Staphylococcal manipulation of host immune responses

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Abstract | *Staphylococcus aureus*, a bacterial commensal of the human nares and skin, is a frequent cause of soft tissue and bloodstream infections. A hallmark of staphylococcal infections is their frequent recurrence, even when treated with antibiotics and surgical intervention, which demonstrates the bacterium's ability to manipulate innate and adaptive immune responses. In this Review, we highlight how *S. aureus* virulence factors inhibit complement activation, block and destroy phagocytic cells and modify host B cell and T cell responses, and we discuss how these insights might be useful for the development of novel therapies against infections with antibiotic resistant strains such as methicillin-resistant *S. aureus*.

Abscesses
The pathological product of *Staphylococcus aureus* infection: the harbouring of a staphylococcal abscess community within a pseudocapsule of fibrin deposits that is surrounded by layers of infiltrating immune cells destroying physiological organ tissue.

Recurrence
The propensity of *S. aureus* infections to reoccur when surgery and/or antibiotic therapy are initially effective.

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Approximately 30% of the human population is continuously colonized with *Staphylococcus aureus*, whereas some individuals are hosts for intermittent colonization[1]. *S. aureus* typically resides in the nares but is also found on the skin and in the gastrointestinal tract. Although colonization is not a prerequisite for staphylococcal disease, colonized individuals more frequently acquire infections[1]. Skin and soft-tissue infections (SSTIs) are the most frequent disease form of *S. aureus*, and these infections can progress to bacteraemia and invasive disease (that is, bloodstream infection, endocarditis or sepsis[2]). In addition, *S. aureus* can cause pneumonia, osteomyelitis, infectious arthritis, abscesses in many organ tissues and infections of surgical wounds or prosthetic materials[2]. Annual attack rates for *S. aureus* disease range between 1–3% and vary with age, ethnicity and geographical location of human populations[2]. At elevated risk for staphylococcal infection are low-birth-weight infants, children, the elderly and patients with indwelling catheters, endotracheal intubation, medical implantation of foreign bodies, trauma, surgical procedures, haemodialysis, diabetes or immunosuppressive or cancer therapy[2]. A key feature of *S. aureus* disease is its recurrence, which occurs for 8–33% of cases of SSTI and bloodstream infections[3]. Prior disease does not elicit protection against subsequent *S. aureus* infection[2].

Neutrophils play a central part in protecting humans against *S. aureus* infection. Staphylococcal entry and replication in host tissues leads to the release of bacterial products (formyl-peptides, lipoproteins or peptidoglycan) and to damaged tissues that produce inflammatory signals (that is, chemoattractants and cytokines[4]). Staphylococcal products are detected by immune cells via Toll-like receptors (TLRs) and G protein-coupled receptors, whereas cytokines activate cognate immune receptors. Neutrophils answer this call, extravasate from blood vessels, and migrate towards the site of infection to phagocytose and kill bacteria or to immobilize and damage the pathogen through NETosis — the release of neutrophil extracellular traps (NETs) comprising DNA and antimicrobial peptides[4]. The importance of neutrophils in controlling *S. aureus* infection has been documented through the study of immune defects. Mutations in genes encoding NADPH oxidase, the enzyme generating bactericidal superoxide in phagocytes, cause chronic granulomatous disease (CGD), which is associated with defects in phagocytic killing of *S. aureus* and frequent infection[5]. Individuals with inborn errors of signal transducer and activator of transcription 1 (STAT1) or STAT3 signalling of immune cells have perturbed interleukin-17 (IL-17) cytokine pathways, which diminishes mucocutaneous immunity and promotes *S. aureus* infection[6]. IL-17-dependent T cell signalling is a key activator of neutrophils and of anti-staphylococcal defences[7]. Finally, cancer patients with diminished blood neutrophil counts are highly susceptible to *S. aureus* infection[8].

Nevertheless, the vast majority of *S. aureus* disease occurs in immune-competent individuals without defects in phagocyte function. To achieve this, *S. aureus* deploys an arsenal of immune-evasive strategies that together prevent phagocytosis and killing by neutrophils. Furthermore, the ability of the pathogen to cause

Box 1 | **Variability of *Staphylococcus aureus* immune evasion determinants**

Genome sequencing of *Staphylococcus aureus* isolates from humans and animals has provided insights into the origin, diversification and spread of the pathogen. Over the past 10,000 years, *S. aureus* evolved as a colonizer and pathogen of humans and their lifestock[142], generating lineages with unique genetic traits and discrete host ranges[143]. Staphylococcal evolution was accompanied by the loss of genes encoding the CRISPR–Cas system, which protect the genome against phages and mobile genetic elements. *S. aureus* relies on horizontal gene transfer mediated by these elements for adaptation, and it preserves its identity through restriction modification systems and satellite phage-encoded pathogenicity islands that block phage replication[143]. When placed under selection in different hosts, *S. aureus* acquires mobile genetic elements that contain genes for antibiotic resistance, immune evasion and adhesion to specific anatomic niches. Analysis of large genome datasets described the core genome, which is common to all *S. aureus* isolates, and found that these core genes contribute to colonization, tissue invasion, establishment of abscess lesions, dissemination, immune evasion and pathogenesis of reiterative disease[36]. Variable genes are associated with *S. aureus* colonization or invasion of specific host species or may be present in subsets of strains associated with increased virulence or specific disease (for example, enterotoxin-mediated gastroenteritis)[93]. Generally, capsular polysaccharide and cell wall-anchored surface proteins are components of the core genome and contribute to immune evasion by synthesizing adenosine (by adenosine synthase A), binding fibrinogen or fibrin (by clumping factors A and B and fibronectin binding proteins A and B) and binding immunoglobulin (by staphylococcal protein A (SpA)). Several secreted proteins are components of the core genome: proteases that cleave host factors (such as aureolysin and staphopain), coagulases that activate prothrombin (such as coagulase and von-Willebrand factor binding protein), toxins that lyse immune cells (such as γ-haemolysin AB, γ-haemolysin CB and leukocidin AB), inhibitors of host proteases (staphylococcal extracellular adherence protein and its homologues) and phenol-soluble modulins, peptides that perturb host cell membranes and trigger neutrophil chemotaxis. Genetic determinants that interfere with neutrophil chemotaxis, phagocytosis, complement activation, promote lysis of immune cells or activate T cells often represent constituents of the variable genome. TABLE 1 summarizes core genome and variable immune evasion factors contributing to staphylococcal disease pathogenesis. Isolates of the CC75 lineage are predominantly found in the South-West Pacific and were originally isolated from superficial skin lesions of individuals from the indigenous communities of Australia[144]. CC75 strains lack the staphyloxanthin gene cluster, retain the CRISPR–Cas system and lack *S. aureus* pathogenicity islands (SaPIs), but they are endowed with genomic island-α and genomic island-β, a unique *coa* gene and a unique *spa* sequence type, which are elements important for staphylococcal evasion of innate and adaptive immune responses[144]. These strains, with the species designation *Staphylococcus argenteus*, may represent an early and terminal branch in the development of *S. aureus* in which mobile genetic elements were not incorporated into the genome because the retained CRISPR–Cas system prevented horizontal gene transfer[144].

recurrent disease implies the presence of mechanisms that effectively block the development of adaptive immune responses. Here, we review recent work on the immune evasive attributes of *S. aureus*, including the subversion of the innate and adaptive immune systems and the killing of immune cells, along with epidemiological features of the corresponding genes. We also discuss how the characterization of bacterial immune evasive factors can have translational effects in the therapy of autoimmune diseases or the development of vaccines and immunotherapeutics against *S. aureus* infection.

Subversion of innate immune responses

Neutrophil extravasation and chemotaxis. Pro-inflammatory signals promote neutrophil adhesion and extravasation across capillary endothelia, relying on reciprocal interactions between endothelial receptors (such as P-selectin, E selectin, intercellular adhesion molecule 1 (ICAM1) and hyaluronan) and ligands on neutrophil surfaces (such as P-selectin glycoprotein ligand 1 (PSGL1), lymphocyte function-associated antigen 1 (LFA1; also known as αLβ2 integrin), αMβ2 integrin and CD44)[9]. Although neutrophils function to migrate towards bacterial invaders, *S. aureus* can interfere with neutrophil extravasation and chemotaxis through the secretion of staphylococcal superantigen-like proteins (SSLs), phenol-soluble modulins (PSMs), chemotaxis inhibitory protein of *S. aureus* (CHIPS), formyl peptide receptor-like 1 inhibitor (FLIPr) and its homologue FLIPr-like (FLIPrL).

