleavage) in the N-terminus of SplA, SplB, and SplC are enough to render the enzymes inactive (296, 298, 299). Therefore, the signal peptides of Spls serve dual functions: 1) to direct the protein secretion, and 2) to serve as pro-peptides to prevent enzyme activation prior to secretion.

SplA, SplB, and SplD have extremely narrow substrate specificities, requiring the recognition of substrate consensus sequences that are 4–5 amino acids in length (Table 3) (298–300). Mucin-16, an O-glycosylated transmembrane protein present in the ocular epithelia, is a substrate for SplA (302). Additionally, searches based on the substrate consensus sequences identified many olfactory receptors as potential Spl substrates, but they remain to be verified experimentally (298–300). Nevertheless, these searches suggest Spls may be important for nasal colonization. SplD contributes to airway inflammation and asthma by promoting IgE production and Th2 responses (303, 304). The role of SplD and SplF in asthma is supported by the identification of these proteins in nasal polyp samples from asthma patient who are also *S. aureus* nasal carriers (303).

However, the role of Spls in *S. aureus* pathogenesis remains unclear. While murine pneumonia and intraperitoneal infection models using Δ*spl* mutants had no effect on host survival or bacterial burden, the absence of *spl* limited the dissemination of bacteria *in vivo*

in a pneumonia model (296, 303). Proteomic analysis of wildtype *S. aureus* and Δ*spl* mutant demonstrated significant changes in many virulence factors important for adhesion and immune evasion, thus Spls have a role in shaping the *S. aureus* proteome (302).

Hyaluronidase—Hyaluronic acid (HA) is a linear polysaccharide comprised of repeating units of N-acetylglucosamine and glucuronic acid linked by alternating β−1,3 and β−1,4 glycosidic bonds (305). HA is a critical component of extracellular matrices (ECM) in vertebrates, providing homeostasis and structural integrity to cells and tissues; it is also important for immune regulation (306, 307). The enzymes that break down HA are collectively known as hyaluronate lyase or hyaluronidase.

In nature, hyaluronidases can be found in vertebrates, invertebrates, and bacteria. Hyaluronidases found in vertebrates and invertebrates break down HA to tetrasaccharides (308). In contrast, bacterial hyaluronidases act as endo-N-aceylhexoaminidases and cleave the β−1,4 linkage in a process known as β-elimination, breaking down HA to unsaturated disaccharides (308).

S. aureus and *S. hyicus* are the only staphylococci known to produce hyaluronidase (309, 310). The activity of staphylococcal hyaluronidase was initially reported by Duran-Reynals in 1933 as a "spreading factor" that increased lesion sizes in a rabbit skin infection model (311). Subsequently, this "spreading factor" was identified by Chain and Duthie in 1940 as hyaluronidase (312). However, it would be in 1995, when the gene encoding for staphylococcal hyaluronidase, *hysA*, was eventually cloned and the corresponding protein purified (313).

As a "spreading factor", hyaluronidase is implicated in the dissemination of bacteria through breaking down HA in ECMs and biofilms. The skin and the lungs are two locations where ECMs are abundant. Deletion of *hysA* resulted in reduced skin and lung pathology and lowered bacterial burden in skin and lung infection models, respectively (314, 315). Deletion of *hysA* was also demonstrated to cause increased biofilm formation and reduced bacterial dissemination (316).

Lipases

Phospholipases.: *S. aureus* can produce two different phospholipases: β-toxin and phosphatidylinositol-specific phospholipase C (PI-PLC). β-toxin is a neutral sphingomylinase with hemolytic and cytolytic activities, previously discussed in the Exotoxin section of this chapter. The other staphylococcal phospholipase, PI-PLC was discovered in 1960s in *S. aureus* culture supernatants where PI-PLC hydrolyzed phosphatidyl inositol (PI) to diglyceride and inositol phosphate (317, 318). Today, *S. aureus* remain the only staphylococci known to produce PI-PLC (319). *S. aureus* membrane does not contain PI, thus *S. aureus* is thought to have acquired PI-PLC to adapt to the host environment (320).

Like other bacterial PI-PLCs, the staphylococcal PI-PLC has an imperfect $(\beta\alpha)_8$-barrel structure (also known as the TIM barrel) (321). The active site of PI-PLC is conserved and is located at the C-terminal end of the β-strands that form the β-barrel (321). The elucidation

of the staphylococcal PI-PLC crystal structure provided explanations for many of PI-PLC's biochemical properties (321). PI-PLC is reported to have an optimum pH between 5.5–6.0 (319). This property can be explained by the unrestricted substrate access to the active site under acidic conditions. In contrast, accessibility of the substrate is restricted under basic conditions (321). PI-PLC is inactivated by NaCl, $HgCl_2$, and Cu_2SO_4 (319). Salt sensitivity of PI-PLC can be explained by the high electropositivity of the barrel rim region and the active site (321). The presence of phosphocholine (PC) enhances the activity of PI-PLC (321). Structural analysis of PI-PLC suggests the presence of PC enables transient dimerization of two PI-PLC monomers, resulting in the enhancement of enzyme activity (322).

Bacterial PI-PLC hydrolyses phosphatidylinositol (PI) in two steps: first, PI is hydrolyzed to diacylglycerol (DAG) and the intermediate product myo-inositol 1,2-cyclic phosphate (cIP). This is followed by a second slower hydrolysis of cIP to myo-inositol 1-phosphate (IP) (323, 324). DAG is an important secondary messenger for activating intracellular pathways in mammalian cells for growth and survival (325). PI-PLC can also release glycosyl-phosphatidylinositol (GPI)-anchored proteins on cell membrane (319). Two such proteins are C8 binding protein and the decay-accelerating factor (DAF) (326, 327). Both proteins are complement regulators normally present on host cells to restrict complement activation on self (328, 329). Recently, PI-PLC has been demonstrated to promote survival of *S. aureus* in human blood and neutrophils (330).

Glycerol ester hydrolases (lipases).: *S. aureus* has two lipases, they are *S. aureus* lipase 1 and 2 (SAL1 & SAL2). SAL1- and SAL2-encoding genes are sometimes annotated as *gehA and gehB*, respectively, for glycerol ester hydrolase (331–333). SAL1 has also been annotated as *lip1* in the literature (331). The two genes are encoded in disparate regions in the *S. aureus* genome, however, they share protein sequence similarity with each other and with other lipases found in other staphylococcal species (332, 333).

The lipases are produced as pre-pro-enzymes (334). The pre-pro-enzyme is processed by signal peptidase I, which cleaves the signal peptide for secretion. The secreted pro-enzyme is cleaved by aureolysin to yield the mature lipase (331). However, cleavage of the pro-peptide is not required and has no effect on the enzymatic activity (331, 335). Utilizing chimeric lipases of *S. hyicus* expressed in *S. carnosus*, the lipase pro-peptides were found to be important for the translocation of the lipases to the extracellular milieu and for stabilizing the proteins to prevent degradation (336, 337).

Enzymatic activities of the lipases are conferred by the conserved catalytic triad, formed by serine, aspartate, and histidine (331, 332). Although sharing a similar catalytic mechanism, SAL1 and SAL2 differ biochemically and have different substrate preferences. SAL1 functions optimally at pH 6.0 and is stable under acidic conditions, but it is inactivated when pH is above 10 (338). Biochemical and molecular analyses showed that Ca^{2+} stabilizes the structure of SAL1 and increases its activity (334, 338). Accordingly, chelators, such as EDTA or EGTA, inhibit SAL1 activity (338). SAL1 has a strong preference for short chain triglycerides, but cannot hydrolyze long chain triglycerides (338).

In contrast, SAL2 functions optimally around pH 8.0 and is inactive under acidic conditions (339). The presence of Ca^{2+} does not enhance the activity of SAL2 (339). As such, chelators have minimal effects on activity. SAL2 prefers long chain triglycerides as substrates (331). However, SAL2 has also been shown to hydrolyze short chain triglycerides, mono- and di-glycerides with lower efficiency and with no apparent positional specificity (331).

The conservation of lipases in staphylococcal species implies their evolutionary importance. However, the contribution of lipases during disease is unclear. *S. aureus* clinical isolates from deep tissue infections produce more lipases than isolates from superficial infections (340). Purified lipases cause aggregation of granulocytes and decrease phagocytosis at high concentration (341, 342). During infection, SAL2 was shown to be important for biofilm formation and contributed to the virulence of *S. aureus* strain RN4220 in a murine intraperitoneal infection model (343). These observations suggest lipases are involved in the overall virulence of *S. aureus* and promote bacterial survival in biofilms and abscesses. Paradoxically, lipase-mediated triglyceride hydrolysis liberates bactericidal free fatty acids, which can interfere with pathogenicity (344). For most lipase-producing strains, these bactericidal fatty acids can be detoxified by fatty acid-modifying enzymes.

Fatty acid modifying enzyme.: Fatty acid modifying enzyme (FAME) was first described in 1992 by Mortensen *et al.* who observed *S. aureus* culture filtrates inhibited the bactericidal activities of host lipids in abscesses (345). Since the initial discovery, FAME activity is well-documented in many staphylococcal species (346, 347). Approximately 80% of *S. aureus* and *S. epidermidis* produce this enzyme (347, 348). Despite its prevalence, the corresponding gene for FAME is not known and the protein has not yet been identified.

FAME promotes staphylococci survival by esterifying the bactericidal free lipids with an alcohol substrate to form alcohol esters. Although FAME can esterify free lipids with methanol, ethanol, 1-propanol, 2-propanol, and 1-butanol, it prefers cholesterol, which is highly abundant in abscesses (345). Saturated and unsaturated fatty acids with 15–19 carbons are efficiently esterified by FAME; however, esterification is also observed for fatty acid chains between 11–24 carbons (349). The optimal pH of the enzyme ranges between 5.0 to 5.5 and has an optimal temperature of about 40°C (345). Enzyme activity is inhibited by di- and tri-glycerides with unsaturated fatty acid side chains (349).

In abscesses, lipases and FAME are thought to complement each other to enhance staphylococci survival (350). While lipases break down triglycerides that inhibit FAME activities, FAME processes the free fatty acids liberated by lipases to protect the staphylococci. This hypothesis is corroborated by the observations that most *S. aureus* strains that carry genes encoding lipases have FAME activities and they are correlated with the invasiveness of the bacteria *in vivo* (345, 348).

Conclusion

S. aureus devotes a significant amount of energy in the production of virulence factors to protect the bacteria from host immune surveillances and to promote bacterial survival in hostile environments. The importance of these virulence factors during infection has been

demonstrated extensively in different *ex vivo* and *in vivo* infection models. Pathogenic *S. aureus* is usually present in hostile host environments with limited resources, thus it follows that the production of many different virulence factors that serve the same purpose can be a waste of limited resources and be disadvantageous for survival. In contrast, this redundancy can ensure protection of the bacteria in case one of the virulence factors is rendered ineffective. Alternatively, the bacteria may have acquired these seemingly redundant virulence factors during its evolution to better adapt to different types of infections or colonization sites.

Many of the exotoxins and secreted enzymes discussed in this chapter share structural and functional similarities. However, closer examinations of these proteins reveal subtle differences that have functional significance. Minor differences in the PFTs lead to the cytolysis of various cell types that are critical for immune defense. Each of the SAgs target different Vβ TCRs resulting in a broad suppression of the T cell repertoire. Proteases, such as the serine proteases, which have structural homology have disparate substrate specificities. Other exotoxins and enzymes have similar functions but differ in when and where they are produced during growth and pathogenesis, suggesting that the complex and seemingly redundant virulence factor repertoire is critical for the success of *S. aureus* as a versatile pathogen. With the rise in antibiotic resistance in microbes, including *S. aureus*, there is an urgent need to develop novel therapeutics and vaccines to combat this deadly pathogen. Understanding the roles these important virulence factors have during diseases can provide the knowledge necessary for designing better therapeutics and identifying vaccine targets.

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References

1. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG, Jr. 2015 Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 28:603–661. [PubMed: 26016486]
2. Kusch H, Engelmann S. 2014 Secrets of the secretome in Staphylococcus aureus. Int J Med Microbiol 304:133–141. [PubMed: 24424242]
3. Gouaux JE, Braha O, Hobaugh MR, Song L, Cheley S, Shustak C, Bayley H. 1994 Subunit stoichiometry of staphylococcal alpha-hemolysin in crystals and on membranes: a heptameric transmembrane pore. Proc Natl Acad Sci U S A 91:12828–12831. [PubMed: 7809129]
4. Song L, Hobaugh MR, Shustak C, Cheley S, Bayley H, Gouaux JE. 1996 Structure of staphylococcal alpha-hemolysin, a heptameric transmembrane pore. Science 274:1859–1866. [PubMed: 8943190]
5. Valeva A, Pongs J, Bhakdi S, Palmer M. 1997 Staphylococcal alpha-toxin: the role of the N-terminus in formation of the heptameric pore -- a fluorescence study. Biochim Biophys Acta 1325:281–286. [PubMed: 9168153]

6. Valeva A, Palmer M, Bhakdi S. 1997 Staphylococcal alpha-toxin: formation of the heptameric pore is partially cooperative and proceeds through multiple intermediate stages. Biochemistry 36:13298–13304. [PubMed: 9341221]
7. Jursch R, Hildebrand A, Hobom G, Tranum-Jensen J, Ward R, Kehoe M, Bhakdi S. 1994 Histidine residues near the N terminus of staphylococcal alpha-toxin as reporters of regions that are critical for oligomerization and pore formation. Infect Immun 62:2249–2256. [PubMed: 8188346]
8. Menzies BE, Kernodle DS. 1994 Site-directed mutagenesis of the alpha-toxin gene of Staphylococcus aureus: role of histidines in toxin activity in vitro and in a murine model. Infect Immun 62:1843–1847. [PubMed: 8168947]
9. Cooper LZ, Madoff MA, Weinstein L. 1966 Heat stability and species range of purified staphylococcal alpha-toxin. J Bacteriol 91:1686–1692. [PubMed: 5937231]
10. Grimminger F, Rose F, Sibelius U, Meinhardt M, Potzsch B, Spriestersbach R, Bhakdi S, Suttorp N, Seeger W. 1997 Human endothelial cell activation and mediator release in response to the bacterial exotoxins Escherichia coli hemolysin and staphylococcal alpha-toxin. J Immunol 159:1909–1916. [PubMed: 9257856]
11. Inoshima I, Inoshima N, Wilke GA, Powers ME, Frank KM, Wang Y, Bubeck Wardenburg J. 2011 A Staphylococcus aureus pore-forming toxin subverts the activity of ADAM10 to cause lethal infection in mice. Nat Med 17:1310–1314. [PubMed: 21926978]
12. Nygaard TK, Pallister KB, DuMont AL, DeWald M, Watkins RL, Pallister EQ, Malone C, Griffith S, Horswill AR, Torres VJ, Voyich JM. 2012 Alpha-toxin induces programmed cell death of human T cells, B cells, and monocytes during USA300 infection. PLoS One 7:e36532. [PubMed: 22574180]
13. Wilke GA, Bubeck Wardenburg J. 2010 Role of a disintegrin and metalloprotease 10 in Staphylococcus aureus alpha-hemolysin-mediated cellular injury. Proc Natl Acad Sci U S A 107:13473–13478. [PubMed: 20624979]
14. Popov LM, Marceau CD, Starkl PM, Lumb JH, Shah J, Guerrera D, Cooper RL, Merakou C, Bouley DM, Meng W, Kiyonari H, Takeichi M, Galli SJ, Bagnoli F, Citi S, Carette JE, Amieva MR. 2015 The adherens junctions control susceptibility to Staphylococcus aureus alpha-toxin. Proc Natl Acad Sci U S A 112:14337–14342. [PubMed: 26489655]
15. Berube BJ, Bubeck Wardenburg J. 2013 Staphylococcus aureus alpha-toxin: nearly a century of intrigue. Toxins (Basel) 5:1140–1166. [PubMed: 23888516]
16. Bhakdi S, Muhly M, Korom S, Hugo F. 1989 Release of interleukin-1 beta associated with potent cytocidal action of staphylococcal alpha-toxin on human monocytes. Infect Immun 57:3512–3519. [PubMed: 2807534]
17. Craven RR, Gao X, Allen IC, Gris D, Bubeck Wardenburg J, McElvania-Tekippe E, Ting JP, Duncan JA. 2009 Staphylococcus aureus alpha-hemolysin activates the NLRP3-inflammasome in human and mouse monocytic cells. PLoS One 4:e7446. [PubMed: 19826485]
18. Suttorp N, Fuhrmann M, Tannert-Otto S, Grimminger F, Bhadki S. 1993 Pore-forming bacterial toxins potently induce release of nitric oxide in porcine endothelial cells. J Exp Med 178:337–341. [PubMed: 8391061]
19. Suttorp N, Seeger W, Dewein E, Bhakdi S, Roka L. 1985 Staphylococcal alpha-toxin-induced PGI2 production in endothelial cells: role of calcium. Am J Physiol 248:C127–134. [PubMed: 3917612]
20. Burnet FM. 1929 The exotoxins of Staphylococcus pyogenes aureus. The Journal of Pathology and Bacteriology 32:717–734.
21. Gill DM. 1982 Bacterial toxins: a table of lethal amounts. Microbiol Rev 46:86–94. [PubMed: 6806598]
22. Bayer AS, Ramos MD, Menzies BE, Yeaman MR, Shen AJ, Cheung AL. 1997 Hyperproduction of alpha-toxin by Staphylococcus aureus results in paradoxically reduced virulence in experimental endocarditis: a host defense role for platelet microbicidal proteins. Infect Immun 65:4652–4660. [PubMed: 9353046]
23. Bramley AJ, Patel AH, O'Reilly M, Foster R, Foster TJ. 1989 Roles of alpha-toxin and beta-toxin in virulence of Staphylococcus aureus for the mouse mammary gland. Infect Immun 57:2489–2494. [PubMed: 2744856]

