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REVIEW

Interaction of host and *Staphylococcus aureus* protease-system regulates virulence and pathogenicity

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Abstract

Staphylococcus aureus causes various health care- and community-associated infections as well as certain chronic TH2 driven inflammatory diseases. It is a potent pathogen with serious virulence and associated high morbidity. Severe pathogenicity is accredited to the *S. aureus* secreted virulence factors such as proteases and host protease modulators. These virulence factors promote adhesion and invasion of bacteria through damage of tight junction barrier and keratinocytes. They inhibit activation and transmigration of various immune cells such as neutrophils (and neutrophil proteases) to evade opsono-phagocytosis and intracellular bacterial killing. Additionally, they protect the bacteria from extracellular killing by disrupting integrity of extracellular matrix. Platelet activation and agglutination is also impaired by these factors. They also block the classical as well as alternative pathways of complement activation and assist in spread of infection through blood and tissue. As these factors are exquisite factors of *S. aureus* mediated disease development, we have focused on review of diversification of various protease-system associated virulence factors, their structural building, diverse role in disease development and available therapeutic counter measures. This review summarises the role of protease-associated virulence factors during invasion and progression of disease.

Keywords *Staphylococcus aureus* · Virulence factors · Proteases · Modulators of proteases · Regulators of immune cells

Introduction

Staphylococcus aureus is gram-positive facultative anaerobe that colonizes human body and acts as a commensal [1, 2]. *S. aureus* causes various acute infections such as health care- and community-associated diseases including pneumonia, cutaneous lesions, toxic shock syndrome and osteomyelitis [3, 4]. *S. aureus* also acts as pathobionts as they are associated with certainchronic TH2-biased inflammatory diseases, such as atopic dermatitis (AD) and chronic rhinosinusitis with nasal polyps [5–7].

Various factors regulate the virulence and pathogenicity of *S. aureus* [8, 9]. These factors activate virulence and metabolic pathways of bacteria to ensure its survival [10, 11]. The host immune responses are actively modulated by these factors to induce immunosuppressive effect on target areas [12, 13]. One of the major factors involved in invasion and colonization of the bacteria is the proteases system [14, 15]. Through 'protease system' we are trying to emphasize on every component of host and microbe that either regulates or regulated by proteases. Proteases can cleave specific components of host immune system, disrupt integrity of extracellular matrix/intercellular connections and counteract epithelia defense actions [16, 17]. Some *S. aureus* secreted factors can bind and regulate host protease precursors to inhibit phagocytic killing by neutrophils in blood as well as macrophages in tissues [18, 19].

Therefore, to understand the mechanism of host–microbe interaction it is necessary to study the interplay of protease system in host and bacteria. Our goal is to address the recent development and research made in this area to the

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best of our knowledge to compile a comprehensive review. Our emphasis will be to highlight the importance of proteases, their inhibitors or activators and the potential ways to regulate virulence as well as pathogenicity through factors secreted by bacteria that modulate host proteases for disease development.

Regulatory locus of virulence factors

Staphylococcus aureus virulence factors are governed directly or indirectly by various regulatory locus such as *agr* (accessory gene regulator), *sarA* (*S. aureus* accessory regulatory) homologues, *sae* (*S. aureus* exoprotein expression) and *SigB* (alternative sigma factor B) [20]. A brief description is given below.

agr locus

Staphylococcus aureus agr locus is a quorum-sensing gene cluster that regulates various virulence and cell wall associated factors [21]. In a study by Ziebandt et al. [22] showed that *agr* modulates expression of over 70 genes in *S. aureus* and 23 of them (including various proteases such as Aur, Spls, SspA and SspB) are known to directly involved in its virulence. The locus contains mainly two transcripts RNAII and RNAIII. RNAII covers the *agr*ABCD operon (involves in secretion, maturation, export of auto-inducing peptide—AIP that activates a classical two-component signal transduction system—TCSTS) and is regulated by P2 promoter. RNAIII (encodes δ-hemolysin—hld) is the effector molecule of agr operon and is regulated by P3 promoter [23]. *agr* locus is also activated by the RAP–TRAP (RNAIII activating protein-target of RAP) two-component system while it is inhibited by RIP- RNAIII inhibiting peptide [24]. RNAIII upregulates various genes associated with bacterial invasion such as leukocidins, enterotoxins (α-toxin, β-hemolysin, Toxic shock syndrome toxin 1, leucotoxins), exoproteases and lipases while downregulates genes involved in colonization such as surface adhesins, protein A, coagulase and fibronectin binding protein [25]. RNAIII regulates expression of these virulence factors both at the transcriptional and translational levels [24].

sar locus

The *sar* locus contains three overlapping transcripts (sarA ~ 0.56 Kb, sarC ~ 0.8 Kb and sarB ~ 1.2 Kb) encoding sarA ORF [26, 27]. *sar* upstream region contains three distinct promoters (P2, P3 and P1) from which these transcripts originate. *sarA* is a DNA binding protein that promotes *agr* mediated regulation of virulence factors by specifically binding to the P2 promoter region of *agr* and to a lesser extent to P3 promoter region. It forms a homodimer that interacts with the conserved A/T-rich recognition motifs present in the promoters of target genes. *sar* also works independent of *agr* and interact directly wth the SAR boxes of the promoters of various cell wall-associated proteins (protein A, fibronectin-binding proteins, collagen adhesin) and exoproteins [24]. It up-regulates α-hemolysin and represses V8 protease [28]. According to Dunman et al. [29] *sarA* regulates directly or indirectly over 120 genes. Some *sarA* homologus regulatory locus are also present in *S. aureus* such as *sarR, sarS, sarT* and *sarU* that regulates various virulence factors.

sae locus

sae is an important regulator of various virulence factors associated with bacterial adhesion, immune modulation or toxicity [30, 31]. Various studies including microarray and proteomics analysis revealed *coa* (coagulase), *hla* (α-toxin) and or *fnbA* (fibronectin-binding protein A) are regulated by *sae* [32]. *sae* locus regulates gene expression primarily at the transcriptional level unlike *agr* [24]. Transcriptional analysis showed that *sae* locus constitutes a four-component regulator system with protein-encoding regions (saeR, saeS, ORF3 and ORF4). saeS is a membrane-spanning sensor histidine kinases (composed of a signal binding domain and an autokinase domain) and saeR (composed of an N-terminal regulatory domain and the C-terminal effector domain) is a cognate cytosolic response regulator [33]. saeS-saeR acts as a bacterial two-component system in which saeS autophosphorylates upon appropriate signal and then activates saeR. In addition, three overlapping transcripts (T1 ~ 3.0 Kb, T2 ~ 2.4 Kb and T3 ~ 2.0 Kb) and one independent transcript (T4 ~ 0.7 Kb) are found in the *sae* operon. *sae* upstream region consists of 2 distinct functional promoters (P1 and P3). *sae* transcription is mainly driven P1 promoter that is strongly autoregulated, activated by *agr*, and repressed by sigma factor B [34]. On the other hand, *sae* does not affect the expression of *agr* or *sarA*. *sae* locus is reported to be influenced by environmental factors (low pH, high NaCl concentration and sub-inhibitory concentrations of antibiotics) [35].

SigB locus

It is a stress response and stationary-phase sigma factor that enhances transcription of genes that can confer resistance to heat, oxidative and antibiotic stresses [36]. SigB directly controls expression sarA promoters, and indirectly of *agr*. SigB also exerts a negative effect on agr expression (RNAIII) independently of SarA [37]. SigB is also necessary for bacterial aggregation and it is mediated by regulating expression of the genes such as clumping factor and adhesins [24].

Types of *S. aureus* proteases and their role in disease development

To manipulate the host immune responses *S. aureus* secretes various proteases such as cysteine proteases, serine proteases, serine protease–like proteins and metallo-proteinases. Earlier it was thought that these proteases assist in the acquisition of nutrients but recent reports have indicated that they interact with neutrophils, plasma proteins and antimicrobial peptides to weaken host immunity [38, 39]. Expression and synthesis of these proteases is mainly modulated by two global regulatory elements, one is *sarA* (staphylococcal accessory regulator) and other one is *agr* (accessory gene regulator). *agr* expression is directly or indirectly regulated by *sarA* which in turn modulates virulence determinant synthesis [40].

Cysteine proteases

These are papain-like cysteine proteases (staphopains) that belong to the family C47 of clan CA of cysteine peptidases. Three-dimensional structure shows a papain-like fold with two domains L- and R-. The N-terminal part represents the L-domain that contains the active site helix carrying a nucleophilic cysteine. The R domain is built around a size-stranded antiparallel pseudobarrel that carries the catalytic histidine and asparagine residues [41]. Staphopains can directly or indirectly damage the epithelium as well as underlying connective tissue [42]. There are two cysteine proteases identified staphopain A (ScpA) and staphopainB (SspB).

