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Sortases, Surface Proteins, and Their Roles in *Staphylococcus aureus* Disease and Vaccine Development

OLAF SCHNEEWIND[1] and DOMINIQUE MISSIAKAS[1]
[1]Department of Microbiology, University of Chicago, Chicago, IL 60637

ABSTRACT Sortases cleave short peptide motif sequences at the C-terminal end of secreted surface protein precursors and either attach these polypeptides to the peptidoglycan of Gram-positive bacteria or promote their assembly into pilus structures that are also attached to peptidoglycan. Sortase A, the enzyme first identified in the human pathogen *Staphylococcus aureus*, binds LPXTG motif sorting signals, cleaves between threonine (T) and glycine (G) residues, and forms an acyl enzyme between its active-site cysteine thiol and the carboxyl group of threonine (T). Sortase A acyl enzyme is relieved by the nucleophilic attack of the cross bridge amino group within lipid II, thereby generating surface protein linked to peptidoglycan precursor. Such products are subsequently incorporated into the cell wall envelope by enzymes of the peptidoglycan synthesis pathway. Surface proteins linked to peptidoglycan may be released from the bacterial envelope to diffuse into host tissues and fulfill specific biological functions. *S. aureus* sortase A is essential for host colonization and for the pathogenesis of invasive diseases. Staphylococcal sortase-anchored surface proteins fulfill key functions during the infectious process, and vaccine-induced antibodies targeting surface proteins may provide protection against *S. aureus*. Alternatively, small-molecule inhibitors of sortase may be useful agents for the prevention of *S. aureus* colonization and invasive disease.

INTRODUCTION

Prior to bacterial genome sequencing and the genetic analysis of pathogenesis, microbiologists identified molecules on microbial surfaces and studied their role in disease processes (1). The ultimate goal of this research was the identification of molecular formulations inciting antibody responses in vaccine recipients that prevented disease yet would otherwise not cause harm (2). Oswald Avery's discovery of the pneumococcus capsule and the demonstration that capsular polysaccharide vaccine protects against pneumococcal pneumonia represent an important paradigm (3, 4). Another was Rebecca Lancefield's characterization of M protein as the determinant of type-specific immunity against *Streptococcus pyogenes*, the causative agent of streptococcal pharyngitis and rheumatic fever (2). Lancefield and Sjöquist required proteases or peptidoglycan (murein) hydrolases, but not membrane detergents, to solubilize surface proteins of Gram-positive bacteria (2, 5, 6). The underlying reason for this biochemical phenomenon is that surface proteins are covalently linked to peptidoglycan at their C-terminal ends (7, 8).