24. Bubeck Wardenburg J, Bae T, Otto M, Deleo FR, Schneewind O. 2007 Poring over pores: alpha-hemolysin and Panton-Valentine leukocidin in Staphylococcus aureus pneumonia. Nat Med 13:1405–1406. [PubMed: 18064027]
25. Kennedy AD, Bubeck Wardenburg J, Gardner DJ, Long D, Whitney AR, Braughton KR, Schneewind O, DeLeo FR. 2010 Targeting of alpha-hemolysin by active or passive immunization decreases severity of USA300 skin infection in a mouse model. J Infect Dis 202:1050–1058. [PubMed: 20726702]
26. Kielian T, Cheung A, Hickey WF. 2001 Diminished virulence of an alpha-toxin mutant of Staphylococcus aureus in experimental brain abscesses. Infect Immun 69:6902–6911. [PubMed: 11598065]
27. O'Callaghan RJ, Callegan MC, Moreau JM, Green LC, Foster TJ, Hartford OM, Engel LS, Hill JM. 1997 Specific roles of alpha-toxin and beta-toxin during Staphylococcus aureus corneal infection. Infect Immun 65:1571–1578. [PubMed: 9125532]
28. Woodin AM. 1960 Purification of the two components of leucocidin from Staphylococcus aureus. Biochem J 75:158–165. [PubMed: 13845860]
29. Woodin AM. 1959 Fractionation of a leucocidin from Staphylococcus aureus. Biochem J 73:225–237. [PubMed: 13845859]
30. Alonzo F, 3rd, Torres VJ. 2014 The bicomponent pore-forming leucocidins of Staphylococcus aureus. Microbiol Mol Biol Rev 78:199–230. [PubMed: 24847020]
31. Seilie ES, Bubeck Wardenburg J. 2017 Staphylococcus aureus pore-forming toxins: The interface of pathogen and host complexity. Semin Cell Dev Biol 72:101–116. [PubMed: 28445785]
32. Spaan AN, van Strijp JAG, Torres VJ. 2017 Leukocidins: staphylococcal bi-component pore-forming toxins find their receptors. Nat Rev Microbiol 15:435–447. [PubMed: 28420883]
33. Yamashita K, Kawai Y, Tanaka Y, Hirano N, Kaneko J, Tomita N, Ohta M, Kamio Y, Yao M, Tanaka I. 2011 Crystal structure of the octameric pore of staphylococcal gamma-hemolysin reveals the beta-barrel pore formation mechanism by two components. Proc Natl Acad Sci U S A 108:17314–17319. [PubMed: 21969538]
34. Yamashita D, Sugawara T, Takeshita M, Kaneko J, Kamio Y, Tanaka I, Tanaka Y, Yao M. 2014 Molecular basis of transmembrane beta-barrel formation of staphylococcal pore-forming toxins. Nat Commun 5:4897. [PubMed: 25263813]
35. Koop G, Vrieling M, Storisteanu DM, Lok LS, Monie T, van Wigcheren G, Raisen C, Ba X, Gleadall N, Hadjirin N, Timmerman AJ, Wagenaar JA, Klunder HM, Fitzgerald JR, Zadoks R, Paterson GK, Torres C, Waller AS, Loeffler A, Loncaric I, Hoet AE, Bergstrom K, De Martino L, Pomba C, de Lencastre H, Ben Slama K, Gharsa H, Richardson EJ, Chilvers ER, de Haas C, van Kessel K, van Strijp JA, Harrison EM, Holmes MA. 2017 Identification of LukPQ, a novel, equid-adapted leukocidin of Staphylococcus aureus. Sci Rep 7:40660. [PubMed: 28106142]
36. Vrieling M, Boerhout EM, van Wigcheren GF, Koymans KJ, Mols-Vorstermans TG, de Haas CJ, Aerts PC, Daemen IJ, van Kessel KP, Koets AP, Rutten VP, Nuijten PJ, van Strijp JA, Benedictus L. 2016 LukMF' is the major secreted leukocidin of bovine Staphylococcus aureus and is produced in vivo during bovine mastitis. Sci Rep 6:37759. [PubMed: 27886237]
37. Yamada T, Tochimaru N, Nakasuji S, Hata E, Kobayashi H, Eguchi M, Kaneko J, Kamio Y, Kaidoh T, Takeuchi S. 2005 Leukotoxin family genes in Staphylococcus aureus isolated from domestic animals and prevalence of lukM-lukF-PV genes by bacteriophages in bovine isolates. Vet Microbiol 110:97–103. [PubMed: 16112825]
38. Yoong P, Torres VJ. 2013 The effects of Staphylococcus aureus leukotoxins on the host: cell lysis and beyond. Curr Opin Microbiol 16:63–69. [PubMed: 23466211]
39. Badarau A, Rouha H, Malafa S, Logan DT, Hakansson M, Stulik L, Dolezilkova I, Teubenbacher A, Gross K, Maierhofer B, Weber S, Jagerhofer M, Hoffman D, Nagy E. 2015 Structure-function analysis of heterodimer formation, oligomerization, and receptor binding of the Staphylococcus aureus bi-component toxin LukGH. J Biol Chem 290:142–156. [PubMed: 25371205]
40. Yanai M, Rocha MA, Matolek AZ, Chintalacharuvu A, Taira Y, Chintalacharuvu K, Beenhouwer DO. 2014 Separately or combined, LukG/LukH is functionally unique compared to other staphylococcal bicomponent leukotoxins. PLoS One 9:e89308. [PubMed: 24586678]

41. Noda M, Kato I, Hirayama T, Matsuda F. 1982 Mode of action of staphylococcal leukocidin: effects of the S and F components on the activities of membrane-associated enzymes of rabbit polymorphonuclear leukocytes. Infect Immun 35:38–45. [PubMed: 6274802]

42. Staali L, Monteil H, Colin DA. 1998 The staphylococcal pore-forming leukotoxins open Ca2+ channels in the membrane of human polymorphonuclear neutrophils. J Membr Biol 162:209–216. [PubMed: 9543493]

43. Perret M, Badiou C, Lina G, Burbaud S, Benito Y, Bes M, Cottin V, Couzon F, Juruj C, Dauwalder O, Goutagny N, Diep BA, Vandenesch F, Henry T. 2012 Cross-talk between Staphylococcus aureus leukocidins-intoxicated macrophages and lung epithelial cells triggers chemokine secretion in an inflammasome-dependent manner. Cell Microbiol 14:1019–1036. [PubMed: 22329718]

44. Spaan AN, Vrieling M, Wallet P, Badiou C, Reyes-Robles T, Ohneck EA, Benito Y, de Haas CJ, Day CJ, Jennings MP, Lina G, Vandenesch F, van Kessel KP, Torres VJ, van Strijp JA, Henry T. 2014 The staphylococcal toxins gamma-haemolysin AB and CB differentially target phagocytes by employing specific chemokine receptors. Nat Commun 5:5438. [PubMed: 25384670]

45. Holzinger D, Gieldon L, Mysore V, Nippe N, Taxman DJ, Duncan JA, Broglie PM, Marketon K, Austermann J, Vogl T, Foell D, Niemann S, Peters G, Roth J, Loffler B. 2012 Staphylococcus aureus Panton-Valentine leukocidin induces an inflammatory response in human phagocytes via the NLRP3 inflammasome. J Leukoc Biol 92:1069–1081. [PubMed: 22892107]

46. Melehani JH, James DB, DuMont AL, Torres VJ, Duncan JA. 2015 Staphylococcus aureus Leukocidin A/B (LukAB) Kills Human Monocytes via Host NLRP3 and ASC when Extracellular, but Not Intracellular. PLoS Pathog 11:e1004970. [PubMed: 26069969]

47. Munoz-Planillo R, Franchi L, Miller LS, Nunez G. 2009 A critical role for hemolysins and bacterial lipoproteins in Staphylococcus aureus-induced activation of the Nlrp3 inflammasome. J Immunol 183:3942–3948. [PubMed: 19717510]

48. Kaneko J, Kimura T, Kawakami Y, Tomita T, Kamio Y. 1997 Panton-valentine leukocidin genes in a phage-like particle isolated from mitomycin C-treated Staphylococcus aureus V8 (ATCC 49775). Biosci Biotechnol Biochem 61:1960–1962. [PubMed: 9404084]

49. Kaneko J, Kimura T, Narita S, Tomita T, Kamio Y. 1998 Complete nucleotide sequence and molecular characterization of the temperate staphylococcal bacteriophage phiPVL carrying Panton-Valentine leukocidin genes. Gene 215:57–67. [PubMed: 9666077]

50. McCarthy AJ, Witney AA, Lindsay JA. 2012 Staphylococcus aureus temperate bacteriophage: carriage and horizontal gene transfer is lineage associated. Front Cell Infect Microbiol 2:6. [PubMed: 22919598]

51. Boakes E, Kearns AM, Ganner M, Perry C, Hill RL, Ellington MJ. 2011 Distinct bacteriophages encoding Panton-Valentine leukocidin (PVL) among international methicillin-resistant Staphylococcus aureus clones harboring PVL. J Clin Microbiol 49:684–692. [PubMed: 21106787]

52. Brown ML, O'Hara FP, Close NM, Mera RM, Miller LA, Suaya JA, Amrine-Madsen H. 2012 Prevalence and sequence variation of panton-valentine leukocidin in methicillin-resistant and methicillin-susceptible staphylococcus aureus strains in the United States. J Clin Microbiol 50:86–90. [PubMed: 22090402]

53. Boakes E, Kearns AM, Badiou C, Lina G, Hill RL, Ellington MJ. 2012 Do differences in Panton-Valentine leukocidin production among international methicillin-resistant Staphylococcus aureus clones affect disease presentation and severity? J Clin Microbiol 50:1773–1776. [PubMed: 22205815]

54. Hamilton SM, Bryant AE, Carroll KC, Lockary V, Ma Y, McIndoo E, Miller LG, Perdreau-Remington F, Pullman J, Risi GF, Salmi DB, Stevens DL. 2007 In vitro production of panton-valentine leukocidin among strains of methicillin-resistant Staphylococcus aureus causing diverse infections. Clin Infect Dis 45:1550–1558. [PubMed: 18190315]

55. Tromp AT, Van Gent M, Abrial P, Martin A, Jansen JP, De Haas CJC, Van Kessel KPM, Bardoel BW, Kruse E, Bourdonnay E, Boettcher M, McManus MT, Day CJ, Jennings MP, Lina G, Vandenesch F, Van Strijp JAG, Jan Lebbink R, Haas PA, Henry T, Spaan AN. 2018 Human CD45 is an F-component-specific receptor for the staphylococcal toxin Panton-Valentine leukocidin. Nat Microbiol doi:10.1038/s41564-018-0159-x.

56. Diep BA, Le VT, Badiou C, Le HN, Pinheiro MG, Duong AH, Wang X, Dip EC, Aguiar-Alves F, Basuino L, Marbach H, Mai TT, Sarda MN, Kajikawa O, Matute-Bello G, Tkaczyk C, Rasigade

JP, Sellman BR, Chambers HF, Lina G. 2016 IVIG-mediated protection against necrotizing pneumonia caused by MRSA. Sci Transl Med 8:357ra124.

57. Diep BA, Chan L, Tattevin P, Kajikawa O, Martin TR, Basuino L, Mai TT, Marbach H, Braughton KR, Whitney AR, Gardner DJ, Fan X, Tseng CW, Liu GY, Badiou C, Etienne J, Lina G, Matthay MA, DeLeo FR, Chambers HF. 2010 Polymorphonuclear leukocytes mediate Staphylococcus aureus Panton-Valentine leukocidin-induced lung inflammation and injury. Proc Natl Acad Sci U S A 107:5587–5592. [PubMed: 20231457]
58. Spaan AN, Henry T, van Rooijen WJ, Perret M, Badiou C, Aerts PC, Kemmink J, de Haas CJ, van Kessel KP, Vandenesch F, Lina G, van Strijp JA. 2013 The staphylococcal toxin Panton-Valentine Leukocidin targets human C5a receptors. Cell Host Microbe 13:584–594. [PubMed: 23684309]
59. Spaan AN, Schiepers A, de Haas CJ, van Hooijdonk DD, Badiou C, Contamin H, Vandenesch F, Lina G, Gerard NP, Gerard C, van Kessel KP, Henry T, van Strijp JA. 2015 Differential Interaction of the Staphylococcal Toxins Panton-Valentine Leukocidin and gamma-Hemolysin CB with Human C5a Receptors. J Immunol 195:1034–1043. [PubMed: 26091719]
60. Cremieux AC, Dumitrescu O, Lina G, Vallee C, Cote JF, Muffat-Joly M, Lilin T, Etienne J, Vandenesch F, Saleh-Mghir A. 2009 Panton-valentine leukocidin enhances the severity of community-associated methicillin-resistant Staphylococcus aureus rabbit osteomyelitis. PLoS One 4:e7204. [PubMed: 19779608]
61. Graves SF, Kobayashi SD, Braughton KR, Whitney AR, Sturdevant DE, Rasmussen DL, Kirpotina LN, Quinn MT, DeLeo FR. 2012 Sublytic concentrations of Staphylococcus aureus Panton-Valentine leukocidin alter human PMN gene expression and enhance bactericidal capacity. J Leukoc Biol 92:361–374. [PubMed: 22581932]
62. Kobayashi SD, Malachowa N, Whitney AR, Braughton KR, Gardner DJ, Long D, Bubeck Wardenburg J, Schneewind O, Otto M, Deleo FR. 2011 Comparative analysis of USA300 virulence determinants in a rabbit model of skin and soft tissue infection. J Infect Dis 204:937–941. [PubMed: 21849291]
63. Chi CY, Lin CC, Liao IC, Yao YC, Shen FC, Liu CC, Lin CF. 2014 Panton-Valentine leukocidin facilitates the escape of Staphylococcus aureus from human keratinocyte endosomes and induces apoptosis. J Infect Dis 209:224–235. [PubMed: 23956440]
64. McCarthy AJ, Lindsay JA. 2013 Staphylococcus aureus innate immune evasion is lineage-specific: a bioinfomatics study. Infect Genet Evol 19:7–14. [PubMed: 23792184]
65. von Eiff C, Friedrich AW, Peters G, Becker K. 2004 Prevalence of genes encoding for members of the staphylococcal leukotoxin family among clinical isolates of Staphylococcus aureus. Diagn Microbiol Infect Dis 49:157–162. [PubMed: 15246504]
66. Cooney J, Kienle Z, Foster TJ, O'Toole PW. 1993 The gamma-hemolysin locus of Staphylococcus aureus comprises three linked genes, two of which are identical to the genes for the F and S components of leukocidin. Infect Immun 61:768–771. [PubMed: 8423103]
67. Spaan AN, Reyes-Robles T, Badiou C, Cochet S, Boguslawski KM, Yoong P, Day CJ, de Haas CJ, van Kessel KP, Vandenesch F, Jennings MP, Le Van Kim C, Colin Y, van Strijp JA, Henry T, Torres VJ. 2015 Staphylococcus aureus Targets the Duffy Antigen Receptor for Chemokines (DARC) to Lyse Erythrocytes. Cell Host Microbe 18:363–370. [PubMed: 26320997]
68. Reyes-Robles T, Lubkin A, Alonzo F, 3rd, Lacy DB, Torres VJ. 2016 Exploiting dominant-negative toxins to combat Staphylococcus aureus pathogenesis. EMBO Rep 17:428–440. [PubMed: 26882549]
69. Siqueira JA, Speeg-Schatz C, Freitas FI, Sahel J, Monteil H, Prevost G. 1997 Channel-forming leucotoxins from Staphylococcus aureus cause severe inflammatory reactions in a rabbit eye model. J Med Microbiol 46:486–494. [PubMed: 9350201]
70. Nilsson IM, Hartford O, Foster T, Tarkowski A. 1999 Alpha-toxin and gamma-toxin jointly promote Staphylococcus aureus virulence in murine septic arthritis. Infect Immun 67:1045–1049. [PubMed: 10024541]
71. Supersac G, Piemont Y, Kubina M, Prevost G, Foster TJ. 1998 Assessment of the role of gamma-toxin in experimental endophthalmitis using a hlg-deficient mutant of Staphylococcus aureus. Microb Pathog 24:241–251. [PubMed: 9533895]

72. Malachowa N, Whitney AR, Kobayashi SD, Sturdevant DE, Kennedy AD, Braughton KR, Shabb DW, Diep BA, Chambers HF, Otto M, DeLeo FR. 2011 Global changes in Staphylococcus aureus gene expression in human blood. PLoS One 6:e18617. [PubMed: 21525981]

73. Gravet A, Colin DA, Keller D, Girardot R, Monteil H, Prevost G. 1998 Characterization of a novel structural member, LukE-LukD, of the bi-component staphylococcal leucotoxins family. FEBS Lett 436:202–208. [PubMed: 9781679]

74. Morinaga N, Kaihou Y, Noda M. 2003 Purification, cloning and characterization of variant LukE-LukD with strong leukocidal activity of staphylococcal bi-component leukotoxin family. Microbiol Immunol 47:81–90. [PubMed: 12636257]

75. Alonzo F, 3rd, Benson MA, Chen J, Novick RP, Shopsin B, Torres VJ. 2012 Staphylococcus aureus leucocidin ED contributes to systemic infection by targeting neutrophils and promoting bacterial growth in vivo. Mol Microbiol 83:423–435. [PubMed: 22142035]

76. Alonzo F, Kozhaya L, Rawlings SA, Reyes-Robles T, DuMont AL, Myszka DG, Landau NR, Unutmaz D, Torres VJ. 2013 CCR5 is a receptor for Staphylococcus aureus leukotoxin ED. Nature 493:51–55. [PubMed: 23235831]

77. Reyes-Robles T, Alonzo F, 3rd, Kozhaya L, Lacy DB, Unutmaz D, Torres VJ. 2013 Staphylococcus aureus leukotoxin ED targets the chemokine receptors CXCR1 and CXCR2 to kill leukocytes and promote infection. Cell Host Microbe 14:453–459. [PubMed: 24139401]

78. Dumont AL, Nygaard TK, Watkins RL, Smith A, Kozhaya L, Kreiswirth BN, Shopsin B, Unutmaz D, Voyich JM, Torres VJ. 2011 Characterization of a new cytotoxin that contributes to Staphylococcus aureus pathogenesis. Mol Microbiol 79:814–825. [PubMed: 21255120]

79. Ventura CL, Malachowa N, Hammer CH, Nardone GA, Robinson MA, Kobayashi SD, DeLeo FR. 2010 Identification of a novel Staphylococcus aureus two-component leukotoxin using cell surface proteomics. PLoS One 5:e11634. [PubMed: 20661294]

80. DuMont AL, Yoong P, Liu X, Day CJ, Chumbler NM, James DB, Alonzo F, 3rd, Bode NJ, Lacy DB, Jennings MP, Torres VJ. 2014 Identification of a crucial residue required for Staphylococcus aureus LukAB cytotoxicity and receptor recognition. Infect Immun 82:1268–1276. [PubMed: 24379286]

81. Malachowa N, Kobayashi SD, Braughton KR, Whitney AR, Parnell MJ, Gardner DJ, Deleo FR. 2012 Staphylococcus aureus leukotoxin GH promotes inflammation. J Infect Dis 206:1185–1193. [PubMed: 22872735]

82. DuMont AL, Yoong P, Day CJ, Alonzo F, 3rd, McDonald WH, Jennings MP, Torres VJ. 2013 Staphylococcus aureus LukAB cytotoxin kills human neutrophils by targeting the CD11b subunit of the integrin Mac-1. Proc Natl Acad Sci U S A 110:10794–10799. [PubMed: 23754403]

83. DuMont AL, Yoong P, Surewaard BG, Benson MA, Nijland R, van Strijp JA, Torres VJ. 2013 Staphylococcus aureus elaborates leukocidin AB to mediate escape from within human neutrophils. Infect Immun 81:1830–1841. [PubMed: 23509138]

84. Thomsen IP, Dumont AL, James DB, Yoong P, Saville BR, Soper N, Torres VJ, Creech CB. 2014 Children with invasive Staphylococcus aureus disease exhibit a potently neutralizing antibody response to the cytotoxin LukAB. Infect Immun 82:1234–1242. [PubMed: 24379282]

85. Chadha AD, Thomsen IP, Jimenez-Truque N, Soper NR, Jones LS, Sokolow AG, Torres VJ, Creech CB. 2016 Host response to Staphylococcus aureus cytotoxins in children with cystic fibrosis. J Cyst Fibros 15:597–604. [PubMed: 26821814]

86. Rainard P, Corrales JC, Barrio MB, Cochard T, Poutrel B. 2003 Leucotoxic activities of Staphylococcus aureus strains isolated from cows, ewes, and goats with mastitis: importance of LukM/LukF'-PV leukotoxin. Clin Diagn Lab Immunol 10:272–277. [PubMed: 12626454]

87. Vrieling M, Koymans KJ, Heesterbeek DA, Aerts PC, Rutten VP, de Haas CJ, van Kessel KP, Koets AP, Nijland R, van Strijp JA. 2015 Bovine Staphylococcus aureus Secretes the Leukocidin LukMF' To Kill Migrating Neutrophils through CCR1. MBio 6:e00335. [PubMed: 26045537]

88. Fromageau A, Cunha P, Gilbert FB, Rainard P. 2011 Purified Staphylococcus aureus leukotoxin LukM/F' does not trigger inflammation in the bovine mammary gland. Microb Pathog 51:396–401. [PubMed: 21951578]

89. Janzon L, Arvidson S. 1990 The role of the delta-lysin gene (hld) in the regulation of virulence genes by the accessory gene regulator (agr) in Staphylococcus aureus. EMBO J 9:1391–1399. [PubMed: 2328718]

90. Wang R, Braughton KR, Kretschmer D, Bach TH, Queck SY, Li M, Kennedy AD, Dorward DW, Klebanoff SJ, Peschel A, DeLeo FR, Otto M. 2007 Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. Nat Med 13:1510–1514. [PubMed: 17994102]

91. Queck SY, Khan BA, Wang R, Bach TH, Kretschmer D, Chen L, Kreiswirth BN, Peschel A, Deleo FR, Otto M. 2009 Mobile genetic element-encoded cytolysin connects virulence to methicillin resistance in MRSA. PLoS Pathog 5:e1000533. [PubMed: 19649313]

92. Kaito C, Saito Y, Nagano G, Ikuo M, Omae Y, Hanada Y, Han X, Kuwahara-Arai K, Hishinuma T, Baba T, Ito T, Hiramatsu K, Sekimizu K. 2011 Transcription and translation products of the cytolysin gene psm-mec on the mobile genetic element SCCmec regulate Staphylococcus aureus virulence. PLoS Pathog 7:e1001267. [PubMed: 21304931]

93. Somerville GA, Cockayne A, Durr M, Peschel A, Otto M, Musser JM. 2003 Synthesis and deformylation of Staphylococcus aureus delta-toxin are linked to tricarboxylic acid cycle activity. J Bacteriol 185:6686–6694. [PubMed: 14594843]

