HHS Public Access

Author manuscript

Microbiol Spectr. Author manuscript; available in PMC 2019 February 22.

Published in final edited form as:

Microbiol Spectr. 2019 January ; 7(1): . doi:10.1128/microbiolspec.PSIB-0004-2018.

Sortases, surface proteins and their roles in *Staphylococcus aureus* disease and vaccine development

Olaf Schneewind and **Dominique Missiakas**

Department of Microbiology, University of Chicago, Chicago, Illinois, USA

Chapter Summary

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 crossbridge 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 *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). Ultimate goal of this research was the identification 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, represents 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,

Contact information: Department of Microbiology, University of Chicago, Cummings Life Sciences Center 607b, 920 East 58th Street, Chicago, IL 60637, USA; oschnee@bsd.uchicago.edu; dmissiak@bsd.uchicago.edu.

6). The underlying reason for this biochemical phenomenon is that surface proteins are covalently linked to peptidoglycan at their C-terminal ends (7, 8).

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 in 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 crossbridge of the bacterial cell wall via their C-terminal threonine 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 membrane anchor) cleaves the LPXTG motif of the sorting signal between its threonine (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 crossbridge 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 at their 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 or MSCRAMMs (29). These include ClfA, ClfB, Cna, FnbpA, FnbpB, and presumably also Pls, SraP, SasG, SrdC and SdrD, albeit that the identify 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 paralogue IsdI cleave the tetrapyrrole ring of heme-iron to liberate iron as a bacterial nutrient and enzyme co-factor (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 co-workers isolated *S. aureus* Newman mutants with insertional lesions 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 are 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, non-redundant contributions towards colonization, invasion of host tissues or the establishment of abscess lesions.

Staphylococcal protein A (SpA)

All clinical *S. aureus* isolates harbor the *spa* gene, which generates a precursor comprised of an N-terminal YSIRK/GXXS signal peptide, followed by 4–5 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 & IgG4) and mouse (IgG1, IgG2a-c & 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 is 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 (ClfA and ClfB)

Vascular damage triggers blood coagulation, a process whereby soluble fibrinogen, a 340 kDa dimer of trimers (α-,β-, 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 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 395–411), 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 229–545) 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 homologue 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 and contributes to *S. aureus* colonization of nasopharynx of mice (102).

Adenosine Synthase A (AdsA)

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 (Nuc) 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* bind to the ClfA N3 domain, inhibit fibrinogen binding (111, 112) and provide 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 of 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 five-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, 127, 128). However, most screens for sortase inhibitors have been conducted with assays measuring SrtA cleavage of LPXTG peptide (129). 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 (130). 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 (131, 132). 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 (131).

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 (133, 134). Based on structural features and substrate specificity, sortase homologs have been classified into six distinct classes A–F (135). Amongst 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 (136, 137).These genes encode pilus component: adhesin and pilin subunits. Class C sortases link adhesin and pilin subunits together to construct a pilus (136–140). 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 (141–143). Pilin protomers are joined progressively to the pilus base; a housekeeping sortase terminates polymerization by transferring the whole structure to the peptidoglycan (142, 144). For additional information on the different classes of sortases and their distribution among various phyla, the reader is referred to a recently published review (135).

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.

Acknowledgements

We thank laboratory members past and present for their contributions to the field of *S. aureus* sortases and surface proteins. Work on *Staphylococcus aureus* in the laboratories of the authors is supported by grants AI038897, AI052474, and AI110937 from the National Institute of Allergy and Infectious Diseases. The authors declare conflicts of interest as inventors of patents under commercial license for *S. aureus* vaccine development. The authors declare no further competing financial interests.

References

1. Lancefield RC. 1928 The antigenic complex of *Streptococcus hemolyticus*. I. Demonstration of a type-specific substance in extracts of *Streptococcus hemolyticus*. J Exp Med 47:91–103. [PubMed: 19869404]
2. Lancefield R 1962 Current knowledge of type-specific M antigens of group A streptococci. J Immunol 89:307–313. [PubMed: 14461914]
3. Avery OT. 1915 A further study on the biologic classification of pneumococci. J Exp Med 22:804–819. [PubMed: 19867961]
4. MacLeod CM, Hodges RG, Heidelberger M, Bernhard WG. 1945 Prevention of pneumococcal pneumonia by immunization with specific capsular polysaccharides. J Exp Med 82:445–465.
5. Sjöquist J, Meloun B, Hjelm H. 1972 Protein A isolated from *Staphylococcus aureus* after digestion with lysostaphin. Eur J Biochem 29:572–578. [PubMed: 4628411]
6. Fischetti VA. 1989 Streptococcal M protein: molecular design and biological behavior. Clin Microbiol Rev 2:285–314. [PubMed: 2670192]
7. Schneewind O, Fowler A, Faull KF. 1995 Structure of the cell wall anchor of surface proteins in *Staphylococcus aureus*. Science 268:103–106. [PubMed: 7701329]
8. Marraffini LA, DeDent AC, Schneewind O. 2006 Sortases and the art of anchoring proteins to the envelopes of gram-positive bacteria. Microbiol Mol Biol Rev 70:192–221. [PubMed: 16524923]
9. Musser JM, Shelburne SAr. 2009 A decade of molecular pathogenomic analysis of group A *Streptococcus*. J Clin Invest 119:2455–2463. [PubMed: 19729843]
10. Schneewind O, Model P, Fischetti VA. 1992 Sorting of protein A to the staphylococcal cell wall. Cell 70:267–281. [PubMed: 1638631]
11. Mazmanian SK, Liu G, Ton-That H, Schneewind O. 1999 *Staphylococcus aureus* sortase, an enzyme that anchors surface proteins to the cell wall. Science 285:760–763. [PubMed: 10427003]

