

Structural and functional role of *Staphylococcus aureus* surface components recognizing adhesive matrix molecules of the host

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Staphylococcus aureus is a versatile and harmful human pathogen in both hospital- and community-acquired infections. *S. aureus* can initiate host infection by adhering to components of the extracellular matrix. Adherence is mediated by a variety of protein adhesins of the microbial surface component recognizing adhesive matrix molecule (MSCRAMM) family. In this article, we describe these MSCRAMMs in terms of structural organization and ligand-binding capacity and discuss their role as a possible target for immunotherapy.

Staphylococcus aureus is regarded as one of the most pathogenic bacterial species owing to its large variety of expressed virulence factors. In fact, it can cause a number of infections, ranging from superficial skin infections to more serious, life-threatening invasive diseases such as endocarditis, bacteremia, osteomyelitis, septic arthritis and postoperative infections [1,2].

The existence of highly virulent methicillin-resistant *S. aureus* strains is an alarming worldwide health threat, and different epidemic strains have developed both in the community and hospitals [3,4]. Meanwhile, even high-level resistance against vancomycin, the most potent drug to which the organism was sensitive, has occurred for clinical *S. aureus* isolates [5,6]. Hence, new therapeutic strategies to circumvent staphylococcal antibiotic resistance need to be introduced.

In the molecular pathogenesis of *S. aureus* infection we can identify steps that are common to other microorganisms. The primary niche for *S. aureus* in humans is the vestibulum nasi, and nasal colonization is a well-documented risk factor for staphylococcal infection in a number of clinical settings [7,8]; other important reservoirs are the inguinal and perineal area, colon and vagina. Once bacteria have gained access to the mucosal regions of the body they disseminate in the extracellular fluids and then spread to deeper host tissues or organs, most notably bone tissue and cardiac valves, where they release toxins and enzymes causing disease.

Hence, during the organism's journey from the initial point of entry to its ultimate destination, *S. aureus* encounters different surfaces

such as mucous, epithelial cells, plasma proteins, endothelial cells and the extracellular matrix (ECM) of the final target.

To colonize these different environments, *S. aureus* is endowed with a variety of surface-associated factors that mediate attachment of the bacterium to the substrate (adhesins). One major class of *S. aureus* adhesins comprises proteins covalently anchored to cell peptidoglycans that specifically attach to plasma or ECM components and collectively are termed microbial surface component recognizing adhesive matrix molecules (MSCRAMMs). These molecules recognize the most prominent components of the ECM or blood plasma such as fibronectin (Fn), collagens and fibrinogen (Fbg) and have an important role as virulence factors [9].

This article focuses on the structure of MSCRAMMs and defines the molecular aspects of their interaction with relevant ligands. We also discuss how these interactions might be targeted as vaccines and in immunoprophylaxis.

The extracellular matrix

General properties

The ECM, the extracellular portion of animal tissue, provides a structural framework for tissues in addition to affecting the cellular physiology of the organism. Blood plasma may be considered a special form of ECM where all the components are present in a soluble form and under specific circumstances such as clot or thrombus formation may become morphologically visible. In general, components of the ECM are produced intracellularly by resident cells and

Keywords

adhesion ■ extracellular matrix ■ MSCRAMM ■ *Staphylococcus aureus* ■ vaccine

secreted into the extracellular milieu [10]. Major constituents identified in ECM include collagens, noncollagenous glycoproteins and proteoglycans [11]. There is tremendous diversity in these molecules due to redundancy of genes that encode isoforms of the same molecule (i.e., collagen) and differential splicing that generates further diversity in glycoproteins such as Fn and thrombospondin. Many ECM glycoproteins are unusually large molecules with extended conformations spanning distances of several hundred nanometers. In general, these macromolecules are composed of distinct structural modules, such as Fn type III, immunoglobulin fold and other domains. As a consequence, they are multifunctional and express distinct binding sites for cells, other ECM glycoproteins, proteoglycans, growth factors and glycolipids. This multidomain organization of ECM components in addition to serving a structural function also affects the cellular physiology of the organism. Cell adhesion, migration, proliferation and differentiation are examples of biological processes influenced by the composition and structural organization of the surrounding ECM [11]. Many of the biological processes regulated by ECM macromolecules involve anchorage of eukaryotic cells. Interactions between eukaryotic cells and the ECM are mediated by specific cellular receptors, of which integrins are the best characterized. Integrins are non-covalently associated heterodimeric glycoprotein complexes expressed on the surface of most cell types that function as receptors for all major extracellular glycoproteins. Each heterodimer consists of one α -chain covalently associated with a β -subunit that usually links many ECM proteins to the cellular cytoskeleton [12,13]. Many pathogens such as *S. aureus*, which spend a large part of their lifecycle in the extracellular milieu, have been shown to use the ECM as a substrate for adhesion and colonization of host tissues through specific bacterial cell-surface MSCRAMMs [9,14,15].

Main components

Collagens

The collagens constitute a superfamily of at least 20 specialized ECM proteins with a structural role as their primary function. The characteristic feature of collagens is a rod-shaped domain composed of a polypeptide triple helix (FIGURE 1). Each polypeptide, also called an α -chain, contains a $(\text{Gly}-\text{X}-\text{Y})_n$ repetitive sequence motif where X is most often proline and Y is usually hydroxyproline and folds into a left-handed helix. Some

collagens are homotrimers of three identical α -chains, while others are heterotrimers of two or three different α -chains.

To be defined as a collagen, a protein must also form fibrils or other assemblies, either alone or in conjunction with other ECM components. Based on the exon structure of their genes, collagens can be divided into several groups: fibrillar collagens (types I, II, III, V and XI) include molecules that form banded fibrils and provide tensile strength to tendons, ligaments, bones and dense connective tissues, thus reinforcing most organs; fibril-associated collagens with interrupted triple-helix collagens (types IX, XII and XIV) are a group of proteins that may serve as molecular linking devices between fibrillar collagens and other macromolecules of the ECM; and sheet-forming collagens (type IV collagens) are proteins that form net-like polymers that assemble in the basal lamina beneath the epithelia. There is also a heterogeneous group of collageneous proteins (types VI, VII and XIII) that do not belong to any of the defined families and that form specialized structures in a variety of tissues [11].

Fibronectins

Fibronectins are glycoproteins present in vertebrates in two forms. The first is a soluble plasma Fn produced and secreted by hepatocytes and the second are insoluble cellular Fns secreted by various types of cells in a soluble form, primarily fibroblasts, which are then assembled into an ECM where they are organized into high molecular weight, insoluble, fibrillar polymers. The primary role of cellular Fns is to attach to all matrices that contain fibrous collagens. Through attachment, Fns promote cell adhesion and spreading, regulate the shape of cells and assembly of the cytoskeleton and are essential for migration and differentiation of many types of cells during embryogenesis (FIGURE 1).