94. Chatterjee SS, Joo HS, Duong AC, Dieringer TD, Tan VY, Song Y, Fischer ER, Cheung GY, Li M, Otto M. 2013 Essential Staphylococcus aureus toxin export system. Nat Med 19:364–367. [PubMed: 23396209]

95. Peschel A, Otto M. 2013 Phenol-soluble modulins and staphylococcal infection. Nat Rev Microbiol 11:667–673. [PubMed: 24018382]

96. Tappin MJ, Pastore A, Norton RS, Freer JH, Campbell ID. 1988 High-resolution 1H NMR study of the solution structure of delta-hemolysin. Biochemistry 27:1643–1647. [PubMed: 3365416]

97. Towle KM, Lohans CT, Miskolzie M, Acedo JZ, van Belkum MJ, Vederas JC. 2016 Solution Structures of Phenol-Soluble Modulins alpha1, alpha3, and beta2, Virulence Factors from Staphylococcus aureus. Biochemistry 55:4798–4806. [PubMed: 27525453]

98. Cheung GY, Duong AC, Otto M. 2012 Direct and synergistic hemolysis caused by Staphylococcus phenol-soluble modulins: implications for diagnosis and pathogenesis. Microbes Infect 14:380–386. [PubMed: 22178792]

99. Rasigade JP, Trouillet-Assant S, Ferry T, Diep BA, Sapin A, Lhoste Y, Ranfaing J, Badiou C, Benito Y, Bes M, Couzon F, Tigaud S, Lina G, Etienne J, Vandenesch F, Laurent F. 2013 PSMs of hypervirulent Staphylococcus aureus act as intracellular toxins that kill infected osteoblasts. PLoS One 8:e63176. [PubMed: 23690994]

100. Surewaard BG, de Haas CJ, Vervoort F, Rigby KM, DeLeo FR, Otto M, van Strijp JA, Nijland R. 2013 Staphylococcal alpha-phenol soluble modulins contribute to neutrophil lysis after phagocytosis. Cell Microbiol 15:1427–1437. [PubMed: 23470014]

101. Surewaard BG, Nijland R, Spaan AN, Kruijtzer JA, de Haas CJ, van Strijp JA. 2012 Inactivation of staphylococcal phenol soluble modulins by serum lipoprotein particles. PLoS Pathog 8:e1002606. [PubMed: 22457627]

102. Kretschmer D, Gleske AK, Rautenberg M, Wang R, Koberle M, Bohn E, Schoneberg T, Rabiet MJ, Boulay F, Klebanoff SJ, van Kessel KA, van Strijp JA, Otto M, Peschel A. 2010 Human formyl peptide receptor 2 senses highly pathogenic Staphylococcus aureus. Cell Host Microbe 7:463–473. [PubMed: 20542250]

103. Periasamy S, Joo HS, Duong AC, Bach TH, Tan VY, Chatterjee SS, Cheung GY, Otto M. 2012 How Staphylococcus aureus biofilms develop their characteristic structure. Proc Natl Acad Sci U S A 109:1281–1286. [PubMed: 22232686]

104. Ebner P, Luqman A, Reichert S, Hauf K, Popella P, Forchhammer K, Otto M, Gotz F. 2017 Non-classical Protein Excretion Is Boosted by PSMalpha-Induced Cell Leakage. Cell Rep 20:1278–1286. [PubMed: 28793253]

105. Merriman JA, Klingelhutz AJ, Diekema DJ, Leung DY, Schlievert PM. 2015 Novel Staphylococcus aureus Secreted Protein Alters Keratinocyte Proliferation and Elicits a Proinflammatory Response In Vitro and In Vivo. Biochemistry 54:4855–4862. [PubMed: 26177220]

106. Spaulding AR, Salgado-Pabon W, Kohler PL, Horswill AR, Leung DY, Schlievert PM. 2013 Staphylococcal and streptococcal superantigen exotoxins. Clin Microbiol Rev 26:422–447. [PubMed: 23824366]

107. Wilson GJ, Seo KS, Cartwright RA, Connelley T, Chuang-Smith ON, Merriman JA, Guinane CM, Park JY, Bohach GA, Schlievert PM, Morrison WI, Fitzgerald JR. 2011 A novel core genome-encoded superantigen contributes to lethality of community-associated MRSA necrotizing pneumonia. PLoS Pathog 7:e1002271. [PubMed: 22022262]

108. Ono HK, Omoe K, Imanishi K, Iwakabe Y, Hu DL, Kato H, Saito N, Nakane A, Uchiyama T, Shinagawa K. 2008 Identification and characterization of two novel staphylococcal enterotoxins, types S and T. Infect Immun 76:4999–5005. [PubMed: 18710864]

109. Fitzgerald JR, Monday SR, Foster TJ, Bohach GA, Hartigan PJ, Meaney WJ, Smyth CJ. 2001 Characterization of a putative pathogenicity island from bovine Staphylococcus aureus encoding multiple superantigens. J Bacteriol 183:63–70. [PubMed: 11114901]

110. Johns MB, Jr., Khan SA. 1988 Staphylococcal enterotoxin B gene is associated with a discrete genetic element. J Bacteriol 170:4033–4039. [PubMed: 2842299]

111. Langley R, Patel D, Jackson N, Clow F, Fraser JD. 2010 Staphylococcal superantigen super-domains in immune evasion. Crit Rev Immunol 30:149–165. [PubMed: 20370627]

112. Lindsay JA, Holden MT. 2006 Understanding the rise of the superbug: investigation of the evolution and genomic variation of Staphylococcus aureus. Funct Integr Genomics 6:186–201. [PubMed: 16453141]

113. Fitzgerald JR, Reid SD, Ruotsalainen E, Tripp TJ, Liu M, Cole R, Kuusela P, Schlievert PM, Jarvinen A, Musser JM. 2003 Genome diversification in Staphylococcus aureus: Molecular evolution of a highly variable chromosomal region encoding the Staphylococcal exotoxin-like family of proteins. Infect Immun 71:2827–2838. [PubMed: 12704157]

114. Lina G, Bohach GA, Nair SP, Hiramatsu K, Jouvin-Marche E, Mariuzza R, International Nomenclature Committee for Staphylococcal S. 2004 Standard nomenclature for the superantigens expressed by Staphylococcus. J Infect Dis 189:2334–2336. [PubMed: 15181583]

115. Mitchell DT, Levitt DG, Schlievert PM, Ohlendorf DH. 2000 Structural evidence for the evolution of pyrogenic toxin superantigens. J Mol Evol 51:520–531. [PubMed: 11116326]

116. Rodstrom KE, Elbing K, Lindkvist-Petersson K. 2014 Structure of the superantigen staphylococcal enterotoxin B in complex with TCR and peptide-MHC demonstrates absence of TCR-peptide contacts. J Immunol 193:1998–2004. [PubMed: 25015819]

117. Kozono H, Parker D, White J, Marrack P, Kappler J. 1995 Multiple binding sites for bacterial superantigens on soluble class II MHC molecules. Immunity 3:187–196. [PubMed: 7648392]

118. Jardetzky TS, Brown JH, Gorga JC, Stern LJ, Urban RG, Chi YI, Stauffacher C, Strominger JL, Wiley DC. 1994 Three-dimensional structure of a human class II histocompatibility molecule complexed with superantigen. Nature 368:711–718. [PubMed: 8152483]

119. Petersson K, Thunnissen M, Forsberg G, Walse B. 2002 Crystal structure of a SEA variant in complex with MHC class II reveals the ability of SEA to crosslink MHC molecules. Structure 10:1619–1626. [PubMed: 12467569]

120. Gunther S, Varma AK, Moza B, Kasper KJ, Wyatt AW, Zhu P, Rahman AK, Li Y, Mariuzza RA, McCormick JK, Sundberg EJ. 2007 A novel loop domain in superantigens extends their T cell receptor recognition site. J Mol Biol 371:210–221. [PubMed: 17560605]

121. Novick RP. 2003 Mobile genetic elements and bacterial toxinoses: the superantigen-encoding pathogenicity islands of Staphylococcus aureus. Plasmid 49:93–105. [PubMed: 12726763]

122. Kim J, Urban RG, Strominger JL, Wiley DC. 1994 Toxic shock syndrome toxin-1 complexed with a class II major histocompatibility molecule HLA-DR1. Science 266:1870–1874. [PubMed: 7997880]

123. Brosnahan AJ, Schaefers MM, Amundson WH, Mantz MJ, Squier CA, Peterson ML, Schlievert PM. 2008 Novel toxic shock syndrome toxin-1 amino acids required for biological activity. Biochemistry 47:12995–13003. [PubMed: 19012411]

124. Arad G, Levy R, Nasie I, Hillman D, Rotfogel Z, Barash U, Supper E, Shpilka T, Minis A, Kaempfer R. 2011 Binding of superantigen toxins into the CD28 homodimer interface is

essential for induction of cytokine genes that mediate lethal shock. PLoS Biol 9:e1001149. [PubMed: 21931534]

125. Spaulding AR, Lin YC, Merriman JA, Brosnahan AJ, Peterson ML, Schlievert PM. 2012 Immunity to Staphylococcus aureus secreted proteins protects rabbits from serious illnesses. Vaccine 30:5099–5109. [PubMed: 22691432]
126. CDC. 2011 Toxic Shock Syndrome (Other Than Streptococcal) (TSS).
127. Davis JP, Chesney PJ, Wand PJ, LaVenture M. 1980 Toxic-shock syndrome: epidemiologic features, recurrence, risk factors, and prevention. N Engl J Med 303:1429–1435. [PubMed: 7432401]
128. Shands KN, Schmid GP, Dan BB, Blum D, Guidotti RJ, Hargrett NT, Anderson RL, Hill DL, Broome CV, Band JD, Fraser DW. 1980 Toxic-shock syndrome in menstruating women: association with tampon use and Staphylococcus aureus and clinical features in 52 cases. N Engl J Med 303:1436–1442. [PubMed: 7432402]
129. Schlievert PM. 1986 Staphylococcal enterotoxin B and toxic-shock syndrome toxin-1 are significantly associated with non-menstrual TSS. Lancet 1:1149–1150.
130. Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, Bost DA, Riehman M, Naidich S, Kreiswirth BN. 1999 Evaluation of protein A gene polymorphic region DNA sequencing for typing of Staphylococcus aureus strains. J Clin Microbiol 37:3556–3563. [PubMed: 10523551]
131. Kim HK, Thammavongsa V, Schneewind O, Missiakas D. 2012 Recurrent infections and immune evasion strategies of Staphylococcus aureus. Curr Opin Microbiol 15:92–99. [PubMed: 22088393]
132. Guss B, Uhlen M, Nilsson B, Lindberg M, Sjoquist J, Sjodahl J. 1984 Region X, the cell-wall-attachment part of staphylococcal protein A. Eur J Biochem 138:413–420. [PubMed: 6697996]
133. Becker S, Frankel MB, Schneewind O, Missiakas D. 2014 Release of protein A from the cell wall of Staphylococcus aureus. Proc Natl Acad Sci U S A 111:1574–1579. [PubMed: 24434550]
134. Peterson PK, Verhoef J, Sabath LD, Quie PG. 1977 Effect of protein A on staphylococcal opsonization. Infect Immun 15:760–764. [PubMed: 870431]
135. Sasso EH, Silverman GJ, Mannik M. 1989 Human IgM molecules that bind staphylococcal protein A contain VHIII H chains. J Immunol 142:2778–2783. [PubMed: 2495325]
136. Romagnani S, Giudizi MG, del Prete G, Maggi E, Biagiotti R, Almerigogna F, Ricci M. 1982 Demonstration on protein A of two distinct immunoglobulin-binding sites and their role in the mitogenic activity of Staphylococcus aureus Cowan I on human B cells. J Immunol 129:596–602. [PubMed: 6979579]
137. Goodyear CS, Silverman GJ. 2003 Death by a B cell superantigen: In vivo VH-targeted apoptotic supraclonal B cell deletion by a Staphylococcal Toxin. J Exp Med 197:1125–1139. [PubMed: 12719481]
138. Graille M, Stura EA, Corper AL, Sutton BJ, Taussig MJ, Charbonnier JB, Silverman GJ. 2000 Crystal structure of a Staphylococcus aureus protein A domain complexed with the Fab fragment of a human IgM antibody: structural basis for recognition of B-cell receptors and superantigen activity. Proc Natl Acad Sci U S A 97:5399–5404. [PubMed: 10805799]
139. Silverman GJ, Goodyear CS. 2006 Confounding B-cell defences: lessons from a staphylococcal superantigen. Nat Rev Immunol 6:465–475. [PubMed: 16724100]
140. Pauli NT, Kim HK, Falugi F, Huang M, Dulac J, Henry Dunand C, Zheng NY, Kaur K, Andrews SF, Huang Y, DeDent A, Frank KM, Charnot-Katsikas A, Schneewind O, Wilson PC. 2014 Staphylococcus aureus infection induces protein A-mediated immune evasion in humans. J Exp Med 211:2331–2339. [PubMed: 25348152]
141. Keener AB, Thurlow LT, Kang S, Spidale NA, Clarke SH, Cunnion KM, Tisch R, Richardson AR, Vilen BJ. 2017 Staphylococcus aureus Protein A Disrupts Immunity Mediated by Long-Lived Plasma Cells. J Immunol 198:1263–1273. [PubMed: 28031339]
142. Falugi F, Kim HK, Missiakas DM, Schneewind O. 2013 Role of protein A in the evasion of host adaptive immune responses by Staphylococcus aureus. MBio 4:e00575–00513. [PubMed: 23982075]

143. Kim HK, Cheng AG, Kim HY, Missiakas DM, Schneewind O. 2010 Nontoxigenic protein A vaccine for methicillin-resistant Staphylococcus aureus infections in mice. J Exp Med 207:1863–1870. [PubMed: 20713595]

144. Winkler KC, de Waart J, Grootsen C. 1965 Lysogenic conversion of staphylococci to loss of beta-toxin. J Gen Microbiol 39:321–333. [PubMed: 4222155]

145. Verkaik NJ, Benard M, Boelens HA, de Vogel CP, Nouwen JL, Verbrugh HA, Melles DC, van Belkum A, van Wamel WJ. 2011 Immune evasion cluster-positive bacteriophages are highly prevalent among human Staphylococcus aureus strains, but they are not essential in the first stages of nasal colonization. Clin Microbiol Infect 17:343–348. [PubMed: 20370801]

146. Aarestrup FM, Larsen HD, Eriksen NH, Elsberg CS, Jensen NE. 1999 Frequency of alpha- and beta-haemolysin in Staphylococcus aureus of bovine and human origin. A comparison between pheno- and genotype and variation in phenotypic expression. APMIS 107:425–430. [PubMed: 10230698]

147. Salgado-Pabon W, Herrera A, Vu BG, Stach CS, Merriman JA, Spaulding AR, Schlievert PM. 2014 Staphylococcus aureus beta-toxin production is common in strains with the beta-toxin gene inactivated by bacteriophage. J Infect Dis 210:784–792. [PubMed: 24620023]

148. Goerke C, Matias y Papenberg S, Dasbach S, Dietz K, Ziebach R, Kahl BC, Wolz C. 2004 Increased frequency of genomic alterations in Staphylococcus aureus during chronic infection is in part due to phage mobilization. J Infect Dis 189:724–734. [PubMed: 14767828]

149. Goerke C, Koller J, Wolz C. 2006 Ciprofloxacin and trimethoprim cause phage induction and virulence modulation in Staphylococcus aureus. Antimicrob Agents Chemother 50:171–177. [PubMed: 16377683]

150. Goerke C, Wirtz C, Fluckiger U, Wolz C. 2006 Extensive phage dynamics in Staphylococcus aureus contributes to adaptation to the human host during infection. Mol Microbiol 61:1673–1685. [PubMed: 16968231]

151. Doery HM, Magnusson BJ, Cheyne IM, Sulasekharam J. 1963 A phospholipase in staphylococcal toxin which hydrolyses sphingomyelin. Nature 198:1091–1092. [PubMed: 14028358]

152. Glenny AT, Stevens MF. 1935 Staphylococcus toxins and antitoxins. The Journal of Pathology and Bacteriology 40:201–210.

153. Low DK, Freer JH, Arbuthnott JP, Mollby R, Wadstrom T. 1974 Consequences of spingomyelin degradation in erythrocyte ghost membranes by staphylococcal beta-toxin (sphingomyelinase C). Toxicon 12:279–285. [PubMed: 4376283]

154. Huseby M, Shi K, Brown CK, Digre J, Mengistu F, Seo KS, Bohach GA, Schlievert PM, Ohlendorf DH, Earhart CA. 2007 Structure and biological activities of beta toxin from Staphylococcus aureus. J Bacteriol 189:8719–8726. [PubMed: 17873030]

155. Huseby MJ, Kruse AC, Digre J, Kohler PL, Vocke JA, Mann EE, Bayles KW, Bohach GA, Schlievert PM, Ohlendorf DH, Earhart CA. 2010 Beta toxin catalyzes formation of nucleoprotein matrix in staphylococcal biofilms. Proc Natl Acad Sci U S A 107:14407–14412. [PubMed: 20660751]

156. Bernheimer AW, Avigad LS, Kim KS. 1974 Staphylococcal sphingomyelinase (beta-hemolysin). Ann N Y Acad Sci 236:292–306. [PubMed: 4371058]

157. Cifrian E, Guidry AJ, Bramley AJ, Norcross NL, Bastida-Corcuera FD, Marquardt WW. 1996 Effect of staphylococcal beta toxin on the cytotoxicity, proliferation and adherence of Staphylococcus aureus to bovine mammary epithelial cells. Vet Microbiol 48:187–198. [PubMed: 9054116]

158. Walev I, Weller U, Strauch S, Foster T, Bhakdi S. 1996 Selective killing of human monocytes and cytokine release provoked by sphingomyelinase (beta-toxin) of Staphylococcus aureus. Infect Immun 64:2974–2979. [PubMed: 8757823]

159. Katayama Y, Baba T, Sekine M, Fukuda M, Hiramatsu K. 2013 Beta-hemolysin promotes skin colonization by Staphylococcus aureus. J Bacteriol 195:1194–1203. [PubMed: 23292775]

160. Tajima A, Iwase T, Shinji H, Seki K, Mizunoe Y. 2009 Inhibition of endothelial interleukin-8 production and neutrophil transmigration by Staphylococcus aureus beta-hemolysin. Infect Immun 77:327–334. [PubMed: 18936175]

161. Herrera A, Vu BG, Stach CS, Merriman JA, Horswill AR, Salgado-Pabon W, Schlievert PM. 2016 Staphylococcus aureus beta-Toxin Mutants Are Defective in Biofilm Ligase and Sphingomyelinase Activity, and Causation of Infective Endocarditis and Sepsis. Biochemistry 55:2510–2517. [PubMed: 27015018]

162. Hayashida A, Bartlett AH, Foster TJ, Park PW. 2009 Staphylococcus aureus beta-toxin induces lung injury through syndecan-1. Am J Pathol 174:509–518. [PubMed: 19147831]

163. Kondo I, Sakurai S, Sarai Y. 1973 Purification of exfoliatin produced by Staphylococcus aureus of bacteriophage group 2 and its physicochemical properties. Infect Immun 8:156–164. [PubMed: 4269383]

164. Yamaguchi T, Hayashi T, Takami H, Nakasone K, Ohnishi M, Nakayama K, Yamada S, Komatsuzawa H, Sugai M. 2000 Phage conversion of exfoliative toxin A production in Staphylococcus aureus. Mol Microbiol 38:694–705. [PubMed: 11115106]

165. O'Reilly M, Dougan G, Foster TJ, Arbuthnott JP. 1981 Plasmids in epidermolytic strains of Staphylococcus aureus. J Gen Microbiol 124:99–107. [PubMed: 6275008]

166. Yamaguchi T, Nishifuji K, Sasaki M, Fudaba Y, Aepfelbacher M, Takata T, Ohara M, Komatsuzawa H, Amagai M, Sugai M. 2002 Identification of the Staphylococcus aureus etd pathogenicity island which encodes a novel exfoliative toxin, ETD, and EDIN-B. Infect Immun 70:5835–5845. [PubMed: 12228315]

167. Sato H, Matsumori Y, Tanabe T, Saito H, Shimizu A, Kawano J. 1994 A new type of staphylococcal exfoliative toxin from a Staphylococcus aureus strain isolated from a horse with phlegmon. Infect Immun 62:3780–3785. [PubMed: 8063394]