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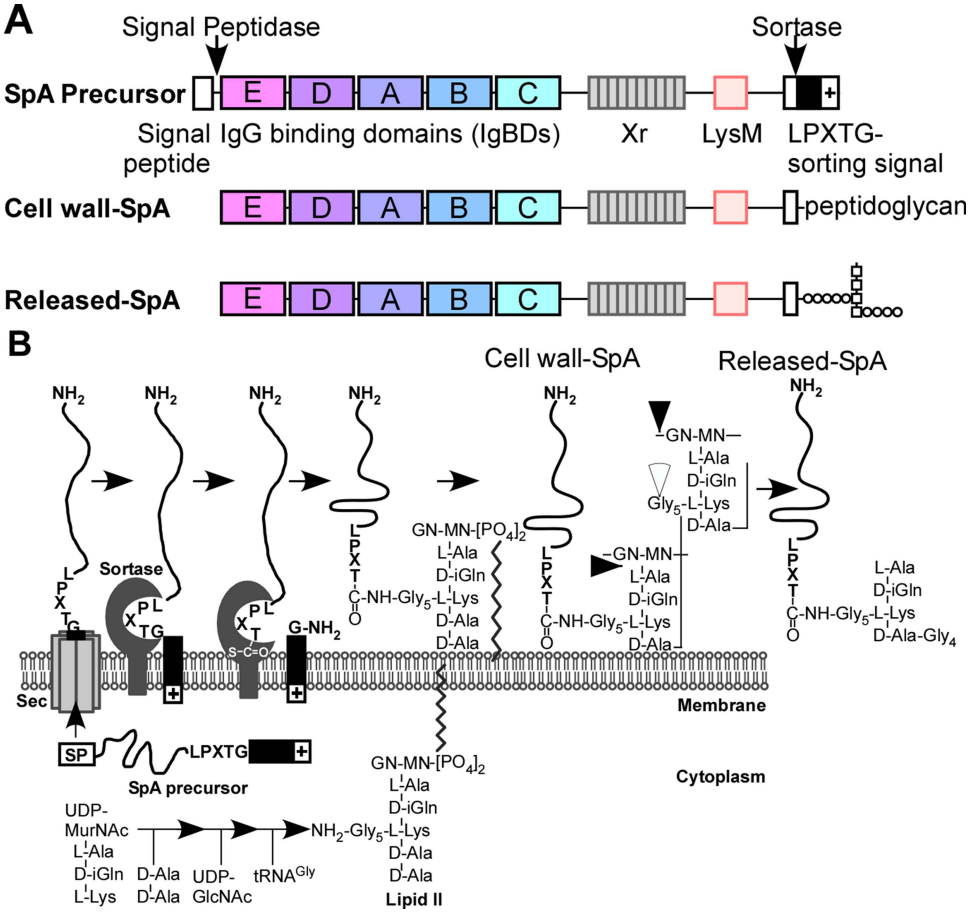
Correspondence: Olaf Schneewind, oschnee@bsd.uchicago.edu; Dominique Missiakas, dmissiak@bsd.uchicago.edu

Whole-genome sequencing enabled bioinformatic studies providing rapid answers about the universality of genetic traits among pathogens or about sequence variation in response to host adaptive immune (antibody) responses (9). While bioinformatic analyses have had tremendous impact on supporting or refuting hypotheses about surface proteins in Gram-positive bacteria, experimental work represents the bedrock for hypothesis testing and for the alignment of arguments supporting bacterial vaccine development.

STAPHYLOCOCCAL SORTASES AND THEIR SURFACE PROTEIN SUBSTRATES

Surface proteins of *S. aureus* are amide linked to the pentaglycine cross bridge of the bacterial cell wall via their C-terminal threonine (T) residue (8). Precursors of staphylococcal surface proteins are synthesized in the bacterial cytoplasm with N-terminal signal peptides for Sec-mediated secretion and C-terminal LPXTG motif sorting signals that promote cell wall anchoring (Fig. 1A) (10). Sortase A, a type II membrane protein (N-terminal

FIGURE 1 Sortase-mediated anchoring to the cell wall envelope of *Staphylococcus aureus* using SpA as a model substrate. **(A)** Drawing to illustrate the primary structure of the SpA precursor with its N-terminal signal peptide and signal peptidase cleavage site, the five immunoglobulin binding domains (IgBDs), region X (Xr) LysM domain, and C-terminal LPXTG motif sorting signal with cleavage site for sortase A. Cell wall SpA is linked to peptidoglycan via an amide bond between the carboxyl group of the C-terminal threonine and the amino group of the pentaglycine cross bridge. Released SpA is liberated from the cell wall envelope via the action of several murein hydrolases. **(B)** Drawing to illustrate *S. aureus* secretion of SpA precursor, sortase-mediated cleavage of SpA precursor and acyl enzyme formation, resolution of the acyl enzyme by lipid II to generate SpA linked to lipid II, incorporation of SpA into the cell wall via the transpeptidation and transglycosylation reaction, and release of SpA from the cell wall envelope by murein hydrolases. Released SpA bears the overall structure L-Ala–D-iGln–L-Lys(SpA-LPET-Gly_5)–D-$AlaGly_4$.



membrane anchor), cleaves the LPXTG motif of the sorting signal between its T and glycine (G) residues to form a thioester-linked acyl enzyme intermediate with its active-site cysteine thiol (11, 12) (Fig. 1B). The acyl enzyme is relieved by the nucleophilic attack of the amino group of the pentaglycine cross bridge within lipid II, the precursor to peptidoglycan biosynthesis (13, 14) (Fig. 1B). Surface protein linked to lipid II is subsequently incorporated into the cell wall envelope via the transglycosylation and transpeptidation reactions of bacterial cell wall synthesis (15–18) (Fig. 1B). *S. aureus srtA* (sortase A) mutants cannot assemble surface proteins into the cell wall envelope (19). The mechanism of action of *S. aureus* sortase A was validated for *Listeria monocytogenes* and *Bacillus anthracis* (20–22) and is considered to be universal in Gram-positive bacteria (23).