SSLs are a family of secreted proteins with structural homology to staphylococcal superantigens[10–12]. The *ssl* genes are arranged as tandem repeats in genomic island-α (GIα; for *ssl1–ssl11*) and in the immune evasion cluster 2 (IEC2; for *ssl12–ssl14*) on the bacterial chromosome[13]. GIα-encoded *ssl* genes vary between lineages as does the coding sequence of individual *ssl* genes; the number of different alleles ranges from 1 to 13 and most alleles are uniquely associated with specific *S. aureus* lineages[13]. *ssl1*, *ssl2*, *ssl3*, *ssl11*, *ssl12*, *ssl13* and *ssl14* are found in all *S. aureus* isolates[13] (BOX 1). Purified, recombinant SSL5 and SSL11 bind PSGL1 on leukocytes and, when assayed *in vitro*, interfere with the binding of neutrophils to P-selectin and neutrophil adhesion and rolling[14,15] (FIG. 1a). SSL5 also interferes with chemokine- and anaphylatoxin-mediated activation of neutrophils by binding to the glycosylated amino termini of G protein-coupled receptors[14,16]. Moreover, SSL5 has been shown to activate platelets and support their adhesion involving platelet surface receptors GPIbα and GPVI[17,18]. Intravenous administration of SSL5 caused intravascular platelet-rich thrombi and increased bleeding of C57Bl/6 mice[19]. Other work demonstrated SSL5-mediated inactivation of leukocyte matrix metalloproteinase 9 (REF. 20). The affinity of SSL5 for different host factors is mediated via its glycan binding pocket, an attribute that is shared by other members of the SSL family[21]. SSL3 binds to TLR2 and blocks immune cell recognition of staphylococcal lipoproteins and peptidoglycan via TLR1–TLR2 and TLR2–TLR6 heterodimers[22], and SSL10 blocks C-X-C chemokine receptor 4 (CXCR4)-mediated

Superantigen
Molecules that crosslink B cell receptors (that is, IgM) or T cell receptors and major histocompatibility complexes to trigger lymphocyte proliferation, thereby diverting adaptive immune responses.

Anaphylatoxin
Protein fragments generated during complement activation of C3a and C5a that trigger immune responses via C3a and C5a receptors on immune cells.

responses on lymphocytes, interfering with the chemoattraction of neutrophils[4] (FIG. 1a). Recent work suggests that SelX, a staphylococcal enterotoxin (superantigen)-like protein, also binds glycosylated PSGL1, and that SSL6 binds to CD47 (also known as integrin-associated protein), a common receptor on most host tissues that promotes migration, anti-phagocytosis and proliferation[23]. Thus, SSLs presumably associate with a wide range of glycoproteins on leukocytes and/or platelets to implement immune-evasive attributes. Most SSLs display species specificity for human but not animal host factors, and SSL-mediated contributions towards *S. aureus* pathogenesis cannot be measured in animal experiments. *S. aureus* also inhibits leukocyte migration via the extracellular adherence protein (Eap). Eap is composed of four β-grasp-like domains and associates with ICAM1 to inhibit leukocyte migration[24]. The *eap* gene is located in the *eap–hlb* locus, the attachment site for *hlb*-converting phages carrying the IEC1 gene cluster[13] (BOX 1).

PSMs are a family of short formyl-peptides that are secreted via an ABC transporter and interfere with the physiological functions of immune cells, specifically neutrophils[25]. PSMα1–PSMα4 and PSMβ1, and PSMβ2, whether formylated or not, activate formyl-peptide receptor 2 (FPR2) on human and mouse neutrophils and stimulate cytokine release[26]. Of note, PSMα1–PSMα4 and PSMβ1 and PSMβ2 are neutralized by binding to serum lipoproteins, and reactive oxygen species (ROS) of activated neutrophils are reported to neutralize PSM signalling[27]. Recent work demonstrated that PSMα3N22Y, a variant with diminished FPR2-binding activity that is secreted by the clonal complex 30 (CC30) methicillin-resistant *S. aureus* (MRSA) lineage, is associated with diminished FPR2 signalling and diminished cytotoxicity, while enhancing bacterial replication and the establishment of abscess lesions in renal tissues[28]. The activation of FPRs via the formyl moiety of PSMs and via their direct binding to FPR2 stimulates chemotaxis. Thus, a key attribute of formylated PSMs seems to be the stimulation of neutrophil chemotaxis via FPRs.

S. aureus also counters neutrophil chemotaxis, which occurs via the secretion of CHIPS, FLIPr and FLIPrL[4] (FIG. 1a). CHIPS is encoded by the *chp* gene in IEC1, which is carried on *hlb*-converting phages[29]. *chp* is found in most human *S. aureus* isolates but not in livestock-associated strains[13]. Secreted CHIPS binds to human formyl-peptide receptor 1 (FPR1) and human C5aR, the complement receptor of neutrophils, but not to mouse FPR1 or C5aR[30,31]. FLIPr and FLIPrL are encoded by genes in the IEC2 locus; at least 9 different alleles are known for these genes, which are found in many, but not all, human *S. aureus* isolates[13] (BOX 1). FLIPrL inhibits FPR1 signalling, whereas FLIPr and FLIPrL bind to FPR2 and inhibit its receptor signalling function[32,33]. Finally, staphopain (ScpA), a secreted cysteine protease, cleaves CXCR2-binding chemokines to block neutrophil migration towards staphylococci[34] (FIG. 1a). The lifestyle of *S. aureus* — invasion of host tissues, replication in abscess lesions and dissemination upon purulent drainage of lesions — requires recruitment of immune cells to the site of infection[35,36]. However, staphylococcal products manipulate infiltrating immune cells to limit their capacity for chemotaxis, phagocytosis and bacterial killing, thereby ensuring the successful outcome of infection.

Complement activation and phagocytosis. Complement, a key component of innate host defences, is composed of >30 proteins that have broad functions in host defence against microorganisms, inflammation, haemostasis and wound repair. Complement is activated by any one of three routes: the classical pathway (which entails antibody and C1q deposition on the staphylococcal surface), the lectin pathway (which involves the association of mannose-binding lectin (MBL)–MBL-associated serine protease (MASP) complexes with staphylococcal carbohydrates) and the alternative pathway (which involves spontaneous breakdown of the complement protein C3 in the serum). The three pathways converge in the assembly of a C3 convertase (C4b2a for the classical and lectin pathways, and C3bBb for the alternative pathway). C3 convertase cleaves C3 into C3a and C3b; C3b becomes covalently linked to the staphylococcal surface (known as opsonization), and C3a is released as a chemoattractant for phagocytes[37]. At high local concentrations of C3b, the C5 convertase cleaves C5 into C5a, another chemoattractant, and C5b, the surface deposition of which promotes membrane attack complex (MAC) formation[37]. The MAC is effective at killing Gram-negative bacteria; however, it is not effective against *S. aureus*, which has a thick peptidoglycan layer that prevents access to the bacterial membrane[38].

In addition to the thick peptidoglycan layer, many clinical *S. aureus* strains express genes for the production of one of two types of capsular polysaccharide, type 5 or type 8 (REF. 39). *In vitro* phagocytosis assays suggested that capsule expression protects staphylococci from neutrophil phagocytosis in the presence of opsonins, and that capsule expression contributes to the pathogenesis of *S. aureus* infection in mice[39]. Capsule-induced protection from phagocytosis may be strain specific, as capsule mutations in other *S. aureus* isolates do not affect the pathogenesis of bloodstream infections in mice[36]. USA300, the current pandemic clone of community-acquired MRSA infections, carries a mutation that abrogates capsule expression[40] (TABLE 1).

S. aureus secretes several proteins that interfere with the deposition of complement on the bacterial surface (FIG. 1b). Aureolysin, a secreted zinc-dependent metalloproteinase, cleaves C3 to generate functionally active C3a and C3b. Complement factor I (fI) and factor H (fH) degrade or bind C3b, which prevents its accumulation on the staphylococcal surface[41]. The aureolysin gene, *aur*, is polymorphic and specific alleles are associated with different *S. aureus* lineages. An *in vivo* phenotype for *S. aureus aur* mutants has not yet been described.

Staphylococcal complement inhibitor (SCIN) associates with and inhibits C3 convertase (C3bBb), thereby preventing the production of C3a, C3b and C5a and interfering with complement activation[42] (FIG. 1b). The structural gene for SCIN, *scn*, is also located on *hlb*-converting phages together with *chp* and *sak* (which encodes staphylokinase, see below). Two polymorphic

Enterotoxin
A staphylococcal superantigen that crosslinks major histocompatibility complex class II molecules and T cell receptors, thereby triggering T cell proliferation, anergy and cytokine storms.

Opsonization
Deposition of complement components on bacterial surfaces to promote recognition, phagocytosis and killing by host phagocytes.