Fibronectin circulating in the blood forms insoluble matrices when bound to fibrin clots. Once immobilized Fn binds, through its exposed central sequence RGD, to integrins expressed on passing, activated platelets; this results in platelet localization to damaged regions of blood vessels and increased expansion of the clot.

Fibronectins are dimers of two similar polypeptides held together by a pair of disulfide bonds at their C-termini. Each subunit is made up largely of three types of repeating modules, which in turn form structural and functional domains specialized in binding to cell-surface integrin receptors or other ECM molecules such as denatured forms of collagen, fibrin or

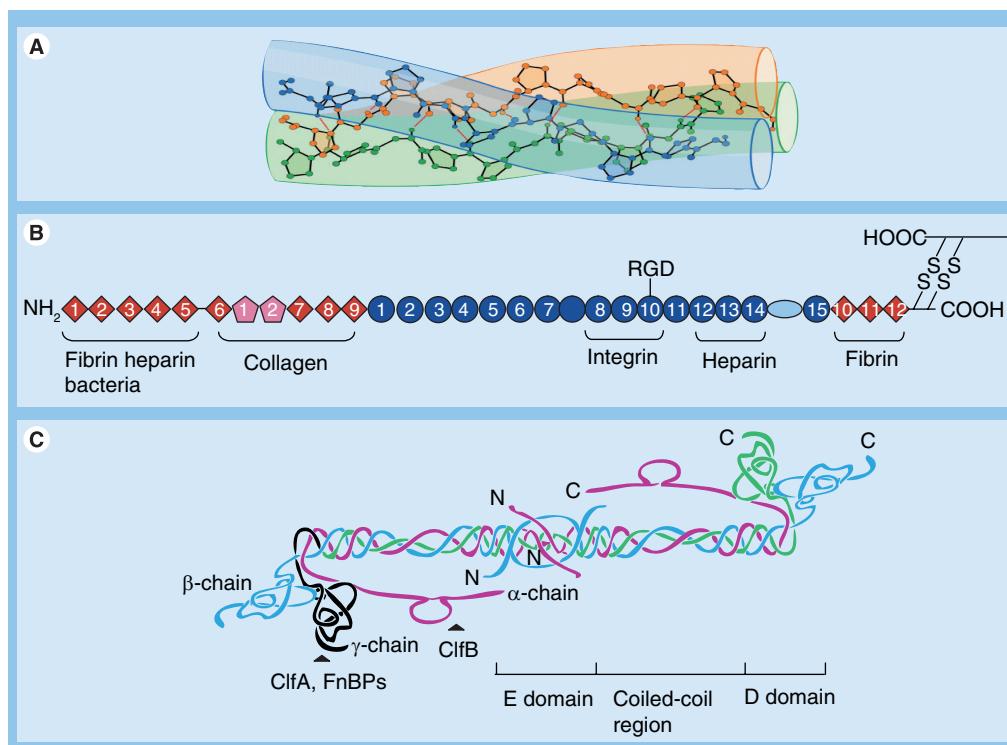


Figure 1. Structural organization of the main components of the extracellular matrix.

(A) Molecular structure of the triple-helical conformation of collagen; three left-handed helices form a right-handed superhelix. **(B)** Domain organization of fibronectin. Scheme shows the modular structure of one fibronectin subunit composed of three types of repeat. Disulfide bonds near the C-termini covalently link two almost identical subunits in the dimeric molecule. The binding sites for molecules and for cells are indicated. **(C)** Schematic model of the structural organization of fibrinogen. Pairs of α - α , β - β and γ - γ chains are linked by interchain disulphide bonds. The E domain, corresponding to the N-termini of the three pairs of chains, is connected to the C-terminal D domains by the coiled-coil regions. The C-termini of the α -chains extend away from the D domain region. Ligand-binding sites are indicated.

Clf: Clumping factor; FnBP: Fibronectin-binding protein.

(A) Reproduced with permission from [127]. **(C)** Adapted with permission from [128].

heparan sulfate. At least 20 different Fn chains have been identified, all of which arise from alternative splicing of the RNA transcript of a single gene [11].

Fibrinogen

Fibrinogen, a soluble plasma protein, is synthesized by hepatocytes and is composed of three pairs of nonidentical chains, α , β and γ , reciprocally linked by a number of disulfide bonds to form an elongated trinodular rod (FIGURE 1). Interchain disulfide bonds involving the N-terminal regions of the three polypeptides cooperate to form the central nodular E domain, while the C-termini of β - and γ -chains represent the D domain. D and E domains are connected by the coiled-coil regions of the three chains, while the α C-terminus appears as a flexible appendix of the oblong molecule. Fbg is converted by thrombin into fibrin through the hydrolysis of four Arg–Gly peptide bonds

in the central globular region. The resulting product spontaneously forms a long, insoluble homopolymeric structure, the fibrin clot. Fbg is the most abundant ligand for the integrin GPIIb/IIIa on the surface of platelets. The binding of Fbg to this integrin on activated platelets results in platelet aggregation and the formation of platelet–fibrin thrombi [11].

Staphylococcal MSCRAMMs

Definition & properties

A characteristic feature of many Gram-positive and -negative bacteria, including *S. aureus*, is the expression of adhesin proteins that specifically bind host ECM components and/or plasma proteins. These proteins either remain associated with the surface of bacteria or are released into the extracellular milieu. Accordingly, as reported above, the former have been termed MSCRAMMs [9], whereas the latter are referred to as secreted expanded repertoire adhesive

molecules (SERAMs) [16]. Both types of protein are involved in colonizing host tissues and in evasion of the host immune response.

Staphylococcus aureus can express up to 21 different surface proteins [17], mainly MSCRAMMs, which are addressed to discrete locations in the bacterial envelope by a mechanism requiring recognition of specific signal peptides and are then covalently anchored to the cell wall [18]. Covalent attachment of surface proteins is catalyzed by sortase transpeptidase enzymes, which join proteins bearing a highly conserved Leu-Pro-X-Thr-Gly (LPXTG, where X is any amino acid) sorting signal to the peptidoglycan scaffold. The sortase A enzyme of *S. aureus*, the prototypical member of the sortase enzyme family, cleaves the LPXTG motif between the threonine and glycine residues, forming a thioacyl-linked acyl-enzyme intermediate, and catalyzes the formation of an amide bond between the carboxyl group of the threonine and the amino group of a glycine residue in peptidoglycan cross bridges [19,20].

The interaction of a MSCRAMM with an ECM molecule involves a typical receptor-ligand interaction where the MSCRAMM serves as a receptor and the ECM molecule as the ligand. As a receptor, MSCRAMM is characterized by a binding specificity and reasonable affinity for its relevant ligand.