Genome sequences of all clinical *S. aureus* isolates harbor two sortase genes, *srtA* and *srtB*; however, the number of surface protein genes is variable (Table 1) (24–26). Sortase A substrates bear the LPXTG motif sorting signal

TABLE 1 *Staphylococcus aureus* cell wall-anchored surface proteins[a]

Protein	Name(s)	GenBank accession number	aa[b]	Ligand(s)[c]	YSIRK motif[d]	Sorting motif[e]	Reference(s)
Sortase A-anchored proteins							
Adenosine synthase A	AdsA (SasH)	ABD22278.1	772	Adenosine and dAdo synthesis	No	LPKTG	106, 108
Clumping factor A	ClfA	ABD20644.1	933	Fibrinogen (γ chain) factor I	Yes	LPDTG	144, 145
Clumping factor B	ClfB	ABD21326.1	899	Fibrinogen (α chain), cytokeratins 8 and 10, loricrin	Yes	LPETG	97–102
Collagen adhesin	Cna	BAF45800.1	1,183	Collagen C1q	No	LPKTG	146, 147
Factor affecting methicillin resistance in Triton X-100 B	FmtB (SasB)	ATC68490.1	2,478	Unknown	Yes	LPDTG	148
Fibronectin binding protein A	FnbpA	ABD21634.1	1,018	Fibronectin, fibrinogen (γ chain), elastin	Yes	LPETG	30
Fibronectin binding protein B	FnbpB	ABD22827.1	940	Fibronectin, fibrinogen (α chain), elastin	Yes	LPETG	30
Iron-regulated surface determinant A	IsdA (SasE)	ABD21627.1	350	Heme transferred from IsdB/H	No	LPKTG	35
Iron-regulated surface determinant B	IsdB (SasJ)	ABD21843.1	645	Hemoglobin heme	Yes	LPQTG	36–39
Iron-regulated surface determinant H	IsdH (SasI/HarA)	ABD20516.1	895	Haptoglobin-hemoglobin heme	Yes	LPKTG	36–40
Plasmin-sensitive surface protein	Pls	AAD09131.1	1,637	Unknown	Yes	LPDTG	149, 150
S. aureus surface protein C	SasC	ABD21355.1	2,186	Promotes intercellular adhesion	Yes	LPNTG	151
S. aureus surface protein D	SasD	ABD21427.1	241	Unknown	No	LPAAG	
S. aureus surface protein F	SasF	ABD21199.1	635	Unknown	No	LPKAG	
S. aureus surface protein G	SasG	BAU36055.1	1,115	Unknown	Yes	LPKTG	
S. aureus surface protein K	SasK	ADC38744.1	211	Unknown	No	LPKTG	
Serine aspartic repeat protein C	SdrC	ABD21592.1	947	β-Neurexin, homophylic bonds	Yes	LPETG	152, 153
Serine aspartic repeat protein D	SdrD	ABD20874.1	1,381	Desmoglein 1	Yes	LPETG	154
Serine aspartic repeat protein E	SdrE	ABD22410.1	1,154	Factor H	Yes	LPETG	155
S. aureus protein A	SpA	ABD22331.1	508	Immunoglobulin (Fcγ, Fab V_H3)	Yes	LPETG	70, 71, 156, 157
Serine-rich adhesin for platelets	SraP (SasA)	ABD21900.1	2,271	Salivary agglutinin (gp340)	Possibly	LPDTG	34, 158
Sortase B-anchored protein							
Iron-regulated surface determinant C	IsdC	ABD20415.1	227	Heme transferred from IsdA	No	NPQTN	28

