Chapter 7
Adhesion Mechanisms of Staphylococci

Christine Heilmann

Abstract Staphylococcal adherence to an either biotic or abiotic surface is the critical first event in the establishment of an infection with these serious pathogens. Especially *Staphylococcus aureus* harbours a variety of proteinaceous and non-proteinaceous adhesins that mediate attachment to a multitude of host factors, such as extracellular matrix and plasma proteins and human host cells, or intercellular adhesion, which is essential for biofilm accumulation. Proteinaceous adhesins may be classified in covalently surface-anchored proteins of the MSCRAMM (microbial surface components recognizing adhesive matrix molecules) family or in proteins that are surface-associated by different means, such as ionic or hydrophobic interactions. Non-covalently surface-associated proteins include the autolysin/adhesins, proteins of the SERAM (secretable expanded repertoire adhesive molecules) family, or membrane-spanning proteins. Non-proteinaceous adhesins comprise the polysaccharide PIA (polysaccharide intercellular adhesin) and wall teichoic and lipoteichoic acids. The features and functions of surface and surface-associated protein adhesins as well as of non-proteinaceous adhesins are discussed.

7.1 Introduction

Besides being harmless inhabitants of the human skin and mucous membranes, the Gram-positive staphylococci belong to the most important pathogens causing diseases ranging from mild skin infections to serious and life-threatening syndromes, such as endocarditis, osteomyelitis, pneumonia, and sepsis (Ziebuhr, 2001). The coagulase-positive species *Staphylococcus aureus* usually causes more acute infections associated with the colonization of the host tissue, but is also a common cause of foreign body-associated infections and known to cause persisting and relapsing infections (Lentino, 2003). Infections due to coagulase-negative species

C. Heilmann (✉)
Institute for Medical Microbiology, University Hospital of Münster, 48149 Münster, Germany
e-mail: heilmac@uni-muenster.de

D. Linke, A. Goldman (eds.), *Bacterial Adhesion*, Advances in Experimental Medicine and Biology 715, DOI 10.1007/978-94-007-0940-9_7,

typically are more subacute or even chronic and usually require a predisposed or immunocompromised host, for example patients with indwelling medical devices. *Staphylococcus epidermidis* is the most frequent coagulase-negative species isolated from medical device-associated infections, such as prosthetic heart valves and orthopaedic implants (Lentino, 2003). The fact that *S. aureus* is more virulent is reflected by its production of numerous virulence factors, i.e. a variety of adhesins, extracellular enzymes, and toxins. In contrast, the capacity of *S. epidermidis* to produce adhesins and especially to secrete extracellular enzymes and toxins is much less pronounced.

To become a pathogen, staphylococci have to gain access to the human host usually by adhering to biotic surfaces, such as components of the extracellular matrix or host tissue, or to abiotic surfaces, such as those of medical devices. Upon adherence, the bacteria proliferate and colonize the respective biotic or abiotic surface by forming a biofilm. The formation of a biofilm can be differentiated in two phases: the primary adherence phase, followed by the accumulation phase. The latter requires intercellular adherence to form the multilayered biofilm. Intercellular adherence may be mediated by polysaccharide- or protein-factors. Within the biofilms, the staphylococci are embedded in a polysaccharide and/or proteinaceous matrix with wall teichoic acids, host factors and extracellular DNA in addition. All these protect the bacteria against the host immune system as well as antibiotic treatment (Heilmann and Götz, 2010). As a consequence, removal of the medical device is frequently necessary to eradicate the infection in implant-associated infections. Some staphylococcal adhesins are not only involved in adherence and colonization, but also in internalization by human host cells. Internalized staphylococci are protected against the human immune system as they "hide" within the host cells representing a potential reservoir for recurrent infections.

Compared to *S. aureus*, much less is known about adhesins and adhesive mechanisms in coagulase-negative staphylococci except for some aspects of *S. epidermidis* biofilm formation. Therefore, this chapter mainly deals with the presentation of *S. aureus* adherence mechanisms. The adhesive mechanisms involved in *S. aureus* biofilm formation on abiotic or biotic surfaces are summarized in Fig. 7.1.