Initially, MSCRAMMs were thought to be monospecific for a given host protein. Now, a different picture has emerged, since more than one ligand has been identified for individual adhesins. Additionally, different ligands may bind to distinct domains or, alternatively, to overlapping sites in the same MSCRAMM. In most MSCRAMM-ligand systems, the sole purpose of both components appears to be reciprocal binding. However, it is possible that upon binding the ligand induces a conformational change in the receptor and *vice versa*. Insights supporting reciprocal structural changes have been obtained in studies on the interaction of Fn with the MSCRAMM Fn-binding protein (FnBP)A [21] or with Fn-binding adhesin BBK32 from *Borrelia burgdorferi* [22]. These changes may result in a specific response (effector specificity) such as activation of Fn-binding sites for the $\alpha_5\beta_1$ integrin and the consequent triggering of bacterial internalization into the host cell (see below).

Members of the MSCRAMM family

Typical members of the MSCRAMM family are staphylococcal protein A (SpA), FnBPA and -B, collagen-binding protein (CNA) and clumping factor (Clf)A and B proteins (TABLE 1).

Clumping factor A

Clumping factor A is the major staphylococcal Fbg-binding protein and is responsible for the observed clumping of *S. aureus* in blood plasma. Starting from the N-terminus, ClfA contains a signal sequence followed by the ligand-binding A region composed of three subdomains (N1, N2 and N3), the serine aspartate repeat domain (R region) and C-terminal features required for cell-wall anchoring such as the LPXTG motif, a transmembrane segment and a short cytoplasmic segment (FIGURE 2) [23]. The crystal structure of the Fbg-binding segment (residues 221–559), which includes two of the domains (N2–N3), demonstrates that each domain adopts an IgG-like fold [24].

Serine aspartate dipeptide repeat protein (Sdr) G is a Fbg-binding protein from *Staphylococcus epidermidis* that has structural similarity with ClfA and FnBPA. On the basis of a comparison of the crystal structure for SdrG N1N2N3 as an apoprotein and in complex with a Fbg β -chain peptide, a molecular model for SdrG-peptide binding has been proposed and termed 'dock, lock and latch'. In the dock, lock and latch model, the apo form of the protein adopts an open conformation that allows the Fbg ligand access to a binding groove located between the N2 and N3 subdomains. As the ligand peptide docks into the groove, a flexible C-terminal extension of the N3 domain undergoes a conformational change so that it is reoriented to cover the ligand peptide and interacts with the N2 subdomain, forming a β -strand complementary to the β -sheet in the N2 domain. This inserted β -strand functions as a latch to form a stable MSCRAMM-ligand complex [25]. Of note, in accordance with the almost identical structure between the A domains of SdrG and ClfA (FIGURE 2), a similar mechanism is operational when region A of ClfA interacts with Fbg. However, there are important differences between Fbg binding to ClfA and to SdrG. First, SdrG binds the N-terminal segment of the β -chain, while ClfA interacts with the extreme C-terminal end of the γ -chain of Fbg, which forms a flexible extention of the globule γ -module. Secondly, the opposite orientation of the bound ligand peptide to ClfA and SdrG is observed when Fbg is positioned in the cleft. The C-terminal residues of the ligand dock between the N2 and N3 in ClfA and create a parallel β -sheet complementary to a strand of the N3 subdomain, whereas in SdrG, the N-terminal residues of the ligand dock between the N2 and N3 subdomains and form an antiparallel

β -sheet with a strand of N3. Another difference is that ClfA does not require an open conformation for ligand binding, while Fbg cannot bind to the closed conformation of SdrG (FIGURE 3) [26].

Almost all *S. aureus* clinical strains carry the *clfA* gene [27]. The *clfA* gene is transcribed weakly during the exponential phase and is stimulated in a SigB-dependent manner during the stationary phase [28,29]. ClfA functions as a virulence factor in the mouse model of septic arthritis [30] and in rabbit and rat models of infective endocarditis [31–33]. Importantly, the Fbg-binding activity of ClfA is critical for the ability of *S. aureus* to provoke disease manifestations [34]. Additionally, as a cofactor, ClfA may bind factor I and enhance cleavage of C3b into inactive iC3b, contributing to immune evasion and pathogenesis [35]. Expression of ClfA on *S. aureus* hampers phagocytosis by polymorphonuclear leukocytes and macrophages [36–38]. Finally, ClfA expressed on the surface of *S. aureus* promotes rapid platelet activation and aggregation by engaging the resting platelet integrin GPIIb/IIIa through a Fbg bridge and the IgG Fc γ RIIa receptor through specific antibodies for the ClfA A domain [32,39].

Analysis of a mutant ClfA lacking Fbg-binding activity revealed a second, although less efficient, mechanism of platelet activation. Binding of immune IgG to the mutated ClfA and assembly of complement proteins on the staphylococcal surface promote an interaction with Fc γ RIIa and a complement receptor on the platelets, resulting in platelet activation and aggregation, respectively. This ClfA-mediated activity of *S. aureus* may be important in the pathogenesis of invasive diseases such as infective endocarditis [39].

Clumping factor B

The ability of *S. aureus* to adhere to Fbg-coated substrates and to form clumps in a solution containing Fbg is also mediated by ClfB, a surface protein related to ClfA. The structural organization of ClfB comprises an N-terminal secretory signal sequence, the ligand-binding A region, and a dipeptide repeat region R, mainly composed of Ser and Asp residues. Sequences involved in anchoring the protein to the surface are located towards the C-terminus of the protein (FIGURE 2) [40].

Although the structural organization for the A domains of both ClfA and ClfB is divided into three similar, independently folded subdomains N1, N2 and N3, their amino acid sequences are only 27% identical. Differently from ClfA, which recognizes the flexible peptide that

Table 1. Major staphylococcal MSCRAMMs and their ligands.