[a]The numbers of cell wall-anchored surface proteins vary among strains of *S. aureus* (26). For example, in strain *Staphylococcus aureus* subsp. *aureus* USA300_FPR3757, genes for Cna, SasK, and Pls are missing; the presence of stop codons results in truncated FmtB (SasB), SasC, and SasG products.
[b]aa, protein length in amino acids.
[c]Molecular component(s) recognized and bound by protein, or molecules synthesized in case of AdsA.
[d]Consensus motif found in some signal sequences which presumably accounts for secretion of proteins at the cross walls (62).
[e]Consensus motif recognized by sortases and present in C-terminal cell wall sorting signal.

at the C-terminal end (Table 1) (27). Sortase B cleaves the NPQTN sorting signal of IsdC (iron-regulated surface determinant C), a protein that is linked to the cell wall when staphylococci are grown under iron starvation conditions, as occurs during host invasion (28). Several sortase A substrates have been described as microbial surface components recognizing adherence matrix molecules (MSCRAMMs) (29). These include ClfA, ClfB, Cna, FnbpA, FnbpB, and presumably also Pls, SraP, SasG, SrdC, and SdrD, although the identity of surface protein ligands in the latter group of proteins remains unclear (Table 1). Each MSCRAMM represents a mosaic of modular domains (30, 31). A surface-exposed, N-terminal A domain is generally endowed with ligand-binding activity. Repeat structural modules allow MSCRAMMs to span the thick peptidoglycan layer of staphylococci (30, 31). ClfA, ClfB, SrdC, SdrD, SdrE, Pls, and SraP each encompass extensively glycosylated serine-aspartate (SD) repeat domains (32–34) (Table 1).

The *srtB* and *isdC* genes are located in the *isd* locus, which also encodes sortase A-anchored products IsdA and IsdB, the membrane transporter IsdEF, and the cytoplasmic protein IsdG (35). The structural gene for sortase A-anchored IsdH is located outside of the *isd* locus (36). IsdB and IsdH function as hemophores to remove heme iron from hemoglobin and haptoglobin when hemoproteins are released from lysed host cells (36–39). IsdH competes with macrophage receptor CD163, the host recycling system for free hemoglobin, for the capture of heme from haptoglobin-hemoglobin (40). Bound heme iron is transferred from the NEAT (near-iron transporter) domains of IsdB or IsdH to the NEAT domain of IsdA for subsequent passage across the cell wall to IsdC and IsdEF-mediated import across the membrane (35). IsdG and its paralog IsdI cleave the tetrapyrrole ring of heme iron to liberate iron as a bacterial nutrient and enzyme cofactor (37, 41, 42). The sortase B-IsdC acyl enzyme intermediate is resolved by the nucleophilic attack of assembled peptidoglycan instead of lipid II (43). This mechanism ensures that IsdC is attached to peptidoglycan in the vicinity to the IsdEF membrane transporter, whereas IsdA and IsdB are deposited across the peptidoglycan layer (44).

SORTASES AND SURFACE PROTEIN CONTRIBUTIONS TO *S. AUREUS* COLONIZATION AND DISEASE PATHOGENESIS

S. aureus srtA mutants cannot colonize the nasopharynx and gastrointestinal tract of mice (45, 46). Further, staphylococcal *srtA* mutants cannot form abscess lesions or survive in mouse tissues (19, 47). Following intravenous *S. aureus* inoculation to precipitate lethal bacteremia in mice or guinea pigs, *srtA* mutants are avirulent and cannot cause disease (48, 49). In the mouse skin abscess lesion and pneumonia models, *S. aureus srtA* mutants display smaller reductions in virulence. We attribute the smaller phenotypic defects to the models' requirements for large bacterial inocula and α-hemolysin secretion (50–52). *S. aureus srtB* mutants exhibit small but significant reductions in virulence in the mouse renal abscess, bloodstream, and infectious arthritis models; these defects are additive with those of sortase A mutants (53).