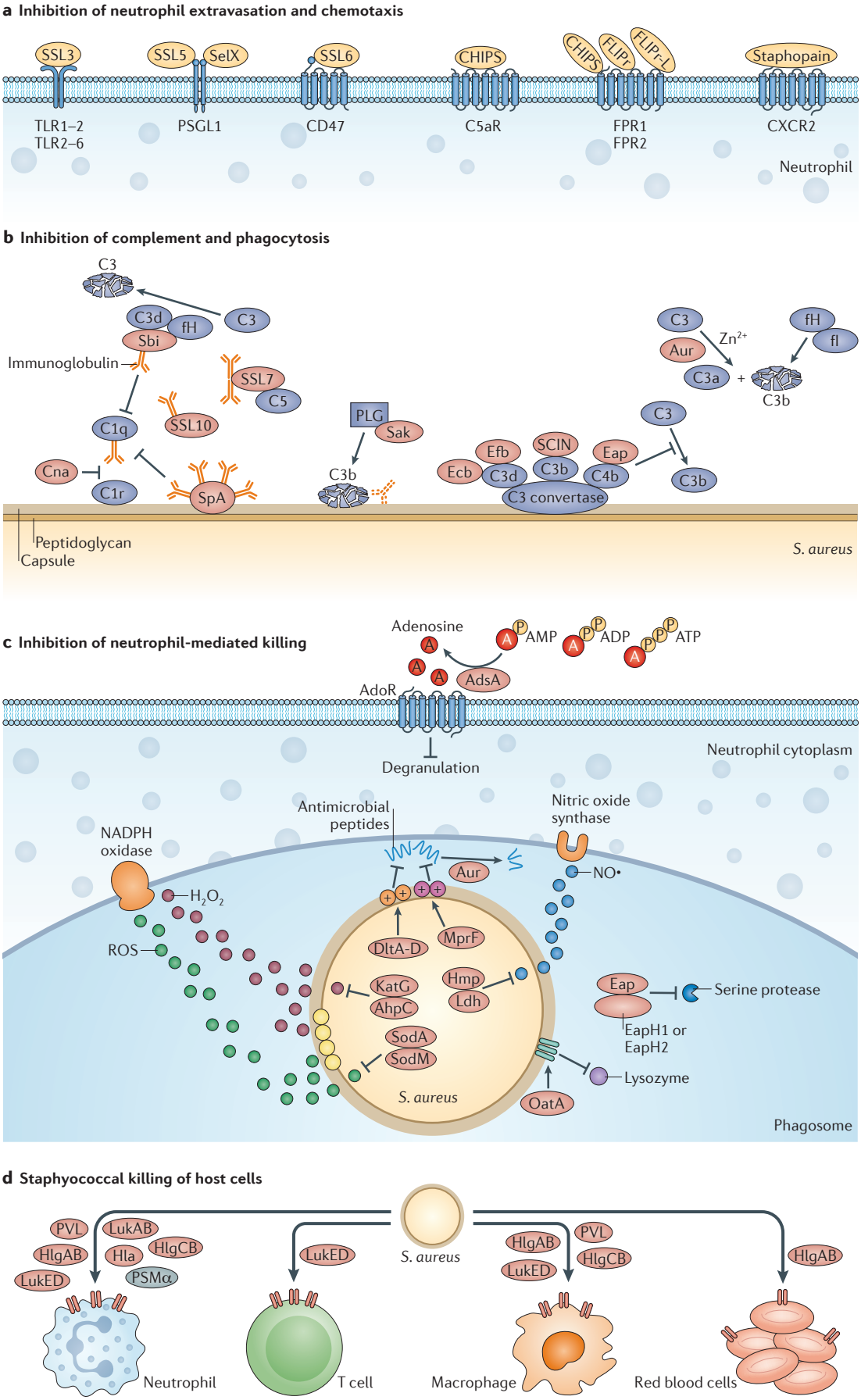

Figure 1 | ***Staphylococcus aureus* interference with chemotaxis, complement and killing by phagocytes.** **a** | Neutrophil extravasation and chemotaxis is inhibited by *Staphylococcus aureus* through the secretion of staphylococcal superantigen-like (SSL) molecules. SSL3 inhibits Toll-like receptor (TLR) heterodimers, SSL5, SSL11 and SelX inhibit PSGL1 signalling (SSL11 is not shown), and SSL6 inhibits the G protein-coupled receptor CD47. Other secreted proteins include chemotaxis inhibitory protein of *S. aureus* (CHIPS), which inhibits the complement receptor C5aR and formyl-peptide receptor 1 (FPR1) and FPR2, formyl peptide receptor-like 1 inhibitor (FLIPr) and FLIPr-like (FLIPrL), which inhibit FPR1 and FPR2, and staphopain, which inhibits signalling from the chemokine receptor C-X-C chemokine receptor (CXCR2). **b** | Complement activation and phagocytosis of staphylococci are blocked through the secretion of inhibitory factors that interfere with opsonization. Collagen adhesin (Cna) blocks the association of complement factor C1q bound to immunoglobulin with complement receptor C1r. Staphylococcal protein A (SpA) and staphylococcal binder of immunoglobulin (Sbi) binding to immunoglobulin blocks its association with C1q. Sbi, SpA, SSL7 and SSL10 sequester immunoglobulins to block their ability to promote complement activation. Sbi (when associated with the host factors C3d and factor H (fH)) and SSL7 also inactivate the complement factors C3 and C5, respectively. Sak associates with plasminogen (PLG) and activates the zymogen to cleave complement factor C3b and immunoglobulin. Extracellular complement-binding protein (Ecb), extracellular fibrinogen-binding protein (Efb), staphylococcal complement inhibitor (SCIN) and extracellular adherence protein (Eap) inhibit C3 convertases, and aureolysin (Aur) cleaves the complement factor C3, which compromises opsonization because the cleavage product C3b is degraded by a complex of the host proteins fI and fH. **c** | *S. aureus* inhibits neutrophil-mediated killing of phagocytosed bacteria by expressing several enzymes and inhibitors. The adenosine-synthesizing enzyme AdsA enables the inhibition of granulation via adenosine receptor (AdoR) signalling. Staphyloxanthin, superoxide dismutase A (SodA) and SodM, the catalase KatG and alkylhydroperoxide reductase (AhpC) are antioxidants that reduce oxidative stress caused by phagosomal reactive oxygen species (ROS) and H_2O_2 generation. Aureolysin (Aur) cleaves antimicrobial peptides and DltA–DltD promote D-alanyl esterification of teichoic acids to protect staphylococci from antimicrobial peptides. MprF modifies phosphatidylglycerol with alanine or lysine, another mechanism to protect staphylococci against antimicrobial peptides. L-lactate dehydrogenase (Ldh) and flavohaemoglobin (Hmp) inhibit nitrosative stress, Eap and its homologues EapH1 and EapH2 inhibit neutrophil serine proteases, and OatA *O*-acetylates peptidoglycan, which prevents its lysozymal degradation. **d** | Secreted β-barrel pore forming toxins (β-PFTs) bind specific receptors on immune cells to impair immune cell functions or promote cell lysis. These β-PFTs include leukocidin ED (LukED) (which binds to neutrophils, T cells and macrophages), γ-haemolysin AB (HlgAB) (which binds to neutrophils, macrophages and red blood cells), HlgCB and Panton–Valentine leukocidin (PVL) (which bind to neutrophils and macrophages), and LukAB and α-haemolysin (Hla) (which bind to neutrophils). Phenol-soluble modulin-α (PSMα), which is another factor secreted by *S. aureus* (but not a β-PFT), can also lyse leukocytes.

Fibrinogen
An abundant glycoprotein of vertebrates that, when cleaved by thrombin or staphylothrombin, self-assembles into fibrin clots.

FcαRI
The IgA Fc receptor, which regulates mucosal immune responses in humans.

Fcγ domain
The portion of antibodies dedicated to C1q complement and Fc-receptor activation.

Core genome
The portion of the genome shared by all members of a bacterial species.

homologues of SCIN, designated SCIN-B and SCIN-C, are encoded by genes in the IEC2 locus. *scn*, *scnB* and *scnC* are found in many, but not all, human clinical isolates (BOX 1); SCIN, SCIN-B and SCIN-C associate with C3 convertase from humans but not with that of other vertebrates[43].

The genes encoding extracellular fibrinogen-binding protein (Efb) and its homologue, extracellular complement-binding protein (Ecb), are also located on IEC2. Both Efb and Ecb bind to C3d (a cleavage product of C3b that activates innate and adaptive responses by binding to complement receptor 2 (CR2)) and inhibit C3bBb and the C5 convertases[43,44] (FIG. 1b). Ecb associates with both fH and C3b to facilitate the complement inhibitory attributes of fH[45]. Efb also binds fibrinogen and prevents fibrinogen interaction with αMβ2, an integrin on neutrophils that activates pro-inflammatory responses, as well as fibrinogen-mediated platelet activation[46,47]. Efb and Ecb inhibitory activities have been observed for human and mouse convertases and fibrinogen. In the mouse intravenous challenge model, the *S. aureus* Δ*efb*Δ*ecb* mutant displayed reduced time-to-death and increased survival, as well as diminished abscess formation in organ tissues[48]. The *ecb* gene is found in all *S. aureus* genomes sequenced to date, whereas *efb* is found in many, but not all, human clinical isolates[13].

SSLs also interfere with complement activation and phagocytosis. For example, SSL7 binds to human IgA and complement C5, interfering with IgA binding to FcαRI, the production of C5a and the oxidative burst of phagocytes *in vitro*; the *in vivo* contributions of SSL7 towards *S. aureus* pathogenesis are not known[49]. SSL10 binds to human and non-human primate IgG1, but not to immunoglobulins of lower vertebrates, and inhibits IgG1 binding to Fcγ receptors and the *in vitro* phagocytosis of IgG1-opsonized bacteria by immune cells[50,51].