12. Ton-That H, Liu G, Mazmanian SK, Faull KF, Schneewind O. 1999 Purification and characterization of sortase, the transpeptidase that cleaves surface proteins of *Staphylococcus aureus* at the LPXTG motif. Proc Natl Acad Sci USA 96:12424–12429. [PubMed: 10535938]
13. Perry AM, Ton-That H, Mazmanian SK, Schneewind O. 2002 Anchoring of surface proteins to the cell wall of *Staphylococcus aureus*. III. Lipid II is an *in vivo* peptidoglycan substrate for sortase-catalyzed surface protein anchoring. J Biol Chem 277:16241–16248. [PubMed: 11856734]
14. Ton-That H, Mazmanian SK, Faull KF, Schneewind O. 2000 Anchoring of surface proteins to the cell wall of *Staphylococcus aureus*. I. Sortase catalyzed *in vitro* transpeptidation reaction using LPXTG peptide and NH_2-Gly_3 substrates. J Biol Chem 275:9876–9881. [PubMed: 10734144]
15. Ton-That H, Labischinski H, Berger-Bächi B, Schneewind O. 1998 Anchor structure of staphyococcal surface proteins. III. The role of the FemA, FemB, and FemX factors in anchoring surface proteins to the bacterial cell wall. J Biol Chem 273:29143–29149. [PubMed: 9786923]
16. Ton-That H, Schneewind O. 1999 Anchor structure of staphylococcal surface proteins. IV. Inhibitors of the cell wall sorting reaction. J Biol Chem 274:24316–24320. [PubMed: 10446208]
17. Ton-That H, Faull KF, Schneewind O. 1997 Anchor structure of staphylococcal surface proteins. I. A branched peptide that links the carboxyl terminus of proteins to the cell wall. J Biol Chem 272:22285–22292. [PubMed: 9268378]
18. Navarre WW, Ton-That H, Faull KF, Schneewind O. 1998 Anchor structure of staphylococcal surface proteins. II. COOH-terminal structure of muramidase and amidase-solubilized surface protein. J Biol Chem 273:29135–29142. [PubMed: 9786922]
19. Mazmanian SK, Liu G, Jensen ER, Lenoy E, Schneewind O. 2000 *Staphylococcus aureus* mutants defective in the display of surface proteins and in the pathogenesis of animal infections. Proc Natl Acad Sci USA 97:5510–5515. [PubMed: 10805806]
20. Dhar G, Faull KF, Schneewind O. 2000 Anchor structure of cell wall surface proteins in *Listeria monocytogenes*. Biochemistry 39:3725–3733. [PubMed: 10736172]
21. Bierne H, Mazmanian SK, Trost M, Pucciarelli MG, Dehoux P, Jansch L, Portillo FG, G. L, Schneewind O, Cossart P. 2002 Inactivation of the *srtA* gene in *Listeria monocytogenes* inhibits anchoring of surface proteins and affects virulence. Mol Microbiol 43:869–881. [PubMed: 11929538]
22. Gaspar AH, Marraffini LA, Glass EM, DeBord KL, Ton-That H, Schneewind O. 2005 Bacillus anthracis sortase A (SrtA) anchors LPXTG motif-containing surface proteins to the cell wall envelope. J Bacteriol 187:4646–4655. [PubMed: 15968076]
23. Mazmanian SK, Ton-That H, Schneewind O. 2001 Sortase-catalyzed anchoring of surface proteins to the cell wall of *Staphylococcus aureus*. Mol Microbiol 40:1049–1057. [PubMed: 11401711]
24. Baba T, Bae T, Schneewind O, Takeuchi F, Hiramatsu K. 2007 Genome sequence of *Staphylococcus aureus* strain Newman and comparative analysis of staphylococcal genomes. J Bacteriol 190:300–310. [PubMed: 17951380]
25. Kuroda M, Ohta T, Uchiyama I, Baba T, Yuzawa H, Kobayashi I, Cui L, Oguchi A, Aoki K, Nagai Y, Lian J, Ito T, Kanamori M, Matsumaru H, Maruyama A, Murakami H, Hosoyama A, Mitsutani-Ui Y, Kobayashi N, Sawano T, Inoue R, Kaito C, Sekimizu K, Hirakawa H, Kuhara S, Goto S, Yabuzaki J, Kanehisa M, Yamashita A, Oshima K, Furuya K, Yoshino C, Shiba T, Hattori M, Ogasawara N, Hayashi H, Hiramatsu K. 2001 Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. Lancet 357:1225–1240. [PubMed: 11418146]
26. McCarthy AJ, Lindsay JA. 2010 Genetic variation in Staphylococcus aureus surface and immune evasion genes is lineage associated: implications for vaccine design and host-pathogen interactions. BMC Microbiol 10:173. [PubMed: 20550675]
27. Schneewind O, Mihaylova-Petkov D, Model P. 1993 Cell wall sorting signals in surface protein of Gram-positive bacteria. EMBO 12:4803–4811.
28. Mazmanian SK, Ton-That H, Su K, Schneewind O. 2002 An iron-regulated sortase enzyme anchors a class of surface protein during *Staphylococcus aureus* pathogenesis. Proc Natl Acad Sci USA 99:2293–2298. [PubMed: 11830639]
29. Patti JM, Allen BL, McGavin MJ, Hook M. 1994 MSCRAMM-mediated adherence of microorganisms to host tissues. Annu Rev Microbiol 48:585–617. [PubMed: 7826020]

30. Foster TJ. 2016 The remarkably multifunctional fibronectin binding proteins of Staphylococcus aureus. Eur J Clin Microbiol Infect Dis 35:1923–1931. [PubMed: 27604831]
31. Foster TJ, Geoghegan JA, Ganesh VK, Hook M. 2014 Adhesion, invasion and evasion: the many functions of the surface proteins of Staphylococcus aureus. Nat Rev Microbiol 12:49–62. [PubMed: 24336184]
32. Thomer L, Becker S, Emolo C, Quach A, Kim HK, Rauch S, Faull KF, Schneewind O, Missiakas DM. 2014 *N*-acetylglucosaminylation of serine-aspartate repeat proteins promotes *Staphylococcus aureus* blood stream infection. J Biol Chem 289:3478–3486. [PubMed: 24344128]
33. Bleiziffer I, Eikmeier J, Pohlentz G, McAulay K, Xia G, Hussain M, Peschel A, Foster S, Peters G, Heilmann C. 2017 The Plasmin-Sensitive Protein Pls in Methicillin-Resistant Staphylococcus aureus (MRSA) Is a Glycoprotein. PLoS Pathog 13:e1006110. [PubMed: 28081265]
34. Siboo IR, Chambers HF, Sullam PM. 2005 Role of SraP, a Serine-Rich Surface Protein of Staphylococcus aureus, in binding to human platelets. Infect Immun 73:2273–80. [PubMed: 15784571]
35. Mazmanian SK, Skaar EP, Gaspar AH, Humayun M, Gornicki P, Jelenska J, Joachmiak A, Missiakas DM, Schneewind O. 2003 Passage of heme-iron across the envelope of *Staphylococcus aureus*. Science 299:906–909. [PubMed: 12574635]
36. Dryla A, Gelbmann D, von Gabain A, Nagy E. 2003 Identification of a novel iron regulated staphylococcal surface protein with haptoglobin-haemoglobin binding activity. Mol Microbiol 49:37–53. [PubMed: 12823809]
37. Skaar EP, Humayun M, Bae T, DeBord KL, Schneewind O. 2004 Iron-source preference of *Staphylococcus aureus* infections. Science 305:1626–1628. [PubMed: 15361626]
38. Pishchany G, Sheldon JR, Dickson CF, Alam MT, Read TD, Gell DA, Heinrichs DE, Skaar EP. 2014 IsdB-dependent hemoglobin binding is required for acquisition of heme by Staphylococcus aureus. J Infect Dis 209:1764–72. [PubMed: 24338348]
39. Choby JE, Skaar EP. 2016 Heme Synthesis and Acquisition in Bacterial Pathogens. J Mol Biol 428:3408–28. [PubMed: 27019298]
40. Saederup KL, Stodkilde K, Graversen JH, Dickson CF, Etzerodt A, Hansen SW, Fago A, Gell D, Andersen CB, Moestrup SK. 2016 The Staphylococcus aureus Protein IsdH Inhibits Host Hemoglobin Scavenging to Promote Heme Acquisition by the Pathogen. J Biol Chem 291:23989–23998. [PubMed: 27681593]
41. Skaar EP, Gaspar AH, Schneewind O. 2004 IsdG and IsdI, heme degrading enzymes in the cytoplasm of *Staphylococcus aureus*. J Biol Chem 279:436–443. [PubMed: 14570922]
42. Reniere ML, Ukpabi GN, Harry SR, Stec DF, Krull R, Wright DW, Bachmann BO, Murphy ME, Skaar EP. 2010 The IsdG-family of haem oxygenases degrades haem to a novel chromophore. Mol Microbiol 75:1529–1538. [PubMed: 20180905]
43. Marraffini LA, Schneewind O. 2005 Anchor structure of staphylococcal surface proteins. V. Anchor structure of the sortase B substrate IsdC. J Biol Chem 280:16263–16271. [PubMed: 15718231]
44. Maresso AW, Schneewind O. 2006 Iron acquisition and transport in *Staphylococcus aureus*. Biometals 19:193–203. [PubMed: 16718604]
45. Kiser KB, Cantey-Kiser JM, Lee JC. 1999 Development and characterization of a *Staphylococcus aureus* nasal colonization model in mice. Infect Immun 67:5001–5006. [PubMed: 10496870]
46. Sun Y, Emolo CE, Holtfreter S, Wiles S, Kreiswirth B, Missiakas D, Schneewind O. 2018 Staphylococcal protein A is required for persistent colonization of mice with *Staphylococcus aureus*. J Bacteriol EPub:ahead of press.
47. Cheng AG, Kim HK, Burts ML, Krausz T, Schneewind O, Missiakas DM. 2009 Genetic requirements for *Staphylococcus aureus* abscess formation and persistence in host tissues. FASEB J 23:3393–3404. [PubMed: 19525403]
48. McAdow M, Kim HK, DeDenta AC, Hendrickx APA, Schneewind O, Missiakas DM. 2011 Preventing *Staphylococcus aureus* sepsis through the inhibition of its agglutination in blood. PLoS Pathog 7:e1002307. [PubMed: 22028651]