168. Bukowski M, Wladyka B, Dubin G. 2010 Exfoliative toxins of Staphylococcus aureus. Toxins (Basel) 2:1148–1165. [PubMed: 22069631]

169. Lyell A 1967 A review of toxic epidermal necrolysis in Britain. Br J Dermatol 79:662–671. [PubMed: 6064936]

170. Lyell A 1983 The staphylococcal scalded skin syndrome in historical perspective: emergence of dermopathic strains of Staphylococcus aureus and discovery of the epidermolytic toxin. A review of events up to 1970. J Am Acad Dermatol 9:285–294. [PubMed: 6350386]

171. Melish ME, Glasgow LA. 1970 The staphylococcal scalded-skin syndrome. N Engl J Med 282:1114–1119. [PubMed: 4245327]

172. Cavarelli J, Prevost G, Bourguet W, Moulinier L, Chevrier B, Delagoutte B, Bilwes A, Mourey L, Rifai S, Piemont Y, Moras D. 1997 The structure of Staphylococcus aureus epidermolytic toxin A, an atypic serine protease, at 1.7 A resolution. Structure 5:813–824. [PubMed: 9261066]

173. Vath GM, Earhart CA, Monie DD, Iandolo JJ, Schlievert PM, Ohlendorf DH. 1999 The crystal structure of exfoliative toxin B: a superantigen with enzymatic activity. Biochemistry 38:10239–10246. [PubMed: 10441117]

174. Vath GM, Earhart CA, Rago JV, Kim MH, Bohach GA, Schlievert PM, Ohlendorf DH. 1997 The structure of the superantigen exfoliative toxin A suggests a novel regulation as a serine protease. Biochemistry 36:1559–1566. [PubMed: 9048539]

175. Amagai M, Matsuyoshi N, Wang ZH, Andl C, Stanley JR. 2000 Toxin in bullous impetigo and staphylococcal scalded-skin syndrome targets desmoglein 1. Nat Med 6:1275–1277. [PubMed: 11062541]

176. Amagai M, Yamaguchi T, Hanakawa Y, Nishifuji K, Sugai M, Stanley JR. 2002 Staphylococcal exfoliative toxin B specifically cleaves desmoglein 1. J Invest Dermatol 118:845–850. [PubMed: 11982763]

177. Hanakawa Y, Selwood T, Woo D, Lin C, Schechter NM, Stanley JR. 2003 Calcium-dependent conformation of desmoglein 1 is required for its cleavage by exfoliative toxin. J Invest Dermatol 121:383–389. [PubMed: 12880431]

178. Hanakawa Y, Schechter NM, Lin C, Nishifuji K, Amagai M, Stanley JR. 2004 Enzymatic and molecular characteristics of the efficiency and specificity of exfoliative toxin cleavage of desmoglein 1. J Biol Chem 279:5268–5277. [PubMed: 14630910]

179. Nishifuji K, Sugai M, Amagai M. 2008 Staphylococcal exfoliative toxins: "molecular scissors" of bacteria that attack the cutaneous defense barrier in mammals. J Dermatol Sci 49:21–31. [PubMed: 17582744]

180. Nagasaka T, Nishifuji K, Ota T, Whittock NV, Amagai M. 2004 Defining the pathogenic involvement of desmoglein 4 in pemphigus and staphylococcal scalded skin syndrome. J Clin Invest 114:1484–1492. [PubMed: 15545999]

181. Becker K, Heilmann C, Peters G. 2014 Coagulase-negative staphylococci. Clin Microbiol Rev 27:870–926. [PubMed: 25278577]

182. Loeb L 1903 The Influence of certain Bacteria on the Coagulation of the Blood. J Med Res 10:407–419. [PubMed: 19971581]

183. Bjerketorp J, Jacobsson K, Frykberg L. 2004 The von Willebrand factor-binding protein (vWbp) of Staphylococcus aureus is a coagulase. FEMS Microbiol Lett 234:309–314. [PubMed: 15135538]

184. Watanabe S, Ito T, Takeuchi F, Endo M, Okuno E, Hiramatsu K. 2005 Structural comparison of ten serotypes of staphylocoagulases in Staphylococcus aureus. J Bacteriol 187:3698–3707. [PubMed: 15901693]

185. McCarthy AJ, Lindsay JA. 2010 Genetic variation in Staphylococcus aureus surface and immune evasion genes is lineage associated: implications for vaccine design and host-pathogen interactions. BMC Microbiol 10:173. [PubMed: 20550675]

186. Viana D, Blanco J, Tormo-Mas MA, Selva L, Guinane CM, Baselga R, Corpa J, Lasa I, Novick RP, Fitzgerald JR, Penades JR. 2010 Adaptation of Staphylococcus aureus to ruminant and equine hosts involves SaPI-carried variants of von Willebrand factor-binding protein. Mol Microbiol 77:1583–1594. [PubMed: 20860091]

187. Cheng AG, McAdow M, Kim HK, Bae T, Missiakas DM, Schneewind O. 2010 Contribution of coagulases towards Staphylococcus aureus disease and protective immunity. PLoS Pathog 6:e1001036. [PubMed: 20700445]

188. McAdow M, Missiakas DM, Schneewind O. 2012 Staphylococcus aureus secretes coagulase and von Willebrand factor binding protein to modify the coagulation cascade and establish host infections. J Innate Immun 4:141–148. [PubMed: 22222316]

189. Friedrich R, Panizzi P, Fuentes-Prior P, Richter K, Verhamme I, Anderson PJ, Kawabata S, Huber R, Bode W, Bock PE. 2003 Staphylocoagulase is a prototype for the mechanism of cofactor-induced zymogen activation. Nature 425:535–539. [PubMed: 14523451]

190. Bjerketorp J, Nilsson M, Ljungh A, Flock JI, Jacobsson K, Frykberg L. 2002 A novel von Willebrand factor binding protein expressed by Staphylococcus aureus. Microbiology 148:2037–2044. [PubMed: 12101292]

191. Kroh HK, Panizzi P, Bock PE. 2009 Von Willebrand factor-binding protein is a hysteretic conformational activator of prothrombin. Proc Natl Acad Sci U S A 106:7786–7791. [PubMed: 19416890]

192. Bock PE, Panizzi P, Verhamme IM. 2007 Exosites in the substrate specificity of blood coagulation reactions. J Thromb Haemost 5 Suppl 1:81–94. [PubMed: 17635714]

193. Peetermans M, Verhamme P, Vanassche T. 2015 Coagulase Activity by Staphylococcus aureus: A Potential Target for Therapy? Semin Thromb Hemost 41:433–444. [PubMed: 25973589]

194. Vanassche T, Verhaegen J, Peetermans WE, Hoylaerts MF, Verhamme P. 2010 Dabigatran inhibits Staphylococcus aureus coagulase activity. J Clin Microbiol 48:4248–4250. [PubMed: 20810780]

195. Hijikata-Okunomiya A, Kataoka N. 2003 Argatroban inhibits staphylothrombin. J Thromb Haemost 1:2060–2061. [PubMed: 12941055]

196. Thomer L, Emolo C, Thammavongsa V, Kim HK, McAdow ME, Yu W, Kieffer M, Schneewind O, Missiakas D. 2016 Antibodies against a secreted product of Staphylococcus aureus trigger phagocytic killing. J Exp Med 213:293–301. [PubMed: 26880578]

197. Loof TG, Goldmann O, Naudin C, Morgelin M, Neumann Y, Pils MC, Foster SJ, Medina E, Herwald H. 2015 Staphylococcus aureus-induced clotting of plasma is an immune evasion mechanism for persistence within the fibrin network. Microbiology 161:621–627. [PubMed: 25533444]

198. Ekstedt RD, Yotis WW. 1960 Studies on staphylococci. II. Effect of coagulase on the virulence of coagulase negative strains. J Bacteriol 80:496–500. [PubMed: 13726269]

199. Zapotoczna M, McCarthy H, Rudkin JK, O'Gara JP, O'Neill E. 2015 An Essential Role for Coagulase in Staphylococcus aureus Biofilm Development Reveals New Therapeutic Possibilities for Device-Related Infections. J Infect Dis 212:1883–1893. [PubMed: 26044292]

200. Guggenberger C, Wolz C, Morrissey JA, Heesemann J. 2012 Two distinct coagulase-dependent barriers protect Staphylococcus aureus from neutrophils in a three dimensional in vitro infection model. PLoS Pathog 8:e1002434. [PubMed: 22253592]

201. de Haas CJ, Veldkamp KE, Peschel A, Weerkamp F, Van Wamel WJ, Heezius EC, Poppelier MJ, Van Kessel KP, van Strijp JA. 2004 Chemotaxis inhibitory protein of Staphylococcus aureus, a bacterial antiinflammatory agent. J Exp Med 199:687–695. [PubMed: 14993252]

202. Coleman DC, Sullivan DJ, Russell RJ, Arbuthnott JP, Carey BF, Pomeroy HM. 1989 Staphylococcus aureus bacteriophages mediating the simultaneous lysogenic conversion of beta-lysin, staphylokinase and enterotoxin A: molecular mechanism of triple conversion. J Gen Microbiol 135:1679–1697. [PubMed: 2533245]

203. Sako T, Sawaki S, Sakurai T, Ito S, Yoshizawa Y, Kondo I. 1983 Cloning and expression of the staphylokinase gene of Staphylococcus aureus in Escherichia coli. Mol Gen Genet 190:271–277. [PubMed: 6224069]

204. Kondo I, Fujise K. 1977 Serotype B staphylococcal bacteriophage singly converting staphylokinase. Infect Immun 18:266–272. [PubMed: 144703]

205. Bokarewa MI, Jin T, Tarkowski A. 2006 Staphylococcus aureus: Staphylokinase. Int J Biochem Cell Biol 38:504–509. [PubMed: 16111912]

206. Behnke D, Gerlach D. 1987 Cloning and expression in Escherichia coli, Bacillus subtilis, and Streptococcus sanguis of a gene for staphylokinase--a bacterial plasminogen activator. Mol Gen Genet 210:528–534. [PubMed: 3123893]

207. Horii T, Yokoyama K, Barua S, Odagiri T, Futamura N, Hasegawa T, Ohta M. 2000 The staphylokinase gene is located in the structural gene encoding N-acetylmuramyl-L-alanine amidase in methicillin-resistant Staphylococcus aureus. FEMS Microbiol Lett 185:221–224. [PubMed: 10754251]

208. Borchardt SA, Babwah AV, Jayaswal RK. 1993 Sequence analysis of the region downstream from a peptidoglycan hydrolase-encoding gene from Staphylococcus aureus NCTC8325. Gene 137:253–258. [PubMed: 7905453]

209. Rabijns A, De Bondt HL, De Ranter C. 1997 Three-dimensional structure of staphylokinase, a plasminogen activator with therapeutic potential. Nat Struct Biol 4:357–360. [PubMed: 9145104]

210. Grella DK, Castellino FJ. 1997 Activation of human plasminogen by staphylokinase. Direct evidence that preformed plasmin is necessary for activation to occur. Blood 89:1585–1589. [PubMed: 9057640]

211. Lijnen HR, Van Hoef B, Collen D. 1993 Interaction of staphylokinase with different molecular forms of plasminogen. Eur J Biochem 211:91–97. [PubMed: 8425556]

212. Collen D, Schlott B, Engelborghs Y, Van Hoef B, Hartmann M, Lijnen HR, Behnke D. 1993 On the mechanism of the activation of human plasminogen by recombinant staphylokinase. J Biol Chem 268:8284–8289. [PubMed: 8463338]

213. Schlott B, Guhrs KH, Hartmann M, Rocker A, Collen D. 1997 Staphylokinase requires NH2-terminal proteolysis for plasminogen activation. J Biol Chem 272:6067–6072. [PubMed: 9038231]

214. Gase A, Hartmann M, Guhrs KH, Rocker A, Collen D, Behnke D, Schlott B. 1996 Functional significance of NH2- and COOH-terminal regions of staphylokinase in plasminogen activation. Thromb Haemost 76:755–760. [PubMed: 8950786]

215. Parry MA, Fernandez-Catalan C, Bergner A, Huber R, Hopfner KP, Schlott B, Guhrs KH, Bode W. 1998 The ternary microplasmin-staphylokinase-microplasmin complex is a proteinase-cofactor-substrate complex in action. Nat Struct Biol 5:917–923. [PubMed: 9783753]

216. Lijnen HR, Van Hoef B, De Cock F, Okada K, Ueshima S, Matsuo O, Collen D. 1991 On the mechanism of fibrin-specific plasminogen activation by staphylokinase. J Biol Chem 266:11826–11832. [PubMed: 2050679]

217. Rooijakkers SH, van Wamel WJ, Ruyken M, van Kessel KP, van Strijp JA. 2005 Anti-opsonic properties of staphylokinase. Microbes Infect 7:476–484. [PubMed: 15792635]

218. Santala A, Saarinen J, Kovanen P, Kuusela P. 1999 Activation of interstitial collagenase, MMP-1, by Staphylococcus aureus cells having surface-bound plasmin: a novel role of plasminogen receptors of bacteria. FEBS Lett 461:153–156. [PubMed: 10567688]

219. Jin T, Bokarewa M, Foster T, Mitchell J, Higgins J, Tarkowski A. 2004 Staphylococcus aureus resists human defensins by production of staphylokinase, a novel bacterial evasion mechanism. J Immunol 172:1169–1176. [PubMed: 14707093]

220. Braff MH, Jones AL, Skerrett SJ, Rubens CE. 2007 Staphylococcus aureus exploits cathelicidin antimicrobial peptides produced during early pneumonia to promote staphylokinase-dependent fibrinolysis. J Infect Dis 195:1365–1372. [PubMed: 17397009]

221. Kwiecinski J, Peetermans M, Liesenborghs L, Na M, Bjornsdottir H, Zhu X, Jacobsson G, Johansson BR, Geoghegan JA, Foster TJ, Josefsson E, Bylund J, Verhamme P, Jin T. 2016 Staphylokinase Control of Staphylococcus aureus Biofilm Formation and Detachment Through Host Plasminogen Activation. J Infect Dis 213:139–148. [PubMed: 26136471]

222. Peetermans M, Vanassche T, Liesenborghs L, Claes J, Vande Velde G, Kwiecinksi J, Jin T, De Geest B, Hoylaerts MF, Lijnen RH, Verhamme P. 2014 Plasminogen activation by staphylokinase enhances local spreading of S. aureus in skin infections. BMC Microbiol 14:310. [PubMed: 25515118]

223. Kwiecinski J, Jacobsson G, Karlsson M, Zhu X, Wang W, Bremell T, Josefsson E, Jin T. 2013 Staphylokinase promotes the establishment of Staphylococcus aureus skin infections while decreasing disease severity. J Infect Dis 208:990–999. [PubMed: 23801604]

224. Cunningham L, Catlin BW, de Garilhe MP. 1956 A Deoxyribonuclease of Micrococcus pyogenes1. Journal of the American Chemical Society 78:4642–4645.

225. Cuatrecasas P, Fuchs S, Anfinsen CB. 1967 Catalytic properties and specificity of the extracellular nuclease of Staphylococcus aureus. J Biol Chem 242:1541–1547. [PubMed: 4290246]

226. Tang J, Zhou R, Shi X, Kang M, Wang H, Chen H. 2008 Two thermostable nucleases coexisted in Staphylococcus aureus: evidence from mutagenesis and in vitro expression. FEMS Microbiol Lett 284:176–183. [PubMed: 18510563]

227. Kuroda M, Ohta T, Uchiyama I, Baba T, Yuzawa H, Kobayashi I, Cui L, Oguchi A, Aoki K, Nagai Y, Lian J, Ito T, Kanamori M, Matsumaru H, Maruyama A, Murakami H, Hosoyama A, Mizutani-Ui Y, Takahashi NK, Sawano T, Inoue R, Kaito C, Sekimizu K, Hirakawa H, Kuhara S, Goto S, Yabuzaki J, Kanehisa M, Yamashita A, Oshima K, Furuya K, Yoshino C, Shiba T, Hattori M, Ogasawara N, Hayashi H, Hiramatsu K. 2001 Whole genome sequencing of meticillin-resistant Staphylococcus aureus. Lancet 357:1225–1240. [PubMed: 11418146]

228. Kiedrowski MR, Crosby HA, Hernandez FJ, Malone CL, McNamara JO, 2nd, Horswill AR. 2014 Staphylococcus aureus Nuc2 is a functional, surface-attached extracellular nuclease. PLoS One 9:e95574. [PubMed: 24752186]

229. Davis A, Moore IB, Parker DS, Taniuchi H. 1977 Nuclease B. A possible precursor of nuclease A, an extracellular nuclease of Staphylococcus aureus. J Biol Chem 252:6544–6553. [PubMed: 893427]

230. Kiedrowski MR, Kavanaugh JS, Malone CL, Mootz JM, Voyich JM, Smeltzer MS, Bayles KW, Horswill AR. 2011 Nuclease modulates biofilm formation in community-associated methicillin-resistant Staphylococcus aureus. PLoS One 6:e26714. [PubMed: 22096493]

231. Mann EE, Rice KC, Boles BR, Endres JL, Ranjit D, Chandramohan L, Tsang LH, Smeltzer MS, Horswill AR, Bayles KW. 2009 Modulation of eDNA release and degradation affects Staphylococcus aureus biofilm maturation. PLoS One 4:e5822. [PubMed: 19513119]

232. Olson ME, Nygaard TK, Ackermann L, Watkins RL, Zurek OW, Pallister KB, Griffith S, Kiedrowski MR, Flack CE, Kavanaugh JS, Kreiswirth BN, Horswill AR, Voyich JM. 2013 Staphylococcus aureus nuclease is an SaeRS-dependent virulence factor. Infect Immun 81:1316–1324. [PubMed: 23381999]

233. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. 2004 Neutrophil extracellular traps kill bacteria. Science 303:1532–1535. [PubMed: 15001782]

234. Berends ET, Horswill AR, Haste NM, Monestier M, Nizet V, von Kockritz-Blickwede M. 2010 Nuclease expression by Staphylococcus aureus facilitates escape from neutrophil extracellular traps. J Innate Immun 2:576–586. [PubMed: 20829609]

235. Thammavongsa V, Missiakas DM, Schneewind O. 2013 Staphylococcus aureus degrades neutrophil extracellular traps to promote immune cell death. Science 342:863–866. [PubMed: 24233725]

236. Arvidson S 1973 Studies on extracellular proteolytic enzymes from Staphylococcus aureus. II. Isolation and characterization of an EDTA-sensitive protease. Biochim Biophys Acta 302:149–157. [PubMed: 4632563]

237. Arvidson S, Holme T, Lindholm B. 1972 The formation of extracellular proteolytic enzymes by Staphylococcus aureus. Acta Pathol Microbiol Scand B Microbiol Immunol 80:835–844. [PubMed: 4630255]

238. Banbula A, Potempa J, Travis J, Fernandez-Catalan C, Mann K, Huber R, Bode W, Medrano F. 1998 Amino-acid sequence and three-dimensional structure of the Staphylococcus aureus metalloproteinase at 1.72 A resolution. Structure 6:1185–1193. [PubMed: 9753696]

239. Potempa J, Dubin A, Korzus G, Travis J. 1988 Degradation of elastin by a cysteine proteinase from Staphylococcus aureus. J Biol Chem 263:2664–2667. [PubMed: 3422637]

240. Nickerson NN, Joag V, McGavin MJ. 2008 Rapid autocatalytic activation of the M4 metalloprotease aureolysin is controlled by a conserved N-terminal fungalysin-thermolysin-propeptide domain. Mol Microbiol 69:1530–1543. [PubMed: 18673454]

241. Bjoorklind A, Jornvall H. 1974 Substrate specificity of three different extracellular proteolytic enzymes from Staphylococcus aureus. Biochim Biophys Acta 370:524–529. [PubMed: 4613383]

242. Drapeau GR. 1978 Role of metalloprotease in activation of the precursor of staphylococcal protease. J Bacteriol 136:607–613. [PubMed: 711676]

243. Nickerson NN, Prasad L, Jacob L, Delbaere LT, McGavin MJ. 2007 Activation of the SspA serine protease zymogen of Staphylococcus aureus proceeds through unique variations of a trypsinogen-like mechanism and is dependent on both autocatalytic and metalloprotease-specific processing. J Biol Chem 282:34129–34138. [PubMed: 17878159]