Staphylococcal binder of immunoglobulin (Sbi) is a secreted protein with two immunoglobulin binding domains (IgBDs; designated Sbi-I and Sbi-II), which are triple-helical bundles that associate with the Fcγ domain of human and vertebrate immunoglobulin (BOX 2). Sbi-I and Sbi-II interfere with C1q binding to immunoglobulin and block the classical complement pathway[52,53] (FIG. 1b). The Sbi-III and Sbi-IV domains associate with C3 and fH to form tripartite complexes that inhibit the alternative pathway[54,55] (FIG. 1b). The *sbi* gene is located in the *sbi–hlg* locus of the core genome of all isolates[13]. Staphylokinase forms enzymatically active complexes with plasminogen, cleaving fibrin, defensins, human IgG, C3b and its proteolytically inactivated product iC3b on bacterial surfaces, thereby blocking complement activation[56–58] (FIG. 1b). Collagen adhesin (Cna), a surface protein expressed by some *S. aureus* isolates, binds C1q and interferes with classical pathway activation, blocking the association between C1q and C1r[59].

Neutrophil-mediated killing. Once phagocytosed, staphylococci are exposed to a variety of toxic products that kill and degrade the engulfed bacteria: antimicrobial peptides, nitric oxide (NO), ROS (that is, hydrogen peroxide, superoxide and hydroxyl radicals), cell wall hydrolases and proteolytic enzymes[4]. However, *S. aureus* has evolved a number of strategies to survive in this environment (FIG. 1c). Peptidoglycan acetylation (by the protein OatA), D-alanylation of teichoic acids (by the DltABCD complex), and lysyl- or alanyl-phosphatidylglycerol synthesis (by the protein MprF) provide staphylococcal resistance against lysozyme- and antimicrobial peptide-mediated killing by blocking enzymes (such as lysozyme) or peptides binding to the envelope target[60–62]. Staphyloxanthin, a carotenoid pigment synthesized by all *S. aureus* isolates[63], provides resistance against hydrogen peroxide and/or hydroxyl radicals, the bactericidal compounds of neutrophils[64] (this is not the case for CC75 isolates but we consider these to belong to a separate species, *Staphylococcus argenteus* (BOX 1)). Similarly, two superoxide dismutases (SodA and SodM), fulfill overlapping functions in eliminating neutrophil superoxide[65], whereas catalase (KatG) and alkylhydroperoxide reductase (AhpC) protect staphylococci against hydrogen peroxide[66].

Table 1 | ***Staphylococcus aureus*** **immune evasion determinants, their function and epidemiology**

Name	Gene	Genome	Proposed Function	Target	Alleles[13]
Adenosine synthase	*adsA*	core	Immune suppression	Adenosine, dAdo synthesis	1
Aureolysin	*aur*	core	Zinc protease	C3	1
Capsule	*cpsA–cpsN*	core	Phagocytosis inhibition	Not known	2
CHIPS	*chp*	IEC1 (var)	Chemotaxis inhibition	FPR1 and C5aR	1
ClfA	*clfA*	core	Phagocytosis inhibition	γ-fibrinogen and factor I	1
ClfB	*clfB*	core	Adherence	α-fibrinogen, keratin 10 and loricrin	1
Cna	*cna*	variable	Collagen adhesion and C1q binding	C1q	1
Coagulase	*coa*	core	Phagocytosis inhibition	Thrombin and fibrinogen	14
δ-toxin	*hld*	core	Mast cell activation	Not known	1
Eap	*eap*	core	Phagocytic killing inhibition	ICAM1, C4b, elastase, cathepsin G and proteinase 3	15
EapH1	*eapH1*	core	Phagocytic killing inhibition	Elastase, cathepsin G and proteinase 3	1
EapH2	*eapH2*	core	Phagocytic killing inhibition	Elastase, cathepsin G and proteinase 3	1
Enterotoxin B	*seb*	SaPI	T cell superantigen	Vβ TCR	1
Enterotoxin C	*sec*	SaPI	T cell superantigen	Vβ TCR	1
Enterotoxin like X	*selX*	core	T cell superantigen	PSGL1	17
Ecb	*ecb*	IEC2 (con)	Complement inhibition	C3d	2
Efb	*efb*	IEC2 (var)	Complement inhibition	C3d and αMβ2 integrin	2
FLIPr	*flipr*	IEC2 (var)	Chemotaxis inhibition	FPR2	9
FLIPrL	*fliprl*	IEC2 (var)	Chemotaxis inhibition	FPR1 and FPR2	9
FnBPA	*fnbpA*	core	Phagocytosis inhibition and invasion	γ-fibrinogen and fibronectin	7
FnBPB	*fnbpB*	core	Invasion and adherence	α-fibrinogen and fibronectin	7
HlgAB	*hlgAB*	*sbi–hlg* (con)	Phagosome escape	CXCR1, CXCR2 and CCR2	2 or 3
HlgCB	*hlgCB*	*sbi–hlg* (con)	Phagosome escape	C5aR and C5L2	3 or 3
LukAB (also known as LukGH)	*lukAB*	*hlb–lukAB*	PMN lysis and NETosis activation	αM integrin	1 or 1
LukED	*lukED*	GIβ (var)	PMN lysis	CCR5, CXCR1 and CXCR2	1 or 1
LukMF	*lukMF*	GIβ (var)	PMN lysis	Not known	
PSMα1	*psma1*	core	Chemotaxis and PMN lysis	FPR2	1
PSMα2	*psma2*	core	Chemotaxis and PMN lysis	FPR2	1
PSMα3	*psma3*	core	Chemotaxis and PMN lysis	FPR2	1
PSMα4	*psma4*	core	Chemotaxis and PMN lysis	FPR2	1
PSMβ1	*psmb1*	PSMb (con)	Chemotaxis and PMN lysis	FPR2	1
PSMβ1	*psmb2*	PSMb (var)	Chemotaxis and PMN lysis	FPR2	1
PVL	*lukFS*	PVL phage	PMN lysis	C5aR	1

In response to nitrosative stress, *S. aureus* expresses flavohaemoglobin (Hmp), which detoxifies NO, and L-lactate dehydrogenase, which maintains redox-haemostasis and survival within neutrophils by producing L-lactate[67]. In addition to its role in inhibiting complement activation, Eap and two structural homologues, EapH1 and EapH2 (which do not inhibit complement), promote *S. aureus* survival by inhibiting neutrophil serine proteases (such as elastase, cathepsin G and proteinase 3)[68]. In the intravenous mouse challenge model, the *S. aureus* Δ*eap* mutant displays a moderate virulence defect[36]; however, the Δ*eap*Δ*eapH1*Δ*eapH2* mutant displays reduced bacterial load and increased mouse survival[68].

Staphylococcal killing of host cells. In addition to its ability to inhibit phagocyte-mediated killing, *S. aureus* also manipulates innate immune responses by inducing the killing of innate immune cells via PSMs and different toxins (FIG. 1d). The PSMα locus includes *psma1–psma4*, whereas PSMβ includes *psmb1* and *psmb2*; *psmb2* is found in only some, but not all, *S. aureus* strains[13]. Peptides similar to PSMα1–PSMα4 and PSMβ1 are expressed by *Staphylococcus epidermidis*, a commensal of the human skin that cannot cause abscess lesions or bloodstream infections in immune competent individuals[25]. Mutations that delete *psma1–psma4* and *psmb1* and *psmb2* interfere with *in vitro* biofilm formation of *S. aureus* mutants

Table 1 (cont.) | ***Staphylococcus aureus* immune evasion determinants, their function and epidemiology**

Name	Gene	Genome	Proposed Function	Target	Alleles[13]
Staphylokinase	*sak*	IEC1 (var)	Phagocytosis inhibition	Plasminogen, fibronectin, C3 and IgG	1
Sbi	*sbi*	*sbi–hlg* (con)	Phagocytosis inhibition	IgG Fcγ, C3 and factor H	4
SCIN	*scn*	IEC1 (var)	Complement inhibition	C3bBb	None
SCIN-B	*scnB*	IEC2 (var)	Complement inhibition	C3bBb	7
SCIN-C	*scnC*	IEC2 (var)	Complement inhibition	C3bBb	7
SpA	*spa*	core	Phagocytosis inhibition and B cell superantigen	Ig Fcγ and Ig Fab (V_H3)	Xr (SpA typing)
SSL3	*ssl3*	Glα (var)	TLR signalling inhibition	TLR2	13
SSL5	*ssl5*	Glα (var)	Chemotaxis and platelet inhibition	PSGL1, GPCRs, GPIbα and GPVI	5
SSL6	*ssl6*	Glα (var)	Chemotaxis inhibition	PSGL1	2
SSL7	*ssl7*	Glα (var)	Phagocytosis inhibition	IgA and C5	4
SSL10	*ssl10*	Glα (var)	Phagocytosis inhibition	IgG, fibrinogen, fibronectin, thrombin and factor Xa	4
SSL11	*ssl11*	Glα (con)	Chemotaxis inhibition	PSGL1	10
Staphyopain	*scpA*	core	Chemotaxis inhibition	CXCR2	1
TSST1	*tst*	SaPI1	T cell superantigen	Vβ2 TCR and MHC class II α-chain	2
vWbp	*vwb*	core	Phagocytosis inhibition	Thrombin, fibrinogen, factor XIII and fibronectin	2