Cheng and coworkers (47) isolated *S. aureus* Newman mutants with insertional lesions in any one gene encoding LPXTG motif surface proteins. Unlike *srtA* variants, all mutants retained the ability to cause renal abscess lesions and lethal bacteremia in mice (47, 48). However, loss of *spa* (staphylococcal protein A), *isdA*, and *isdB* resulted in significant reductions in the number of abscess lesions (47). Mutations in the genes for clumping factor A (*clfA*) or adenosine synthase A (*adsA*) caused significant delays in time to death in the murine model for *S. aureus* bacteremia (48). When analyzed with human nasal epithelial cells, cotton rats, or mice as models for *S. aureus* colonization, *srtA* mutants have been shown to be unable to colonize the nasopharynx and gastrointestinal tract (54–56). In these models, clumping factor B (ClfB) and IsdA stand out as key contributors to *S. aureus* colonization (55, 57, 58). Thus, compared to any other virulence gene, *srtA* mutations exhibit the largest reduction in the ability of *S. aureus* to colonize and invade its hosts. Further, the sortase substrates AdsA, ClfA, ClfB, IsdA, IsdB, and SpA make important, nonredundant contributions towards colonization, invasion of host tissues, or the establishment of abscess lesions.

Staphylococcal Protein A

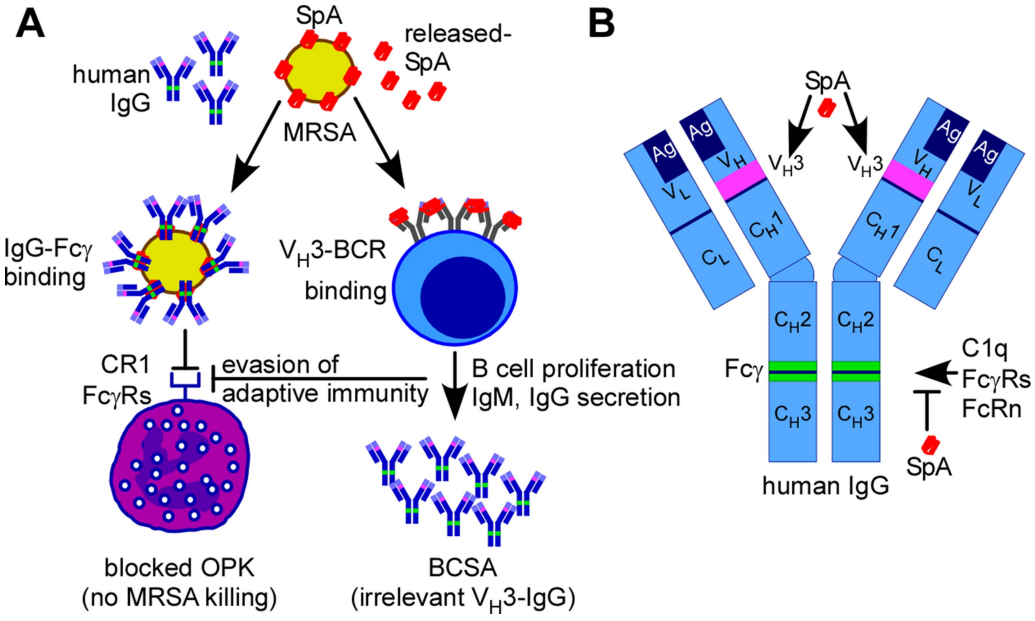
All clinical *S. aureus* isolates harbor the *spa* gene, which generates a precursor comprised of an N-terminal YSIRK/GXXS signal peptide, followed by 4 or 5 immunoglobulin binding domains (IgBDs), the region X repeats (Xr), LysM domain, and LPXTG sorting signal (23, 59, 60) (Fig. 1). SpA precursors enter the secretory pathway at septal membranes via their YSIRK/GXXS signal peptide (61–63). Once SpA is deposited into the cross wall, septal peptidoglycan is split and the cross wall assumes one-half of the spherical surface of *S. aureus* cells (61, 63). Staphylococci divide perpendicular to previous cell division planes, resulting in rapid SpA distribution over the entire bacterial surface (61). During cell division, dedicated