7.2 Surface Adhesion Proteins of *Staphylococcus aureus*

S. aureus is able to directly adhere to host tissue, such as the host epithelium or endothelium and binds to a multitude of components of the extracellular matrix, such as fibronectin (Fn), fibrinogen (Fg), vitronectin (Vn), thrombospondin, bone-sialoprotein, elastin, collagen, and von Willebrand factor. Moreover, implanted material rapidly becomes coated with plasma and extracellular matrix proteins or platelets. Thus, all these host factors could serve as specific receptors for colonizing bacteria. Proteinaceous surface adhesins of *S. aureus* can either be covalently linked to the cell wall peptidoglycan or surface-associated by different means, such as ionic or hydrophobic interactions (summarized in Table 7.1).

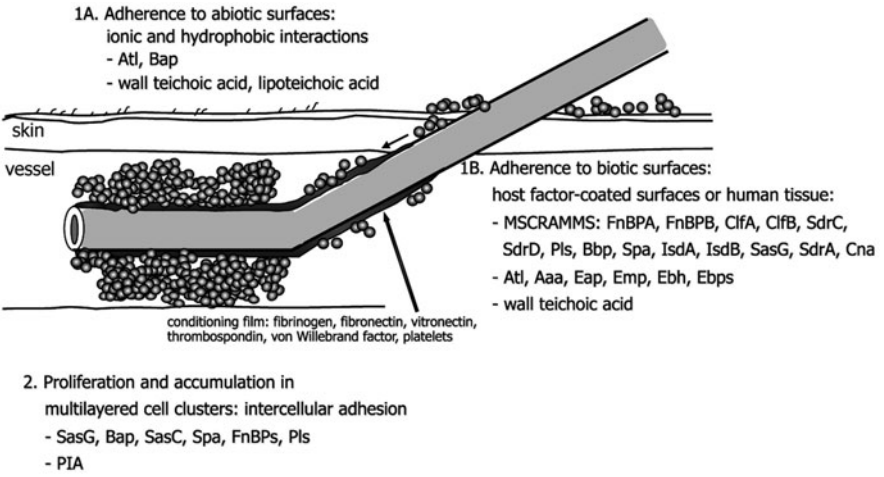

Fig. 7.1 Overview on the complex adhesive mechanisms involved in the different phases of *S. aureus* biofilm formation. *S. aureus* factors involved in the colonization of a catheter surface or host tissue, such as the extracellular matrix and the human endothelium or epithelium are given

7.2.1 Covalently-Linked Cell Surface Proteins (MSCRAMMs)

Most *S. aureus* surface adhesins are covalently linked to the cell wall peptidoglycan and belong to the MSCRAMM (microbial surface components recognizing adhesive matrix molecules) protein family (Clarke and Foster, 2006). Covalently-linked MSCRAMMs have a common overall organization including an N-terminal signal peptide, an exposed ligand-binding domain, which is often followed or interrupted by direct repeated sequences, a characteristic hydrophobic cell wall- and membrane-spanning domain, a C-terminal LPXTG motif responsible for cell-wall anchorage, and a positively charged tail. In most cases, cell-wall anchorage is mediated by a membrane-bound transpeptidase, called sortase (SrtA) that cleaves the peptide bond between the threonine and glycin of the LPXTG motif and covalently links the carboxyl group of threonine with the amino group of peptidoglycan cross-bridges (Schneewind et al., 1993). *S. aureus* genomes contain more than 20 genes encoding surface-anchored adhesins. In contrast, there are only 12 genes encoding covalently-linked MSCRAMMs in the *S. epidermidis* RP62A genome. MSCRAMMs can bind to one or more host extracellular matrix and plasma protein and a given host factor can be bound by more than one staphylococcal adhesin. However, not the ligands or functions of all MSCRAMMs have been identified yet.