C5aR, C5a receptor; CCR, CC-chemokine receptor; CHIPS, chemotaxis inhibitory protein of *S. aureus*; Clf, clumping factor; con, conserved; Cna, Collagen adhesin; dAdo, deoxyadenosine; CXCR, C-X-C chemokine receptor; Eap, extracellular adherence protein; Ecb, extracellular complement-binding protein; Efb, extracellular fibrinogen-binding protein; FLIPr, formyl peptide receptor-like 1 inhibitor; FLIPrL, FLIPr-like; FPR, formyl-peptide receptor; FnBP, fibronectin-binding protein; GI, genomic island; GPCR, G protein-coupled receptor; Hlg, γ-haemolysin; ICAM1, intercellular adhesion molecule 1; IEC, immune evasion cluster; Ig, immunoglobulin; Luk, leukocidin; MHC, major histocompatibility complex; PMN, polymorphonuclear leukocyte; PSGL1, P-selectin glycoprotein ligand 1; PSM, phenol-soluble modulin; PVL, Panton–Valentine leukocidin; SaPI, *S. aureus* pathogenicity island; Sbi, staphylococcal binder of immunoglobulin; SCIN, staphylococcal complement inhibitor; SpA, staphylococcal protein A; SSL, *S. aureus* superantigen-like; TCR, T cell receptor; TLR, Toll-like receptor; TSST1, toxic shock syndrome toxin 1; var, variable; vWbp, von Willebrand factor-binding protein.

and with the expression of virulence factors, including α-haemolysin[25]. *S. aureus* Δ*psma1–psma4* mutants are attenuated in the mouse bloodstream infection model[69], a phenotype that may be due to defects in biofilm formation, virulence gene expression and/or contributions of PSMα1–PSMα4 towards lysis of immune cells, presumably via membrane insertion and pore formation[70].

β-barrel pore-forming toxins (β-PFTs) are secreted by the bacterium as soluble monomers and, on association with receptors on cell surfaces, assemble into multimeric pore structures, penetrating the lipid bilayer to invoke alterations in the physiology of injured cells or their outright lysis[71]. α-haemolysin (Hla), the prototype β-PFT of *S. aureus*, is encoded by the *hla* gene, which is located within IEC2. Although conserved among all *S. aureus* isolates, some lineages of *S. aureus* carry a nonsense mutation that blocks *hla* expression[72]. Hla binds to its receptor on host cells, ADAM10, and assembles into a heptameric pore; through the metalloproteinase activity of ADAM10, Hla modulates the function of immune cells, including neutrophils, or triggers lysis of epithelial cells[73,74]. *S. aureus hla* mutants display defects in disease severity in mouse models for lethal pneumonia, bacteraemia and SSTI; however, *hla* is not required for the establishment of *S. aureus* abscess lesions[75–77]. Based on ADAM10 expression on the surface of myeloid cells, organ epithelia and the vascular endothelium, Hla causes global, as well as organ-specific, changes to host physiology during *S. aureus* infection[74].

Leukocidins
Bacterial secreted toxins targeting white blood cells (leukocytes) for destruction.

Leukocidins are other β-PFTs secreted by *S. aureus* (FIG. 1d). Following leukocidin association with receptors on myeloid cells and erythrocytes, these toxins assemble from two different subunits (F and S) into an octameric pore structure[78]. All *S. aureus* strains produce at least three leukocidins, γ-haemolysin AB (HlgAB), HlgCB and leukocidin AB (LukAB; also known as LukGH), whereas other strains may also secrete Panton–Valentine leucocidin (PVL; which is encoded by *lukPV*) and LukED or LukMF[79] (BOX 1). The operon encoding LukAB is located immediately adjacent to *hlb*, whereas the operon encoding γ-haemolysin (*hlgABC*) is part of the *sbi–hlg* locus. LukAB binds to the I domain of human, but not mouse, αM integrin on myeloid cells[80]. Purified LukAB can trigger human neutrophils to release NETs that, at least temporarily, ensnare staphylococci[81]. LukAB has also been reported to promote *S. aureus* escape from the phagosome of neutrophils[82]. Purified HlgAB γ-haemolysin, but not purified HlgCB γ-haemolysin, is able to lyse human and rabbit red blood cells[83]. HlgAB binds to chemokine receptors CXCR1, CXCR2 and CC-chemokine receptor 2 (CCR2), whereas HlgCB uses complement receptors C5aR and C5L2 to associate with target cells[84]. Following staphylococcal inoculation into human blood, *hlgABC* is upregulated 34–145-fold[85], and the *S. aureus* Δ*hlgABC* mutant displays reduced survival, presumably because HlgAB and HlgCB promote release of iron-compounds from erythrocytes, thereby enabling bacterial acquisition of this essential nutrient[83].

Box 2 | **Structural features of immune evasion factors**

Crystallographic analysis of *Staphylococcus aureus* immune evasion determinants revealed five discrete structural domains that enable specific interactions with the host's immune system: oligonucleotide-binding (OB) fold, β-grasp domain, triple-helical bundle (THB), leukocidin domain and immunoglobulin-like fold. Varying the amino acid sequence for these domains has created panoplies of ligands that interact with the defence molecules of infected hosts at the places that matter most[4,145]. Thus, the study of *S. aureus* immune evasion factors laid bare the most intricate workings of the human immune system and identified new avenues for the therapy of autoimmune and inflammatory diseases. Examples for immune-evasion factors with OB fold and β-grasp domains include the staphylococcal T cell superantigens (SEA, SEB, SEC1–3, SED, SEE, SEG, SHE, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SER, SEU, toxic shock syndrome toxin 1 (TSST1), TSST2 and SelX) and the staphylococcal superantigen-like (SSL) family (SSL1–SSL13)[125]. Chemotaxis inhibitory protein of *S. aureus* (CHIPS), formyl peptide receptor-like 1 inhibitor (FLIPr), FLIPr-like (FLIPrL), extracellular adherence protein (Eap), EapH1 and EapH2 have β-grasp domains but not an OB fold[68,146]. Immune evasion factors with triple helical bundles include extracellular complement-binding protein (Ecb), extracellular fibrinogen-binding protein (Efb), staphylococcal protein A (SpA), staphylococcal binder of immunoglobulin (Sbi), staphylococcal complement inhibitor (SCIN), SCIN-B and SCIN-C[147], whereas members of the β-PFT family (γ-haemolysin proteins, leukocidin proteins, Panton–Valentine leukocidin (PVL) and Hla) have leukocidin domains[79]. Surface proteins with IgG-like domains include the immune evasion factor clumping factor A (ClfA) and its relatives ClfB, fibronectin-binding protein A (FnBPA) and FnBPB[102] (TABLE 1).

Both purified HlgAB and HlgCB promote lysis of neutrophils, monocytes and macrophages from humans, as well as non-human primates, and to a lesser degree rabbits and mice[83]. In a mouse intravenous challenge model, animals infected with a *S. aureus* Δ*lukAB* mutant displayed increased time-to-death and survival. Using subcutaneous inoculation in mice or rabbits, the *S. aureus lukAB* mutant did not display defects in skin abscess formation[86]. The Δ*hlgAB* mutant displayed a virulence defect in the intraperitoneal challenge model in mice[84].

lukED is present in the GIβ locus of ~70% of clinical *S. aureus* isolates[13] (BOX 1). Purified LukED triggers lysis of macrophages, dendritic cells and T cells from many different vertebrates, as the toxin binds to the chemokine receptors CCR5, CXCR1 and CXCR2 (REFS 87,88). For *S. aureus* strain Newman, which harbours GIβ, the Δ*lukED* mutation increased the time-to-death and survival of mice following intravenous challenge with mutant staphylococci[89]. PVL is secreted by *S. aureus* lysogenized with PVL phage[90]. PVL binds to the C5aR on neutrophils, monocytes and macrophages, but its activity is restricted towards human and rabbit cells[9]. By virtue of binding C5aR, PVL not only exerts its lytic activity on target host cells but can also facilitate the priming of human polymorphonuclear leukocytes by pro-inflammatory stimuli (for example, formyl peptides). Injection of purified recombinant PVL leads to increased immune cell recruitment and increased architectural destruction of the lung, owing to toxin-mediated recruitment and subsequent lysis of immune cells[9]. Only ~2% of *S. aureus* isolates secrete PVL; however, community-acquired MRSA isolates frequently harbour PVL phages, and PVL expression is also associated with necrotizing pneumonia[91]. *S. aureus* Δ*lukPV* variants display defects in the pathogenesis of SSTIs and lung infections in rabbits, but not in mice, which seems to be due to neutrophil-mediated inflammatory responses and tissue distruction[76,92]. *lukMF*, genes for another phage-encoded leukocidin, are found in *S. aureus* isolates associated with bovine mastitis[13].