ScpA

ScpA is a 20 kDa protein that degrades elastin, collagen, fibrinogen, fibronectin and kininogen present in tissues [43, 44]. It is synthesized as zymogen and then this inactive precursor is self-activated outside the cell [45]. ScpA significantly affects neutrophils (major component of the innate immune response regulated by cytokines and chemokines), which plays an important role in the interaction of *S. aureus* and host [46, 47]. ScpA impairs movement as well as the activation of neutrophils towards CXCR2 chemokines (interleukin-1 to -7 that possess an E-L-R amino acid motif and a CXC motif, which binds to the CXCR2 integral neutrophil membrane protein, Fig. 1a). It does soby cleaving N-termini of CXCR2 integral protein present on neutrophil membranes [48, 49]. It also inactivates α-1-protease inhibitor and α-1-antichymotrypsin as shown in Fig. 1a [50]. ScpA is reported to inhibit the classical

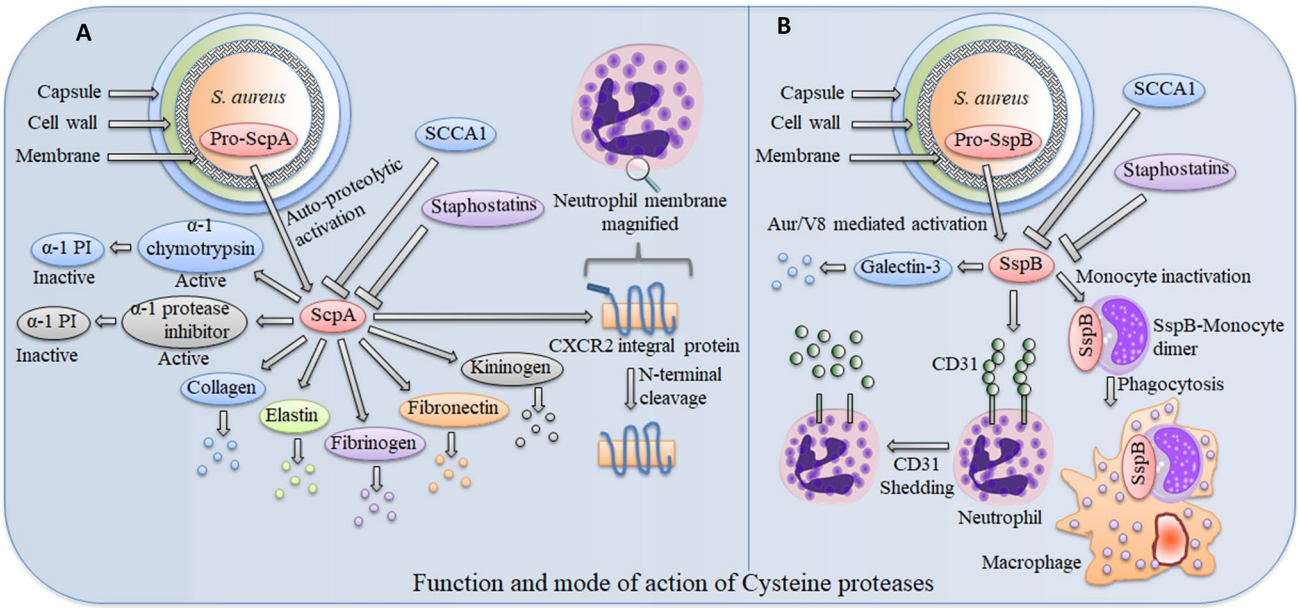

Fig. 1 Function and mode of action of Cysteine proteases. **a** Role of ScpA in host immune regulation. Immature ScpA (Pro-ScpA) is secreted out of pathogen and processed through autocatlytic activation. Active enzyme cleaves CXCR2 integral membrane protein of neutrophils and impairs neutrophil migration. It inactivates protease inhibitors (α-1-protease inhibitor and α-1-antichymotrypsin), proteins of extracellular matrix (elastin, collagen, fibrinogen and fibronectin) and kininogen. It is inhibited by endogenous staphostatins and human serpin SCCA1 (Squamous Cell Carcinoma Antigen 1). **b** Role of SspB in host immune regulation. Immature SspB (Pro-SspB) is secreted out of pathogen and processed through Aur mediated activation. Active enzyme sheds of CD31 from neutrophils imparing removal of aged neutrophils. It promotes monocyte inactivation and phagocytosis by macrophages by forming a monocyte-SspB dimer. It also inhibits immune responsive protein galectin-3. Its inhibitors are staphostatins and SCCA1

as well as alternative pathways of complement activation [51]. Human epithelial-derived serpin SCCA1 (Squamous Cell Carcinoma Antigen 1) can inhibit the activity of staphopains *in-vivo* at epithelial surfaces infected by *S. aureus* (Fig. 1a). SCCA1 interacts with ScpA forming a covalent complex in which the enzyme and inhibitor are linked by a thio-ester bond. Then ScpA cleaves the peptide bond present between Gly354-Ser355 and releases the C-terminal 4.5 kDa serpin fragment. This suicide substrate mechanism is called reactive site loop (RSL) cleavage of the inhibitor by the protease that makes the enzyme inactive [52]. Another endogenous novel class of ScpA protease inhibitors is low molecular weight analogues of staphostatins (A & B, Fig. 1a). These highly specific homologues occlude the active site of ScpA in a substrate-like manner forming a long-lived inhibitor-enzyme complex and rendering the enzyme inactive [52]. Therefore, anlagos of these endogenous protease inhibitors can be screened and potent anti-ScpA drugs can be developed for abrogating the activity of these proteases, and thus virulence development.

SspB

SspB is also 20 kDa and structurally similar to ScpA though having significant difference in their nucleotide sequence. SspB is too produced as zymogen but its activation depends on Aur (aureolysin- a metalloproteinase) mediated proteolytic activation of a serine protease V8 or SspA [45]. As shown in Fig. 1b it interacts with the neutrophils and sheds of CD31 (cluster of differentiation 31, also known as Platelet endothelial cell adhesion molecule- PECAM1, involved in transmigration of leukocyte, angiogenesis, removal of aged neutrophils and activation of integrin) from its surface [39, 53]. It causes imbalance in the homeostasis of host immune cells in the inflamed tissue. SspB also hampers the antibacterial response of monocytes by repressing their chemotactic activity and by forming a dimer of SspB-monocyte [9]. These SspB conjugated monocytes are subsequently phagocytized by macrophages (Fig. 1b) leading to further dampening of immune system and vigorous colonization of bacteria [39]. Human galectin-3 is an immune responsive β-galactoside-binding lectin protein [54]. It provides antimicrobial defense by activating neutrophil NADPH oxidase and inducing ROS production in neutrophils [55]. SspB is reported to hydrolyze galectin-3 and inhibit its opsonizing and bacteriostatic properties (Fig. 1b) [56]. Similar to ScpA, SspB activity is also inhibited by SCCA1 and staphostatins as shown in Fig. 1b.

Serine protease

SspA

The serine protease (SspA or V8 or GluV8) was first purified from *S. aureus* strain V8 that belong to the family of glutamyl endopeptidase [57]. SspA is similar to pancreatic serine proteases without the disulphide bridges. It also shares high structural homology of the active site with other serine proteases such as epidermolytic toxins and trypsin [58]. It is secreted as an inactive precursor and Aur is required for activation as well as maturation of this protease (Fig. 2) [45, 59]. SspA specifically targets peptide bonds present on the carboxyl side of glutamate (and aspartate, to a lesser extent) residues of all immunoglobulin classes [9]. As shown in Fig. 2 SspA degrades the Fc region of immunoglobulins that disturbs the effector function and leads to partial loss of antigenic determinants of the antibody. It blocks and impairs the interaction of cell surface antigens to immune effector cells mediated by immunoglobulins [60]. Cleavage of IgG hinge by SspA hinders cellular and humoral immune responses induced by pathogens [61, 62]. Staphylococcal binder of immunoglobulin (Sbi) also interacts with the Fc region of IgG and enhances this immune-suppressor response (Fig. 2) [63, 64]. To counteract this invasion tactic of pathogens, host releases anti-hinge autoantibodies that specifically interact with cleaved IgGs and restore Fc-mediated cell-killing functions [65, 66]. It is reported that the production of anti-hinge antibodies can be boosted by an immunization strategy of pathogen-mediated proteolysis of host IgGs. When animals were immunized with peptide analogs of the cleaved IgG hinge, significant reduction in *S. aureus* colony formation was observed. This substrate specificity is attributed to the positively charged N-terminus of the enzyme. Broad-spectrum inhibitor Pefabloc SC is reported to inhibit activity of SspA (Fig. 2) [56]. It is reported that a single mutation in SspA impart a modest impact on the daptomycin antibiotic tolerance in MRSA [67].

It is reported that SspA is significantly secreted during the development of AD (a common chronic relapsing inflammatory skin disease) infection [68]. SspA affects skin integrity by damaging stratum corneum in murine model in in-vivo conditions [40, 69, 70]. Recently it is shown that tight junction damage and impairment of barrier integrity by SspA is caused by keratinocyte damage as shown in Fig. 2 [71]. AD induces the expression of proinflammatory cytokine IL-1β that in turn activates production of antimicrobial/host defense peptides (HDPs) such as β-Defensins (human β-defensin 2- hBD2) [72]. Soluble protective factor hBD2 inhibits SsspA (Fig. 2) and is sufficient to protect keratinocytes against SspA mediated damage [68]. This protective phenotype of hBD2 against SspA-induced skin barrier dysfunction can be targeted for therapeutic applications instead of corticosteroids to restore skin lesion.