49. Kim HK, Falugi F, Thomer L, Missiakas DM, Schneewind O. 2015 Protein A suppresses immune responses during Staphylococcus aureus bloodstream infection in guinea pigs. mBio 6:e02369–14. [PubMed: 25564466]
50. Bubeck-Wardenburg J, Patel R, Schneewind O. 2007 Surface proteins and exotoxins are required for the pathogenesis of *Staphylococcus aureus* pneumonia. Infect Immun 74:1040–1044.
51. Bubeck Wardenburg J, Schneewind O. 2008 Vaccine protection against *Staphylococcus aureus* pneumonia. J Exp Med 205:287–294. [PubMed: 18268041]
52. Kennedy AD, Bubeck-Wardenburg J, Gardner DJ, Long D, Whitney AR, Braughton KR, Schneewind O, DeLeo FR. 2010 Targeting of alpha-hemolysin by active or passive immunization decreases severity of USA300 skin infection in a mouse model. J Infect Dis 202:1050–1058. [PubMed: 20726702]
53. Jonsson IM, Mazmanian SK, Schneewind O, Bremell T, Tarkowski A. 2003 The role of *Staphylococcus aureus* sortase A and sortase B in murine arthritis. Microb Infect 5:775–780.
54. Corrigan RM, Miajlovic H, Foster TJ. 2009 Surface proteins that promote adherence of *Staphylococcus aureus* to human desquamated nasal epithelial cells. BMC Microbiol 9:22. [PubMed: 19183486]
55. Schaffer AC, Solinga RM, Cocchiaro J, Portoles M, Kiser KB, Risley A, Randall SM, Valtulina V, Speziale P, Walsh E, Foster T, Lee JC. 2006 Immunization with *Staphylococcus aureus* clumping factor B, a major determinant in nasal carriage, reduces nasal colonization in a murine model. Infect Immun 74:2145–2153. [PubMed: 16552044]
56. Misawa Y, Kelley KA, Wang X, Wang L, Park WB, Birtel J, Saslowsky D, Lee JC. 2015 *Staphylococcus aureus* colonization of the mouse gastrointestinal tract Is modulated by wall teichoic acid, capsule, and surface proteins. PLoS Pathog 11:e1005061. [PubMed: 26201029]
57. Clarke SR, Brummell KJ, Horsburgh MJ, McDowell PW, Mohamad SA, Stapleton MR, Acevedo J, Read RC, Day NP, Peacock SJ, Mond JJ, Kokai-Kun JF, Foster SJ. 2006 Identification of *in vivo*-expressed antigens of *Staphylococcus aureus* and their use in vaccinations for protection against nasal carriage. J Infect Dis 193:1098–1108. [PubMed: 16544250]
58. Wertheim HF, Walsh E, Choudhurry R, Melles DC, Boelens HA, Miajlovic H, Verbrugh HA, Foster T, van Belkum A. 2008 Key role for clumping factor B in *Staphylococcus aureus* nasal colonization of humans. PLoS Med 5:e17. [PubMed: 18198942]
59. Forsgren A 1970 Significance of protein A production by staphylococci. Infect Immun 2:672–673. [PubMed: 16557894]
60. Votintseva AA, Fung R, Miller RR, Knox K, Godwin H, Wyllie DH, Bowden R, Crook DW, Walker AS. 2014 Prevalence of Staphylococcus aureus protein A (spa) mutants in the community and hospitals in Oxfordshire. BMC Microbiol 14:63. [PubMed: 24621342]
61. DeDent AC, McAdow M, Schneewind O. 2007 Distribution of protein A on the surface of *Staphylococcus aureus*. J Bacteriol 189:4473–4484. [PubMed: 17416657]
62. DeDent AC, Missiakas DM, Schneewind O. 2008 Signal peptides direct surface proteins to two distinct envelope locations of *Staphylococcus aureus*. EMBO J 27:2656–2668. [PubMed: 18800056]
63. Yu W, Missiakas D, Schneewind O. 2018 Septal secretion of protein A in *Staphylococcus aureus* requires SecA and lipoteichoic acid synthesis. Elife 7:e34092. [PubMed: 29757141]
64. Frankel MB, Hendrickx AP, Missiakas DM, Schneewind O. 2011 LytN, a murein hydrolase in the cross-wall compartment of *Staphylococcus aureus*, is involved in proper bacterial growth and envelope assembly. J Biol Chem 286:32593–32605. [PubMed: 21784864]
65. Frankel MB, Schneewind O. 2012 Determinants of murein hydrolase targeting to cross-wall of *Staphylococcus aureus* peptidoglycan. J Biol Chem 287:10460–10471. [PubMed: 22303016]
66. Becker S, Frankel MB, Schneewind O, Missiakas DM. 2014 Release of protein A from the cell wall envelope of *Staphylococcus aureus*. Proc Natl Acad Sci USA 111:1574–1579. [PubMed: 24434550]
67. Kim HK, Falugi F, Missiakas D, Schneewind O. 2016 Peptidoglycan-linked protein A promotes T-cell dependent antibody expansion during *Staphylococcus aureus* infection. Proc Natl Acad Sci USA 113:5718–5723. [PubMed: 27140614]