Staphylococcal MSCRAMMs	Ligands	Ref.
Bone sialoprotein-binding protein	Bone sialoprotein	[49]
Clumping factor A	Fibrinogen and complement regulator factor I	[23,26,35]
Clumping factor B	Fibrinogen and cytokeratin 10	[40,45]
Collagen-binding protein	Collagen	[69]
Fibronectin-binding protein A	Fibronectin, fibrinogen and elastin	[21,50,58]
Fibronectin-binding protein B	Fibronectin and elastin	[56]
Iron-regulated surface determinant A	Fibronectin and fibrinogen	[105]
Protein A	IgG, IgM and von Willebrand factor	[84,97]

MSCRAMM: Microbial surface component recognizing adhesive matrix molecule.

extends from the γ -module at the C-terminus of the γ -chain of Fbg [41], ClfB binds to repeat region 5 within the flexible connector region of the α -chain of Fbg [42]. In addition to Fbg, ClfB binds to cytokeratin (CK)10 exposed on the surface of desquamated epithelial cells and represents the major determinant in the ability of *S. aureus* to adhere to squamous cells during the anterior nare colonization process [43,44]. Fbg and CK10 have the same or overlapping binding sites of ClfB and the mechanism of ClfB binding to Fbg is thought to be similar to CK10 binding [45]. In contrast to ClfA, which is present on bacterial cells at all stages of the growth cycle, ClfB is expressed on the cell surface only during the exponential growth phase. This suggests that transcription of the *clfB* gene ceases during the exponential phase and that surface protein may be degraded by proteases or shed into the growth medium [46]. As reported for ClfA, ClfB expressed on the surface of *S. aureus* can aggregate platelets [47,48]. However, bacteria expressing ClfB aggregate platelets with a longer lag time than ClfA and by distinct Fbg- and complement-mediated mechanisms. In the Fbg-dependent mechanism, Fbg acts as a bridge between the platelet GPIIb/IIIa and the bacterium, while specific anti-ClfB antibodies can bind the Fc receptor on platelets to cause activation through receptor clustering. Staphylococci expressing a mutated form of ClfB that does not bind Fbg are still able to cause aggregation in a complement-dependent manner. In this event, complement proteins bound to the bacterium can presumably interact with a complement receptor on the surface of platelets and anti-ClfB antibodies bound to the staphylococcal surface form the second link to platelets via the Fc γ RIIa receptor [47,48].

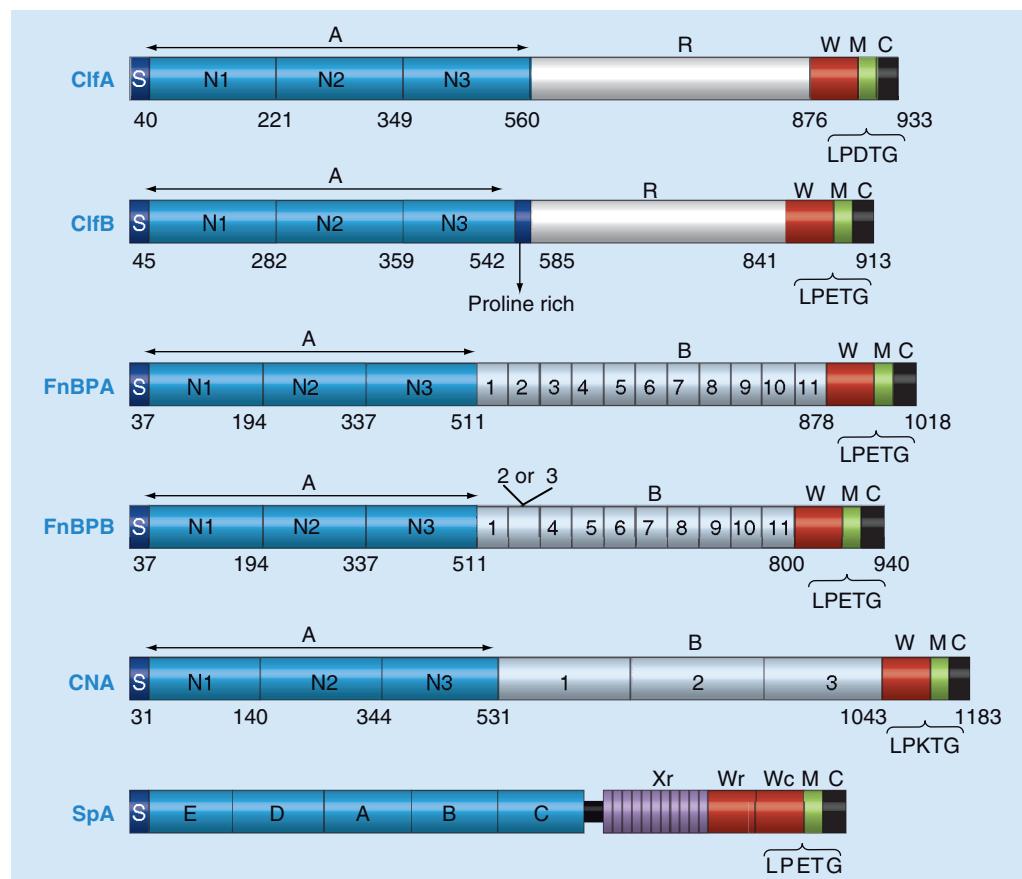


Figure 2. Domain organization of MSCRAMMs in *Staphylococcus aureus*. ClfA, ClfB, FnBPA/FnBPB and CNA have a common structural organization including a signal sequence (S) followed by the N-terminal ligand binding A region in which three subdomains, N1, N2 and N3, can be identified. At the C-terminus, the cell-wall-binding region (W), the membrane-spanning segment (M) and the charged C-terminal tail (C) are present. ClfA and ClfB contain the Ser–Asp dipeptide repeat R region. ClfB also contains an additional proline-rich sequence linking the A and R region. FnBPA/FnBPB contain the unique fibronectin-binding repeat region between the A domain and the C-terminus. E,A,B,C and D are the ligand-binding repeats of SpA. Xr represents the short sequence-repeat region made of variable numbers of octapeptide motifs. The positions of the cell-wall-spanning region (Wr and Wc), the membrane-spanning region M and the positively charged cytoplasmic tail are indicated. The positions of the LPXTG motif are indicated. The amino acid residue number identifying the boundary between each MSCRAMM subdomain is indicated.

Clf: Clumping factor; CNA: Collagen-binding protein; FnBP: Fibronectin-binding protein; MSCRAMM: Microbial surface component recognizing adhesive matrix molecule; SpA: Staphylococcal protein A.

Bone sialoprotein-binding protein

The *S. aureus* chromosome also encodes a protein with 1171 amino acids, called bone sialoprotein-binding protein (Bbp). It has been suggested that the Bbp protein may be important in the localization of bacteria to bone tissue, and thus might be of relevance in the pathogenicity of osteomyelitis [49]. Bbp, along with ClfA and ClfB, belongs to a large family of structurally related proteins characterized by an R domain containing Sdr proteins. The A domains of the Sdr proteins are of similar size (~500 amino acid residues) and present a 20–30% identity.

Fibronectin-binding proteins (FnBPA/FnBPB)

Fibronectin-binding protein A is a multi-functional adhesin that binds to Fbg or elastin with its N-terminal portion (the A domain) [50], whereas its C-terminal moiety binds Fn (FIGURE 2) [21]. The C-terminal domain is organized into 11 tandem repeats, each interacting with the Fn N-terminal domain (NTD), which is composed of five type I modules. Six of the 11 sites bind the ligand with high affinity; other sites bind more weakly. *S. aureus* contains a second Fn-binding protein, FnBPB, which shows 68% sequence homology with FnBPA. Based on sequence alignment,

it has been predicted that FnBPB contains ten rather than 11 repeats, but it contains the same number (six) of high-affinity binding sites for the NTD of Fn as FnBPA (FIGURE 2) [SPEZIALE PETAL., UNPUBLISHED DATA] [21].