Epidermin leader peptide processing serine protease (EpiP)

It acts as a subtilisin-like serine protease that can cleave both casein and collagen (Fig. 2) [73, 74]. It possesses a

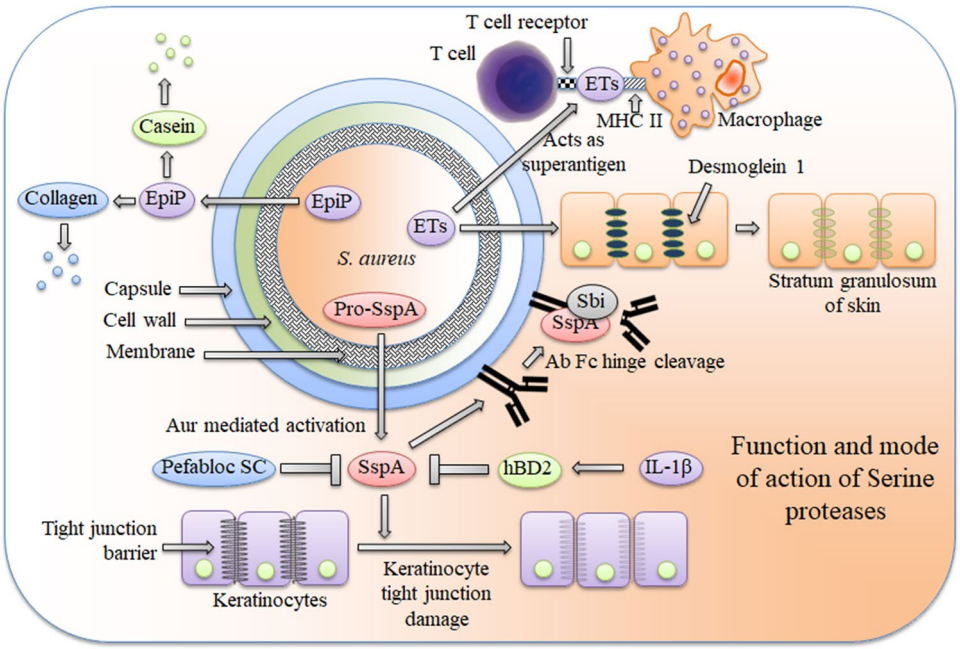

Fig. 2 Function and mode of action of serine proteases. Immature SspA (Pro-SspA) is secreted out of pathogen and processed through Aur mediated activation. Active enzymealong with Sbi (Staphylococcal binder of immunoglobulin) blocks and impairs immunoglobulins by degrading their Fc region. It dampens barrier integrity by damaging tight junction in the karatinocytes. Antibiotic pefabloc-Sc and defense peptide hBD2 (human β-defensin 2) inhibits SspA activity. ETs acts as superantigens and mediate interaction between T-cell receptor and MHC-II of macrophages. ETs also cleave Desmoglein 1 required for cell-to-cell keratinocyte adhesion impairing membrane integrity. EpiP cleaves casein and collagen

peptidase-S8 domain carrying the catalytic triad of Aspartic acid, Histidine, and Serine. It also has an N-terminal prodomain (having 4 β strands flanked by 2 α helices) and a C-terminal protease domain (having subtilisin-like serine protease fold). In the extracellular matrix it is secreted as a zymogen and then processed by an autocatalytical intramolecular mechanism. It shows homology to the extracellular epidermin leader peptidase of *S. epidermidis* that mediates proteolytic processing of the mature l-antibiotic (peptide antibiotics/bacteriocins containing polycyclic thioether amino acids—lanthionine or methyllanthionine) epidermin. Though epidermin synthesis is not sure in *S. aureus*, EpiP provide increased virulence through some unknown mechanism. Vaccination with a recombinant EpiP (rEpiP) and a noncleaving mutant (rEpiP-S393A) peptide significantly reduced *S. aureus* infection in mice [74]. EpiP also shows homology with *Streptococcus pyogenes* SpyCEP protease that impairs IL-8 by cleaving its C-terminal fragment. It leads to inhibition of neutrophil recruitment at the locus of bacterial infection [75, 76].

Exfoliative toxins (ET)

These are serine proteases carrying chymotrypsin family catalytic triad of Serine, Histidine and Aspartate residues [77]. *S. aureus* possesses two main biologically, antigenically and serologically distinct ETs namely ETA (26.9 kDa, heat stable, located on chromosome) and ETB (27.2 kDa, heat-labile and located in large plasmid). X-ray crystallography structure shows two main domains (S1 and S2) made of six-strand β-barrels and a C-terminal α-helix [78, 79]. The active site carrying the catalytic triad is located on the interface of two barrels [80]. They possess esterolytic activity (intrinsic *N*-t-butyloxycarbonyl-L-glutamic acid alpha-phenyl esterase activity) and mitogenic activity but their mitogenic activity is independent of their proteolytic activity [81]. ETs reported to cause skin infections such as staphylococcal scalded skin syndrome in newborns, old people and immunocompromised patients [82]. Broad-spectrum serine protease inhibitor DFP (diisopropylphosphorofluoridate) impairs esterolytic activity of ETB but not ETA. As shown in Fig. 2, ETs are also reported to be superantigens (proteins that interact simultaneously with the T-cell receptor and to the major histocompatibility complex class II, leading to induction of T cells expressing specific Vβ subsets of the TCR repertoire). ETs specifically cleave Desmoglein 1 (a desmosomal glycoprotein of cadherin superfamily that mediates keratinocyte cell-to-cell adhesion in the stratum granulosum of the skin) but not Desmoglein 3 (Fig. 2) [83, 84]. Hydrolysis of Desmoglein 1 depends upon its proper conformation as unfolded protein is not cleaved [85]. ETs cause staphylococcal scalded skin syndrome (SSSS) and bullous impetigo. Symptoms of

the diseases include severe loss of superficial skin layers, blister formation, dehydration and secondary infections [86]. ETs share high homology with SspA and both probably act together to disrupt stability and barrier function of the skin through desmoglein1 degradation [87].

Serine protease–like proteins (Spls)

S. aureus secretes 6 serine protease–like proteins (SplA-SplF) that are encoded in a single operon known as νSaβ pathogenicity island [88, 89]. All Spls share high sequence homology and they are specific for *S. aureus* only [90, 91]. They are referred as serine protease–like proteins because they show significant amino acid homology with SspA protease and ETs [90, 92, 93]. Spls are directly regulated by sae (*S. aureus* exoprotein expression locus that modulates various virulence factors) regulatory system [94–96]. They posses a signal peptide and chymotrypsin-like fold that contains two domains consisting of six antiparallel β strands folded into a β barrel. Active site of Spls carries Histidine, Aspartic acid and Serine in the interface of two β barrels [97, 98]. Upon docking of a substrate containing the consensus sequence motif Tryptophan-Glutamic acid-Leucine-Glutamine, Spls form a oxyanion hole in their three dimensional structure [99]. Spls are reported to have individual and very unique protease activity as they target specific proteins involved in host-pathogen interaction rather than general bacterial nutrition [93, 98, 99]. Spls trigger TH2 cytokines and elicit production of IgE antibody in response to allergens [100]. They are released as soluble proteins in considerable amounts so that they can be sequestered from the adjuvant bacterial cells, which can drive a TH1/TH17 response (that may override the Spl effects).

Spls are reported to be active in various common chronic diseases such as asthma and pneumonia causing disseminated lung damage [9]. These proteases along with α-hemolysin (pore-forming toxin) lead to dysfunction of epithelial barrier facilitating allergen invasion and subsequent allergy response. In a study systematic analysis of virulence factors identified Spls that impose strongest and frequent induction of human immune memory of proteins mostly allergen-specific IgE antibodies [101]. Spls significantly aggravate chronic allergic inflammation by enhancing the pre-existing TH2-bias and cytokine (mainly IL-4, IL-5, and IL-13) response. It induces anti-inflammatory cytokine IL-10 but do not elicit IFN-g, IL-6, TNF, and IL-17 [101]. Spls also elicit IgG4 antibody activity. Analysis of Hla (an immunomodulatory virulence factor and a potent cytolysin) activity in Δspl::erm (allelic replacement spl operon mutant) reveals that hemolysis is unaffected by the Spls [102, 103]. Analysis of surface proteomic of wild type and Δspl::erm revealed various virulence factors (complement inhibitors, hemolysins, adhesins) regulated by Spls [104].

SplA

SplA is reported to promote bacterial invasion and spread in the lung tissues by cleaving a glycosylated cell surface protein mucin 16 [104]. Mucin 16 is a ~ 25-MDa, cell surface protein that is heavily glycosylated and acts as a protective barrier against pathogens through lubrication of epithelia [105]. Analysis of 3D structure and investigation of substrate cleavage site revealed that SplA has specific preference for W/Y-L-Y↓T/S (Trp/Tyr-Leu-Tyr-Thr/Ser) motif. It cleaves mucin 16 at Y-L-Y-S site and induces shedding of mucin 16 from epithelia (Fig. 3a) [106, 107]. Mucin 16 is also present in epithelial cells of ocular surface, airway and female reproductive tract epithelia [108]. Mucin 16 impaired human epithelial cell lines showed more susceptibility towards bacterial colonization and invasion [109]. When treated with a serine protease inhibitor 3,4-DCI (3,4-dichloroisocoumarin), it significantly reduced this shedding effect. This shedding property of SplA upsets integrity of airway epithelial barrier and promotes disease development [110].