68. Pauli NT, Kim HK, Falugi F, Huang M, Dulac J, Dunand CH, Zheng NY, Kaur K, Andrews S, Huang Y, Dedent A, Frank K, Charnot-Katsikas A, Schneewind O, Wilson PC. 2014 *Staphylococcus aureus* infection induces protein A-mediated immune evasion in humans. J Exp Med 211:2331–2339. [PubMed: 25348152]
69. Falugi F, Kim HK, Missiakas DM, Schneewind O. 2013 The role of protein A in the evasion of host adaptive immune responses by Staphylococcus aureus mBio 4:e00575–13. [PubMed: 23982075]
70. Forsgren A, Sjöquist J. 1966 Protein A from *S. aureus*. I. Pseudo-immune reaction with human gamma-globulin. J Immunol 97:822–827. [PubMed: 4163007]
71. Forsgren A 1968 Protein A from *Staphylococcus aureus*. VI. Reaction with subunits from guinea pig γ1- and γ2-globulin. J Immunol 100:927–930. [PubMed: 4171943]
72. Sasso EH, Silverman GJ, Mannik M. 1989 Human IgM molecules that bind staphylococcal protein A contain VHIII H chains. J Immunol 142:2778–2783. [PubMed: 2495325]
73. Kim HK, Cheng AG, Kim H-Y, Missiakas DM, Schneewind O. 2010 Non-toxigenic protein A vaccine for methicillin-resistant *Staphylococcus aureus* infections. J Exp Med 207:1863–1870. [PubMed: 20713595]
74. Verkaik NJ, Lebon A, de Vogel CP, Hooijkaas H, Verbrugh HA, Jaddoe VW, Hofman A, Moll HA, van Belkum A, van Wamel WJ. 2010 Induction of antibodies by *Staphylococcus aureus* nasal colonization in young children. Clin Microbiol Infect 16:1312–1317. [PubMed: 19832714]
75. Swierstra J, Debets S, de Vogel C, Lemmens-den Toom N, Verkaik N, Ramdani-Bouguessa N, Jonkman MF, van Dijl JM, Fahal A, van Belkum A, van Wamel W. 2015 IgG4 subclass-specific responses to *Staphylococcus aureus* antigens shed new light on host-pathogen interaction. Infect Immun 83:492–501. [PubMed: 25404029]
76. Holtfreter S, Jursa-Kulesza J, Masiuk H, Verkaik NJ, de Vogel C, Kolata J, Nowosiad M, Steil L, van Wamel W, van Belkum A, Völker U, Giedrys-Kalemba S, Bröker BM. 2011 Antibody responses in furunculosis patients vaccinated with autologous formalin-killed *Staphylococcus aureus*. Eur J Clin Microbiol Infect Dis 30:707–717. [PubMed: 21509478]
77. Kluytmans J, van Belkum A, Verburgh H. 1997 Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 10:505–520. [PubMed: 9227864]
78. Weinstein HJ. 1959 The relation between nasal-staphylococcal-carrier state and the incidence of postoperative complications. N Engl J Med 260:1303–1308. [PubMed: 13666958]
79. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL. 2005 The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis 5:751–62. [PubMed: 16310147]
80. Forsgren A, Quie PG. 1974 Effects of staphylococcal protein A on heat labile opsonins. J Immunol 112:1177–1180. [PubMed: 4590699]
81. Adams RL, Bird RJ. 2009 Review article: Coagulation cascade and therapeutics update: relevance to nephrology. Part 1: Overview of coagulation, thrombophilias and history of anticoagulants. Nephrology 14:462–70. [PubMed: 19674315]
82. Doolittle RF. 2003 Structural basis of the fibrinogen-fibrin transformation: contributions from X-ray crystallography. Blood Rev 17:33–41. [PubMed: 12490209]
83. Ware S, Donahue JP, Hawiger J, Anderson WF. 1999 Structure of the fibrinogen gamma-chain integrin binding and factor XIIIa cross-linking sites obtained through carrier protein driven crystallization. Protein Sci 8:2663–2671. [PubMed: 10631982]
84. Levi M, Keller TT, van Gorp E, ten Cate H. 2003 Infection and inflammation and the coagulation system. Cardiovasc Res 60:26–39. [PubMed: 14522404]
85. Much H 1908 Über eine Vorstufe des Fibrinfermentes in Kulturen von *Staphylokokkus aureus*. Biochem Z 14:143–155.
86. Cheng AG, McAdow M, Kim HK, Bae T, Missiakas DM, Schneewind O. 2010 Contribution of coagulases towards *Staphylococcus aureus* disease and protective immunity. PLoS Pathog 6:e1001036. [PubMed: 20700445]
87. Thomer L, Schneewind O, Missiakas D. 2016 Pathogenesis of Staphylococcus aureus Bloodstream Infections. Annu Rev Pathol 11:343–64. [PubMed: 26925499]

88. O'Connell DP, Nanavaty T, McDevitt D, Gurusidappa S, Hook M, Foster TJ. 1998 The fibrinogen-binding MSCRAMM (clumping factor) of *Staphylococcus aureus* has a Ca2+-dependent inhibitory site. J Biol Chem 273:6821–6829. [PubMed: 9506984]

89. Strong DD, Laudano AP, Hawiger J, Doolittle RF. 1982 Isolation, characterization and synthesis of peptides from human fibrinogen that block the staphylococcal clumping reaction and construction of a synthetic clumping particle. Biochemistry 21:1414–1420. [PubMed: 7074096]

90. Ganesh VK, Rivera JJ, Smeds E, Ko Y-P, Bowden MG, Wann ER, Gurusidappa S, Fitzgerald JR, Höök M. 2008 A structural model of the *Staphylococcus aureus* ClfA-fibrinogen interaction opens new avenues for the design of anti-staphylococcal therapeutics. PLoS Pathog 4:e1000226. [PubMed: 19043557]

91. Ponnuraj K, Bowden MG, Davis S, Gurusiddappa S, Moore D, Choe D, Xu Y, Hook M, Narayana SV. 2003 A "dock, lock, and latch" structural model for a staphylococcal adhesin binding to fibrinogen. Cell 115:217–228. [PubMed: 14567919]

92. Bowden MG, Heuck AP, Ponnuraj K, Kolosova E, Choe D, Gurusiddappa S, Narayana SV, Johnson AE, Höök M. 2008 Evidence for the "dock, lock, and latch" ligand binding mechanism of the staphylococcal microbial surface component recognizing adhesive matrix molecules (MSCRAMM) SdrG. J Biol Chem 283:638–647. [PubMed: 17991749]

93. Flick MJ, Du X, Prasad JM, Raghu H, Palumbo JS, Smeds E, Höök M, Degen JL. 2013 Genetic elimination of the binding motif on fibrinogen for the *S. aureus* virulence factor ClfA improves host survival in septicemia. Blood in press.

94. O'Brien L, Kerrigan SW, Kaw G, Hogan M, Penades J, Litt D, Fitzgerald DJ, Foster TJ, Cox D. 2002 Multiple mechanisms for the activation of human platelet aggregation by Staphylococcus aureus: roles for the clumping factors ClfA and ClfB, the serine-aspartate repeat protein SdrE and protein A. Molecular microbiology 44:1033–44. [PubMed: 12010496]

95. Loughman A, Fitzgerald JR, Brennan MP, Higgins J, Downer R, Cox D, Foster TJ. 2005 Roles for fibrinogen, immunoglobulin and complement in platelet activation promoted by Staphylococcus aureus clumping factor A. Mol Microbiol 57:804–18. [PubMed: 16045623]

96. Ní Eidhin D, Perkins S, Francois P, Vaudaux P, Höök M, Foster TJ. 1998 Clumping factor B (ClfB), a new surface-located fibrinogen-binding adhesin of *Staphylococcus aureus*. Mol Microbiol 30:245–257. [PubMed: 9791170]

97. Walsh EJ, Miajlovic H, Gorkun OV, Foster TJ. 2008 Identification of the Staphylococcus aureus MSCRAMM clumping factor B (ClfB) binding site in the alphaC-domain of human fibrinogen. Microbiology 154:550–558. [PubMed: 18227259]

98. Perkins S, Walsh EJ, Deivanayagam CC, Narayana SV, Foster TJ, Hook M. 2001 Structural organization of the fibrinogen-binding region of the clumping factor B MSCRAMM of Staphylococcus aureus. J Biol Chem 276:44721–8. [PubMed: 11568183]