Staphylococcal agglutination. Coagulation, the conversion of fibrinogen to a crosslinked fibrin meshwork by activated thrombin, is an innate defence of all vertebrates that immobilizes microbial invaders and attracts immune cells for phagocytic clearance of bacteria. Therefore, every successful bacterial pathogen must evolve mechanisms for escape from fibrin entrapment and subsequent phagocytosis by infiltrating immune cells. A hallmark of all *S. aureus* isolates is the secretion of two coagulases: coagulase (Coa) and von Willebrand factor-binding protein (vWbp)[93]. Coa and vWbp associate with prothrombin, a zymogen, to generate enzymatically active staphylothrombin, which cleaves the A and B peptides of fibrinogen to generate fibrin fibrils[94] (FIG. 2). As staphylothrombin does not cleave other substrates of thrombin, it avoids the activation of clotting and inflammatory factors that ordinarily accompany fibrin polymerization[95]. Staphylothrombin activity is not subject to feedback inhibition through host antithrombin. However, staphylothrombin is blocked by dabigatran and other direct thrombin inhibitors of the same family[96]. The staphylothrombin-generated fibrin meshwork protects *S. aureus* from phagocytes and contributes to the formation of staphylococcal abscess lesions and lethal bacteraemia in mice[97]. Activation of prothrombin is mediated by the D1 and D2 domains in the N-terminal region of Coa and is blocked by specific antibodies, which provide protection from *S. aureus* bloodstream infection in the mouse model[98]. Perhaps owing to purifying selection, *coa* is one of the most variable genes in the core genome of *S. aureus*, with >50% sequence variation in the coding sequence for its D1–D2 domains and 14 distinct isoforms[93] (TABLE 1). vWbp also has conserved D1–D2 domains for association with prothrombin, but this complex generates fibrin at a reduced rate and contributes to abscess formation without affecting staphylococcal escape from phagocytosis[99]. The gene encoding vWbp, *vwb,* has limited sequence variability[98].

S. aureus agglutinates with Coa- or vWbp-derived fibrin fibrils, which requires clumping factor A (ClfA), a glycosylated, sortase-anchored surface protein, the immunoglobulin-like domains of which bind to

Dabigatran
A small molecule that directly binds and inhibits thrombin as well as staphylothrombin, the complex formed between coagulase or von Willebrand Factor-binding protein and prothrombin.

Sortase
The bacterial transpeptidase responsible for anchoring surface proteins to the cell wall envelope.

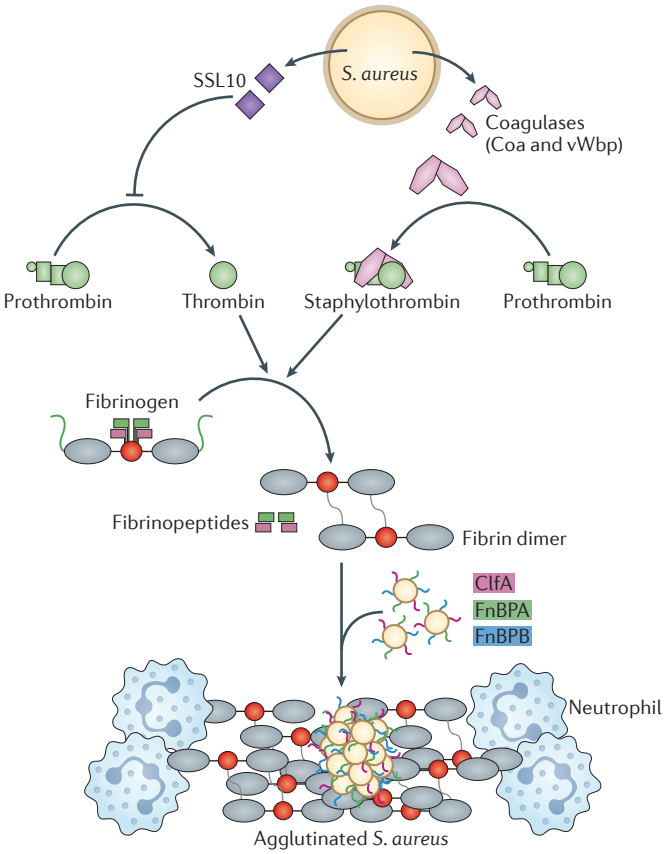

Figure 2 | ***Staphylococcus aureus* agglutination with fibrin provides protection against phagocytes.** Physiological host defences immobilize bacteria through the activation of the serine protease zymogens prothrombin (also known as factor II) and factor X (not shown). In the contact-activation pathway, surface contact results in the autocleavage of prothrombin, thereby generating thrombin (also known as factor IIa). *Staphylococcus aureus* superantigen-like 10 (SSL10) inhibits prothrombin autoactivation, whereas the *S. aureus* coagulases, coagulase (Coa) and von Willebrand factor-binding protein (vWbp) convert prothrombin to staphylothrombin. Both thrombin and staphylothrombin cleave fibrinopeptides A and B from fibrinogen to generate fibrin, which self-assembles and polymerizes into cable structures that immobilize bacteria. Thrombin activation results in the activation of additional haemostasis factors that facilitate the simultaneous attraction of phagocytes to immobilized bacteria, which is thus inhibited by SSL10 secretion. However, staphylothrombin cleaves fibrinopeptides from fibrinogen without activation of other haemostasis factors and promotes fibrin polymer assembly on the staphylococcal surface, where it protects the bacterium from neutrophils and phagocytic clearance. Fibrin agglutination on the staphylococcal surface also involves the *S. aureus* surface proteins clumping factor A (ClfA), fibronectin-binding protein A (FnBPA) and FnBPB, which bind to the fibrinogen γ-chains.

Fibronectin
A high molecular weight glycoprotein of the extracellular matrix of vertebrates that associates with integrins on cell surfaces.

Factor Xa
The activated serine protease that cleaves prothrombin to activate the clotting cascade of vertebrates; also known as thrombokinase.

the carboxy-terminal end of the γ-chain in fibrinogen and fibrin (that is, the D domain)[96,100,101] (FIG. 2). Thus, ClfA acts synergistically with Coa or vWbp in protecting staphylococci from opsonophagocytic killing, and Δ*clfA* mutants display defects in the pathogenesis of lethal bloodstream infections in mice[96].

Four other sortase-anchored surface proteins use their immunoglobulin-like domains to bind fibrinogen or fibrin: ClfB (which binds to the fibrinogen α-chain), fibronectin-binding protein A (FnBPA) and FnBPB (which bind to the C-terminal end of the fibrinogen γ-chain), and bone sialoprotein binding protein (Bbp; also known as SdrE isoform; which binds to the fibrinogen α-chain)[102] (FIG. 2). These surface proteins display functional redundancy for the *S. aureus* agglutination pathway and contribute to the pathogenesis of bloodstream infections[103].

Purified, recombinant SSL10 has also been reported to bind human fibrinogen and fibronectin, as well as pig prothrombin and factor Xa[51]. The association of SSL10 with prothrombin and factor Xa occurs via the γ-carboxylic acid (Gla) domain and interferes with calcium-activated blood clotting but not with staphylothrombin-mediated fibrin formation[51] (FIG. 2). *ssl7* and *ssl10* are not found in all *S. aureus* isolates[13].

Staphylokinase activates human, but not mouse, plasminogen and may solubilize coagulase-induced fibrin deposits, thereby aiding *S. aureus* in generating purulent lesions for dissemination to new hosts (FIG. 1b). Of note, binding of surface proteins by fibrinogen or fibrin deposits has also been reported to influence staphylococcal interference with platelet aggregation and innate immune functions[104,105]. In addition to their role in binding to fibrin and fibrinogen, some surface proteins have been reported to bind additional host ligands, at least *in vitro*. Binding to these additional host ligands, which include complement factor I (by the surface protein ClfA), fibronectin (by the surface proteins FnBPA and FnBPB), keratin 10 (by the surface protein ClfB) and loricrin (by the surface protein ClfB), may contribute to staphylococcal immune evasion, invasion of host cells or colonization of squamous epithelia[102]. *fnbpA* and *fnbpB*, which are components of the *S. aureus* core genome, have sequence polymorphisms, and seven isotypes with discrete antigenicity have been described[106] (BOX 1).

Adenosine and deoxyadenosine signalling and NETosis. Adenosine is a potent mediator of immune responses and, under physiological conditions, is synthesized following hypoxia, exposure to ROS and cell lysis associated with tissue damage. Adenosine elicits its biological effects by binding to one or more of four G protein-coupled receptors[107]: A1, A2A, A2B and A3. Adenosine receptor interaction triggers anti-inflammatory signalling cascades that inhibit platelet aggregation, neutrophil superoxide burst, neutrophil degranulation, T cell activation and release of the cytokines IL-1α and IL-10 (REF. 108). *S. aureus* increases the concentrations of extracellular adenosine during infection by expressing adenosine synthase A (AdsA), a sortase-anchored protein that catalyses the dephosphorylation of adenosine mono-, di- and triphosphates[109] (FIG. 3a). Both *ex vivo* and during mouse infection, the Δ*adsA* mutation increases killing of staphylococci by blood neutrophils, while decreasing extracellular adenosine[109]. Thus, AdsA-mediated synthesis of adenosine promotes survival of *S. aureus* within neutrophils, presumably by inhibiting superoxide burst and/or degranulation[109]. Furthermore, adenosine decreases major histocompatibility complex (MHC) class II expression by macrophages and dendritic cells and dampens IL-12 production, a pivotal stimulus for T helper 1-type immune responses[107]. Staphylococcal enhancement of adenosine production may therefore interfere with T cell effector mechanisms and adaptive immune responses in infected hosts[109].