244. McAleese FM, Walsh EJ, Sieprawska M, Potempa J, Foster TJ. 2001 Loss of clumping factor B fibrinogen binding activity by Staphylococcus aureus involves cessation of transcription, shedding and cleavage by metalloprotease. J Biol Chem 276:29969–29978. [PubMed: 11399757]

245. Gonzalez DJ, Okumura CY, Hollands A, Kersten R, Akong-Moore K, Pence MA, Malone CL, Derieux J, Moore BS, Horswill AR, Dixon JE, Dorrestein PC, Nizet V. 2012 Novel phenol-soluble modulin derivatives in community-associated methicillin-resistant Staphylococcus aureus identified through imaging mass spectrometry. J Biol Chem 287:13889–13898. [PubMed: 22371493]

246. Cassat JE, Hammer ND, Campbell JP, Benson MA, Perrien DS, Mrak LN, Smeltzer MS, Torres VJ, Skaar EP. 2013 A secreted bacterial protease tailors the Staphylococcus aureus virulence repertoire to modulate bone remodeling during osteomyelitis. Cell Host Microbe 13:759–772. [PubMed: 23768499]

247. Sieprawska-Lupa M, Mydel P, Krawczyk K, Wojcik K, Puklo M, Lupa B, Suder P, Silberring J, Reed M, Pohl J, Shafer W, McAleese F, Foster T, Travis J, Potempa J. 2004 Degradation of human antimicrobial peptide LL-37 by Staphylococcus aureus-derived proteinases. Antimicrob Agents Chemother 48:4673–4679. [PubMed: 15561843]

248. Potempa J, Watorek W, Travis J. 1986 The inactivation of human plasma alpha 1-proteinase inhibitor by proteinases from Staphylococcus aureus. J Biol Chem 261:14330–14334. [PubMed: 3533918]

249. Potempa J, Fedak D, Dubin A, Mast A, Travis J. 1991 Proteolytic inactivation of alpha-1-anti-chymotrypsin. Sites of cleavage and generation of chemotactic activity. J Biol Chem 266:21482–21487. [PubMed: 1793458]

250. Laarman AJ, Ruyken M, Malone CL, van Strijp JA, Horswill AR, Rooijakkers SH. 2011 Staphylococcus aureus metalloprotease aureolysin cleaves complement C3 to mediate immune evasion. J Immunol 186:6445–6453. [PubMed: 21502375]

251. Holers VM. 2014 Complement and its receptors: new insights into human disease. Annu Rev Immunol 32:433–459. [PubMed: 24499275]
252. Beaufort N, Wojciechowski P, Sommerhoff CP, Szmyd G, Dubin G, Eick S, Kellermann J, Schmitt M, Potempa J, Magdolen V. 2008 The human fibrinolytic system is a target for the staphylococcal metalloprotease aureolysin. Biochem J 410:157–165. [PubMed: 17973626]
253. Wegrzynowicz Z, Heczko PB, Drapeau GR, Jeljaszewicz J, Pulverer G. 1980 Prothrombin activation by a metalloprotease from Staphylococcus aureus. J Clin Microbiol 12:138–139. [PubMed: 6785302]
254. Burlak C, Hammer CH, Robinson MA, Whitney AR, McGavin MJ, Kreiswirth BN, Deleo FR. 2007 Global analysis of community-associated methicillin-resistant Staphylococcus aureus exoproteins reveals molecules produced in vitro and during infection. Cell Microbiol 9:1172–1190. [PubMed: 17217429]
255. Rice K, Peralta R, Bast D, de Azavedo J, McGavin MJ. 2001 Description of staphylococcus serine protease (ssp) operon in Staphylococcus aureus and nonpolar inactivation of sspA-encoded serine protease. Infect Immun 69:159–169. [PubMed: 11119502]
256. Drapeau GR, Boily Y, Houmard J. 1972 Purification and properties of an extracellular protease of Staphylococcus aureus. J Biol Chem 247:6720–6726. [PubMed: 4627743]
257. Arvidson S, Holme T, Lindholm B. 1973 Studies on extracellular proteolytic enzymes from Staphylococcus aureus. I. Purification and characterization of one neutral and one alkaline protease. Biochim Biophys Acta 302:135–148. [PubMed: 4632562]
258. Prasad L, Leduc Y, Hayakawa K, Delbaere LT. 2004 The structure of a universally employed enzyme: V8 protease from Staphylococcus aureus. Acta Crystallogr D Biol Crystallogr 60:256–259. [PubMed: 14747701]
259. Yoshikawa K, Tsuzuki H, Fujiwara T, Nakamura E, Iwamoto H, Matsumoto K, Shin M, Yoshida N, Teraoka H. 1992 Purification, characterization and gene cloning of a novel glutamic acid-specific endopeptidase from Staphylococcus aureus ATCC 12600. Biochim Biophys Acta 1121:221–228. [PubMed: 1599945]
260. Drapeau GR. 1978 Unusual COOH-terminal structure of staphylococcal protease. J Biol Chem 253:5899–5901. [PubMed: 681326]
261. Carmona C, Gray GL. 1987 Nucleotide sequence of the serine protease gene of Staphylococcus aureus, strain V8. Nucleic Acids Res 15:6757. [PubMed: 3306605]
262. Yabuta M, Ochi N, Ohsuye K. 1995 Hyperproduction of a recombinant fusion protein of Staphylococcus aureus V8 protease in Escherichia coli and its processing by OmpT protease to release an active V8 protease derivative. Appl Microbiol Biotechnol 44:118–125. [PubMed: 8579825]
263. McGavin MJ, Zahradka C, Rice K, Scott JE. 1997 Modification of the Staphylococcus aureus fibronectin binding phenotype by V8 protease. Infect Immun 65:2621–2628. [PubMed: 9199429]
264. Karlsson A, Saravia-Otten P, Tegmark K, Morfeldt E, Arvidson S. 2001 Decreased amounts of cell wall-associated protein A and fibronectin-binding proteins in Staphylococcus aureus sarA mutants due to up-regulation of extracellular proteases. Infect Immun 69:4742–4748. [PubMed: 11447146]
265. Murphy J, Ramezanpour M, Stach N, Dubin G, Psaltis AJ, Wormald PJ, Vreugde S. 2017 Staphylococcus Aureus V8 protease disrupts the integrity of the airway epithelial barrier and impairs IL-6 production in vitro. Laryngoscope doi:10.1002/lary.26949.
266. Prokesova L, Potuznikova B, Potempa J, Zikan J, Radl J, Hachova L, Baran K, Porwit-Bobr Z, John C. 1992 Cleavage of human immunoglobulins by serine proteinase from Staphylococcus aureus. Immunol Lett 31:259–265. [PubMed: 1372285]
267. Rousseaux J, Rousseaux-Prevost R, Bazin H, Biserte G. 1983 Proteolysis of rat IgG subclasses by Staphylococcus aureus V8 proteinase. Biochim Biophys Acta 748:205–212. [PubMed: 6354271]
268. Hirasawa Y, Takai T, Nakamura T, Mitsuishi K, Gunawan H, Suto H, Ogawa T, Wang XL, Ikeda S, Okumura K, Ogawa H. 2010 Staphylococcus aureus extracellular protease causes epidermal barrier dysfunction. J Invest Dermatol 130:614–617. [PubMed: 19812593]
269. Shaw L, Golonka E, Potempa J, Foster SJ. 2004 The role and regulation of the extracellular proteases of Staphylococcus aureus. Microbiology 150:217–228. [PubMed: 14702415]

270. Chan PF, Foster SJ. 1998 Role of SarA in virulence determinant production and environmental signal transduction in Staphylococcus aureus. J Bacteriol 180:6232–6241. [PubMed: 9829932]

271. Takeuchi S, Matsunaga K, Inubushi S, Higuchi H, Imaizumi K, Kaidoh T. 2002 Structural gene and strain specificity of a novel cysteine protease produced by Staphylococcus aureus isolated from a diseased chicken. Vet Microbiol 89:201–210. [PubMed: 12243897]

272. Kalinska M, Kantyka T, Greenbaum DC, Larsen KS, Wladyka B, Jabaiah A, Bogyo M, Daugherty PS, Wysocka M, Jaros M, Lesner A, Rolka K, Schaschke N, Stennicke H, Dubin A, Potempa J, Dubin G. 2012 Substrate specificity of Staphylococcus aureus cysteine proteases--Staphopains A, B and C. Biochimie 94:318–327. [PubMed: 21802486]

273. Rzychon M, Sabat A, Kosowska K, Potempa J, Dubin A. 2003 Staphostatins: an expanding new group of proteinase inhibitors with a unique specificity for the regulation of staphopains, Staphylococcus spp. cysteine proteinases. Mol Microbiol 49:1051–1066. [PubMed: 12890028]

274. Massimi I, Park E, Rice K, Muller-Esterl W, Sauder D, McGavin MJ. 2002 Identification of a novel maturation mechanism and restricted substrate specificity for the SspB cysteine protease of Staphylococcus aureus. J Biol Chem 277:41770–41777. [PubMed: 12207024]

275. Nickerson N, Ip J, Passos DT, McGavin MJ. 2010 Comparison of Staphopain A (ScpA) and B (SspB) precursor activation mechanisms reveals unique secretion kinetics of proSspB (Staphopain B), and a different interaction with its cognate Staphostatin, SspC. Mol Microbiol 75:161–177. [PubMed: 19943908]

276. Filipek R, Potempa J, Bochtler M. 2005 A comparison of staphostatin B with standard mechanism serine protease inhibitors. J Biol Chem 280:14669–14674. [PubMed: 15644332]

277. Hofmann B, Schomburg D, Hecht HJ 1993 Crystal Structure of a Thiol Proteinase from Staphylococcus Aureus V-8 in the E-64 Inhibitor Complex. Acta Crystallogr, SectA 49:102.

278. Kantyka T, Shaw LN, Potempa J. 2011 Papain-like proteases of Staphylococcus aureus. Adv Exp Med Biol 712:1–14. [PubMed: 21660655]

279. Kantyka T, Pyrc K, Gruca M, Smagur J, Plaza K, Guzik K, Zeglen S, Ochman M, Potempa J. 2013 Staphylococcus aureus proteases degrade lung surfactant protein A potentially impairing innate immunity of the lung. J Innate Immun 5:251–260. [PubMed: 23235402]

280. Laarman AJ, Mijnheer G, Mootz JM, van Rooijen WJ, Ruyken M, Malone CL, Heezius EC, Ward R, Milligan G, van Strijp JA, de Haas CJ, Horswill AR, van Kessel KP, Rooijakkers SH. 2012 Staphylococcus aureus Staphopain A inhibits CXCR2-dependent neutrophil activation and chemotaxis. EMBO J 31:3607–3619. [PubMed: 22850671]

281. Imamura T, Tanase S, Szmyd G, Kozik A, Travis J, Potempa J. 2005 Induction of vascular leakage through release of bradykinin and a novel kinin by cysteine proteinases from Staphylococcus aureus. J Exp Med 201:1669–1676. [PubMed: 15897280]

282. Sonesson A, Przybyszewska K, Eriksson S, Morgelin M, Kjellstrom S, Davies J, Potempa J, Schmidtchen A. 2017 Identification of bacterial biofilm and the Staphylococcus aureus derived protease, staphopain, on the skin surface of patients with atopic dermatitis. Sci Rep 7:8689. [PubMed: 28821865]

283. Smagur J, Guzik K, Bzowska M, Kuzak M, Zarebski M, Kantyka T, Walski M, Gajkowska B, Potempa J. 2009 Staphylococcal cysteine protease staphopain B (SspB) induces rapid engulfment of human neutrophils and monocytes by macrophages. Biol Chem 390:361–371. [PubMed: 19284294]

284. Smagur J, Guzik K, Magiera L, Bzowska M, Gruca M, Thogersen IB, Enghild JJ, Potempa J. 2009 A new pathway of staphylococcal pathogenesis: apoptosis-like death induced by Staphopain B in human neutrophils and monocytes. J Innate Immun 1:98–108. [PubMed: 20375568]

285. Kulig P, Zabel BA, Dubin G, Allen SJ, Ohyama T, Potempa J, Handel TM, Butcher EC, Cichy J. 2007 Staphylococcus aureus-derived staphopain B, a potent cysteine protease activator of plasma chemerin. J Immunol 178:3713–3720. [PubMed: 17339469]

286. Kubica M, Guzik K, Koziel J, Zarebski M, Richter W, Gajkowska B, Golda A, Maciag-Gudowska A, Brix K, Shaw L, Foster T, Potempa J. 2008 A potential new pathway for Staphylococcus aureus dissemination: the silent survival of S. aureus phagocytosed by human monocyte-derived macrophages. PLoS One 3:e1409. [PubMed: 18183290]

287. Schindler D, Gutierrez MG, Beineke A, Rauter Y, Rohde M, Foster S, Goldmann O, Medina E. 2012 Dendritic cells are central coordinators of the host immune response to Staphylococcus aureus bloodstream infection. Am J Pathol 181:1327–1337. [PubMed: 22885107]

288. Loughran AJ, Atwood DN, Anthony AC, Harik NS, Spencer HJ, Beenken KE, Smeltzer MS. 2014 Impact of individual extracellular proteases on Staphylococcus aureus biofilm formation in diverse clinical isolates and their isogenic sarA mutants. Microbiologyopen 3:897–909. [PubMed: 25257373]

289. Mootz JM, Malone CL, Shaw LN, Horswill AR. 2013 Staphopains modulate Staphylococcus aureus biofilm integrity. Infect Immun 81:3227–3238. [PubMed: 23798534]

290. Ohbayashi T, Irie A, Murakami Y, Nowak M, Potempa J, Nishimura Y, Shinohara M, Imamura T. 2011 Degradation of fibrinogen and collagen by staphopains, cysteine proteases released from Staphylococcus aureus. Microbiology 157:786–792. [PubMed: 21081759]

291. Kantyka T, Plaza K, Koziel J, Florczyk D, Stennicke HR, Thogersen IB, Enghild JJ, Silverman GA, Pak SC, Potempa J. 2011 Inhibition of Staphylococcus aureus cysteine proteases by human serpin potentially limits staphylococcal virulence. Biol Chem 392:483–489. [PubMed: 21476872]

292. Rzychon M, Filipek R, Sabat A, Kosowska K, Dubin A, Potempa J, Bochtler M. 2003 Staphostatins resemble lipocalins, not cystatins in fold. Protein Sci 12:2252–2256. [PubMed: 14500882]

293. Filipek R, Rzychon M, Oleksy A, Gruca M, Dubin A, Potempa J, Bochtler M. 2003 The Staphostatin-staphopain complex: a forward binding inhibitor in complex with its target cysteine protease. J Biol Chem 278:40959–40966. [PubMed: 12874290]

294. Shaw LN, Golonka E, Szmyd G, Foster SJ, Travis J, Potempa J. 2005 Cytoplasmic control of premature activation of a secreted protease zymogen: deletion of staphostatin B (SspC) in Staphylococcus aureus 8325–4 yields a profound pleiotropic phenotype. J Bacteriol 187:1751–1762. [PubMed: 15716447]

295. Rieneck K, Renneberg J, Diamant M, Gutschik E, Bendtzen K. 1997 Molecular cloning and expression of a novel Staphylococcus aureus antigen. Biochim Biophys Acta 1350:128–132. [PubMed: 9048880]

296. Reed SB, Wesson CA, Liou LE, Trumble WR, Schlievert PM, Bohach GA, Bayles KW. 2001 Molecular characterization of a novel Staphylococcus aureus serine protease operon. Infect Immun 69:1521–1527. [PubMed: 11179322]

297. Baba T, Bae T, Schneewind O, Takeuchi F, Hiramatsu K. 2008 Genome sequence of Staphylococcus aureus strain Newman and comparative analysis of staphylococcal genomes: polymorphism and evolution of two major pathogenicity islands. J Bacteriol 190:300–310. [PubMed: 17951380]

298. Dubin G, Stec-Niemczyk J, Kisielewska M, Pustelny K, Popowicz GM, Bista M, Kantyka T, Boulware KT, Stennicke HR, Czarna A, Phopaisarn M, Daugherty PS, Thogersen IB, Enghild JJ, Thornberry N, Dubin A, Potempa J. 2008 Enzymatic activity of the Staphylococcus aureus SplB serine protease is induced by substrates containing the sequence Trp-Glu-Leu-Gln. J Mol Biol 379:343–356. [PubMed: 18448121]

299. Stec-Niemczyk J, Pustelny K, Kisielewska M, Bista M, Boulware KT, Stennicke HR, Thogersen IB, Daugherty PS, Enghild JJ, Baczynski K, Popowicz GM, Dubin A, Potempa J, Dubin G. 2009 Structural and functional characterization of SplA, an exclusively specific protease of Staphylococcus aureus. Biochem J 419:555–564. [PubMed: 19175361]

300. Zdzalik M, Kalinska M, Wysocka M, Stec-Niemczyk J, Cichon P, Stach N, Gruba N, Stennicke HR, Jabaiah A, Markiewicz M, Kedracka-Krok S, Wladyka B, Daugherty PS, Lesner A, Rolka K, Dubin A, Potempa J, Dubin G. 2013 Biochemical and structural characterization of SplD protease from Staphylococcus aureus. PLoS One 8:e76812. [PubMed: 24130791]

301. Popowicz GM, Dubin G, Stec-Niemczyk J, Czarny A, Dubin A, Potempa J, Holak TA. 2006 Functional and structural characterization of Spl proteases from Staphylococcus aureus. J Mol Biol 358:270–279. [PubMed: 16516230]

302. Paharik AE, Salgado-Pabon W, Meyerholz DK, White MJ, Schlievert PM, Horswill AR. 2016 The Spl Serine Proteases Modulate Staphylococcus aureus Protein Production and Virulence in a Rabbit Model of Pneumonia. mSphere 1.

303. Stentzel S, Teufelberger A, Nordengrun M, Kolata J, Schmidt F, van Crombruggen K, Michalik S, Kumpfmuller J, Tischer S, Schweder T, Hecker M, Engelmann S, Volker U, Krysko O, Bachert C, Broker BM. 2017 Staphylococcal serine protease-like proteins are pacemakers of allergic airway reactions to Staphylococcus aureus. J Allergy Clin Immunol 139:492–500e498. [PubMed: 27315768]

304. Teufelberger AR, Nordengrun M, Braun H, Maes T, De Grove K, Holtappels G, O'Brien C, Provoost S, Hammad H, Goncalves A, Beyaert R, Declercq W, Vandenabeele P, Krysko DV, Broker BM, Bachert C, Krysko O. 2017 The IL-33/ST2 axis is crucial in type 2 airway responses induced by Staphylococcus aureus-derived serine protease-like protein D. J Allergy Clin Immunol doi:10.1016/j.jaci.2017.05.004.

305. Laurent TC, Fraser JR. 1992 Hyaluronan. FASEB J 6:2397–2404. [PubMed: 1563592]

306. Monslow J, Govindaraju P, Pure E. 2015 Hyaluronan - a functional and structural sweet spot in the tissue microenvironment. Front Immunol 6:231. [PubMed: 26029216]

307. Lee-Sayer SS, Dong Y, Arif AA, Olsson M, Brown KL, Johnson P. 2015 The where, when, how, and why of hyaluronan binding by immune cells. Front Immunol 6:150. [PubMed: 25926830]

308. Hynes WL, Walton SL. 2000 Hyaluronidases of Gram-positive bacteria. FEMS Microbiol Lett 183:201–207. [PubMed: 10675584]

309. Hart ME, Hart MJ, Roop AJ. 2009 Genotypic and Phenotypic Assessment of Hyaluronidase among Type Strains of a Select Group of Staphylococcal Species. Int J Microbiol 2009:614371. [PubMed: 20130817]

310. Devriese LA, Hajek V, Oeding P, Meyer SA, Schleifer KH. 1978 Staphylococcus-Hyicus (Sompolinsky 1953) Comb Nov and Staphylococcus-Hyicus Subsp Chromogenes Subsp Nov. International Journal of Systematic Bacteriology 28:482–490.

311. Duran-Reynals F 1933 Studies on a Certain Spreading Factor Existing in Bacteria and Its Significance for Bacterial Invasiveness. J Exp Med 58:161–181. [PubMed: 19870187]

312. Chain E, Duthie ES. 1940 Identity of Hyaluronidase and Spreading Factor. British Journal of Experimental Pathology 21:324–338.