99. Haim M, Trost A, Maier CJ, Achatz G, Feichtner S, Hintner H, Bauer JW, Onder K. 2010 Cytokeratin 8 interacts with clumping factor B: a new possible virulence factor target. Microbiology 156:3710–3721. [PubMed: 20817646]

100. Walsh EJ, O'Brien LM, Liang X, Hook M, Foster TJ. 2004 Clumping factor B, a fibrinogen-binding MSCRAMM (microbial surface components recognizing adhesive matrix molecules) adhesin of Staphylococcus aureus, also binds to the tail region of type I cytokeratin 10. J Biol Chem 279:50691–9. [PubMed: 15385531]

101. O'Brien LM, Walsh EJ, Massey RC, Peacock SJ, Foster TJ. 2002 Staphylococcus aureus clumping factor B (ClfB) promotes adherence to human type I cytokeratin 10: implications for nasal colonization. Cell Microbiol 4:759–770. [PubMed: 12427098]

102. Mulcahy ME, Geoghegan JA, Monk IR, O'Keeffe KM, Walsh EJ, Foster TJ, McLoughlin RM. 2012 Nasal colonisation by *Staphylococcus aureus* depends upon clumping factor B binding to the squamous epithelial cell envelope protein loricrin. PLoS Pathog 8:e1003092. [PubMed: 23300445]

103. Ganesh VK, Barbu EM, Deivanayagam CC, Le B, Anderson AS, Matsuka YV, Lin SL, Foster TJ, Narayana SV, Höök M. 2011 Structural and biochemical characterization of *Staphylococcus aureus* clumping factor B/ligand interactions. J Biol Chem 286:25963–25972. [PubMed: 21543319]

104. Cheng AG, DeDent AC, Schneewind O, Missiakas DM. 2011 A play in four acts: *Staphylococcus aureus* abscess formation. Trends Microbiol 19:225–232. [PubMed: 21353779]
105. Berends ET, Horswill AR, Haste NM, Monestier M, Nizet V, von Köckritz-Blickwede M. 2010 Nuclease expression by *Staphylococcus aureus* facilitates escape from neutrophil extracellular traps. J Innate Immun 2:576–586. [PubMed: 20829609]
106. Thammavongsa V, Missiakas DM, Schneewind O. 2013 Staphylococcus aureus conversion of neutrophil extracellular traps into deoxyadenosine promotes immune cell death Science 342:863–866. [PubMed: 24233725]
107. Winstel V, Missiakas D, Schneewind O. 2018 *Staphylococcus aureus* targets the purine salvage pathway to kill phagocytes. Proc Natl Acad Sci USA 115:6846–6851. [PubMed: 29891696]
108. Thammavongsa V, Kern JW, Missiakas DM, Schneewind O. 2009 *Staphylococcus aureus* synthesizes adenosine to escape host immune responses. J Exp Med 206:2417–2427. [PubMed: 19808256]
109. Thammavongsa V, Schneewind O, Missiakas DM. 2011 Enzymatic properties of *Staphylococcus aureus* adenosine synthase (AdsA). BMC Biochem 12:56. [PubMed: 22035583]
110. Josefsson E, Higgins J, Foster TJ, Tarkowski A. 2008 Fibrinogen binding sites P336 and Y338 of clumping factor are crucial for *Staphylococcus aureus* virulence. PLoS One 3:e2206. [PubMed: 18493318]
111. Domanski PJ, Patel PR, Bayer AS, Zhang L, Hall AE, Syribeys PJ, Gorovits EL, Bryant D, Vernachio JH, Hutchins JT, Patti JM. 2005 Characterization of a humanized monoclonal antibody recognizing clumping factor A expressed by *Staphylococcus aureus*. Infect Immun 73:5229–5232. [PubMed: 16041045]
112. Ganesh VK, Liang X, Geoghegan JA, Cohen AL, Venugopalan N, Foster TJ, Hook M. 2016 Lessons from the crystal structure of the S. aureus surface protein clumping factor A in complex with tefibazumab, an inhibiting monoclonal antibody. EBioMedicine 13:328–338. [PubMed: 27789272]
113. Hall AE, Domanski PJ, Patel PR, Vernachio JH, Syribeys PJ, Gorovits EL, Johnson MA, Ross JM, Hutchins JT, Patti JM. 2003 Characterization of a protective monoclonal antibody recognizing *Staphylococcus aureus* MSCRAMM protein clumping factor A. Infect Immun 71:6864–6870. [PubMed: 14638774]
114. Weems JJ, Jr, Steinberg JP, Filler S, Baddley JW, Corey GR, Sampathkumar P, Winston L, John JF, Kubin CJ, Talwani R, Moore T, Patti JM, Hetherington S, Texter M, Wenzel E, Kelley VA, Fowler VG, Jr. 2006 Phase II, randomized, double-blind, multicenter study comparing the safety and pharmacokinetics of Tefibazumab to placebo for treatment of *Staphylococcus aureus* bacteremia. Antimicrob Agents Chemother 50:2751–2755. [PubMed: 16870768]
115. Tkaczyk C, Hamilton MM, Sadowska A, Shi Y, Chang CS, Chowdhury P, Buonapane R, Xiao X, Warrener P, Mediavilla J, Kreiswirth B, Suzich J, Stover CK, Sellman BR. 2016 Targeting alpha toxin and ClfA with a multimechanistic monoclonal-antibody-based approach for prophylaxis of serious *Staphylococcus aureus* disease. MBio 7:e00528–16. [PubMed: 27353753]
116. Tkaczyk C, Hua L, Varkey R, Shi Y, Dettinger L, Woods R, Barnes A, MacGill RS, Wilson S, Chowdhury P, Stover CK, Sellman BR. 2012 Identification of anti-alpha toxin monoclonal antibodies that reduce the severity of *Staphylococcus aureus* dermonecrosis and exhibit a correlation between affinity and potency. Clin Vaccine Immunol 19:377–385. [PubMed: 22237895]
117. Tkaczyk C, Kasturirangan S, Minola A, Jones-Nelson O, Gunter V, Shi YY, Rosenthal K, Aleti V, Semenova E, Warrener P, Tabor D, Stover CK, Corti D, Rainey G, Sellman BR. 2017 Multimechanistic Monoclonal Antibodies (MAbs) Targeting Staphylococcus aureus Alpha-Toxin and Clumping Factor A: Activity and Efficacy Comparisons of a MAb Combination and an Engineered Bispecific Antibody Approach. Antimicrob Agents Chemother 61:e00629–17. [PubMed: 28584141]
118. Creech CB, Frenck RWJ, Sheldon EA, Seiden DJ, Kankam MK, Zito ET, Girgenti D, Severs JM, Immermann FW, McNeil LK, Cooper D, Jansen KU, Gruber W, Eiden J, Anderson AS, Baber J. 2017 Safety, tolerability, and immunogenicity of a single dose 4-antigen or 3-antigen *Staphylococcus aureus* vaccine in healthy older adults: results of a randomised trial. Vaccine 35:385–394. [PubMed: 27866765]

119. Scully IL, Liberator PA, Jansen KU, Anderson AS. 2014 Covering all the bases: preclinical development of an effective *Staphylococcus aureus* vaccine. Front Immunol 5:109. [PubMed: 24715889]

120. Stranger-Jones YK, Bae T, Schneewind O. 2006 Vaccine assembly from surface proteins of *Staphylococcus aureus*. Proc Nat Acad Sci USA 103:16942–16947. [PubMed: 17075065]