SplB

It belongs to the chymotrypsin family of serine proteases (family S1, small subfamily S1B). No known targets of SplB have been identified but transmembrane sensory proteins olfactory receptor could be a potential target of these proteases as they contain W-E-L-Q motif in their sequence [99]. SplB has been shown to stimulate specific antibody production in staphylococcal liquid culture [111]. They are devoid of any propeptides so it is difficult to obtain their potential inhibitors. Though it has a signal peptide that imposes an inhibitory effect [97]. Crystal structure of zymogen and mature enzyme does not offer any major conformational change in the active site. Only a unique network of hydrogen bonds distant form the active site is formed by the new N-terminal glutamic acid of mature SplB [97]. Apart from inhibiting the SplB activity, the signal peptide mediates targeting of SplB into extracellular compartment. Proteolytic activity of SplB is triggered by signal peptidase only upon secretion. Rearrangements due to hydrogen bonding formation orchestrate a substrate binding cleft and an oxyanion hole in the mature enzyme. In a study novel α-aminophosphonic analogues of glutamine and their peptidyl derivatives have been tested. One of the compounds—Cbz-Glu-Leu-$Gln^{P}(OC_6H_4\text{-}4\text{-}O\text{-}CH_3)_2$—displayed an apparent second-order inhibition rate value of 1400 $M^{-1}s^{-1}$ [112].

SplD

Potent activity of SplD is reported during various allergic responses. It causes increased airway hyper-reactivity and greater mucus production in allergic airway in mice. It has

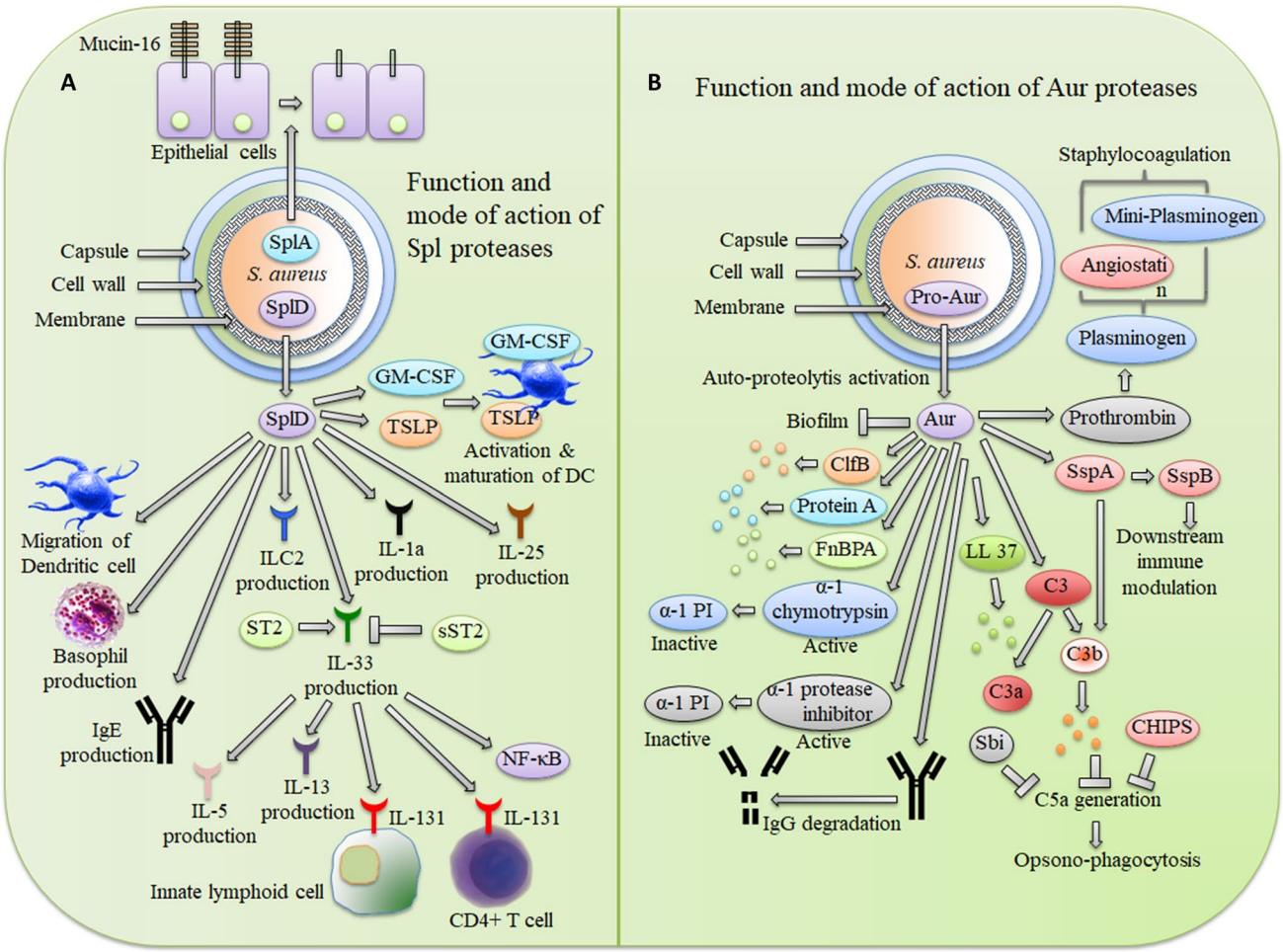

Fig. 3 Function and mode of action of Spl and Aur proteases. **a** Role of Spls in host immune regulation. SplA cleaves glycosylated cell surface protein mucin 16 that acts as a protective barrier against pathogens. SplD mediates allergy response by activating production of IL-1a, IL-25, TSLP (thymic stromal lymphopoietin), GM-CSF(Granulocyte–macrophage colony-stimulating factor), IgE and basophil. SplD also promotes maturation and migration of dendritic cells. SplD induces IL-33 production which then regulates various downstream factors such as NF- κB, IL-5, IL-13, innate lymphoid cells and CD4 + T cells. Il-33 mediated response is induced by ST2 while inhibited sST2 (soluble suppression of tumorigenicity 2). **b** Role of Aur in host immune regulation. Immature Aur (Pro-Aur) is secreted out of pathogen and processed through autocatlytic activation. Active enzyme then activates SspA and SspB. Aur cleaves C3 into C3a and C3b. Then Aur inhibits C3b deposition and C5a generation, and thus inhibits opsono-phagocytosis. CHIPS (chemotaxis inhibitory protein of *S. aureus*) and Sbi also assist in C5a inhibition. Aur also cleaves IgG, FnBPA (Fibronectin protein A), Protein A, ClfB (Clumping factor B), α-1-protease inhibitor and α-1-antichymotrypsin. Aur activates prothrombin to induce staphylocoagulation

the more target specificity than SplA and SplB. It preferentially cleaves targets on R-Y/W-P/L-T/L/I/V↓S residues but can also cleave substrates with slight variation in these residues [93]. During idiopathic or severe asthma *S. aureus* releases enterotoxins (superantigens that have potent mitogenic activity on T cells) that are IgE antibodies specific [113, 114]. Production of SplD-specific IgE (as well as eotaxin, bronchial hyperreactivity, eosinophilia, goblet cell hyperplasia and TH2 cytokine response) was evident in the local draining lymph nodes/airways of mice after intratracheal applications of recombinant SplD without adjuvant [101, 115]. SplD-specific IgE was also found in human airway mucosa. Eosinophilia and increase in TH2 cytokine production is IL-33 dependent, while IgE production and goblet cell metaplasia are independent of this pathway [115]. Cleavage and maturation of IL-33 does not depend on SplD but rather on endogenous neutrophil elastase or cathepsin G from neutrophils, which are released during bacterial infections [116].

SplD-mediated allergic response is also characterized by production of IL-1a, IL-25, TSLP (thymic stromal lymphopoietin that is a pleiotrophic cytokine involved in pathogenesis of asthma) and GM-CSF (Granulocyte–macrophage colony-stimulating factor secreted by immune cells) but it

does not cause degranulation of mast cells (Fig. 3a). Additionally, ILC2s and basophils are also produced [117]. SplD treatment also promotes migration of Dendritic cells (DCs that help in antigen processing and immune response initiation) to lung and local lymph nodes where they probably mediate priming of TH2 cell differentiation from naive T cells (Fig. 3a) [115, 118]. DC maturation is mediated by IL-33 while population of local T- and B-cell is not affected by IL-33 [119, 120]. SplD also induces the secretion of OVA-specific IgE antibodies when given in combination with OVA. OVA is an inert protein but when *S. aureus* enterotoxin B (a superantigen) is present it can activate TH2 response and bronchial hyperreactivity [121, 122]. This response is independent of the IL-1 receptor pathway and mediated by activation of inflammatory DCs and polyclonal T cells [123].

According to Hammad et al. [124] SplD mediated allergic responses are independent of TLR4 (Toll-like receptor 4—a transmembrane protein that also activates NF-κB and immune responses in presence of allergens) [125]. Additionally, SplD did not directly activate TLR2 in-vitro. sST2 (soluble suppression of tumorigenicity 2) inhibit production of PAR2 + cells (that act independently of TLR4) in the lungs after SplD treatment (Fig. 3a) [126]. PARs are cell surface Protease-Activated Receptors that recognizes allergens and play a key role in TH2 cytokine response [127, 128]. They lead to cleavage of intercellular epithelial tight junctions, CXCR1 present on neutrophil surfaceand CD23 as well as CD25 receptors present on immune cells thereby enhancing allergy development [129, 130].

SplC, SplE and SplF

SplC shows structural homology to SspA and ETs [131]. It shows restricted substrate specificity and remains inactive in absence of a substrate. Because of an unusual conformation of the adjacent flexible occulding loop its active triad (His, Asp, Ser) remains blocked. In this case, the catalytic His residue is rotated outside the catalytic cleft due to steric conflict with the adjoining Gly–Gly residues. It is postulated that catalytic activity is retained when a substrate induces conformational switch in the occluding loop developing an oxyanion hole [92]. SplC contains a trypsin like fold with two perpendicular β-barrels interrupted by a short helix and a longer helix at the C terminus. It also has a hydrophobic S1 substrate-binding subsite that can capture non-polar side-chain of the substrate [92].