313. Farrell AM, Taylor D, Holland KT. 1995 Cloning, nucleotide sequence determination and expression of the Staphylococcus aureus hyaluronate lyase gene. FEMS Microbiol Lett 130:81–85. [PubMed: 7557301]

314. Makris G, Wright JD, Ingham E, Holland KT. 2004 The hyaluronate lyase of Staphylococcus aureus - a virulence factor? Microbiology 150:2005–2013. [PubMed: 15184586]

315. Ibberson CB, Jones CL, Singh S, Wise MC, Hart ME, Zurawski DV, Horswill AR. 2014 Staphylococcus aureus hyaluronidase is a CodY-regulated virulence factor. Infect Immun 82:4253–4264. [PubMed: 25069977]

316. Ibberson CB, Parlet CP, Kwiecinski J, Crosby HA, Meyerholz DK, Horswill AR. 2016 Hyaluronan Modulation Impacts Staphylococcus aureus Biofilm Infection. Infect Immun 84:1917–1929. [PubMed: 27068096]

317. Doery HM, Magnusson BJ, Gulasekharam J, Pearson JE. 1965 The properties of phospholipase enzymes in staphylococcal toxins. J Gen Microbiol 40:283–296. [PubMed: 5867760]

318. Magnusson BJ, Gulasekharam J, Doery HM. 1962 Phospholipase Activity of Staphylococcal Toxin. Nature 196:270–&.

319. Daugherty S, Low MG. 1993 Cloning, expression, and mutagenesis of phosphatidylinositol-specific phospholipase C from Staphylococcus aureus: a potential staphylococcal virulence factor. Infect Immun 61:5078–5089. [PubMed: 8225585]

320. Beining PR, Huff E, Prescott B, Theodore TS. 1975 Characterization of the lipids of mesosomal vesicles and plasma membranes from Staphylococcus aureus. J Bacteriol 121:137–143. [PubMed: 1116984]

321. Goldstein R, Cheng J, Stec B, Roberts MF. 2012 Structure of the S. aureus PI-specific phospholipase C reveals modulation of active site access by a titratable pi-cation latched loop. Biochemistry 51:2579–2587. [PubMed: 22390775]

322. Cheng J, Goldstein R, Stec B, Gershenson A, Roberts MF. 2012 Competition between anion binding and dimerization modulates Staphylococcus aureus phosphatidylinositol-specific phospholipase C enzymatic activity. J Biol Chem 287:40317–40327. [PubMed: 23038258]

323. Griffith OH, Ryan M. 1999 Bacterial phosphatidylinositol-specific phospholipase C: structure, function, and interaction with lipids. Biochim Biophys Acta 1441:237–254. [PubMed: 10570252]

324. Heinz DW, Essen LO, Williams RL. 1998 Structural and mechanistic comparison of prokaryotic and eukaryotic phosphoinositide-specific phospholipases C. J Mol Biol 275:635–650. [PubMed: 9466937]

325. Flores-Diaz M, Monturiol-Gross L, Naylor C, Alape-Giron A, Flieger A. 2016 Bacterial Sphingomyelinases and Phospholipases as Virulence Factors. Microbiol Mol Biol Rev 80:597–628. [PubMed: 27307578]

326. Hansch GM, Weller PF, Nicholson-Weller A. 1988 Release of C8 binding protein (C8bp) from the cell membrane by phosphatidylinositol-specific phospholipase C. Blood 72:1089–1092. [PubMed: 3416071]

327. Medof ME, Walter EI, Roberts WL, Haas R, Rosenberry TL. 1986 Decay accelerating factor of complement is anchored to cells by a C-terminal glycolipid. Biochemistry 25:6740–6747. [PubMed: 2432921]

328. Schonermark S, Rauterberg EW, Shin ML, Loke S, Roelcke D, Hansch GM. 1986 Homologous species restriction in lysis of human erythrocytes: a membrane-derived protein with C8-binding capacity functions as an inhibitor. J Immunol 136:1772–1776. [PubMed: 2419413]

329. Walter EI, Ratnoff WD, Long KE, Kazura JW, Medof ME. 1992 Effect of glycoinositolphospholipid anchor lipid groups on functional properties of decay-accelerating factor protein in cells. J Biol Chem 267:1245–1252. [PubMed: 1370460]

330. White MJ, Boyd JM, Horswill AR, Nauseef WM. 2014 Phosphatidylinositol-specific phospholipase C contributes to survival of Staphylococcus aureus USA300 in human blood and neutrophils. Infect Immun 82:1559–1571. [PubMed: 24452683]

331. Cadieux B, Vijayakumaran V, Bernards MA, McGavin MJ, Heinrichs DE. 2014 Role of lipase from community-associated methicillin-resistant Staphylococcus aureus strain USA300 in hydrolyzing triglycerides into growth-inhibitory free fatty acids. J Bacteriol 196:4044–4056. [PubMed: 25225262]

332. Rosenstein R, Gotz F. 2000 Staphylococcal lipases: biochemical and molecular characterization. Biochimie 82:1005–1014. [PubMed: 11099797]

333. Gotz F, Verheij HM, Rosenstein R. 1998 Staphylococcal lipases: molecular characterisation, secretion, and processing. Chem Phys Lipids 93:15–25. [PubMed: 9720246]

334. Nikoleit K, Rosenstein R, Verheij HM, Gotz F. 1995 Comparative biochemical and molecular analysis of the Staphylococcus hyicus, Staphylococcus aureus and a hybrid lipase. Indication for a C-terminal phospholipase domain. Eur J Biochem 228:732–738. [PubMed: 7737171]

335. Rollof J, Normark S. 1992 In vivo processing of Staphylococcus aureus lipase. J Bacteriol 174:1844–1847. [PubMed: 1548232]

336. Demleitner G, Gotz F. 1994 Evidence for importance of the Staphylococcus hyicus lipase pro-peptide in lipase secretion, stability and activity. FEMS Microbiol Lett 121:189–197. [PubMed: 7926670]

337. Liebl W, Gotz F. 1986 Studies on lipase directed export of Escherichia coli beta-lactamase in Staphylococcus carnosus. Mol Gen Genet 204:166–173. [PubMed: 3018441]

338. Simons JW, Adams H, Cox RC, Dekker N, Gotz F, Slotboom AJ, Verheij HM. 1996 The lipase from Staphylococcus aureus. Expression in Escherichia coli, large-scale purification and comparison of substrate specificity to Staphylococcus hyicus lipase. Eur J Biochem 242:760–769. [PubMed: 9022707]

339. Rollof J, Hedstrom SA, Nilsson-Ehle P. 1987 Positional specificity and substrate preference of purified Staphylococcus aureus lipase. Biochim Biophys Acta 921:370–377. [PubMed: 3651494]

340. Rollof J, Hedstrom SA, Nilsson-Ehle P. 1987 Lipolytic activity of Staphylococcus aureus strains from disseminated and localized infections. Acta Pathol Microbiol Immunol Scand B 95:109–113. [PubMed: 3591310]

341. Rollof J, Braconier JH, Soderstrom C, Nilsson-Ehle P. 1988 Interference of Staphylococcus aureus lipase with human granulocyte function. Eur J Clin Microbiol Infect Dis 7:505–510. [PubMed: 3141158]

342. Rollof J, Vinge E, Nilsson-Ehle P, Braconier JH. 1992 Aggregation of human granulocytes by Staphylococcus aureus lipase. J Med Microbiol 36:52–55. [PubMed: 1731059]

343. Hu C, Xiong N, Zhang Y, Rayner S, Chen S. 2012 Functional characterization of lipase in the pathogenesis of Staphylococcus aureus. Biochem Biophys Res Commun 419:617–620. [PubMed: 22369949]

344. Shryock TR, Dye ES, Kapral FA. 1992 The accumulation of bactericidal lipids in staphylococcal abscesses. J Med Microbiol 36:332–336. [PubMed: 1588583]

345. Mortensen JE, Shryock TR, Kapral FA. 1992 Modification of bactericidal fatty acids by an enzyme of Staphylococcus aureus. J Med Microbiol 36:293–298. [PubMed: 1560452]

346. Chamberlain NR, Brueggemann SA. 1997 Characterisation and expression of fatty acid modifying enzyme produced by Staphylococcus epidermidis. J Med Microbiol 46:693–697. [PubMed: 9511818]

347. Long JP, Hart J, Albers W, Kapral FA. 1992 The production of fatty acid modifying enzyme (FAME) and lipase by various staphylococcal species. J Med Microbiol 37:232–234. [PubMed: 1404319]

348. Lu T, Park JY, Parnell K, Fox LK, McGuire MA. 2012 Characterization of fatty acid modifying enzyme activity in staphylococcal mastitis isolates and other bacteria. BMC Res Notes 5:323. [PubMed: 22726316]

349. Kapral FA, Smith S, Lal D. 1992 The esterification of fatty acids by Staphylococcus aureus fatty acid modifying enzyme (FAME) and its inhibition by glycerides. J Med Microbiol 37:235–237. [PubMed: 1404320]

350. Long JP, Kapral FA. 1993 Host response to coagulase-negative staphylococci in abscesses induced within mice. J Med Microbiol 39:191–195. [PubMed: 8366517]

351. Oganesyan V, Peng L, Damschroder MM, Cheng L, Sadowska A, Tkaczyk C, Sellman BR, Wu H, Dall'Acqua WF. 2014 Mechanisms of neutralization of a human anti-alpha-toxin antibody. J Biol Chem 289:29874–29880. [PubMed: 25210036]

352. Roblin P, Guillet V, Joubert O, Keller D, Erard M, Maveyraud L, Prevost G, Mourey L. 2008 A covalent S-F heterodimer of leucotoxin reveals molecular plasticity of beta-barrel pore-forming toxins. Proteins 71:485–496. [PubMed: 18214982]

353. Olson R, Nariya H, Yokota K, Kamio Y, Gouaux E. 1999 Crystal structure of staphylococcal LukF delineates conformational changes accompanying formation of a transmembrane channel. Nat Struct Biol 6:134–140. [PubMed: 10048924]

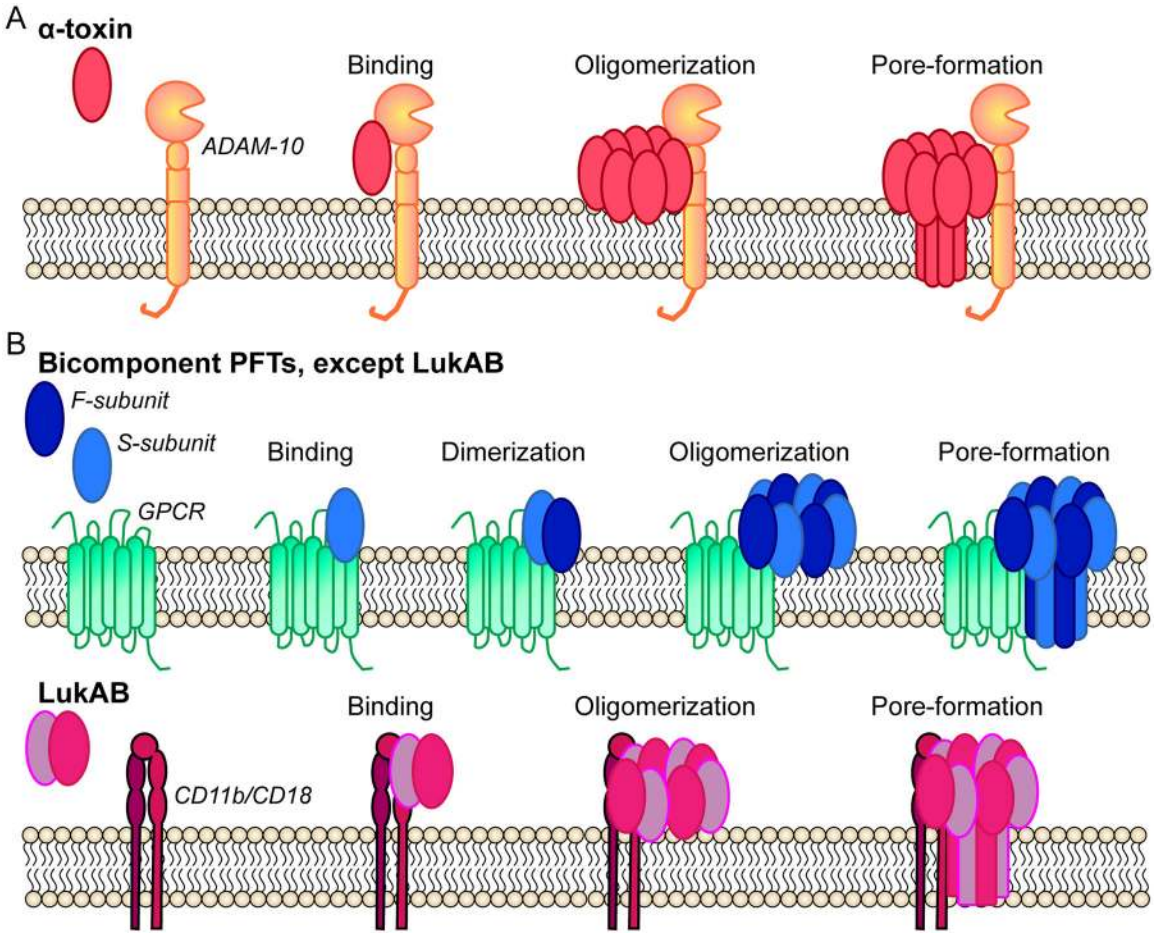

Figure 1:

Current models for PFT pore formation for A) α-toxin and B) the bicomponent PFTs. A) α-toxin is secreted as monomer. Upon binding to the host receptor, ADAM-10, the toxin monomers oligomerize to form a heptameric prepore on the target cell surface. The prestem domains of the prepore then extend to form a β-barrel pore that punctures the target cell membrane. B) The bicomponent PFTs are also secreted as monomers, (except LukAB, which is secreted as dimers). The S-subunit recognizes the target cell by binding to cell surface receptors (LukPQ is an exception, the F-subunit LukQ is the receptor recognition subunit). These receptors are typically GPCRs (except for LukAB, which binds to the integrin, CD11b). Upon receptor binding, the S-subunit dimerizes with the F-subunit, followed by oligomerization of 3 additional leukocidin dimers, resulting in an octameric prepore. Similar to the α-toxin pore formation model, the prestem domains of the prepore extend to form a β-barrel pore, thus disrupting the target cell membrane.

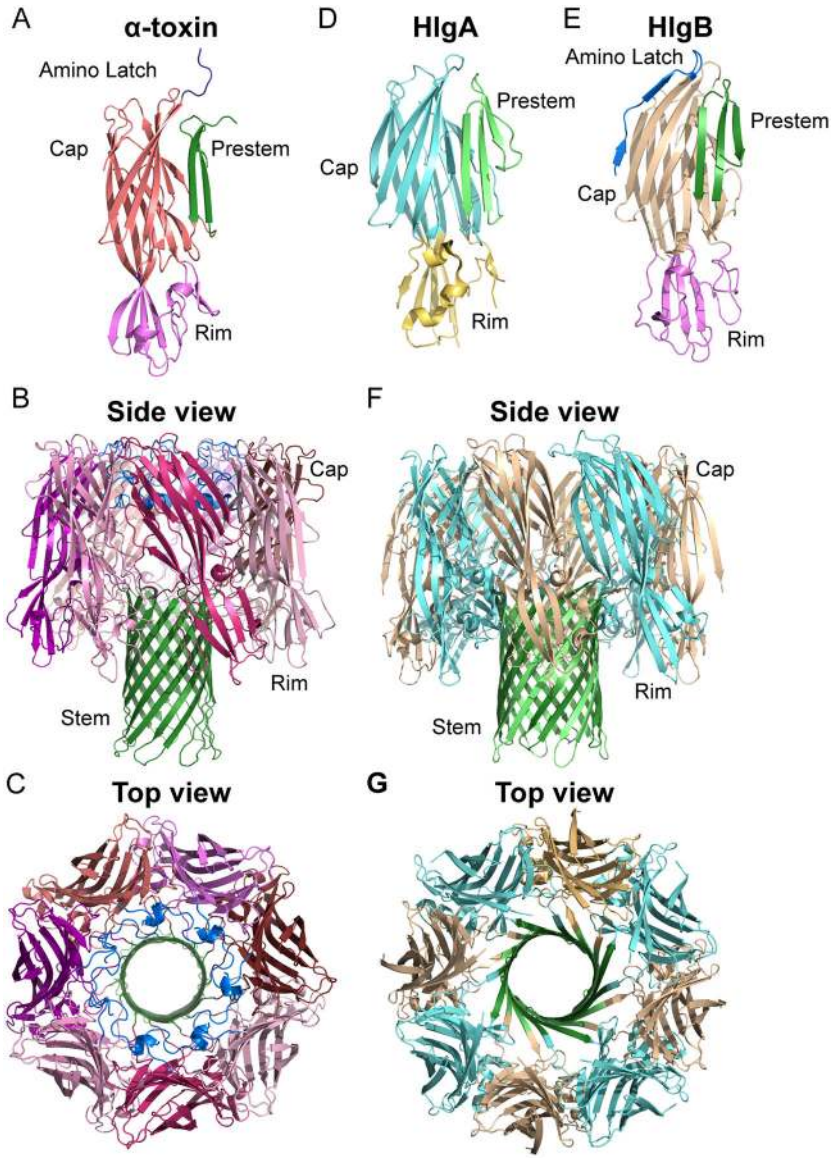

Figure 2:

Structures of (A-C) α-toxin and (D-G) the bicomponent PFT, HlgAB. A) The α-toxin monomer (PDB:4U6V) (351). The amino latch is colored in blue, cap domain in red, rim domain in pink, and prestem domain in green. B-C) The α-toxin heptamer (7AHL) (4), each α-toxin is colored in a different shade of pink to denote individual protomer. The amino latches are highlighted in blue and the β-barrel pore is in green. The monomers of D) HlgA (2QK7) (352) and E) HlgB (1LKF) (353). The amino latch of HlgB is colored in blue; the cap domain for HlgA is in cyan and HlgB is in beige; the rim domains are in yellow for HlgA and pink for HlgB; and the prestem domains are in green. F-G) The HlgAB octamer (3B07) (33). The HlgA protomers are in cyan, the HlgB protomers are in beige, and the β-barrel pore is in green.

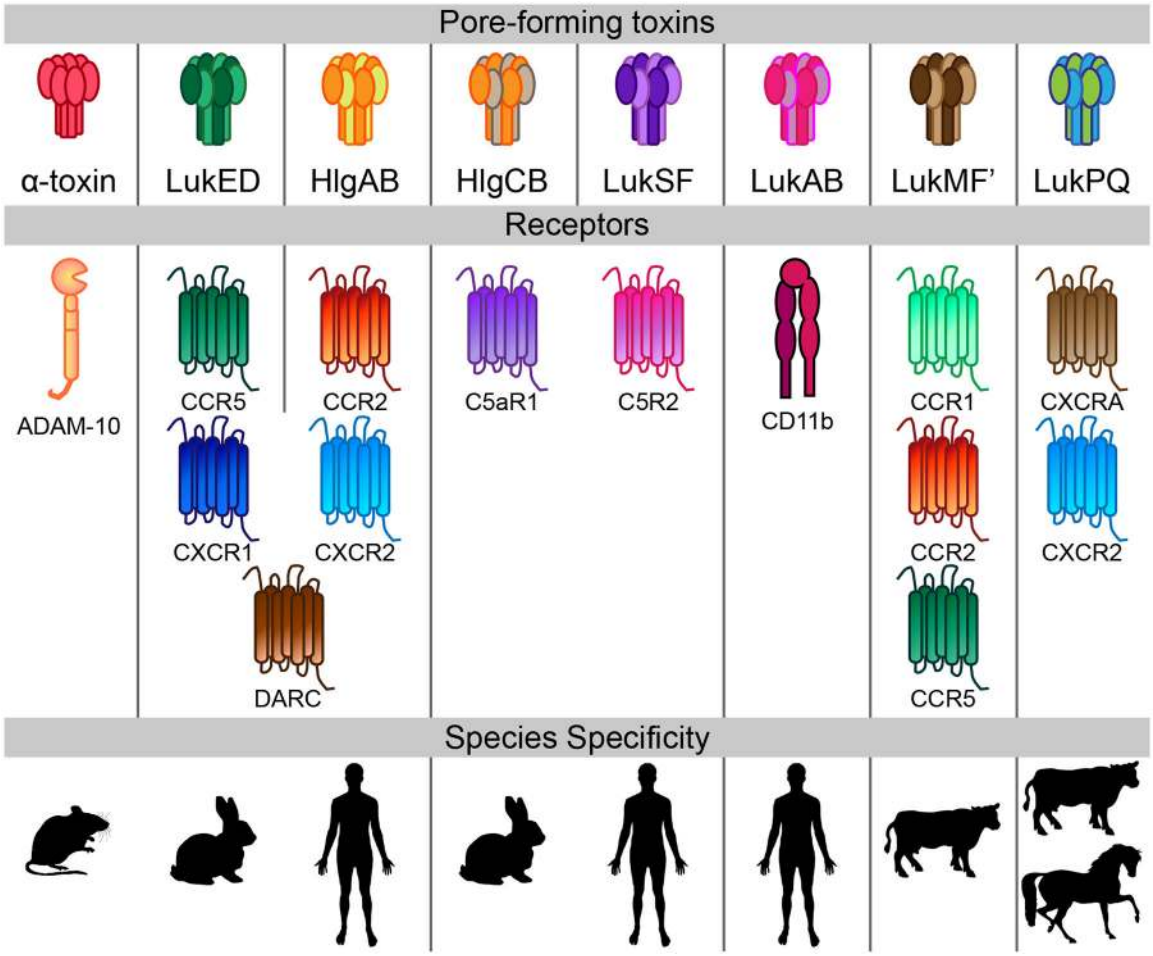

Figure 3:

S. aureus PFTs and their receptor, species, and cell type specificity. A) Currently, *S. aureus* is known to produce 8 different β-barrel PFTs. Each of these PFTs target different cell surface receptors. While some PFTs share the same receptors, they can differ in their species specificity. Collectively, the PFTs exert their sublytic and lytic effects on a variety of cells, including erythrocytes, endothelial cells, epithelial cells, neutrophils, monocytes, macrophages, dendritic cells, and T cells.