121. Kuklin NA, Clark DJ, Secore S, Cook J, Cope LD, McNeely T, Noble L, Brown MJ, Zorman JK, Wang XM, Pancari G, Fan H, Isett K, Burgess B, Bryan J, Brownlow M, George H, Meinz M, Liddell ME, Kelly R, Schultz L, Montgomery D, Onishi J, Losada M, Martin M, Ebert T, Tan CY, Schofield TL, Nagy E, Meineke A, Joyce JG, Kurtz MB, Caulfield MJ, Jansen KU, McClements W, Anderson AS. 2006 A novel *Staphylococcus aureus* vaccine: iron surface determinant B induces rapid antibody responses in rhesus macaques and specific increased survival in a murine *S. aureus* sepsis model. Infect Immun 74:2215–2223. [PubMed: 16552052]

122. Kim HK, DeDent A, Cheng AG, McAdow M, Bagnoli F, Missiakas DM, Schneewind O. 2010 IsdA and IsdB antibodies protect mice against *Staphylococcus aureus* abscess formation and lethal challenge. Vaccine 28:6382–6392. [PubMed: 20226248]

123. Brown M, Kowalski R, Zorman J, Wang X-m, Towne V, Zhao Q, Secore S, Finnefrock AC, Ebert T, Pancari G, Isett K, Zhang Y, Anderson AS, Montgomery D, Cope L, McNeely T 2009 Selection and characterization of murine monoclonal antibodies to *Staphylococcus aureus* iron-regulated surface determinant B with functional activity *in vitro* and *in vivo*. Clin Vaccine Immunol 16:1095–1104. [PubMed: 19553551]

124. Fowler VG, Allen KB, Moreira ED, Moustafa M, Isgro F, Boucher HW, Corey GR, Carmeli Y, Betts R, Hartzel JS, Chan IS, McNeely TB, Kartsonis NA, Guris D, Onorato MT, Smugar SS, DiNubile MJ, Sobanjo-ter Meulen A. 2013 Effect of an investigational vaccine for preventing *Staphylococcus aureus* infections after cardiothoracic surgery: a randomized trial. JAMA 309:1368–1378. [PubMed: 23549582]

125. Kim HK, Emolo C, DeDent AC, Falugi F, Missiakas DM, Schneewind O. 2012 Protein A-specific monoclonal antibodies and the prevention of *Staphylococcus aureus* disease in mice. Infect Immun 80:3460–3470. [PubMed: 22825452]

126. Thammavongsa V, Rauch S, Kim HK, Missiakas DM, Schneewind O. 2015 Protein A-neutralizing monoclonal antibody protects neonatal mice against *Staphylococcus aureus*. Vaccine 33:523–526. [PubMed: 25488332]

127. Ton-That H, Mazmanian H, Faull KF, Schneewind O. 2000 Anchoring of surface proteins to the cell wall of *Staphylococcus aureus*. I. Sortase catalyzed *in vitro* transpeptidation reaction using LPXTG peptide and NH_2-Gly_3 substrates. J Biol Chem 275:9876–9881. [PubMed: 10734144]

128. Ton-That H, Mazmanian SK, Alksne L, Schneewind O. 2002 Anchoring of surface proteins to the cell wall of *Staphylococcus aureus*. II. Cysteine 184 and histidine 120 of sortase A form a thiolate imidazolium ion pair for catalysis. J Biol Chem 277:7447–7452. [PubMed: 11714722]

129. Maresso AW, Wu R, Kern JW, Zhang R, Janik D, Missiakas DM, Duban ME, Joachimiak A, Schneewind O. 2007 Activation of inhibitors by sortase triggers irreversible modification of the active site. J Biol Chem 282:23129–23139. [PubMed: 17545669]

130. Maresso AW, Schneewind O. 2008 Sortase as a target of anti-infective therapy. Pharmacol Rev 60:128–141. [PubMed: 18321961]

131. Zhang J, Liu H, Zhu K, Gong S, Dramsi S, Wang YT, Li J, Chen F, Zhang R, Zhou L, Lan L, Jiang H, Schneewind O, Luo C, Yang CG. 2014 Antiinfective therapy with a small molecule inhibitor of *Staphylococcus aureus* sortase. Proc Natl Acad Sci USA 111:13517–13522. [PubMed: 25197057]

132. Oh KB, Nam KW, Ahn H, Shin J, Kim S, Mar W. 2010 Therapeutic effect of (Z)-3-(2,5-dimethoxyphenyl)-2-(4-methoxyphenyl) acrylonitrile (DMMA) against Staphylococcus aureus infection in a murine model. Biochem Biophys Res Commun 396:440–444. [PubMed: 20433810]

133. Pallen MJ, Lam AC, Antonio M, Dunbar K. 2001 An embarrassment of sortases - a richness of substrates. Trends Microbiol 9:97–101. [PubMed: 11239768]

134. Hendrickx AP, Budzik JM, Oh SY, Schneewind O. 2011 Architects at the bacterial surface - sortases and the assembly of pili with isopeptide bonds. Nat Rev Microbiol 9:166–176. [PubMed: 21326273]

135. Jacobitz AW, Kattke MD, Wereszczynski J, Clubb RT. 2017 Sortase Transpeptidases: Structural Biology and Catalytic Mechanism. Adv Protein Chem Struct Biol 109:223–264. [PubMed: 28683919]
136. Ton-That H, Schneewind O. 2003 Assembly of pili on the surface of *Corynebacterium diphtheriae*. Mol Microbiol 50:1429–1438. [PubMed: 14622427]
137. Mandlik A, Swierczynski A, Das A, Ton-That H. 2007 *Corynebacterium diphtheriae* employs specific minor pilins to target human pharyngeal epithelial cells. Mol Microbiol 64:111–124. [PubMed: 17376076]
138. Budzik JM, Marraffini LA, Souda P, Whitelegge JP, Faull KF, Schneewind O. 2008 Amide bonds assemble pili on the surface of bacilli. Proc Nat Acad Sci USA 105:10215–10220. [PubMed: 18621716]
139. Budzik JM, Oh SY, Schneewind O. 2008 Cell wall anchor structure of BcpA pili in *Bacillus anthracis*. J Biol Chem 283:36676–36686. [PubMed: 18940793]
140. Budzik JM, Oh SY, Schneewind O. 2009 Sortase D forms the covalent bond that links BcpB to the tip of *Bacillus cereus* pili. J Biol Chem 284:12989–12997. [PubMed: 19269972]
141. Ton-That H, Marraffini L, Schneewind O. 2004 Sortases and pilin elements involved in pilus assembly of *Corynebacterium diphtheriae*. Mol Microbiol 53:1147–1156. [PubMed: 15306018]
142. Swaminathan A, Mandlik A, Swierczynski A, Gaspar A, Das A, Ton-That H. 2007 Housekeeping sortase facilitates the cell wall anchoring of pilus polymers in *Corynebacterium diphtheriae*. Mol Microbiol 66:961–974. [PubMed: 17919283]
143. Mandlik A, Das A, Ton-That H. 2008 The molecular switch that activates the cell wall anchoring step of pilus assembly in gram-positive bacteria. Proc Natl Acad Sci USA 105:14147–14152. [PubMed: 18779588]
144. Chang C, Amer BR, Osipiuk J, McConnell SA, Huang IH, Hsieh V, Fu J, Nguyen HH, Muroski J, Flores E, Ogorzalek Loo RR, Loo JA, Putkey JA, Joachimiak A, Das A, Clubb RT, Ton-That H. 2018 In vitro reconstitution of sortase-catalyzed pilus polymerization reveals structural elements involved in pilin cross-linking. Proc Natl Acad Sci USA 115:E5477–E5486. [PubMed: 29844180]
145. McDevitt D, Francois P, Vaudaux P, Foster TJ. 1994 Molecular characterization of the clumping factor (fibrinogen receptor) of Staphylococcus aureus. Mol Microbiol 11:237–48. [PubMed: 8170386]
146. Hair PS, Ward MD, Semmes OJ, Foster TJ, Cunnion KM. 2008 Staphylococcus aureus clumping factor A binds to complement regulator factor I and increases factor I cleavage of C3b. J Infect Dis 198:125–33. [PubMed: 18544012]
147. Zong Y, Xu Y, Liang X, Keene DR, Hook A, Gurusiddappa S, Hook M, Narayana SV. 2005 A 'Collagen Hug' model for Staphylococcus aureus CNA binding to collagen. EMBO J 24:4224–36. [PubMed: 16362049]
148. Kang M, Ko YP, Liang X, Ross CL, Liu Q, Murray BE, Hook M. 2013 Collagen-binding microbial surface components recognizing adhesive matrix molecule (MSCRAMM) of Gram-positive bacteria inhibit complement activation via the classical pathway. J Biol Chem 288:20520–31. [PubMed: 23720782]
149. Komatsuzawa H, Sugai M, Ohta K, Fujiwara T, Nakashima S, Suzuki J, Lee CY, Suginaka H. 1997 Cloning and characterization of the fmt gene which affects the methicillin resistance level and autolysis in the presence of triton X-100 in methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 41:2355–61. [PubMed: 9371333]
150. Kuusela P, Hilden P, Savolainen K, Vuento M, Lyytikainen O, Vuopio-Varkila J. 1994 Rapid detection of methicillin-resistant Staphylococcus aureus strains not identified by slide agglutination tests. J Clin Microbiol 32:143–7. [PubMed: 8126170]
151. Kuusela P, Saksela O. 1990 Binding and activation of plasminogen at the surface of Staphylococcus aureus. Increase in affinity after conversion to the Lys form of the ligand. Eur J Biochem 193:759–65. [PubMed: 1701146]
152. Schroeder K, Jularic M, Horsburgh SM, Hirschhausen N, Neumann C, Bertling A, Schulte A, Foster S, Kehrel BE, Peters G, Heilmann C. 2009 Molecular characterization of a novel