The ligand-binding repeats of FnBPA have an intrinsically disordered structure but, on binding to Fn, these units acquire particular conformations that can be monitored by specific monoclonal antibodies, which recognize so-called ligand-induced binding sites [21]. The presence of ligand-induced binding site-binding antibodies in sera of patients recovering from a *S. aureus* infection suggests that the conformational transition in the adhesins may occur *in vivo* and that *S. aureus*, in fact, interacts with Fn during the infection [51]. A conformational change in FnBPA upon ligand binding can also be demonstrated by circular dichroism spectroscopy [52]. On the basis of the high-resolution NMR structure of a short FnBPA synthetic peptide in complex with ¹F1 ²F1 of NTD, a new protein–protein interaction called the ‘tandem β-zipper’ has been proposed. In the complex structure, short segments of the MSCRAMM-derived peptide form a fourth β-strand complementing the three β-strand sheets present in the NTD modules [53,54]. Importantly, it has been predicted that in the NTD, the majority of the F1 modules interact with the binding repeats of FnBPA in a similar manner. Recently, the crystal structure of the NTD module pairs ²F1 ³F1 and ⁴F1 ⁵F1 in complex with synthetic peptides derived from the FnBPA-1 and FnBPA-5 repeats have been visualized at high resolution, which provided more detail of the NTD–FnBPA interactions [55]. FIGURE 4 shows two F1 module pair/peptide structures that together comprise the structure of the N-terminal repeat (FnBPA-1) in complex with ^{2–5}F1 modules of the NTD. The structure reveals that the peptides establish prevalent contacts with amino acid residues residing in the ²F1 (or ⁴F1) modules of the NTD. Electrostatic interactions are also established between positive patches on ²F1 and ⁴F1 and negatively charged residues at positions 15–16 and 36–37 in the FnBPA-1 unit, respectively. Finally, loops in the MSCRAMM sequence appear between modules ²F1 and ³F1 and also between ³F1 and ⁴F1; conversely, no linker residues were observed between ⁴F1 and ⁵F1.

Residues at the N-terminus of FnBPA (37–511) share 20% identity with the Fbg-binding A domain of ClfA and are predicted to fold into three subdomains, N1, N2 and N3,

similar to ClfA. The A domain of FnBPA, as reported for the A domain of ClfA, binds Fbg at the C-terminus of the γ-chain [50] but, unlike ClfA, it also binds elastin [56] and tropoelastin [57]. Additionally, Fbg and elastin bind to the same site on FnBPA [56]. A structural model of the FnBPA A domain has a conformation similar to the resolved structure of region A of SdrG and ClfA, including the hydrophobic trench. Residues lining this trench are critical for binding of FnBPA to both elastin and Fbg [58].

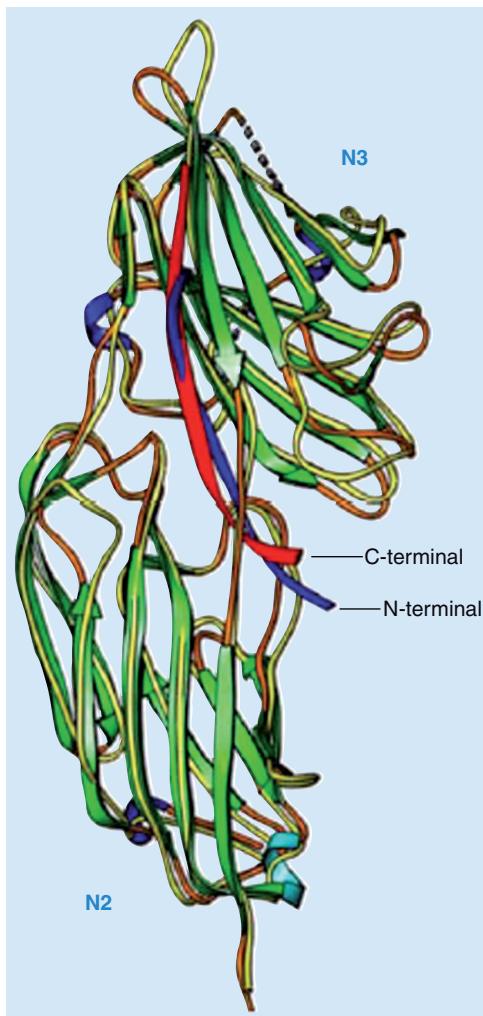


Figure 3. Superpositions of the clumping factor A-peptide and SdrG-peptide complexes. Clumping factor A (N2-N3) is green coloured and serine aspartate dipeptide repeat protein G N2-N3 is a thin yellow uniform coil. The fibrinogen γ-chain analog (γ 395–411) in complex with clumping factor A and the synthetic peptide from fibrinogen β-chain (β 6–20) bound to the serine aspartate dipeptide repeat protein G are indicated as red and blue ribbons, respectively. Note the opposite direction of the bound peptides. Reproduced with permission from [25].

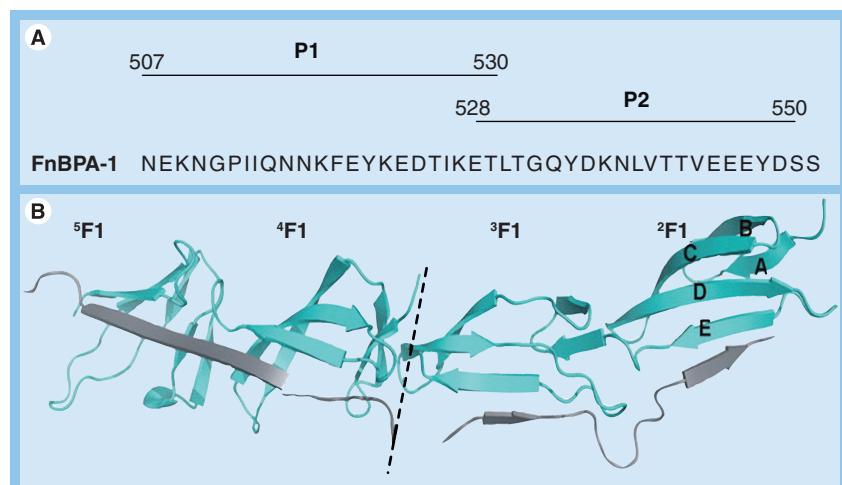


Figure 4. Synthetic peptides spanning the entire sequence of FnBPA-1 in complex with N-terminal domain F1 module pairs from fibronectin.