murein hydrolases release SpA molecules from the peptidoglycan (64, 65). SpA linked to cell wall peptide fragments is thereby released into host tissues (66) (Fig. 1). Released SpA activates V_H3 idiotype B cell receptors (BCRs) and promotes IgG and IgM secretion in activated plasmablasts (67, 68) (Fig. 2A). When displayed in the bacterial envelope, SpA binds to Fcγ, i.e., the effector domain of IgG, and protects staphylococci from opsonophagocytic killing by immune cells (49, 69) (Fig. 2A). The five IgBDs of SpA each bind to Fcγ of human (IgG1, IgG2, and IgG4) and mouse (IgG1, IgG2a to -c, and IgG3) IgG (70, 71) (Fig. 2B). Each IgBD also binds V_H3 heavy chains of human and mouse immunoglobulin, including IgM (BCRs), IgG, IgE, IgD, and IgA (49, 68, 69, 72, 73) (Fig. 2B). Thus, released SpA functions as a B cell superantigen that promotes systemic production of V_H3-clonal IgG and IgM antibodies that do not recognize staphylococcal antigens, thereby preventing the development of pathogen-specific antibodies and the establishment of protective immunity (49, 67, 68). In spite of the B cell superantigen activity of SpA, *S. aureus* colonization and invasive disease in humans are associated with the development of antibody responses against some staphylococcal antigens, predominantly serum IgG4 (74–76). These antibodies are, however, not protective and cannot promote opsonophagocytic killing because they are captured by cell wall-anchored SpA (71, 77–80).

Clumping factors A and B

Vascular damage triggers blood coagulation, a process whereby soluble fibrinogen, a 340-kDa dimer of trimers (α, β, and γ chains), is converted to insoluble fibrin following cleavage of fibrinopeptides A and B from the α and γ chains by thrombin; the prothrombinase complex Va/Xa is responsible for the conversion of prothrombin (PT) to active thrombin (81–83). The hemostatic system

FIGURE 2 Biological functions of staphylococcal protein A (SpA). **(A)** *Staphylococcus aureus* and its antibiotic-resistant isolates (MRSA) harbor SpA in the cell wall envelope or released into the extracellular milieu (released SpA). Cell wall SpA binds Fcγ of human and animal IgG (green segment within blue IgG) and blocks the effector functions of antibodies, thereby preventing opsonophagocytic killing (OPK) of MRSA by immune cells through interference with complement (CR1) and Fcγ receptors (FcγRs). Released SpA cross-links V_H3-clonal B cell receptors (V_H3-BCR on the surface of B cells), triggering B cell proliferation and secretion of V_H3-clonal IgM and IgG (pink segments within blue IgG) without antigen specificity for *S. aureus*. This B cell superantigen activity (BCSA) of SpA produces irrelevant V_H3-clonal IgG and prevents the establishment of protective immunity against *S. aureus*. **(B)** Drawing to illustrate the primary structure of human IgG with variable (V_L and V_H) and conserved (C_L, C_H1, C_H2, and C_H3) light (L) and heavy (H) chains, their antigen-binding paratope (Ag), V_H3, and Fcγ domains. SpA binding sites at V_H3 heavy chains and Fcγ are in pink and green, respectively.