Staphylococcus aureus surface protein (SasC) involved in cell aggregation and biofilm accumulation. PLoS One 4:e7567. [PubMed: 19851500]

153. Barbu EM, Ganesh VK, Gurusiddappa S, Mackenzie RC, Foster TJ, Sudhof TC, Hook M. 2010 beta-Neurexin is a ligand for the Staphylococcus aureus MSCRAMM SdrC. PLoS Pathog 6:e1000726. [PubMed: 20090838]
154. Feuillie C, Formosa-Dague C, Hays LM, Vervaeck O, Derclaye S, Brennan MP, Foster TJ, Geoghegan JA, Dufrene YF. 2017 Molecular interactions and inhibition of the staphylococcal biofilm-forming protein SdrC. Proc Natl Acad Sci U S A 114:3738–3743. [PubMed: 28320940]
155. Askarian F, Ajayi C, Hanssen AM, van Sorge NM, Pettersen I, Diep DB, Sollid JU, Johannessen M. 2016 The interaction between Staphylococcus aureus SdrD and desmoglein 1 is important for adhesion to host cells. Sci Rep 6:22134. [PubMed: 26924733]
156. Zhang Y, Wu M, Hang T, Wang C, Yang Y, Pan W, Zang J, Zhang M, Zhang X. 2017 Staphylococcus aureus SdrE captures complement factor H's C-terminus via a novel 'close, dock, lock and latch' mechanism for complement evasion. Biochem J 474:1619–1631. [PubMed: 28258151]
157. Uhlen M, Guss B, Nilsson B, Gatenbeck S, Philipson L, Lindberg M. 1984 Complete sequence of the staphylococcal gene encoding protein A. A gene evolved through multiple duplications. J Biol Chem 259:1695–702. [PubMed: 6319407]
158. Graille M, Stura EA, Corper AL, Sutton BJ, Taussig MJ, Charbonnier JB, Silverman GJ. 2000 Crystal structure of a Staphylococcus aureus protein A domain complexed with the Fab fragment of a human IgM antibody: structural basis for recognition of B-cell receptors and superantigen activity. Proc Natl Acad Sci U S A 97:5399–404. [PubMed: 10805799]
159. Kukita K, Kawada-Matsuo M, Oho T, Nagatomo M, Oogai Y, Hashimoto M, Suda Y, Tanaka T, Komatsuzawa H. 2013 Staphylococcus aureus SasA is responsible for binding to the salivary agglutinin gp340, derived from human saliva. Infect Immun 81:1870–9. [PubMed: 23439307]