SplE shows unique substrate specificity because of the unusual or distorted organization (sterically restricts unspecific substrate access and oxyanion hole formation) of its S1 subsite. It also shows P1 subsite substrate specificity, which is not found in other spl proteases. It prefers (Y/F/W)-L-(H/Q)↓ consensus motif in its substrates. SplE contains chymotrypsin-like fold consisting of two b barrel domains [132].

SplF is found in human in nasal polyps and airway mucosa released from intramucosal bacteria during allergic responses. In asthma it shows similar inflammatory response to that of SplD such as allergic sensitization and eosinophilia [93, 115]. SplD and SplF share 94.6% similarity, therefore, speculated to share similar properties [90].

Metalloproteinase

Aureolysin (Aur)

They belong to the M4 family of metallopeptidases (contains one zinc ion for enzyme activity and four calcium ions for structural stability). It contains 301 amino acids that folds into a β-pleated N-terminal domain and an α -helical C-terminal domain [133]. Aur is produced as a zymogen that contains an N-terminal fungalysin-thermolysin-propeptide (FTP) domain. During secretion and passage across the outer membrane rapid autocatalytic processing of FTP occurs rendering Aurproteolytic activity (Fig. 3b) [134]. Then Aur initiates staphylococcal proteolyticcascade, which involve activation of serine protease SspA, and cysteine protease SspB (Fig. 3b) [53]. Aur is reported to actively inhibit phagocytosis and killing of bacteria by neutrophils through repression of the classical as well as alternative pathways of complement system [51]. Aur mimics C3 convertase and cleaves C3 (central component of the complement system) in the α-chain yielding C3a and C3b as shown in Fig. 3b [135]. C3b opsonizes bacteria; activate potent anaphylatoxin C5a (attracts and activates phagocytes) and phagocytosis. Aur inhibits C3b deposition by working in collaboration with host factors H and I. Therefore, it also inhibits C5a generation and neutrophil chemotaxis. Another *S. aureus* secreted protein CHIPS (chemotaxis inhibitory protein of *S. aureus*) along with Aur helps in inhibition of neutrophil chemotaxis by impairing function of the C5a and formylated peptide receptors [136, 137]. Inhibition of C3 induced immune response by Aur is also assisted by *S. aureus* Sbi [63, 64]. Aur may act along side SspA to mediate these complement inhibitor responses as well as cleavage of staphylococcal surface-associated proteins such as fibronectin-binding protein and Protein A [40, 135]. It also regulates and cleaves fibrinogen-binding protein clumping factor B (ClfB, Fig. 3b). ClfB is surface-associated protein that is covalently attached to the cell wall and mediate adherence of bacteria to immobilized fibrinogen as well as to the blood clots. Proper reason of this cleavage is unknown but it is hypothesized that cleavage of ClfB may induce detachment of bacterial cells from colonized sites and assist in the spread of infection within the host [138]. Aur is also

reported to inhibit biofilm formation, which is assisted by other proteases such as ScpA, SspA and SspB [139, 140].

Aur affects immune response development by regulating the degradation of Igs, cathelicidin-derived antimicrobial peptide LL-37, serpin-type plasma protease inhibitors α1-proteinase (endogenous protease inhibitor that regulates neutrophil serine protease elastase) as well as α1-antichymotrypsin (Fig. 3b) [50, 84]. Cleavage of antimicrobial peptides by Aur might protect staphylococci inside the phagocytes [141, 142]. It also promotes bacterial escape from the host immune system by converting plasminogen into angiostatin and mini-plasminogen (mini-plasminogen activates urokinase-type plasminogen activator-uPA to hydrolyse fibrin). Aur also targets the activation of prothrombin (that promotes staphylocoagulation) and fibrinolytic system leading to bacterial spread and invasion as shown in Fig. 3b [143]. EDTA that acts as ion chelator is reported to inhibit Aur activity [56]. Thrombin-derived TCPs (C-terminal peptides) acts as HDPs (host defense peptides) that interact with bacterial lipopolysaccharide and provide both anti-inflammatory and anti-microbial activities *in-vitro*, with therapeutic potential against infection and septic shock *in-vivo*. Proteolytic signatures or peptide biomarkers for Aur have been studied which could be explored for the development of novel therapeutics [144].

Autolysins/adhesins (LytM)

These peptidoglycan hydrolases (similar lysostaphin-like metalloproteases or endopeptidase) are secreted from *S. aureus* that is involved in various responses such as bacterial cell wall metabolism, cell division, daughter cell separation, growth, antibiotic mediated cell lysis and pathogenicity [145]. Autolysin Aaa is involved in the adherence of *S. aureus* to surface-adsorbed extracellular matrix proteins fibrinogen and fibronectin. It could not lyse pentaglycine but when fused with catalytic and cell wall binding domains (CWBDs) of lysostaphin (a zinc-dependent 27 kDa glycyl glycine M23 family metalloendopeptidases from *S. simulans* that cleaves the N-acylmuramoyl-Ala bond between the cell wall peptidoglycan and the crosslinking peptide), the chimeric protein could effectively display anti-staphylococcal activity [146–148]. CWBD mediates transport of enzyme to the bacterial outer surface, assisting in the direct contact of catalytic domain with PG substrates. Therefore, LytM can also be targeted for the development of new antibacterial therapeutics. These possess similar active site and core folding motif but have highly divergent folds [149]. It is also zinc-dependent enzyme with N- and C-terminal. LytM synthesis has been shown to be elevated in vancomycin-resistant *S. aureus* [150, 151]. In *S. aureus* its expression is negatively modulated by RAT that is a regulator of autolytic activity [152]. Series of (phenylureido) piperidinyl benzamides have been identified as the prospective inhibitors of Autolysin E [153]. Atl (Major Autolysin/peptidoglycan hydrolase with amidase and glucosaminidase domains) facilitate interaction of *S. aureus* to various host cellular components during infection such as fibronectin, gelatin, thrombospondin 1, vitronectin and heparin [154]. Atl also induces platelet activation and aggregation in whole blood [155]. Targocil is a novel bacteriostatic inhibitor that blocks growth of various *S. aureus* strains by impairing Atl translocation across *S. aureus* membrane. It targets the TarG subunit of the TarGH (WTA-wall teichoic acid translocase) that transports WTA across the membrane to the wall. WTA is essential for maintaining cell surface integrity, growth and tissue colonization during infection [156].

sa0205

This enzyme from *S. aureus* also carries lysostaphin-type endopeptidase catalytic domain [157]. It is 192 amino acid long and possesses a 25 amino acid N-terminal signal sequence that acts as a membrane anchor followed by a short 23 amino acid linker with unknown function [146, 158]. Linker region in LytM and lysostaphin is quite long (159 and 227 amino acids respectively). For catalytic activity it requires a single zinc ion but high concentration of zinc is shown to inhibit enzyme activity. It is regulated by vancomycin-resistance associated sensor/regulator (VraSR) two-component system that modulates synthesis of cell wall positively. Therefore, it is involved in resistance to vancomycin bacterial cell wall synthesis [159].

Types of *S. aureus* factors that modulate host proteases for disease development

For the induction of virulence and pathogenicity, in addition to proteases various other factors are secreted by *S. aureus* that activate or inhibit immune responsive host proteases.

Coagulase (Coa)

The outstanding pathogenic potential of *S. aureus* is attributed to the capacity of bacteria to evade immune responses. One of the mechanisms is the development of a pseudocapsule in the abscesses and the lesion area that protect the bacteria from host immune cells. This is facilitated by clotting factors Coa, a polypeptide that binds and initiates non-proteolytic activation of prothrombin [160]. Coa possesses two N terminal α-helical domains (D1 and D2), linker region and a C-terminal end of tandem repeats [161, 162]. D1 and D2 of Coa (acts as a cofactor) interact with the C-terminal β-chain of prothrombin inducing a conformational change and forming a functional active site [163]. This alteration

in conformation is brought by the insertion of Ile^{1}-Val^{2} of the D1-D2 domain into Ile^{16} pocket of prothrombin. The resulting complex is known as staphylothrombin that induce conversion of fibrinogen to fibrin and fibrinopeptides as shown in Fig. 4a [164]. The formation of fibrin networks promotes clotting of blood or plasma. It also defends *S. aureus* phagocytic as well as neutrophil clearance and facilitates spread of pathogenesis in blood stream [165, 166]. Coa is also regulated by sar/agr locus. Coa mediated activation of prothrombin also promotes direct cleavage of complement component C3 and its activation fragments (Fig. 4a). In addition, thrombin can cleave C5 into C5a independent of C3. Dabigatranandargatroban are reported to be potent inhibitors of staphylothrombin [167, 168].

vonWillebrand factor binding protein (vWbp)

vWbp (a large multifunctional glycoprotein) is another *S. aureus* sectreted protein that facilitates blood clumping. It is 270 kDa in size and exists as homodimer or multimer. It possesses a positively charged N-terminal domain (homologous to D1 and D2 domain of Coa), a unique hydrophobic vWF motif (interacts with the A1-domain of VWF) and a more polar fibrinogen binding site [165, 169]. Similar to Coa it also activates prothrombin to cleave fibrinogen into fibrin. Coa and vWbp works along with clumping factor A (ClfA) to form fibrin cables and induce coagulation cascade as shown in Fig. 4a [170, 171]. It leads to the formation of abscesses, lethal sepsis and bacterial spread in host tissues. vWbp also interacts with fibronectinas well as factor XIII and triggers non-proteolytic activation of FXIII (helps in fibrin crosslinking during blood coagulation) zymogen *in-vitro* [172]. vWbp is also associated with endovascular infections such as endocarditis. VWF in the inflamed or damaged endothelium bridges to the secreted vWbp which in turn interacts with SrtA (Sortase A)-dependent staphylococcal surface protein ClfA (Fig. 4a). *S. aureus* SrtA is a transpeptidase that interacts with MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) such as ClfA with the C-terminal cell wall sorting signal containing a Leu-Pro-X-Thr-Gly (LPXTG) motif. This complex helps bacterial cells to overcome shear forces of flowing blood and anchoring themselves in the vascular endothelium. It promotes bacterial adherence to the host cell and extracellular matrix [173, 174]. Direct thrombin inhibitors are effective against vWbp mediated agglutination cascade [175].