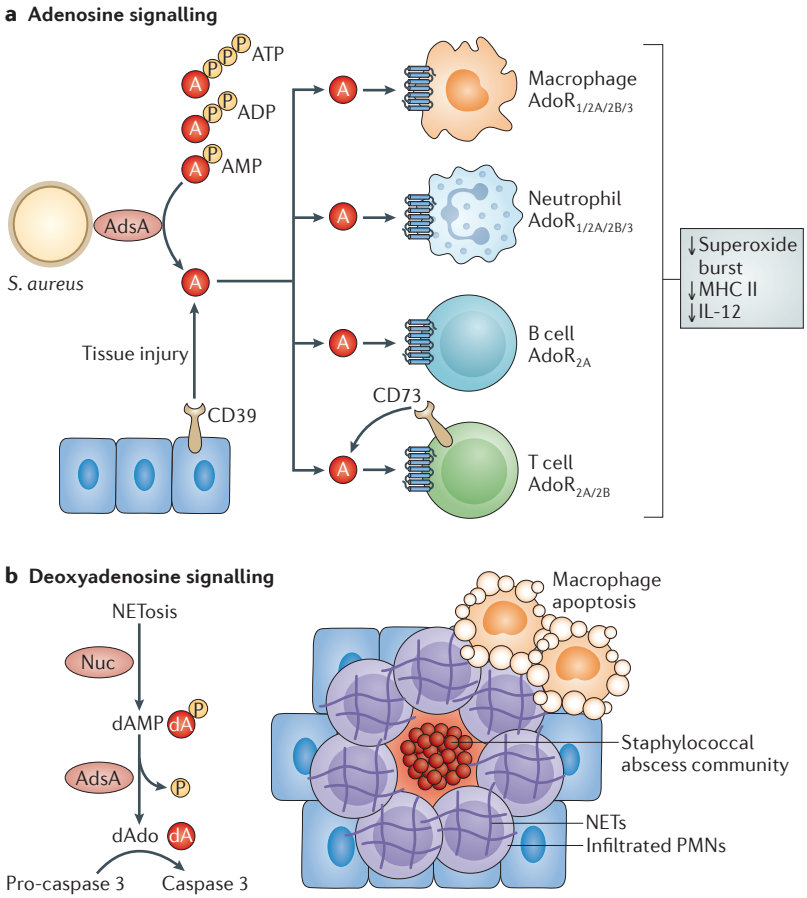

Figure 3 | ***Staphylococcus aureus* AdsA perturbs adenosine and deoxyadenosine signalling. a** | *Staphylococcus aureus* infection and its associated inflammatory damage promote the release of ATP, which is converted by adenosine synthase A (AdsA) into the immune suppressive signalling molecule adenosine (A). Adenosine inhibits activation of B cells, T cells, macrophages and dendritic cells via adenosine receptor (AdoR) signalling by acting on four different receptors ($AdoR_1$, $AdoR_{2A}$, $AdoR_{2B}$ and $AdoR_3$). Under physiological conditions, CD39 and CD73 generate adenosine signals to limit inflammatory responses; CD39 and CD73 are also responsible for the adenosine halo surrounding immune cells and for immune suppressive states involving regulatory T cells (T cells expressing the $FOXP3^+$ marker protein (not shown)). **b** | *S. aureus* induced NETosis of infiltrating neutrophils leads to nuclease-mediated degradation of the DNA fibres that are the major components of neutrophil extracellular traps (NETs) and AdsA-mediated conversion of 5′-monophosphate-deoxyadenosine (dAMP) into deoxyadenosine (dAdo), which promotes autocleavage of the apoptosis factor pro-capsase 3 to caspase 3. Caspase 3 induces macrophage death, thereby protecting *S. aureus* against professional phagocytes. IL-12, interleukin-12; MHC II, major histocompatibility complex class II; Nuc, staphylococcal nuclease; PMN, polymorphonuclear leukocyte.

Fab domains
The portions of antibodies dedicated to antigen binding.

V_H3 clan IgM
IgM derived from one of three clans of variable heavy chain (V_H) genes, the products of which provide the scaffold for the antigen-binding determinants of antibodies.

Plasmablasts
Immature B cells in the blood that secrete antibodies.

AdsA activity also modulates immune responses following the degradation of NETs. During bloodstream infection in mice, *S. aureus* disseminates to many different organ tissues to establish abscess lesions. These lesions are composed of a bacterial nidus, designated as the staphylococcal abscess community (SAC), encased within a pseudocapsule of fibrin deposits, and surrounded by layers of immune cells[97]. In spite of large numbers of infiltrated neutrophils, mice are unable to eliminate staphylococci from abscess lesions and eventually succumb to persistent infection[36]. Although neutrophils use NETosis to entangle staphylococci, NETs are degraded by staphylococcal nuclease (Nuc) and thereby fail to exert bactericidal activities[110] (FIG. 3b). Nucdigestion of NETs releases 5′ and 3′ monophosphate nucleotides that are converted by AdsA into deoxyadenosine[111] (FIG. 3b). Deoxyadenosine production triggers caspase 3 induced apoptosis of macrophages and prevents phagocyte entry into the SAC, the core of staphylococcal abscess lesions, thereby promoting bacterial survival within the lesion[111].

Manipulation of adaptive immune responses

B cell responses. *S. aureus* is capable of manipulating B cell survival and function, especially through production of staphylococcal protein A (SpA), which is a sortase-anchored surface protein with high affinity for vertebrate immunoglobulin, including human IgA, IgD, IgG1–IgG4, IgM and IgE[112]. SpA is initially deposited in the staphylococcal envelope and subsequently released by cell wall hydrolases (LytM)[113]. *spa* is expressed by all clinical *S. aureus* isolates; the immunoglobulin binding domains are conserved in the genomes of these isolates, but region X, the cell wall spanning domain of SpA, is a highly polymorphic sequence[114,115] (BOX 1).

The immunosuppressive attributes of SpA have been ascribed to two distinct binding activities: association with the Fcγ domain and with the Fab domains of antibodies[116,117]. SpA binding to the Fcγ domain of IgG blocks phagocytosis of staphylococci[118], whereas SpA binding to the Fab domains and crosslinking of V_H3 clan IgM promotes B cell superantigen activity[119] (FIG. 4a). Of note, SpA binds specifically to V_H3 clan IgM antibodies, which mediate the predominant antibody responses to infection and immunization, but not to other clan antibodies. In the intravenous challenge model of *S. aureus* infected mice, *spa* expression suppresses antibody responses against many different staphylococcal antigens and provides antiphagocytic attributes, promoting staphylococcal survival in blood[120]. Infection of mice with *S. aureus spa* variants that cannot bind immunoglobulin is associated with attenuated disease and with antibody responses against many different antigens that can protect animals against subsequent lethal challenge with other *S. aureus* isolates[120].

Mice harbour a limited repertoire of V_H3^+ B cells, whereas humans possess large populations of these cells, yet both species cannot develop SpA-neutralizing antibodies during infection[121]. *S. aureus* infection in humans triggers expansions of V_H3 idiotypic plasmablasts (>90% of blood plasmablasts), the antibodies of which (that is, the B cell receptors) associate with SpA via their Fab domains but do not display pathogen-specific binding activities[121] (FIG. 4b). When mice are treated with purified SpA, crosslinking of V_H3 clonal B cells triggers proliferation and apoptotic collapse of expanded populations of B cells[122]. It is not clear, however, whether apoptotic collapse of expanded lymphocyte populations occurs during *S. aureus* infection in mice or in humans.