also immobilizes microbial invaders for destruction by the immune system (84). However, this does not occur with *S. aureus*. All clinical *S. aureus* isolates clot human or animal blood even in the presence of coagulation inhibitors (85). Coagulation is promoted by secreted coagulase (Coa) and von Willebrand factor binding protein (vWbp) bound to PT (86). Coa-PT and vWbp-PT complexes cleave the A and B fibrinopeptides of fibrinogen but do not cut any of the other thrombin substrates (FV, FVIII, FXI, FXIII, protein C, antithrombin, and plasmin) (87). ClfA triggers *S. aureus* agglutination by binding to the C-terminal end of the fibrinogen γ chain (residues 395 to 411), effectively capping and tethering Coa-PT- and vWbp-PT-polymerized fibrin cables to the staphylococcal surface (48). ClfA, the prototypical MSCRAMM, is comprised of an N-terminal A domain with N1, N2, and N3 subdomains, an EF-hand like calcium binding module, and the SD repeat domain with 154 tandem seryl-aspartyl repeats (88). The N2 and N3 domains of ClfA (residues 229 to 545) assume immunoglobulin-like folds and bind their fibrin/fibrinogen ligand via the "dock, lock, and latch" mechanism (89–93). This interaction prevents further binding between fibrin/fibrinogen and the platelet integrin $\alpha_{IIb}\beta_3$ (94, 95). Thus, in addition to binding fibrinogen, ClfA functions as an inhibitor of platelet-fibrin clots. ClfB, which is also conserved among *S. aureus* isolates, represents a homolog of ClfA. The A domains of the two proteins are 26% identical (96), and both proteins use YSIRK/GXXS signal peptides, glycosylated SD repeats, and LPXTG motif sequences as topogenic elements (32, 62). ClfB binds to several host proteins, including the Aα chain of fibrinogen (97, 98), cytokeratin 8 (99), cytokeratin 10 (100, 101), and loricrin (102) (Table 1). These mammalian proteins harbor a motif sequence, GSSGXG, that represents the binding site for ClfB (103) and contributes to *S. aureus* colonization of the nasopharynx of mice (102).

Adenosine Synthase A

S. aureus abscess lesions are composed of a bacterial nidus, the staphylococcal abscess community (SAC), encased within a pseudocapsule of fibrin and surrounded by layers of immune cells (86, 104). In spite of large numbers of infiltrated neutrophils, mice are unable to eliminate staphylococci from abscess lesions and eventually succumb to the persistent infection (47). Although neutrophils use NETosis (extracellular DNA) to entangle staphylococci, NETs are degraded by staphylococcal nuclease and thereby fail to exert bactericidal activities (105). Nuclease digestion of NETs releases 5′ and 3′ monophosphate nucleotides that are converted by *S. aureus* AdsA into deoxyadenosine (dAdo) (106). AdsA-mediated dAdo production triggers caspase-3-induced apoptosis of mouse and human macrophages and prevents phagocyte entry into the SAC (106). Human equilibrative nucleoside transporter 1 is responsible for the uptake of dAdo in phagocytes (107). Conversion of dAdo to dAMP is catalyzed by deoxycytidine kinase and adenosine kinase, and the subsequent formation of dATP triggers caspase-3-induced cell death (107). AdsA also converts adenosine nucleosides and nucleotides released during host cell lysis into adenosine, which binds adenosine receptors and triggers host immune suppression during bloodstream infection (108, 109).

USING SORTASES AND SURFACE PROTEINS FOR VACCINE DEVELOPMENT

The contribution of sortases towards *S. aureus* colonization and invasive disease provoked interest in surface proteins as vaccine antigens. Purified recombinant ClfA (A domain) generates antibodies that neutralize ClfA binding to fibrin(ogen) and provide partial protection against lethal bloodstream infection and infectious arthritis in mice (110). Anti-ClfA mouse hybridoma antibody or its cloned humanized variant tefibazumab binds to the ClfA N3 domain, inhibits fibrinogen binding (111, 112), and provides partial protection against lethal bloodstream infection in mice (113). Administration of clinical-grade tefibazumab was safe in healthy human volunteers and in patients with methicillin-resistant *S. aureus* (MRSA) bacteremia but could not improve the clinical outcomes for these patients (114). Using ClfA-immunized VelocImmune mice, MedImmune investigators isolated monoclonal antibody 11H10, with inhibitory activity for ClfA binding to fibrinogen (115). Human 11H10 IgG1 promotes MRSA opsonophagocytic killing with differentiated HL-60 neutrophils (115) and increases the survival of mice with lethal MRSA bloodstream infection (116, 117). MedImmune seeks to develop 11H10 IgG1 in conjunction with monoclonal antibody against α-hemolysin to improve the outcome of patients with ventilator-associated pneumonia and other invasive diseases (115). Pfizer developed SA4Ag, a multicomponent vaccine composed of ClfA, capsular polysaccharide type 5 and 8 conjugates, and manganese transporter C (118). SA4Ag is currently undergoing clinical efficacy evaluation in patients with instrumented posterior spinal fusion to protect against *S. aureus* surgical site and bloodstream infections (119).