α-toxin

One of the main cytotoxic agents secreted by *S. aureus* is the α-toxin or α-hemolysin (Hla). It belongs to the pore forming beta-barrel toxin family characterized by seven heptameric monomers that forms a pore in the cell membrane [176]. The mature extracellular protein is 33 kDa [177, 178]. It forms a membrane-inserted heptamer by self-assembly to promote host cell lysis and death. Naturally occurring catechin epigallocatechin gallate is reported to impair the haemolytic activity of α-toxin by blocking heptamer self-assembly [179]. It is regulated at transcriptional and translational level by two long non-coding RNAs, SSR42 (effector of *Rsp*-repressor of surface proteins regulator) and RNAIIII (*agr* regulator) [180]. It causes cellular death by necrosis, apoptosis or pyroptosis [181]. In alveolar epithelial cells it interacts with receptor ADAM10 (A Disintegrin and metalloproteinase domain-containing protein 10) that promotes activity of ADAM10 (also known as CDw156 or CD156c) metalloprotease (Fig. 4b) [182]. It has a disintigrin and cysteine-rich domain that is distinct from the active site plays a critical role in the regulation of protease activity and substrate specificity [183]. Their primary function is to cleave membrane proteins such as TNF-alpha and E-cadherin at the cellular surface (Fig. 4b) [184, 185]. TNF-alpha is a cytokine involved in systemic inflammation and produced by immune cells such as macrophages and CD4+ lymphocytes. E-cadherin (calcium-dependent adhesion) is essential for formation of adherens junctions to bind cells with each other [186]. Cleavage of these proteins leads to disruption of epithelial barrier function, increased staphylococcal invasion and disease development [9]. α -toxin causes high tissue toxicity and loss of skin epithelial integrity in human skin model [187]. It is also reported to cause pulmonary infections of cystic fibrosis in mice and rapid chondrocyte death in bovine cartilage during septic arthritis [188, 189]. α -toxin also leads to platelet aggregation (at first prevents *S. aureus* but later damages thrombosis in liver) and multi-organ dysfunction during sepsis. Administration of α-toxin neutralizing antibody (MEDI4893*) did not affect the initial and beneficial platelet recruitment but impaired platelet aggregation and subsequent liver damagein mice [190, 191]. Another human anti- α -toxin IgG1 monoclonal antibody is MEDI4893 (Suvratoxumab) that impairs α -toxin lytic activity *in-vitro* and *S. aureus* fitness [192]. Cerulenin (inhibits fatty acid and steroid biosynthesis) is reported to inhibit secretion of *S. aureus* α-toxin [193]. Flavonoid Lysionotin (5,7-dihydroxy-4′,6,8-methoxyflavone) derived from *Lysionotus pauciflorus* is reported to protect human epithelial cells by inhibiting α-toxin expression. Lysionotin can also protect mice from pneumonia caused by *S. aureus* [194]. AP-1 family mammalian transcriptional regulator c-Jun proto-oncoprotein is phosphorylated and activated by α-toxin. Once activated c-Jun protects Lung Epithelial Cells from α-toxin [195]. It is reported that sub-inhibitory concentration of Mupirocin (pseudomonic acid A, competitive inhibitor of isoleucyl-tRNA synthetase) impairs α-toxin by downregulating *agr, saeRS* and *sarA* [196]. α-toxin is inhibited by another flavonoid Eriodictyol [197]. In recent

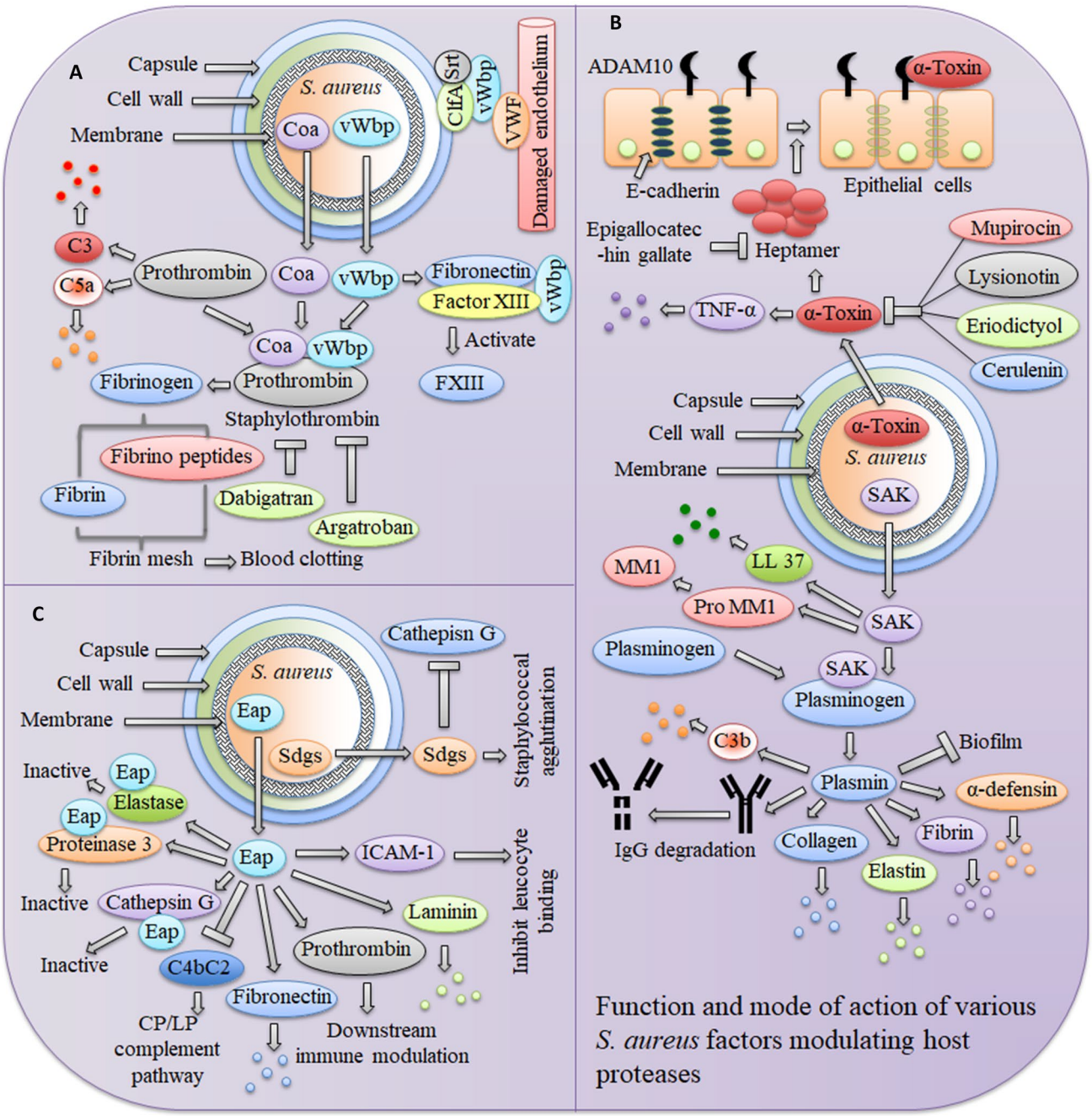

Fig. 4 Function and mode of action of various *S. aureus* viulence factors. **a** Role of vWbp and Coa in host immune regulation. Coa and vWbp induce coagulation cascade by interacting with prothrombin which cleave fibrinogen into fibrin and fibrino peptides. Coa mediated activation of prothrombin also promotes direct cleavage of C3. In addition, thrombin can cleave C5 into C5a independent of C3. Dabigatran and argatroban inhibit interaction of prothrombin with Coa and vWbp. vWbp also promotes association of bacteria with the damaged vascular tissue by forming a complex with VWF (von Willebrand Factor), ClfA and SrtA (Sortase A). vWbp also activates enzyme FXIII by interacting with fibronectin and Factor XIII. **b** Role of α-Toxin and SAK in host immune regulation. α-toxin forms hepatmer to promote host cell lysis which is inhibited by epigallocatechin gallate. It interacts with ADAM10 (A Disintegrin and metalloprotease domain-containing protein 10) and cleaves membrane proteins such as TNF-alpha and E-cadherin. α-toxin is inhibited by Mupirocin, Lysionotin, Eriodictyol and Cerulenin. SAK interacts with plasminogen and activates plasmin which degrades IgG, C3b, elastin, collagen, fibrin, α-defensin and antimicrobial peptide LL-37. SAK also inhibits biofilm formation and activates matrix metalloprotease 1. **c** Role of Eap and Sdgs in host immune regulation. Eap inhibits Elastase C, Proteinase 3 and Cathepisn G. It also inhibits formation of C4bC2. Eap interacts and regulates fibronectin, fibrinogen, laminin and prothrombin. Eap also interacts with ICAM-1 (intercellular adhesion molecule 1) that leads to inhibition of leukocytes binding to endothelial cells. Sdgs inhbit Cathepisn G activity and assist in staphylocoagulation

study biomimetic toxin nanosponges (behaves as toxin decoy and absorb hemolytic toxin) have been developed by coating membranes of human red blood cells onto polymeric nanoparticles to neutralize α-toxin [198].