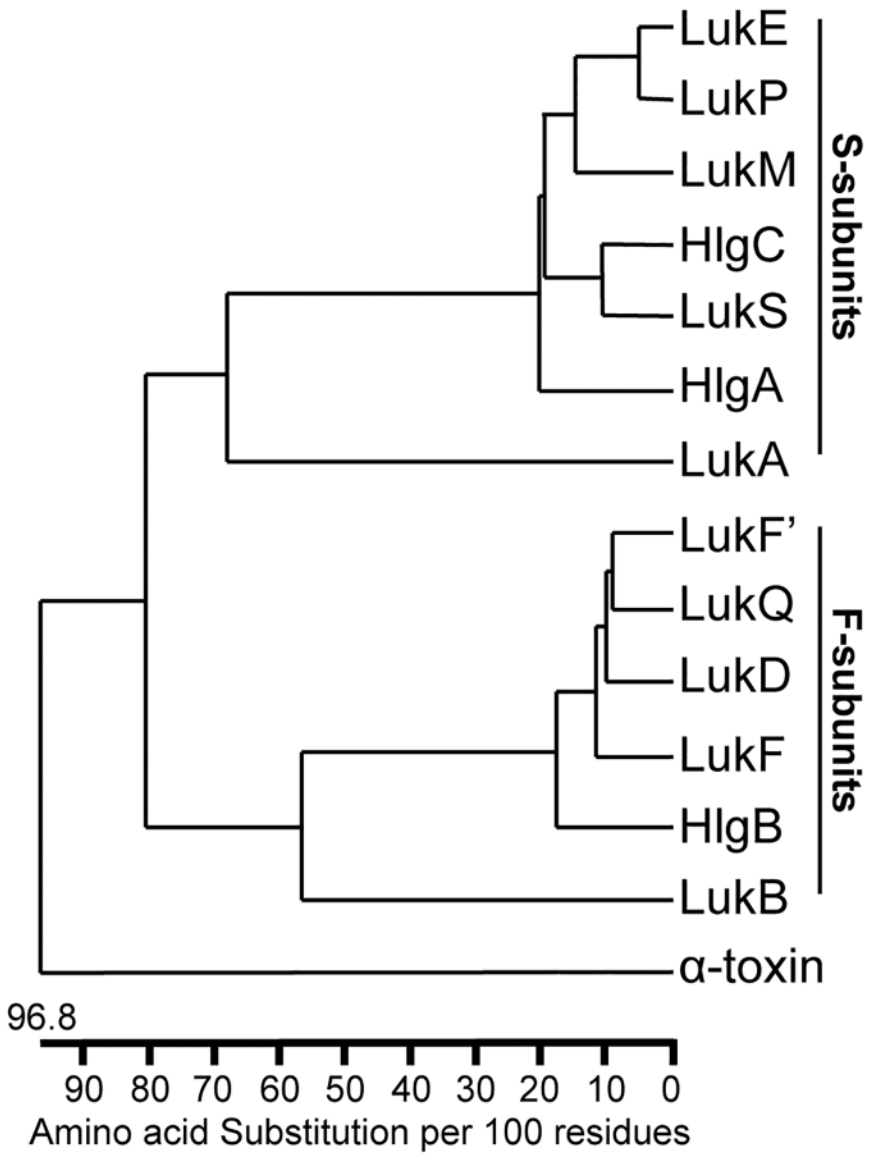

Figure 4:

Phylogenic tree of *S. aureus* PFTs. The tree is constructed based on the mature protein sequences using the DNASTAR MegAlign ClustalW method for multiple sequence alignment.

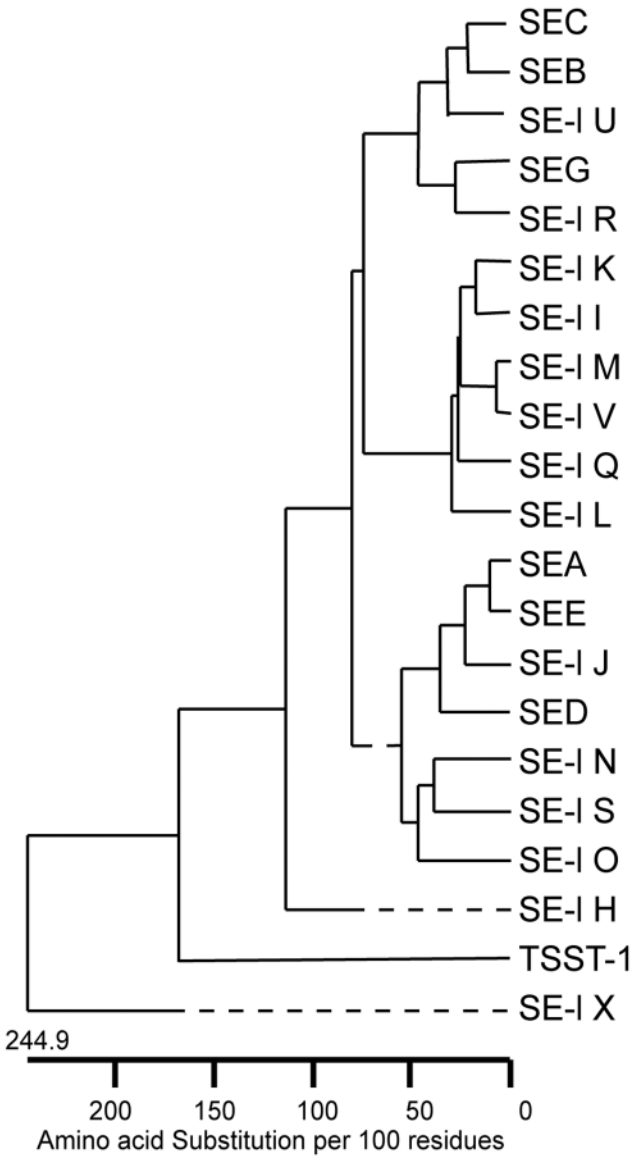

Figure 5:

Phylogenic tree of *S. aureus* SAgs. The tree is constructed based on the mature protein sequences using the DNASTAR MegAlign ClustalW method for multiple sequence alignment.

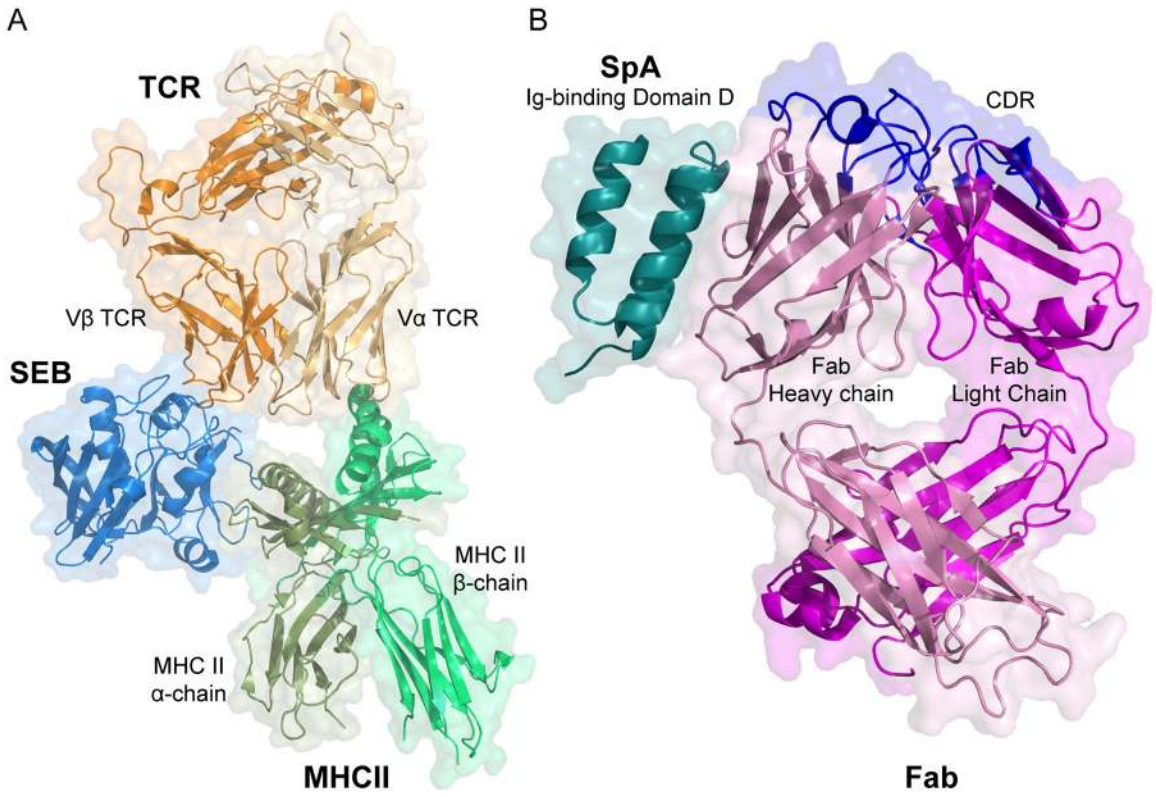

Figure 6:

Crystal structures of *S. aureus* superantigens in complex with their cellular targets. A) The T cell SAg, SEB in complex with TCR and MHC class II molecule (4C56) (116). SEB (blue) crosslinks the α-chain of MHC (dark green) to the Vβ TCR (orange) to induce T cell proliferation that results in T cell anergy and/or apoptosis. B) B cell SAg, SpA (teal) in complex with the Fab fragment (pink/magenta) (1DEE) (138). Conventional antigens bind to BCR at the CDR (blue), a hypervariable region that confer antigen specificities. SpA binds at a constant region of the receptor to activate B cells for supraclonal expansion, which leads to clonal deletion of SpA-activated B cells.

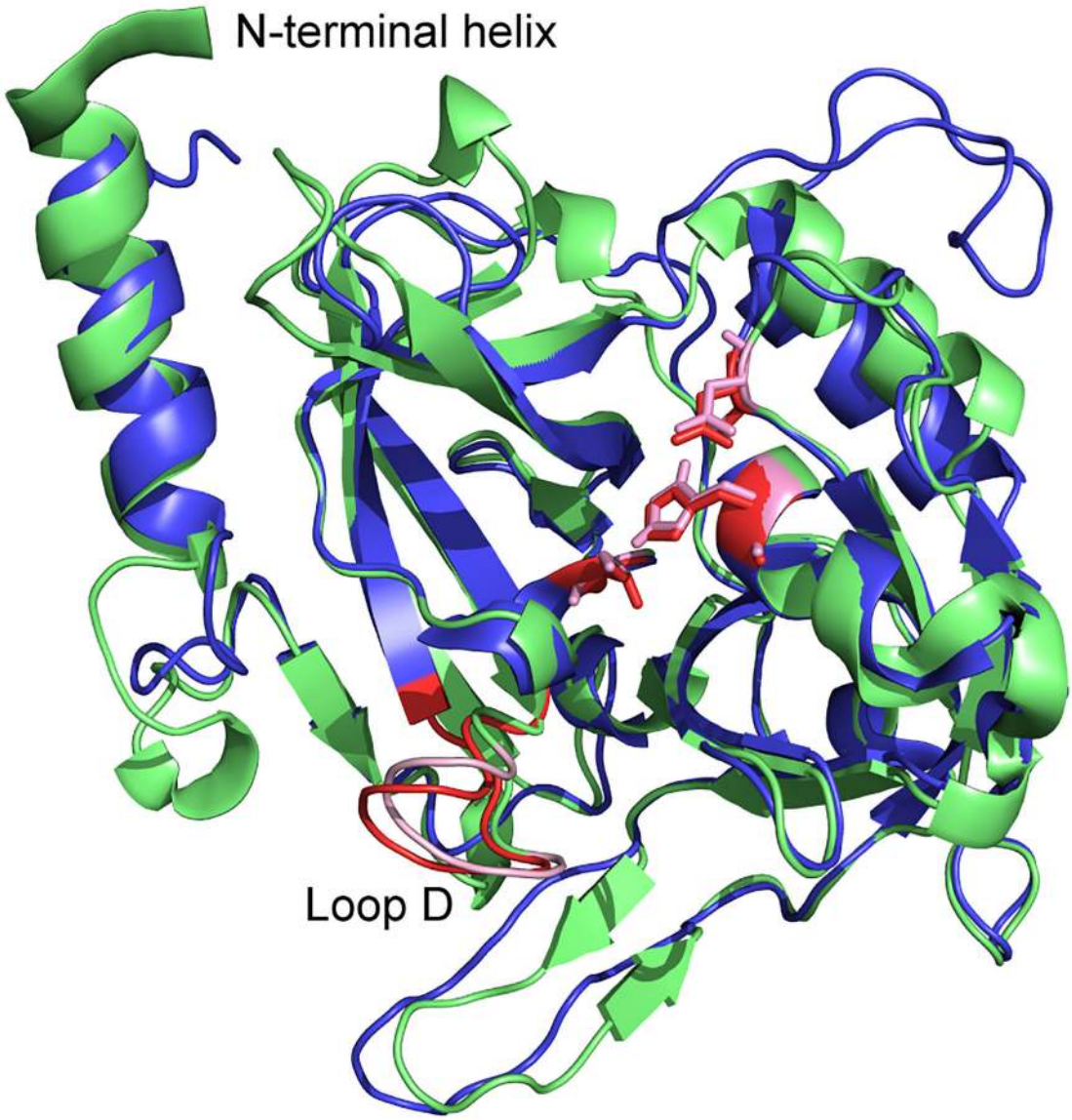

Figure 7:
Overlaid of the crystal structures of ETA and ETB. ETA (1EXF, green) (174) and ETB (1QTF, blue) (173) share high structural identity. ETs cause SSSS by cleaving Dsg1 at the epithelial cell junctions. Both ETs are serine proteases. Loop D and the catalytic triad are highlighted in pink for ETA and red for ETB.

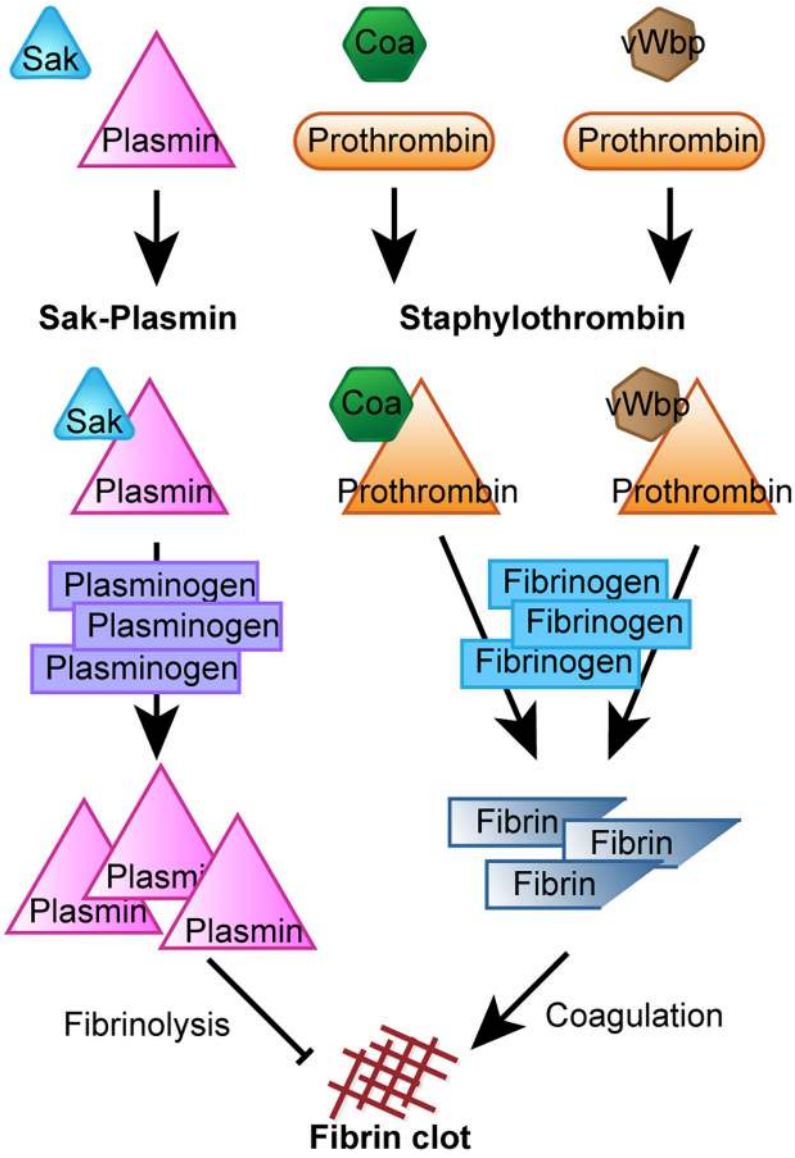

Figure 8:

S. aureus produces cofactors that hijack the host's coagulation system. Coa and vWbp bind to prothrombin and alter the confirmation of the protein to form the complex, staphylothrombin. This complex is highly active and cleaves fibrinogens to fibrins, promoting the formation of fibrinous clots. Sak binds to plasmin to form the Sak-plasmin complex. Sak stabilizes plasmin to enhance enzymatic activity. Sak-plasmin cleaves plasminogen to form plasmin, which breaks down fibrin clots.