Purified IsdB elicits antibodies that block heme iron scavenging and provide partial protection against *S. aureus*

bacteremia in preclinical models (120–122). IsdB-specific antibodies may also promote opsonophagocytosis of *S. aureus* (121, 123). In a phase 3 clinical trial, IsdB (V710) immunization did not protect thoracic surgery patients from *S. aureus* surgical site infections (124). V710 immunization increased the risk for fatal *S. aureus* bacteremia 5-fold over the control cohort; the molecular basis for this safety concern is not known (124).

Humans and mice cannot generate antibodies against the IgBDs of SpA; however, SpA variants, engineered to exhibit reduced immunoglobulin binding, elicit SpA-neutralizing antibody responses (73). Animals with SpA-neutralizing antibodies exhibit dramatic increases in pathogen-specific antibody responses during colonization or invasive disease (46, 49, 69, 73). In fact, the corresponding SpA vaccine can protect against *S. aureus* colonization, renal abscess formation, and lethal bloodstream infection (46, 49, 69, 73). Similarly, SpA-neutralizing monoclonal antibody protects against *S. aureus* colonization and invasive disease in mice (125, 126). SpA vaccines have not yet been subjected to clinical testing.

SORTASE INHIBITORS

The complete transpeptidation reaction that is carried out by sortases can be recapitulated *in vitro* (12, 14, 127). However, most screens for sortase inhibitors have been conducted with assays measuring SrtA cleavage of LPXTG peptide (128). These inhibitors are generally not active *in vivo*, suggesting that in the envelope of *S. aureus*, sortase A may predominantly exist as an acyl enzyme (129). Other inhibitors can block sortase A activity *in vivo*, and such compounds abolish surface protein anchoring to the cell wall envelope of *S. aureus* and protect animals against lethal bloodstream infection (130, 131). Of note, sortase inhibitors may be useful for the prevention of *S. aureus* disease, as they can be expected to block colonization and invasion. Owing to the fact that the compounds cannot kill *S. aureus*, sortase inhibitors are unlikely to exhibit a therapeutic effect in individuals with active infectious disease (130).

SORTASES IN OTHER PATHOGENIC MICROBES

Gram-positive bacteria often harbor homologs of staphylococcal sortase A or class A sortases; only some microbes express sortase B homologs or class B sortases (132, 133). Based on structural features and substrate specificity, sortase homologs have been classified into six distinct classes, A to F (134). Among bacterial pathogens, *Corynebacterium diphtheriae* and *Bacillus anthracis* harbor class C sortase genes, which are clustered with surface protein genes containing LPXTG- and motif-specific sorting signals (135, 136). These genes encode pilus components that include adhesin and pilin subunits. Class C sortases link adhesin and pilin subunits together to construct a pilus (135–139). Class C sortases cleave the LPXTG motif of pilins to form acyl enzyme intermediates that are relieved by the nucleophilic attack of the ε-amino group of a conserved lysine (K) residue within the pilin motif of an incoming subunit (140–142). Pilin protomers are joined progressively to the pilus base; a housekeeping sortase terminates polymerization by transferring the whole structure to the peptidoglycan (141, 143). For additional information on the different classes of sortases and their distribution among various phyla, the reader is referred to a recently published review (134).

In conclusion, sortases are ubiquitous in Gram-positive bacteria, anchoring proteins and pili to peptidoglycan via a conserved transpeptidation mechanism. Sortase-mediated attachment of virulence factors in *S. aureus* has stimulated searches for sortase inhibitors and protective antigens. These strategies may lead to the development of drugs that can prevent hospital-acquired infections or to protective vaccines that can prevent *S. aureus* colonization and/or invasive diseases.

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We declare conflicts of interest as inventors of patents under commercial license for *S. aureus* vaccine development. We declare no further competing financial interests.

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