Staphylokinase (SAK)

It is a 15.5 kDa bacteriophage encoded protein it is positively regulated by agrand negatively by sarA locus [199, 200]. 3D crystallography structure shows a central α-helix, a five-strand β-sheet and the connecting loops [201]. SAK is a thrombolytic agent and it interacts with plasminogen by its α-helix domain leading to its non-proteolytic activation (Fig. 4b) [202, 203]. Dimerization of staphylokinase–plasminogen alters conformation of plasminogen and converts it to broad-spectrumproteolytic enzyme plasmin (Fig. 4b) [200, 204]. Plasmin initiates bacterial penetration into the surrounding tissues and fibrin specific thrombolysis of major opsonins such as IgG as well as C3b in human plasma (Fig. 4b) [205]. Staphylococcal bound plasmin also cleaves zymogen of one of the major interstitial collagenase pro-matrix metalloprotease 1 into active matrix metalloprotease 1 (enhances the lysing effects of plasmin) [206]. SAK-plasminogen complex also hampers biofilm formation [207]. C-terminal of SAK also interacts with α-Defensins (short cationic β-sheet bactericidal human neutrophil peptides) and promotes staphylococcal resistance to host innate immunity (Fig. 4b) [208, 209]. It also interacts and inhibits another 18 kDa antimicrobial peptide LL-37 (also known as CAP-18 for cathelicidin antimicrobial peptide) found in immune cells such as macrophages, leukocytes and keratinocytes (Fig. 4b) [9, 210].

Extracellular adherence protein (Eap)

Eap consist of 4–6 tandem repeats of Eap domain that possesses a core fold of an α-helix lying diagonally across a five-stranded, mixed β-sheet [211]. It belongs to SERAM (secretable expanded repertoire adhesive molecules) family proteins. Eap and its two structural homologues EapH1 and EapH2 impairs the adherence of neutrophils to nonstimulated and TNF-alpha stimulated endothelial cells thereby preventing release of neutrophil serine proteases. It also inhibits extracellular killing of bacteria and transendothelial migration of neutrophils to reduce inflammation responses. It directly interferes with the activity of neutrophil serine proteases such as elastase (cleaves elastin determines the mechanical properties of connective tissue), proteinase 3 (critically associated with proteolytic generation of antimicrobial peptides) and cathepsin G (plays a critical part in elimination of intracellular bacteria and break down of tissues at the inflammatory sites) by occluding their catalytic cleft (Fig. 4c) [212, 213]. Eap inhibits classical and lectin pathways of complement. It prevents interaction of C2 from C4b thus disrupts formation of C4bC2 (CP/LP C3 proconvertase). It does not allow the formation of C3b, and thus prevent *S. aureus* opsonophagocytosis and killing by neutrophils [214]. Eap also interacts with plasma proteins such as fibronectin, fibrinogen, laminin and prothrombin (Fig. 4c) [9, 215]. Its interaction with pro-inflammatory mammalian surface adhesion ICAM-1 (intercellular adhesion molecule 1) leads to inhibition of leukocytes binding to activated endothelial cells as well as movement of leukocytes from bloodstream to the infection site (Fig. 4c) [216, 217]. Eap interacts with platelets and leads to its activation and aggregation in whole blood [155]. Eap is also described as an intrinsic DNA-binding protein that leads to DNA aggregation and thereby inhibit anti-microbial defense response of host. Eap also reduces NETs (neutrophil extracellular traps composed of DNA-histone scaffolds) formation [218]. It also interferes with growth factor-stimulated activation of the MAPK (mitogen-activated protein kinase) pathway that is known to be responsible for cell shape modulation. It leads to altered morphology and adhesive properties of keratinocytes. It impaires proliferation and migration capacities of keratinocytes and inhibits re-epithelialization process during wounding [218].

Serine aspartate glycosyltransferases (SdgA and SdgB)

Sdgs are involved in GlcNAc modification (*N*-Acetylglucosaminylation) of pathogenesis responsive serine-aspartate (SD) repeat proteins such as ClfA. They directly interact and covalently link GlcNAc moieties to the SDR-domain. As shown in Fig. 4c, it inhibits proteolysis of SD repeat proteins by human neutrophil-derived cathepsin G (serine proteases of the chymotrypsin family involved in elimination of intracellular pathogens). This mediates immunogenicity and inhibits degradation of virulence-associated cell wall proteins [219, 220]. SdgB also contribute to staphylococcal agglutination with fibrin fibrils in human plasma [221].

Extracellular fibrinogen binding protein (Efb)

Staphylococcus aureus Efb is a 15.8 kDa potent virulence factor as it inhibits bacterial opsonization and phagocytosis. Efb mimics as an allosteric inhibitor, blocks binding of C3b with complement factor B and the generation of active C3 convertase [222]. It interacts with C3b by its C-terminus and with fibrinogen (Aα-chain of the D fragment) by its N-terminus generating a trimolecular complex that impairs the host resistance (Fig. 5a) [223]. Fibrinogen deposition on the bacterial surface induces the formation of protective pseudocapsule (fibrin network) around bacteria with the help of prothrombin. The fibrinogen-binding domain is similar

to that of coa [224]. N-terminal domain of Efb also induces platelet binding of fibrinogen and impairs platelet activation. This results in inhibition of platelet-monocyte and platelet-granulocyte complex formation involved in innate immune response [225]. It also inhibits Maps (MHC class II analog proteins involved in T cell responses and neutrophil recruitment) and CHIPS that interacts with C5a—as well as formylated peptide receptors to mediate early inflammatory response (Fig. 5a) [136, 137, 226].

Collagen adhesion protein (Cna)

Cna is a prototype cell surface adhesion protein involved in pathogenesis and host immune evasion [227]. This multimodular cell wall anchored protein belongs to MSCRAMM (microbial surface component recognizing adhesive matrix molecules) family of adhesins [228]. Cna possesses an N-terminal signal peptide, a non-repetitive A region with N1, N2 and N3 domains; tandem B repeats and a cell wall-anchoring