Non-toxigenic SpA, designated SpA_{KKAA}, was engineered by substituting twenty amino acid residues essential for its association with the Fcγ and Fab regions[123]. Although SpA_{KKAA} has twenty amino acid substitutions, this antigen elicits antibodies that neutralize SpA when

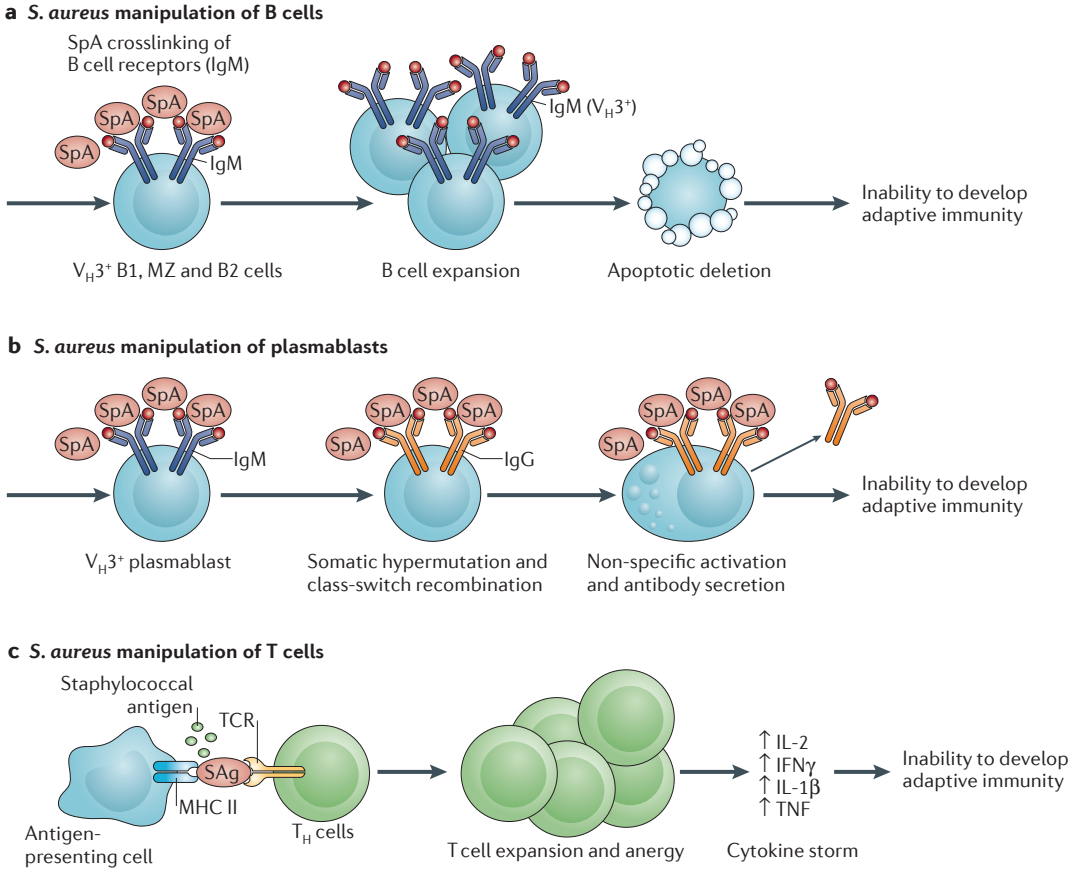

Figure 4 | ***Staphylococcus aureus* manipulates B cell and T cell responses.** **a** | *Staphylococcus aureus* releases staphylococcal protein A (SpA) into host tissues, where it binds to and crosslinks V_H3 clan B cell receptors. In B1 cells, marginal zone (MZ) B cells and B2 cells, SpA crosslinking is associated with proliferative expansion and apoptotic collapse. The death of these cells impedes the development of adaptive immunity during *S. aureus* infections. **b** | In V_H3^+ plasmablasts, SpA crosslinking promotes somatic hypermutation and class switching from IgM antibodies to IgG antibodies, followed by the secretion of antibodies that are not specific for the *S. aureus* antigen. **c** | *S. aureus* secretes T cell superantigen (SAg), which crosslinks major histocompatibility complex class II antigens (MHC II) on the surface of antigen-presenting cells and T cell receptors (TCRs) on the surface of T helper (T_H) cells, triggering T cell expansion and anergy and causing cytokine storms (including interleukin-2 (IL-2), interferon-γ (IFNγ), IL-1β and tumour necrosis factor (TNF)). As a result, a T cell response specific for *S. aureus* antigens is not produced.

injected into animals[123]. The SpA_{KKAA}-derived polyclonal antibodies promote phagocytosis of staphylococci and display adjuvant attributes by suppressing staphylococcal B cell superantigen activity and promoting humoral immune responses against a wide range of *S. aureus* antigens[123]. Studies with mouse monoclonal antibodies corroborate this concept[53].

T cell responses. Staphylococcal T cell superantigens bind to MHC class II molecules on the surface of antigen-presenting cells, providing antigen-independent crosslinking with T cell receptors on T helper cells[124] (FIG. 4c). *S. aureus* strains have been shown to express 23 different enterotoxins and T cell superantigens[125]. Three superantigens are most frequently associated with human disease — toxic shock syndrome toxin 1 (TSST1), staphylococcal enterotoxin B (SEB) and SEC — each providing high-affinity interactions with distinct subsets of Vβ chain T cell receptors[126]. In humans with toxic shock syndrome, *S. aureus* secretion of TSST1 or other enterotoxins trigger expansions of cognate T cell populations, up to 30% of blood lymphocytes and nonspecific release of cytokines, preventing a focused adaptive immune response[127]. Depending on the site and severity of *S. aureus* infection or intoxication, superantigen-mediated activation of T cell responses may be associated with cytokine storms and toxic shock syndrome pathology[128]. Staphylococcal superantigens are also thought to interfere with antigen-specific proliferation of T cells and with antibody responses against specific subsets of staphylococcal antigens, including staphylococcal superantigens[129]. It is not yet known whether superantigens have a crucial role in the suppression of T cell responses in mice that are observed during *S. aureus* bloodstream infections[130].

S. aureus can also manipulate T cell responses by promoting T cell lysis. For example, δ-toxin (Hld; also known as δ-haemolysin), a member of the PSMα family, can lyse T cells[131] and has also been reported to trigger mast cell degranulation, which could be a key factor in

Autologous vaccine
A whole-cell killed *S. aureus* vaccine administered to an infected individual that was derived from the patient's isolate.

the exacerbation of *S. aureus* infected atopic dermatitis lesions, where histamine release is otherwise triggered by antigen-induced crosslinking of IgE bound to FcγRI receptor[132]. Hld is encrypted within the *agr*-regulated RNA III molecule, the regulatory arm of staphylococcal quorum-sensing[133].

Outlook

S. aureus strains secrete many immune evasive molecules and, when placed under selective pressure, acquire mobile genetic elements with additional factors so that staphylococci meet the demands for invasion of host species and replication in specific anatomical niches[13]. These strategies are accomplished through factors that block phagocyte chemotaxis, complement activation, phagocytic uptake and oxidative killing, often redirecting host defences such as fibrin formation or NETosis to favour pathogen replication. Staphylococcal infection is also associated with perturbations of adaptive responses, including the disruptive proliferation of B cells and T cells, which prevents the establishment of protective immune responses. Why do *S. aureus* isolates acquire so many different immune evasion factors when other bacterial pathogens manage with only a small number? Addressing this question, one should consider that *S. aureus* maintains life-long associations with its human hosts, colonizing and reiteratively invading large segments of the population. We know of no other bacterial pathogen able to sustain a similar lifestyle. Staphylococcal capabilities of causing recurrent and reiterative infections probably rely on two mechanisms. First, *S. aureus* manipulation of B cell and T cell responses must be successful, as increased age is not associated with decreased incidence. Second, mobile genetic elements enable acquisition and/or exchange of immune evasive traits between *S. aureus* strains and horizontal gene transfer may implement disease in individuals who did mount successful immune responses against immune evasion determinants. If pressed to predict the future of *S. aureus* as it evolves with a population whose lifespan continues to increase, we would forecast more invasion by mobile genetic elements and more immune evasion determinants.

Considering the formidable weapons of the pathogen against the host's immune defences, development of vaccines against *S. aureus* is a daunting task. Conventional approaches for vaccine development follow the mantra of eliciting specific antibodies that trigger pathogen killing *in vitro* and disease protection in preclinical (animal) models of staphylococcal disease. The simplest means of achieving these goals are whole-cell vaccines, either killed or attenuated preparations. Indeed, use of an autologous vaccine for individuals with recurrent *S. aureus* infection has been practised for many years[134]. This approach can elicit pathogen-specific antibodies; however, it has not been demonstrated to raise protective immunity[135]. Live-attenuated vaccines have been studied in animal models with variable success but not in humans. If one considers that the immune-evasive strategies are often species specific, it seems risky to derive claims on human protective immune responses against *S. aureus* from experiments with animals. What is true for whole-cell vaccines certainly applies to subunit vaccines. Antibodies against capsular polysaccharide, ClfA, IsdB and lipoteichoic acid bind to the surface of the pathogen, which enables phagocyte-mediated killing *in vitro* and provides protection from infection in specific animal models; however, the corresponding vaccines or antibodies did not achieve efficacy in clinical trials[136–138]. It occurred to us that *in vitro* assays for phagocytic killing of *S. aureus* often do not consider key evasion strategies of the pathogen and the corresponding defences of humans. We believe this can be addressed with Lancefield's assay for antibody-mediated killing of bacteria in fresh anti-coagulated human blood[139]. The Lancefield assay can also be used for prospective studies in humans, assessing antibody titres, status of immunity (bactericidal activity of blood) and probability of disease. This approach may identify criteria for protective immunity, stratify patients at risk for *S. aureus* disease and guide applications for immune-therapy or vaccination that reduce the incidence of disease. Previous work targeted B cell and T cell superantigens from *S. aureus* as vaccine antigens to enable the development of broad-spectrum immune responses during each encounter of the host with this pathogen[123,140]. Another promising approach exploited the structural relatedness of immune evasion factors to target multiple molecules with antibodies that recognize conserved structural features[141] (BOX 2). Nonetheless, the efficacy of these approaches has not yet been assessed in human clinical trials.

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Competing interests statement
The authors declare competing interests: see Web version for details.