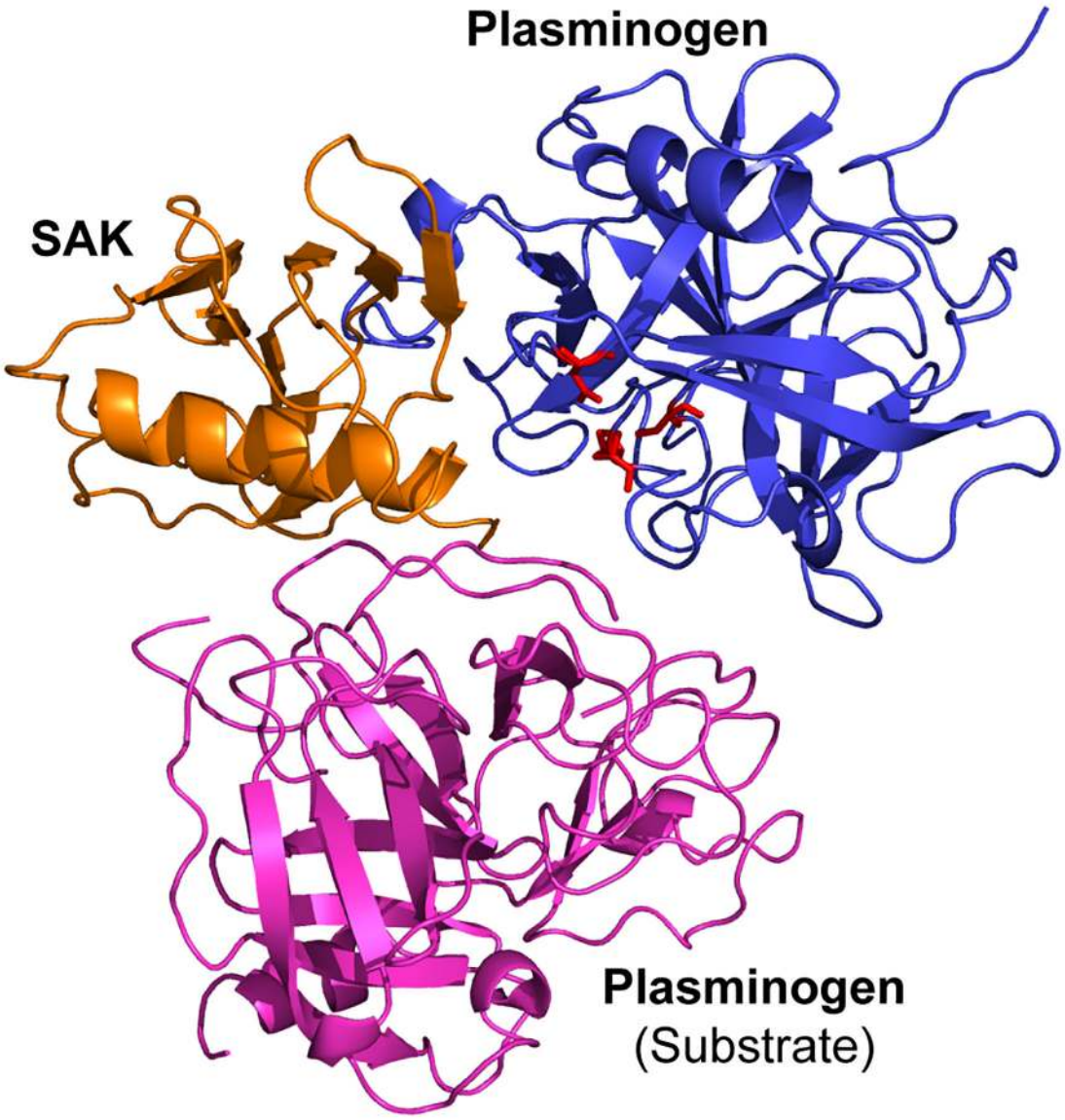

Figure 9:

Crystal structure of Sak in complex with 2 plasminogen molecules (1BUI) (215). While Sak binding to plasminogen does not have enzymatic activity, the trimeric complex captures how Sak may be binding to plasmin to cleave plasminogen. Sak (orange) is in complex with plasminogen (blue), exposing the catalytic site (red). Sak facilitates the docking of the substrate plasminogen (pink) to promote cleavage by plasmin.

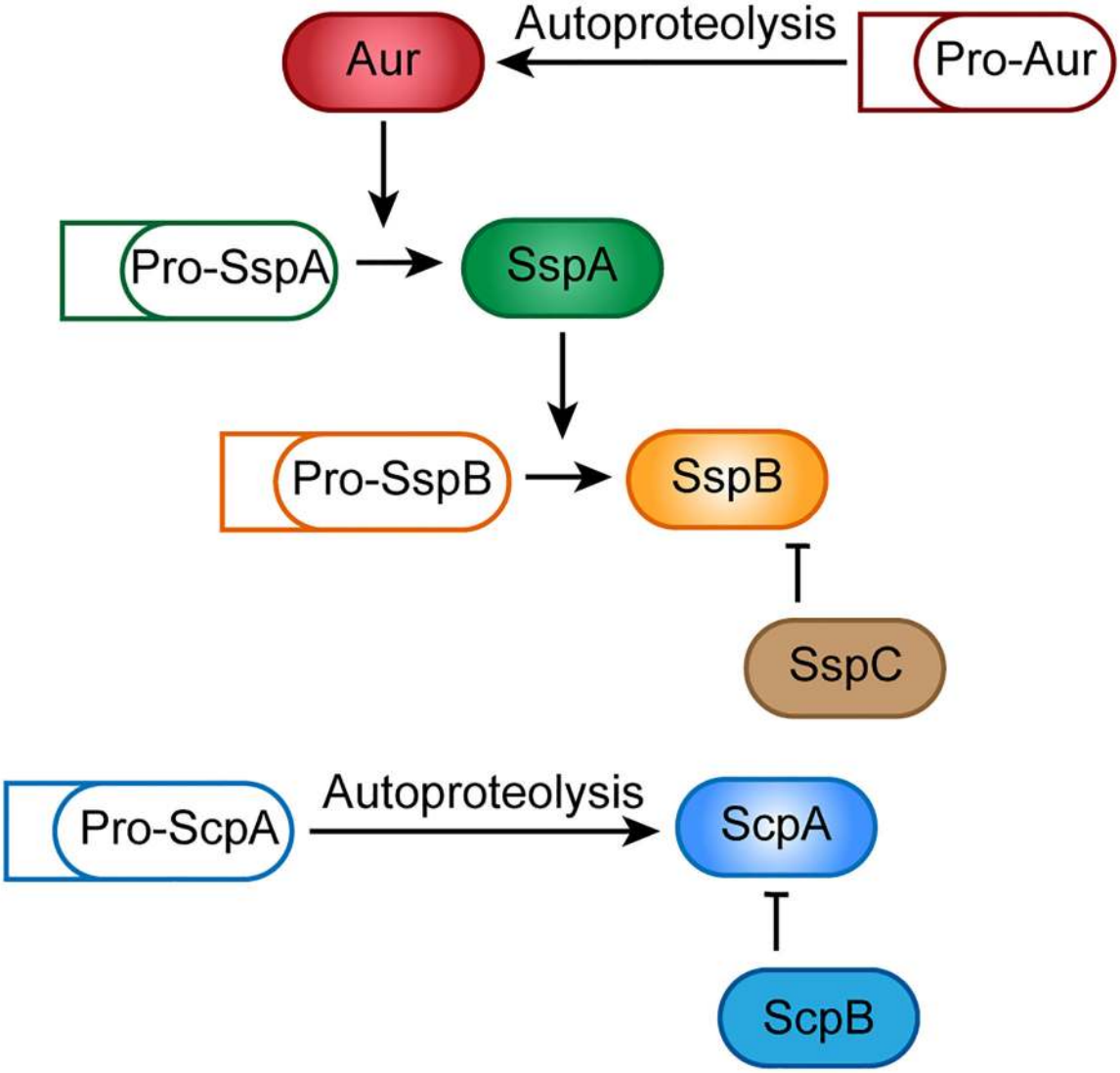

Figure 10:
Staphylococcal protease cascade. The metalloprotease, Aur is activated by autoproteolysis after protein secretion. Aur is required to activated the serine protease, SspA. SspA processes one of staphopains, SspB from zymogen to active enzyme. The other staphopain, ScpA is activated by autoproteolysis. Both staphopains are inhibited by staphostatins prior to secretion. SspB is inhibited by SspC and ScpA is inhibited by ScpB.

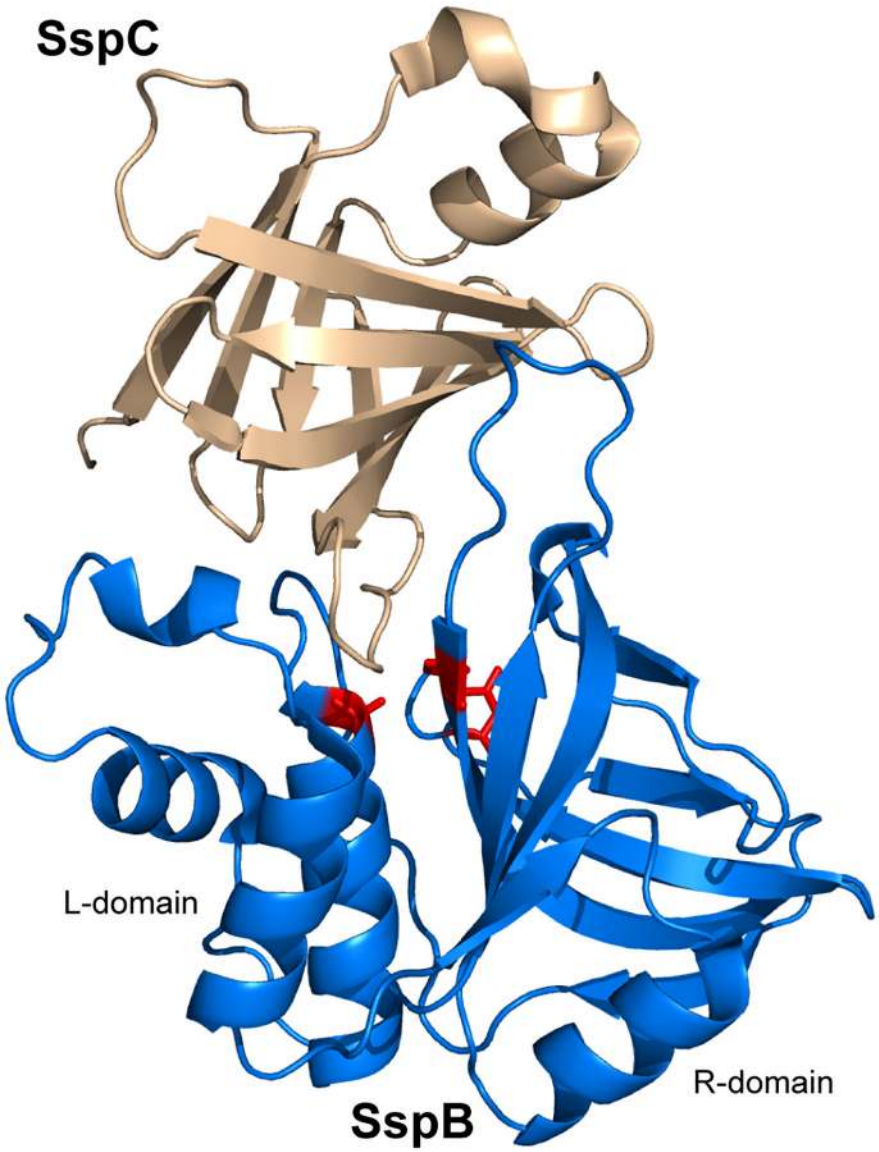

Figure 11:

Staphopain-staphostatin complex (1PXV) (293). Staphopain, SspB (blue) has 2 domains: the L-domain is helical and the R-domain consists of β-strands that fold into a β-barrel-like structure. The catalytic site of SspB is highlighted in red. Staphopain, SspC (beige) is a single domain protein comprised of 8 β-strands forming a single mixed β-barrel domain. SspC is a competitive inhibitor of SspB, directly blocking substrate access to the active site.

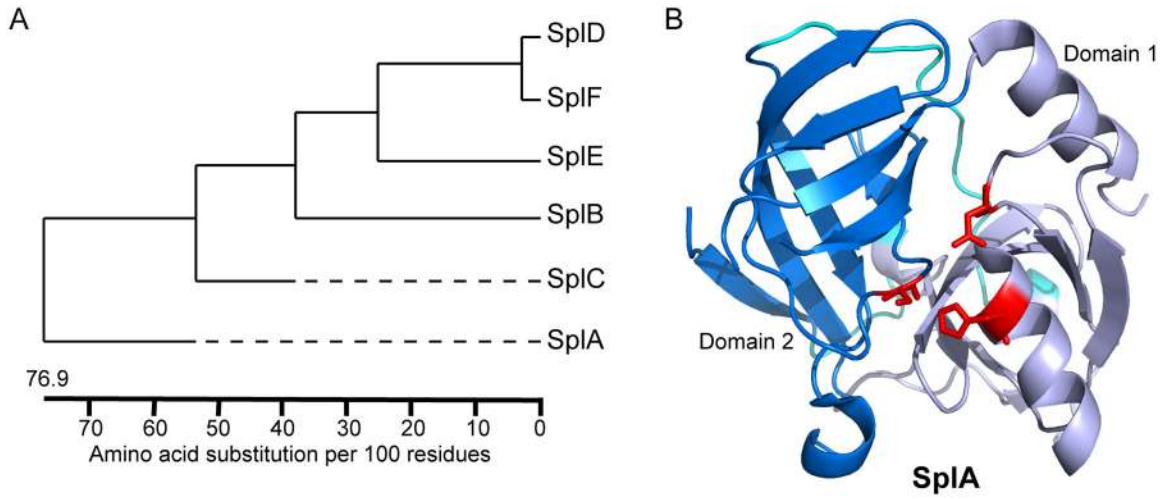

Figure 12:

A) Phylogenic tree of *S. aureus* Spls. The tree is constructed based on the mature protein sequences using the DNASTAR MegAlign ClustalW method for multiple sequence alignment. B) Crystal structure of SplA (2W7S) (299). SplA has 2 domains connected by a linker (cyan). Domain 1 (light purple) consists of α-helices and β-strands and Domain 2 (blue) is comprised of β-strands. Both domains fold into a β-barrel structure. The catalytic triad (red) is located at the center between the two domains.

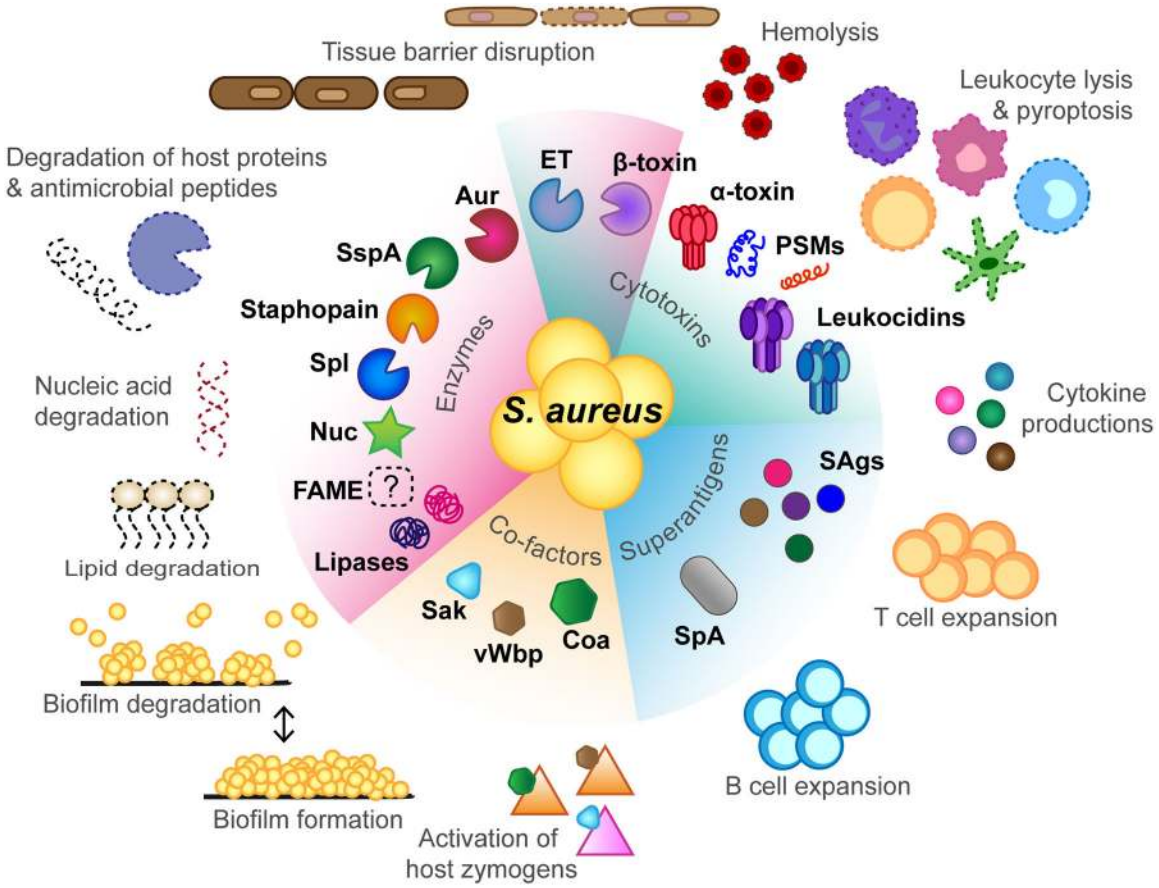

Figure 13:

S. aureus secretes many different toxins and enzymes. Superantigens are proteins that have high mitogenic properties, causing T and B cells expansions that result in clonal deletion and massive cytokine production. Cytotoxins, such as α-toxin and the leukocidins, cause cytokine production, hemolysis, and leukocyte cell deaths through targeting specific cell surface receptors. The amphiphilic PSM peptides mediate cytolysis by inserting into the lipid bilayer of cell membranes. Enzymes, such as β-toxin and the ETs, cause cytotoxicity on mammalian cells, resulting in cell death, inflammation, and tissue barrier disruptions. Other enzymes, including various proteases and nucleases, mediate host protein degradations, thwarting many important host immune surveillance and defense molecules. These enzymes can also act on self-proteins to degrade biofilms for bacterial dissemination. Lipases and FAME work synergistically to degrade lipids in the environment for nutrients. Cofactors, including Coa, vWbp, and Sak, bind and activate host zymogens in the coagulation system to mediate clot formation and dissolution. Altogether, these toxins and enzyme provide critical nutrients (i.e. iron and carbon) that are important for the growth and survival of the bacteria. Importantly, they target various aspects of host immune defenses, thus contributing to the overall virulence of *S. aureus* during infections.

Table 1:

Major exotoxins produced by *S. aureus*.

Exotoxin(s)	Gene(s)	Function(s)
α-toxin	*hla*	pore-forming toxin
PVL (LukSF-PV)	*lukS, lukF*	pore-forming toxin
HlgAB	*hlgA, hlgB*	pore-forming toxin
HlgCB	*hlgC, hlgB*	pore-forming toxin
LukED	*lukE, lukD*	pore-forming toxin
LukAB/HG	*lukA/H, lukB/G*	pore-forming toxin
LukMF'	*lukM, lukF'*	pore-forming toxin
LukPQ	*lukP, lukQ*	pore-forming toxin
PSMα1 to PSMα4	*psmα1* to *psmα 4*	phenol soluble modulins
PSMβ1, PSMβ2	*psmβ1, psmβ2*	phenol soluble modulins
δ-toxin	*hld*	phenol soluble modulins
PSM-mec	*psm-mec*	phenol soluble modulins
ε-toxin	*cytE*	cytotoxin
SEA to SEE, SEG	*sea* to *see*, *seg*	enterotoxins, T cell superantigens
SE-l H to SE-l X	*selh* to *selX*	T cell superantigens
TSST-1	*tst*	T cell superantigens
SpA	*spa*	B cell superantigen
β-toxin	*hlb*	sphingomyelinase, biofilm ligase
Exfoliative toxin A	*eta*	serine protease
Exfoliative toxin B	*etb*	serine protease

Table 2:

Major secreted cofactors and enzymes produced by *S. aureus*.

Cofactor/Enzyme	Gene	Function(s)
Coagulase	*coa*	cofactor, activates prothrombin
vWbp	*vwb*	cofactor, activates prothrombin
Staphylokinase	*sak*	cofactor, activates plasminogen
Nuc (thermonuclease)	*nuc*	nuclease
Aureolysin	*aur*	metalloprotease
ScpA (V8 protease)	*sspA*	serine protease
SplA	*splA*	serine protease
SplB	*splB*	serine protease
SplC	*splC*	serine protease
SplD	*splD*	serine protease
SplE	*splE*	serine protease
SplF	*splF*	serine protease
Exfoliative toxin A	*eta*	serine protease
Exfoliative toxin B	*etb*	serine protease
Staphopain A	*scpA*	cysteine protease
Staphopain B	*sspB*	cysteine protease
Hyaluronidase	*hysA*	lyase
β-toxin	*hlb*	sphingomyelinase, biofilm ligase
PI-PLC	*plc*	phospholipase
SAL1	*lip1*	lipase
SAL2	*geh*	lipase
FAME	unknown	detoxify free fatty acids

Table 3:

Consensus cleavage sequence of Spls.

Spl	Consensus Cleavage Sequence
SplA	Trp/Tyr – Leu – Tyr – Tyr – Ser
SplB	Trp – Glu – Leu – Gln
SplC	To be determined
SplD	Arg – Trp/Tyr – Pro/Leu – The/Leu/Ile/Val
SplE	To be determined
SplF	To be determined