(A) Sequence of the FnBPA-1 repeat. Peptide sequences are indicated by solid bars.
 (B) Ribbon diagram for the structure of ²F1³F1/P1 and ⁴F1⁵F1/P2. Fibronectin modules are indicated in cyan and the FnBPA-1 is indicated in gray.

FnBP: Fibronectin-binding protein.

Reproduced with permission from [53].

The FnBPA A domain occurs in at least seven different isotypes that differ antigenically and exhibit limited immunocrossreactivity, yet retain their ligand-binding functions. It has been suggested that this antigenic variation of the FnBPA A domain may aid *S. aureus* in evading host immune responses [59].

Fibronectin-binding protein A and B are major platelet-activating factors on the surface of *S. aureus* from the exponential phase of growth [60], and have been indicated as crucial virulence determinants in the endocarditis animal model [61,62].

Binding of soluble Fbg and/or Fn molecules present in blood to FnBPA expressed on the surface allows the formation of bridges with integrin GPIIbIIIa on resting platelets. This bacterium–platelet interaction is not in itself sufficient to promote activation. Specific IgG bound to both region A and the repetitive domain crosslinks the complex to the Fc γ RIIa platelet receptor, causing platelet activation and aggregation [60]. Thus, rapid activation of platelets mediated by FnBPs (and ClfA) requires a Fbg or Fn bridge to the integrin GPIIb/IIIa and an immunoglobulin bridge to the Fc γ RIIa receptor.

Besides playing a key role in attachment and colonization of host tissues, FnBPA can act as an invasin. In fact, adhesin binds Fn and staphylococci-bound Fn are then recognized by integrin $\alpha_5\beta_1$ on host cells in a process that leads to cellular invasion [63,64]. Invasion allows bacteria to escape detection by host defence systems

and represents an important virulence strategy, particularly for organisms that cause persistent infections. Thus, *S. aureus*, previously thought to essentially be an extracellular pathogen, might have important intracellular stages.

Fibronectin-mediated interactions between FnBPA/FnBPB and integrin $\alpha_5\beta_1$ on endothelial cells are also critical in promoting *S. aureus* endothelial colonization and invasion [65]. Upon interaction with endothelial cells, FnBPs powerfully induce a variety of proinflammatory endothelial responses including expression of cell adhesion molecules such as ICAM-1 and VCAM-1, as well as secretion of chemokines and cytokines (e.g., IL-8 and monocyte chemoattractant protein 1). Additionally, FnBPA causes a tissue factor-mediated endothelial procoagulant response that synergistically acts with adhering blood monocytes to trigger the coagulation cascade that leads to thrombus formation. These FnBPA effects are mediated by the repetitive Fn-binding region of the MSCRAMM [66].

Recently, a novel *S. aureus* biofilm phenotype promoted by FnBPs has been reported [67]. The biofilm development appears to be independent of the known ligand-binding activity of these multifunctional proteins. Furthermore, the A domain alone, and not the domain required for Fn binding, promoted biofilm formation.

Collagen-binding protein

The collagen-binding MSCRAMM on *S. aureus* termed CNA is composed of an A region and a varying number of B repeats depending on the strain. At the C-terminal end of the CNA there are features required for surface targeting and covalent anchoring of the peptidoglycan (FIGURE 2) [68]. The collagen-binding region is located in the A region [69,70], which is composed of three subdomains: N1 corresponding to residues 31–140, N2 residues 141–344 and N3 residues 345–531. The segment of CNA showing the highest affinity for collagen is composed of the two subdomains N1 and N2 (aa residues 31–344) [71], each adopting a variant IgG fold and a long linker that connects N1 and N2 [72]. Recently, a ‘collagen hug mechanism’ has been proposed, where a collagen triple helix initially associates with the N2 subdomain [72]. The collagen is then wrapped by the N1–N2 linker and the N1 subdomain. The N1 subdomain interacts with the N2 subdomain via a number of hydrophobic bonds and finally the insertion of the latch, representing the extension of the C-terminal end of the N2 subdomain, is introduced into the N1 subdomain to secure the CNA–collagen complex (FIGURE 5) [72]. The

proposed model predicts that CNA only binds to monomeric forms of collagen, implying that the MSCRAMM does not mediate staphylococcal adherence to higher-ordered structures such as fibrillar collagens or denatured collagen [72] but only to triple-helix monomers that are naturally occurring or generated through tissue injury [73,74].

The function of the B repeats is unknown, but this region is thought to form a stalk that displays the A domain on the cell surface [75].

The majority of *S. aureus* strains (>90%) isolated from patients with bone and joint infections express CNA, whereas significantly fewer isolates obtained from wound or soft-tissue infections express this adhesin [68]. The transcription of the *CNA* gene is temporally regulated, with expression being highest in exponentially growing cultures and falling to almost undetectable levels as cultures enter the post-exponential growth phase [76]. Furthermore, the staphylococcal accessory regulator (*sar*) is the primary regulator element controlling transcription of the *CNA* adhesin gene and the regulatory impact of *sar* is independent of the interaction between SarA and accessory gene regulator (*agr*) [77]. CNA participates in the infectious process of pathogenic *S. aureus* and has been demonstrated to be a virulence factor in many different animal models of staphylococcal infections including endocarditis [78], osteomyelitis [79], arthritis [68,71] and keratitis [80], suggesting that its ability to interact with collagen provides a general advantage for the bacteria. Under fluid shear conditions, CNA-mediated interaction of *S. aureus* with collagen type I has been shown to be important in the development of thrombi in whole blood [81].

Protein A

Protein A, the first staphylococcal surface protein to be characterized, is primarily known for its ability to bind the Fc region of mammalian IgG. The structural organization of this protein is somewhat different from that of other characterized cell-wall-associated proteins. The N-terminal region of SpA includes five subdomains, labeled E, D, A, B and C, linked to the cell surface by a region that contains a variable number of proline-rich octapeptide repeats (X_r domain) followed by a nonrepeating segment (FIGURE 2) [82,83]. Each of the IgG-binding subdomains are composed of 58 residue units that form three-helix bundles and that bind with high affinity to the Fc region of immunoglobulin G and to the fragment antigen binding region of heavy-chain variable domain 3 (VH3) on IgM [84,85]. Both the SpA–Fc and

SpA–fragment antigen binding interactions have been analyzed at the molecular level with cocrystallized complexes. One face of the subdomain comprising residues from helices I and II binds IgG Fc, whereas residues from helices II and III on the other face bind IgM [86,87]. Indeed, it is possible for a single SpA domain to bind each ligand simultaneously [88,89].

The interaction of SpA with IgG hinders phagocytosis because bacteria become coated with IgG in a conformation that is not recognized by the Fc receptor on neutrophils [90–92]. Moreover, SpA-bound IgG cannot stimulate complement fixation by the classical pathway. Another consequence of the ability of SpA to bind to B lymphocytes displaying IgM bearing VH3 heavy chains is the induction of proliferation; this results in depletion of a significant portion of the B cell repertoire [93,94].