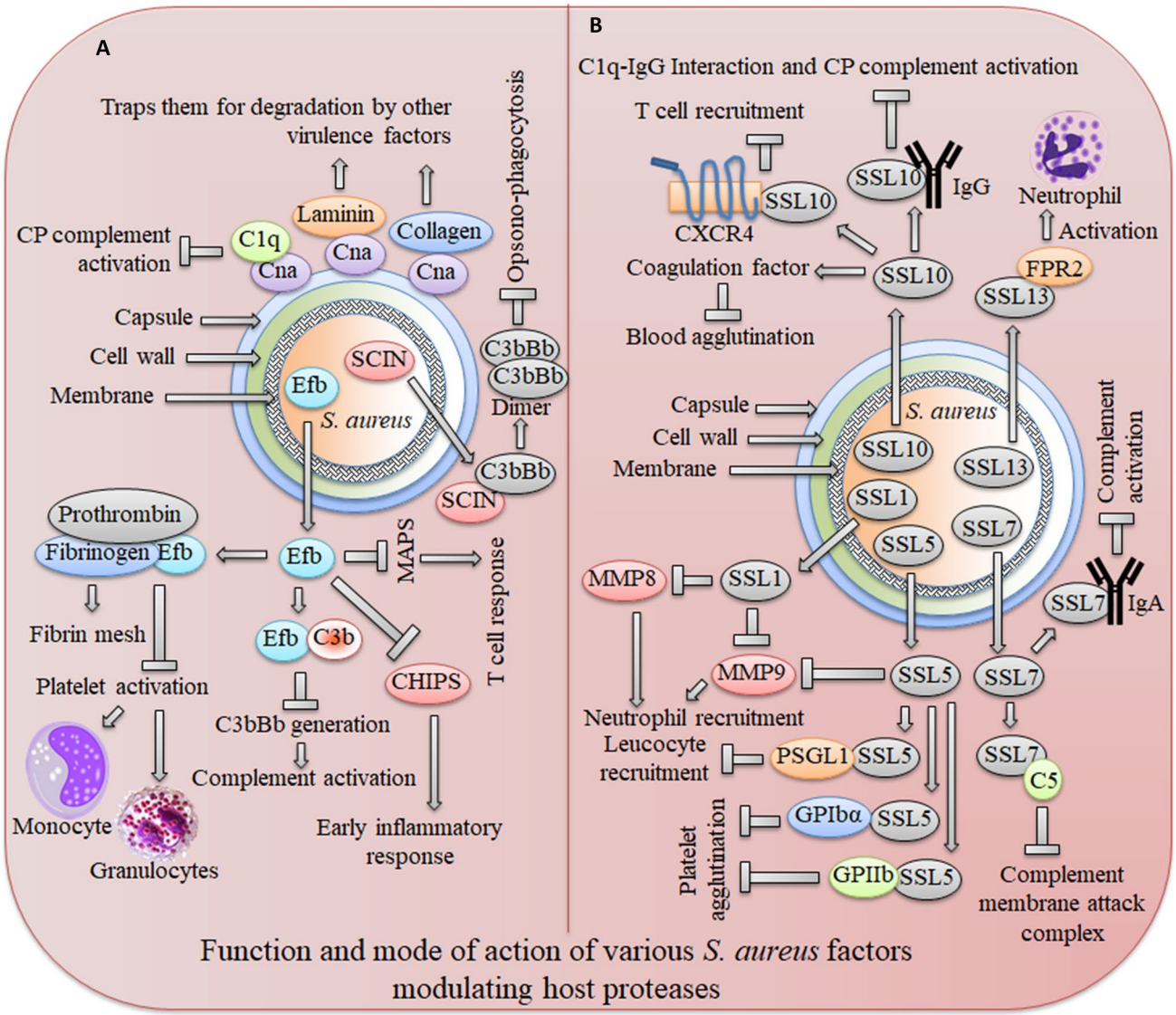

Fig. 5 Function and mode of action of various *S. aureus* viulence factors. **a** Role of Efb, SCIN and Cna in host immune regulation. Efb inhibits C3 convertase generation. Efb interacts with fibrinogen to form protective pseudocapsule (fibrin mesh) for bacteria and impair platelet-monocyte/granulocyte complex formation. Eap also inhibit Maps (MHC class II analog protein) and CHIPS to suppress immune responses. Can interacts with collagen, laminin and C1q to trap them for degradation by other virulence factors. SCIN leads to dimerization of C3bBb (C3 convertase) and inhibits opsonophagocytosis. **b** Role of SSLs in host immune regulation. SSL1 and SSL5 inhibit matrix metalloproteinase MMP8/9 and neutrophil recruitment. SSL5 also interacts with PSGL-1 (P-selectin glycoprotein ligand-1) to inhibit leucocyte recruitment and GPIbα/GPIIb receptors to inhibit platelet agglutination. SSL7 interacts with C5 to inhibit complement membrane attack complex formation and also targets IgA to block complement activation. On the other hand, SSL13 acts as a positive host immune regulator and helps recruit neutrolphils by interacting with FPR2 (formyl-peptide receptor 2)

region containing an LPXTG-domain; a transmembrane segment and a short positively charged cytoplasmic tail. Cna interacts with complement protein C1q (possessing six collagen-like triple helix domains) and inhibit the classical pathway (CP) of complement fixation (Fig. 5a) [229]. This is mediated by inhibition of interaction of C1q with C1r and C1 proteases. This interaction was supposed to induce self-activation of C1r that process proenzyme C1 into an active serine protease leading to cleavage of C4 and C2. The heterotetramer complex C1r2C1s2 then activates CP [230].

As shown in Fig. 5a Cna interacts with ligand (collagen) by collagen hug (multistep binding mechanism in which N-terminal N1–N2 of A domain of Cna wrap around collagen triple-helical rod and B domain provides stability to the interaction). It also interacts with extracellular matrix protein Lam (laminin, an important component of the basement membrane). Both C1q and Lam binding is weak as well as different from collagen hug interaction and can be efficiently inhibited by monoclonal antibodies directed against the collagen-binding domain of Cna [231].

Staphylococcal complement inhibitor (SCIN)

SCIN is another complement-evasion molecule that inhibits C3 convertase (central component of complement pathways). It is a 9.8 kDa protein having triple α-helical bundle structure connected by two short loops [232]. SCIN is regulated by *S. aureus* genetic cluster SaPI5 (*S. aureus* pathogenicity island 5). It leads to dimerization of C3 convertase (C3bBb), blocks C3b deposition, inhibits chemotaxis as well as phagocytosis and impairs C5a generation by C4b2aC3b (Fig. 5a) [233]. Dimeric enzyme inhibits interaction with complement receptor 1 and the complement receptor of the Ig superfamily [234]. It is produced extensively during the early stages of biofilm formation. Human monoclonal antibody humAb 6D4 is reported to specifically interact and inhibit SCIN [235].

Superantigen-like proteins (SSLs)

S. aureus genomic pathogenicity island vSaα encodes 14 SSLs that share structural homology (not functional) with the classical superantigens [236, 237]. They are characterized by the presence of an N-terminal oligonucleotide/oligosaccharide-binding (OB) fold linked to the C-terminal β-grasp domain and lack functional T-cell receptor binding domain [238].

SSL1 is a broad range matrix metalloproteinase (MMP, degrades extracellular matrix and modulate inflammation) inhibitor. It inhibits MMP8 (neutrophil collagenase) and MMP9 (neutrophil gelatinase B). It leads to impairment of neutrophils migration through collagen and MMP-mediated cleavage and potentiation of IL-8 (Fig. 5b). Thus, SSL1 blocks activation, chemotaxis and migration of neutrophil [239]. *S. aureus* escapes TLR2 signaling (Toll-like receptors recognize bacterial lipoproteins and play an important role in the host defense responses) by secreting SSL3 and SSL4 [240]. SSL5 is also reported to interact and inhibit activity of MMP8 as well as MMP9 and thereby inhibit neutrophil transmigration through Matrigel basement membrane (Fig. 5b) [241, 242]. SSL5 is reported to interact with neutrophil surface receptor CD162 [243]. SSL5 also interacts with PSGL-1 (P-selectin glycoprotein ligand-1) that is found on leukocytes and mediates movement of leukocyte on vascular endothelium (Fig. 5b) [244]. SSL5 impairs platelet agglutination by interacting with GPIbα and GPIIb/IIIa receptors (Fig. 5b) [245]. It antagonizes chemo-attractant by interacting with chemokine and anaphylatoxin receptors [244]. SSL7 is reported to interact with C5, and thus impair formation of the complement membrane attack complex. It also targets IgA to block complement activation [243]. It also has a therapeutic potential and can be used as a C5-targeted drug to control acute complement activation in drug-induced immune hemolytic anemia and ABO-incompatible erythrocyte transfusions [246]. SSL10 inhibits blood agglutination by interfering with the activation of coagulation cascade via binding to the Gla (γ-carboxyglutamic acid) domain of coagulation factor but not by directly inhibiting thrombin activity [247]. SSL10 also inhibits recruitment of T cell leukemia via CXCR4 binding (Fig. 5b) [241]. It interacts with human IgG and impairs binding of IgG and C1q as shown in Fig. 5b. It leads to inhibition of CP of complement activation and Fcγ receptor-mediated phagocytosis by neutrophils [14, 248]. SSL13 does not inhibit host immune processes but help recruit human neutrophils to the site of infection. It behaves as a neutrophil chemoattractant and pathogen alarming molecule. SSL13 activates neutrophils by interacting with FPR2(formyl-peptide receptor 2), induces degranulation and oxidative burst in neutrophils [243].

Identification of virulence factors by Tn-seq

Tn-seq (Transposon sequencing) or TraDIS (Transposon Directed Insertion Site Sequencing) analysis is a robust method to identify essential, conditionally essential and non-essential genes under different conditions in *S. aureus* [249]. Transposon libraries are sequenced by high throughput methods to probe the entire *S. aureus* genome with great resolution and sensitivity. In an attempt to identify genes involved in fitness of *S. aureus* in various infected compartments, several fitness and virulence associated genes have been surfaced. It includes SplA, metallo-proteases and host protease modulators such as vWbp and fibrinogen binding protein and δ-hemolysin [249]. Ibberson et al. [250] also has analyzed high throughput Tn-seq data in *S. aureus*

and found fibrinogen binding protein and δ-hemolysin as the essential gene required for polymicrobial infection. In another Tn-seq study hemolysin has been identified as an essential gene for survival and replication in the resence of nitric oxide [251]. This technique should be employed thoroughly as it identifies potential new targeting opportunities in the entire genome of *S. aureus* for the development of novel therapeutics.

Concluding remarks and future perspectives

Staphylococcus aureus mediated disease development is becoming a major issue all over the world. Its severe virulence and pathogenicity is attributed to its ability to evade as well as suppress host immunity. This is regulated by various *S. aureus* secreted proteins known as virulence factors such as proteases and proteins that regulates host proteases. They facilitate entry/invasion through skin, inhibit/evade complement pathway, block/suppress neutrophil protease as well as neutrophil migration, escape/prevent opsonophagocytosis, promotes spread through tissue/blood and assist in disease development/dissemination. This assist in the propagation of TH2-biased diseases, health care- and community-associated infectious diseases causing high morbidity among infected patients. To defeat these detrimental effects of *S. aureus* without compromising the health of patient, new insight is required in the field of host-pathogen interaction. In this review, we add-up the current knowledge and understanding of the crosstalk of host and *S. aureus* proteases as well as protease regulators. We believe that these integrated networks should be studied/analyzed in detail and future research on the combined regulatory pathway should be undertaken keeping the following points into account:

(a) Functional significance of many proteases and their regulators (from *S. aureus* as well as host) is not fully understood. Therefore, to understand the complete mechanism of *S. aureus* mediated disease development mode of action of novel virulence factors should be studied.
(b) Protease system plays an important role in evasion of immune responses. Therefore, they can be targeted for development of new therapeutic approaches for clinical treatments. But proper clinical trial phases should be employed in the process to avoid host cytotoxicity. Drugs should be used in combination with other compounds to add synergistic effect to the therapies.
(c) Extensive microarray, transcriptomic, Tn-seq and proteomic approaches should be undertaken to analyze the entire network involved in virulence as well as pathogenicity. High throughput screening and identification of novel virulence factors would shed light on the process.
(d) The study should be extended to other Staphylococcal strains and similar bacteria to fully decipher the host-bacterial interaction.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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