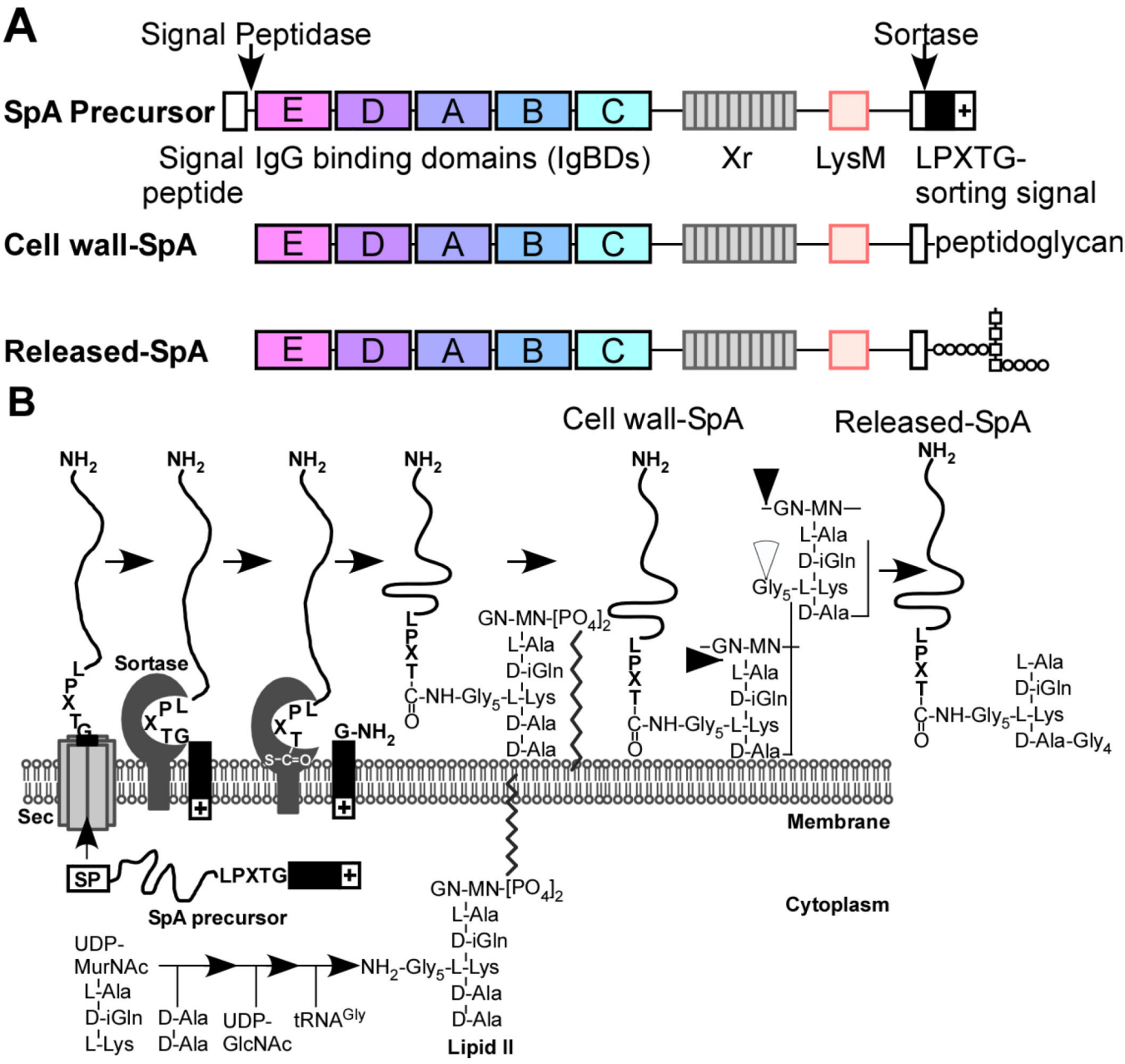

FIG. 1.

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 crossbridge. 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-Ala-Gly_4.

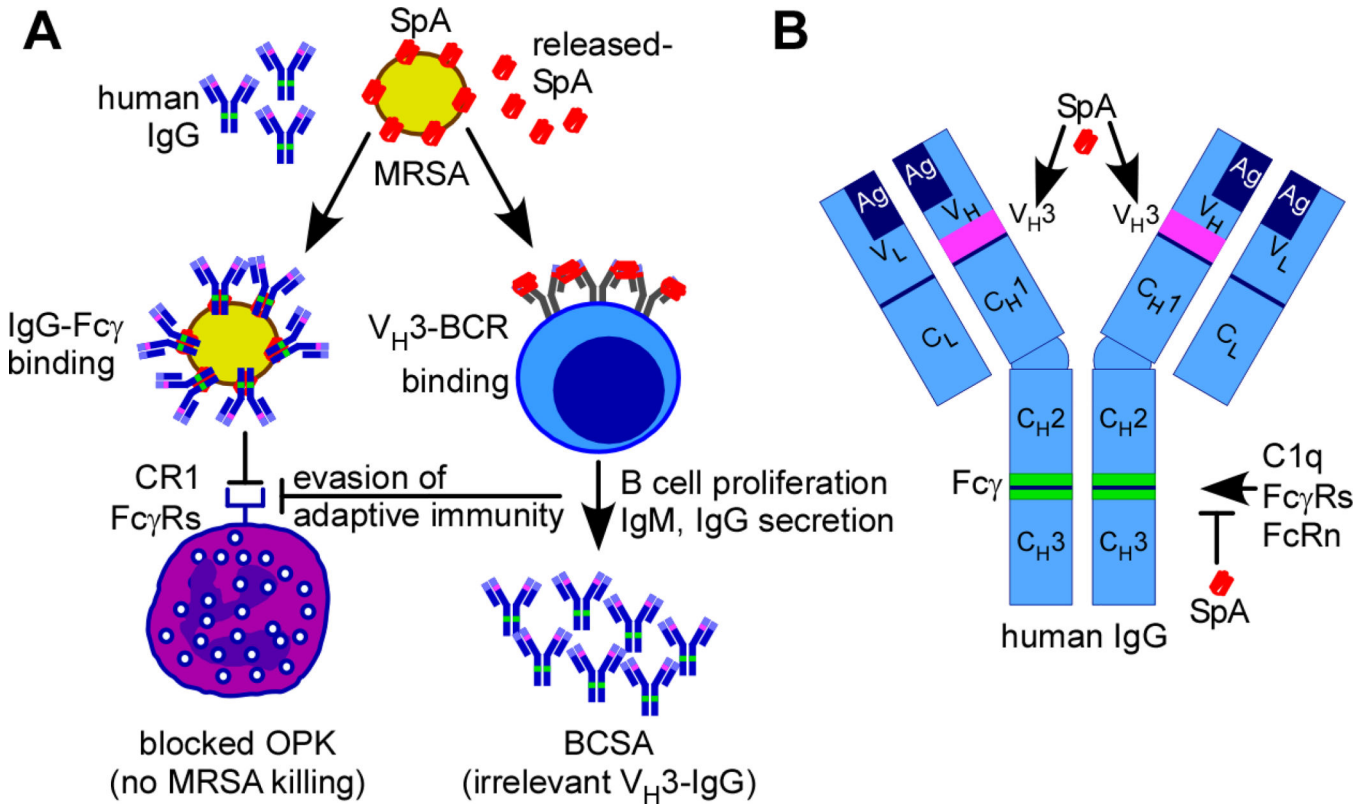

FIG. 2.

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 crosslinks 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 and C_H1, C_H2 & 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 Fc γ are identified in pink and green color, respectively.

TABLE 1.

Staphylococcus aureus cell wall-anchored surface proteins [1].

Sortase A anchored protein	Name(s)	Genbank accession number	aa [2]	Ligand(s) [3]	YSIRK Motif [4]	Sorting Motif [5]	Reference
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	(145, 146)
Clumping Factor B	ClfB	ABD21326.1	899	Fibrinogen (α chain) Cytokeratin 8 & 10 Loricrin	Yes	LPETG	(97–102)
Collagen adhesin	Cna	BAF45800.1	1,183	Collagen C1q	No	LPKTG	(147, 148)
Factor affecting methicillin resistance in Triton X-100 B	FmtB (SasB)	ATC68490.1	2,478	Unknown	Yes	LPDTG	(149)
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	HaptoglobinHemoglobin Heme	Yes	LPKTG	(36–40)
Plasmin sensitive surface protein	Pls	AAD09131.1	1,637	Unknown	Yes	LPDTG	(150, 151)
S. aureus surface -protein C	SasC	ABD21355.1	2,186	Promotes intercellular adhesion	Yes	LPNTG	(152)
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	(153, 154)
Serine aspartic repeat -protein D	SdrD	ABD20874.1	1,381	Desmoglein 1	Yes	LPETG	(155)
Serine aspartic repeat -protein E	SdrE	ABD22410.1	1,154	Factor H	Yes	LPETG	(156)
S. aureus protein A	SpA	ABD22331.1	508	Immunoglobulin (Fcγ, Fab V_H3)	Yes	LPETG	(70, 71, 157, 158)
Serine-rich adhesin for platelets	SraP (SasA)	ABD21900.1	2,271	Salivary agglutinin (gp340)	Possibly	LPDTG	(34, 159)
Sortase B anchored protein	**Name(s)**	**Genbank accession number**	**aa** [1]	**Ligand(s)** [2]	**YSIRK motif** [3]	**Sorting motif** [4]	**Reference**
Iron-regulated surface determinant C	IsdC	ABD20415.1	227	Heme transferred from IsdA	No	NPQTN	(28)

[1]The number of cell wall-anchored surface proteins varies among strains 272 of *S. aureus* (26). For example, in strain *S. 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.

[2]aa, protein length in amino acids.

[3]Molecular component(s) recognized and bound by protein, or molecules synthesized in case of AdsA.

[4]Consensus motif found in some signal sequences which presumably accounts for secretion of proteins at the cross walls (62).

[5]Consensus motif recognized by sortases and present in C-terminal cell wall sorting signal.