SpA is also known to bind the von Willebrand factor (vWF), a protein essential for hemostasis [95]. The ability of *S. aureus* to bind vWF could contribute to the adherence of the bacterium to platelets or to damaged blood vessels and play an important role in mediating interactions with platelets at high shear rates [96]. Mutagenesis studies have demonstrated that protein A binding to vWF is mediated by conserved IgG-binding subdomains, between helices I and II, which is also the region responsible for the Fc interaction [97].

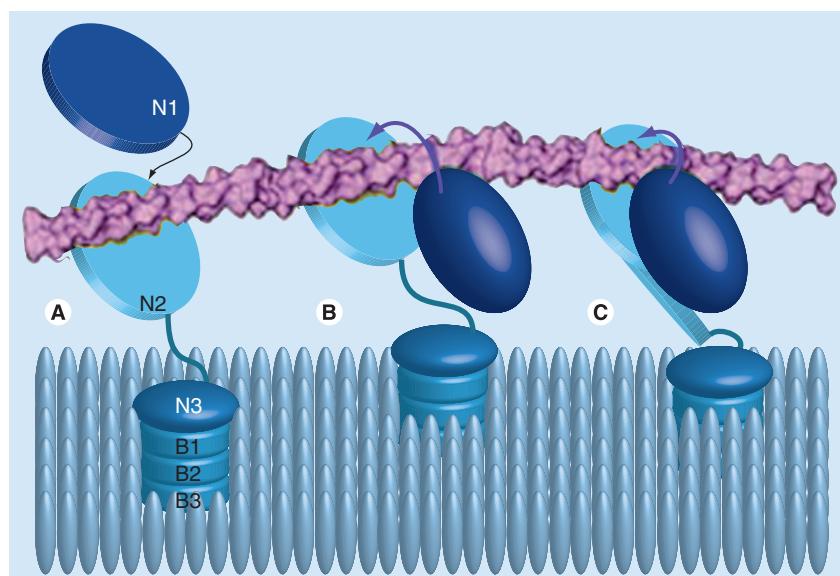


Figure 5. Representation of a hypothetical ‘collagen hug’ model.

(A) Collagen molecule binds the N2 subdomain. (B) Collagen is wrapped by the linker connecting the N1 and N2 subdomains. (C) N1 interacts with N2 subdomain by hydrophobic interactions and its position in the complex is stabilized by contacts with the C-terminal latch of N2.

Reproduced with permission from [71].

Staphylococcal protein A also recognizes gClqR/p33, a complement receptor exposed on the surface of activated platelets. This binding has been indicated as an additional mechanism for bacterial cell adhesion at sites of vascular injury and thrombosis [98].

Gómez *et al.* have shown that TNFR1, a receptor for TNF- α widely distributed in the airway epithelium, binds to protein A [99]. This interaction, mediated by the subdomain that binds IgG, is essential for the pathogenesis of *S. aureus* pneumonia [100] and for activation of TNFR1 and subsequent proinflammatory signaling. Protein A also induces TNFR1 shedding from the surface of epithelial cells and macrophages, which serves to limit TNF- α signaling [101]. *S. aureus*, through the repeated Xr sequences of SpA, activates known components of the type I IFN cascade, particularly in the lung, and this activation contributes to the virulence of the respiratory pathogen [102]. Protein A has been identified as an essential component of staphylococcal biofilm [103]. Contribution of protein A to biofilm development does not require the covalent anchorage of the protein to the bacterial cell wall. Additionally, in contrast to accumulation-associated protein (Aap)- and *Staphylococcus aureus* surface protein G (SasG)-mediated biofilm development that must undergo proteolytic processing to render the protein active for cell–cell interaction (see later), protein A also appears to induce biofilm formation under conditions where the level of proteases is low [103].

Iron-regulated surface determinant A

Recently, a novel locus, iron-regulated surface determinant (*isd*), has been identified in *S. aureus* [104]. This *isd* locus, present in all sequenced *S. aureus* genomes, includes nine genes encoded by separate iron-regulated transcriptional units. The Isd pathway consists of nine proteins that work in concert to acquire heme. One of the gene products encoded by the *isd* locus is IsdA. Although the IsdA protein was initially suggested to play a role in iron acquisition, under iron-limiting conditions, this protein has broad-spectrum ligand-binding activity, including Fbg and Fn [105]. Remarkably, IsdA binds to involucrin, loricrin and CK10, all proteins present in the cornified envelope of human desquamated epithelial cells [106]. IsdA contributes to the ability of *S. aureus* to colonize skin through its negative charge, which reduces susceptibility to bactericidal lipids and cationic antimicrobial peptides [107]. Additionally, IsdA

protects *S. aureus* against the bactericidal protease activity of apolactoferrin [108]. Furthermore, IsdA has been shown to be involved in adherence to human desquamated nasal epithelial cells [109] and to enhance colonization of the anterior nares of cotton rats [110].

MSCRAMMs as therapeutic targets

For an ideal vaccine, a bacterial antigen and, more specifically, a MSCRAMM must be located on the surface, be expressed at high levels both *in vitro* and *in vivo* and play a crucial role as a virulence factor. Moreover, antibodies against the MSCRAMM should prevent microbial adherence and/or facilitate clearance of the organism through phagocytosis. In several cases the above-mentioned properties regarding MSCRAMMs and the relevant antibodies have been demonstrated [111–117].

Hence, it is not surprising that a large number of trials have been performed to test the value of *S. aureus* MSCRAMMs individually or in combination as a vaccine. In an initial study, Nilsson *et al.* vaccinated mice with the recombinant fragment including the collagen-binding domain of CNA and found that animals had a significantly higher survival rate than control mice when challenged with a virulent clinical strain of *S. aureus*. To determine if the protective effect was antibody mediated, the researchers passively immunized naive mice with adhesin-specific antibodies. Similar to the active immunization strategy, passive transfer of these antibodies protected mice against sepsis-induced death. Additionally, staphylococci opsonized for phagocytic uptake with sera from collagen-binding-domain-immunized animals were ingested more efficiently and intracellular killing was enhanced [111].

In a similar study, a recombinant form of region A of ClfA was used to generate strong immune responses and has shown potential as a vaccine component in active and passive immunization studies. ClfA immunization showed a reduction in arthritis and lethality induced with *S. aureus*, although protection was strain dependent [30]. In a more recent study, therapy with a murine monoclonal antibody 12–9 against ClfA, known as tefibazumab (Aurexis[®]), protected mice in a lethal sepsis model [113]. In a rabbit model of endocarditis, the humanized version of this antibody, given in combination with vancomycin, drastically reduced bacteremia and bacterial load in parenchymatous organs as compared with the animals treated with vancomycin alone [114,118].

An experimental hyperimmune IgG preparation named Veronate®, produced by plasmapheresis from donors with high IgG titers against ClfA, was positively tested for protecting very low birth weight infants from staphylococcal infections [115] and significantly decreased the infection rates in a catheter-induced endocarditis infection model [116]. However, further development of this promising approach has been placed on hold owing to failure to demonstrate efficacy in a completed Phase III study.

Considering the relatively high binding affinity of ClfA for Fbg and the high concentration of Fbg in plasma, it has been hypothesized that the ClfA–Fbg interaction during the immunization phase could decrease antigen presentation by reducing the visibility of critical epitopes on the ClfA molecule and consequently decrease production of potentially important antibodies. Thus, it has been proposed that ClfA mutated forms lacking the capacity to interact with Fbg might be a better vaccine candidate than wild-type ClfA [36]. Schaffer *et al.* evaluated nasal immunization with killed *S. aureus* grown under conditions to optimally express ClfB and systemic and mucosal vaccination in mice with recombinant ClfB. Animals immunized with killed staphylococcal cells exhibited reduced nasal colonization compared with control mice. In addition, passive immunization with a monoclonal antibody that inhibits the interaction of ClfB with CK10 protected mice from staphylococcal nasal colonization [117]. Likewise, there was a significant reduction in colony-forming units of *S. aureus* isolated from the nose of mice immunized systemically or intranasally with a recombinant region A of ClfB with respect to control groups [119]. Other surface proteins with potential MSCRAMM activity investigated as vaccine targets include Isd proteins. Purified IsdB induced a rapid antibody response in rhesus macaques and protection in a mouse model of infection. The vaccine conferred a significant reduction in mortality relative to control mice immunized with adjuvant alone [120]. It has also been reported that vaccination of cotton rats with IsdA and IsdH might prevent and protect against *S. aureus* nasal colonization [110].

More promising results from novel approaches based on the combination of several selected MSCRAMMs have been reported. Active immunization of mice with a combination of the A domains of ClfA, FnBPA and FnBPB or passive immunization with monoclonal antibodies against ClfA and FnBPA resulted in a higher

level of protection than that obtained by vaccination with a single protein or antibody in an aortic patch murine model [121].

A more recent study evaluated a multivalent vaccine containing MSCRAMM (e.g., IsdA) and surface proteins with potential MSCRAMM activity (e.g., IsdB, SdrD and SdrE antigens) in a mouse challenge model. Among 19 surface proteins tested, the authors found that the combination of four proteins, IsdA, IsdB, SdrD and SdrE, administered to mice provided excellent protection against challenge with different pathogenic strains of *S. aureus* [122]. In conclusion, owing to the multiple redundant expression of MSCRAMMs, an effective vaccine against staphylococci should be multicomponent, incorporating several MSCRAMMs.

MSCRAMM DNA vaccines have been developed with similar results. Gaudreau *et al.* immunized mice with plasmids expressing ClfA, FnBPA and the enzyme sortase as single proteins or as a multivalent polyprotein [123]. When mice were challenged with a virulent strain of *S. aureus*, 55% of vaccinated animals survived as compared with 15% of control mice. However, vaccination did not promote a significant reduction in clinical signs of septic arthritis. Castigliuolo *et al.* [124] used three MSCRAMMs (ClfA, FnBPA and CNA) and the secreted extracellular Fbg-binding protein (Efb) as targets in a DNA vaccine. Intranasal immunization with a plasmid DNA mixture encoding the four adhesins triggered significant levels of serum and mucosal IgG and a strong splenocyte proliferative response. Of note, immunized mice were also protected against intramammary challenge with *S. aureus*. These studies confirm that DNA vaccination formulated to encode a combination of several MSCRAMMs is as effective as the protein formulation described by Stranger-Jones *et al.* [122].

Conclusion

Considerable progress has been made in our understanding of ECM recognition by *S. aureus* in the last few years. Several *S. aureus* MSCRAMMs have been identified and characterized and the crystal structures of some of these in complex with their ligands have been resolved. Of the *S. aureus* MSCRAMMs reviewed herein, many have proven to be involved in staphylococcal pathogenesis, whereas others have been shown to interact *in vitro* with host molecules in a fashion that predicts their contribution to the disease process. The identified MSCRAMMs show remarkable antigenic

properties and valid protective activity; thus, they appear to be promising candidates for an effective antistaphylococcal vaccine.

Future perspective

Over the last few years, the systematic use of genome-sequence databases, gene-expression technology, x-ray crystallography and high-quality animal studies have increased our appreciation of the structures and functions of *S. aureus* MSCRAMMs. Despite these efforts, recent discoveries should not underestimate our ignorance regarding other aspects of *S. aureus* MSCRAMM-ECM interactions. For example, the specific targets of important presumptive MSCRAMMs such as SdrC, D and E remain to be identified. Similarly, with the exception of the repeating region of FnBPA/FnBPB, the function of the repeat domains of the MSCRAMMs is still unknown. Furthermore, we have only just begun to explore the consequences of MSCRAMM-promoted adherence on both *S. aureus* and different host-cell phenotypes [125,126]. The role of staphylococcal

adhesins as virulence factors and how this relates to the complex sequence of events in the initiation and progression of disease is also not completely clear. Finally, MSCRAMMs appear to be valid protective agents and promising targets for immunotherapy in mice, but it is not known whether a successful MSCRAMM-based vaccine would be effective in humans. Thus, challenging questions remain to be answered in the biochemistry, molecular biology and immunology of MSCRAMMs before an optimal approach to combat and neutralize this formidable pathogen can be devised.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- The extracellular matrix (ECM) of the host is defined as the extracellular material comprising secreted macromolecules forming an integrated system that regulates anchoring, development and function of mammalian cells. The ECM is also the attachment substrate for extracellular pathogens such as *Staphylococcus aureus*.
- Adherence to the ECM and subsequent colonization of host tissues by *S. aureus* occurs through specific bacterial cell-surface adhesins called microbial surface component recognizing adhesive matrix molecules (MSCRAMMs).
- MSCRAMMs are multidomain proteins characterized by common features within the N-and C-terminal sequences. The ligand-binding activities reside in the N-terminal and/or the central region of the MSCRAMMs.
- Examples of well-known MSCRAMMs include the clumping factors A and B, fibronectin-binding proteins A and B, which also bind to fibrinogen, the collagen-binding protein and protein A, which binds IgG and the von Willebrand factor.
- MSCRAMMs appear to have a specific role in attachment and colonization of tissues and in several cases have been confirmed as critical virulence determinants of infectious disease.
- Individual MSCRAMMs are promising vaccine candidates and suitable for immunoprophylaxis.

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