7.2.1.1 Fibronectin-Binding Proteins (FnBPA and FnBPB)

S. aureus produces two closely related covalently-linked Fn-binding MSCRAMMs, FnBPA and FnBPB, which are encoded by *fnbA* and *fnbB*, respectively (Jönsson

Table 7.1 Surface and surface-associated proteins of *S. aureus*

Gene	Product	Ligand specificity/Function	Reference
Covalently-linked surface proteins			
bbp	Bbp	Bone sialoprotein	Tung et al. (2000)
bap	Bap	Attachment to polystyrene; intercellular adhesion; prevents binding to Fg, Fn, and host tissue and internalization	Cucarella et al. (2002)
clfA	ClfA	Fg; platelet aggregation	McDevitt et al. (1997), O'Brien et al. (2002a)
clfB	ClfB	Fg, cytokeratin 10, desquamated nasal epithelial cells; platelet aggregation	Corrigan et al., (2009), Ni Eidhin et al. (1998), O'Brien et al. (2002a, b)
cna	Cna	collagen	Patti et al. (1995)
fnbA	FnBPA	Fn, Fg, elastin; intercellular adhesion	Signas et al. (1989), Roche et al. (2004), Wann et al. (2000), Vergara-Irigaray et al. (2009)
fnbB	FnBPB	Fn, elastin; intercellular adhesion	Jönsson et al. (1991), Roche et al. (2004), Vergara-Irigaray et al. (2009)
isdA	IsdA	Fg, Fn, fetuin, haemoglobin, transferrin, haemin, desquamated nasal epithelial cells	Clarke and Foster (2006), Mazmanian et al. (2003)
isdB	IsdB	haemoglobin, haemin, platelet integrin GPIIb/IIIa	Mazmanian et al. (2003), Miajlovic et al. (2010)
isdC	IsdC	Haemin	Mazmanian et al. (2003)
isdH	IsdH	Haptoglobulin, haptoglobulin-haemoglobin complex	Dryla et al. (2003)
sasA (*sraP*)	SasA (SraP)	Binding to platelets	Siboo et al. (2008)
sasB	SasB	unknown	Roche et al. (2003a)
sasC	SasC	Attachment to polystyrene; intercellular adhesion	Schroeder et al. (2009)
sasD	SasD	unknown	Roche et al. (2003a)
sasF	SasF	unknown	Roche et al. (2003a)

Table 7.1 (continued)

Gene	Product	Ligand specificity/Function	Reference
sasG	SasG	Binding to nasal epithelial cells; intercellular adhesion; prevents binding to Fg, Fn, cytokeratin 10, IgG	Corrigan et al. (2007), Roche et al. (2003b)
sasK	SasK	unknown	Roche et al. (2003a)
sasH	SasH	unknown	Roche et al. (2003a)
sdrC	SdrC	Binding to nasal epithelial cells and β-neurexin	Barbu et al. (2010), Corrigan et al. (2009)
sdrD	SdrD	Binding to nasal epithelial cells	Corrigan et al. (2009)
sdrE	SdrE	Platelet aggregation	O'Brien et al. (2002a)
spa	Protein A (Spa)	IgG, IgM, von Willebrand Factor, platelet receptor gC1qR; bacterial cell aggregation	Chavakis et al. (2005), Nguyen et al. (2000), Merino et al. (2009)
pls	Pls	Promotes binding to nasal epithelial cells, glycolipids; intercellular adhesion; prevents binding to IgG and Fn and internalization	Huesca et al. (2002), Roche et al. (2003b), Hussain et al. (2009)
Non covalently-linked surface-associated proteins			
atl	Atl	Attachment to polystyrene, Fg, Fn, Vn, endothelial cells	Hirschhausen et al. (2010)
aaa	Aaa	Fg, Fn, Vn	Heilmann et al. (2005)
eap	Eap (Map, P70)	Fg, Fn, Vn, collagen, ICAM-1, eukaryotic cell surfaces, staphylococcal cells; promotes uptake of *S. aureus* by eukaryotic cells	Chavakis et al. (2005)
emp	Emp	Fg, Fn, Vn, collagen	Chavakis et al. (2005)
ebh	Ebh	Fn	Clarke and Foster (2006)
ebps	Ebps	Elastin	Downer et al. (2002)

et al., 1991; Signas et al., 1989). The Fn-binding activity of both proteins has been localized to a C-terminally located and highly conserved repeat domain (D repeats) that is composed of an approximately 40-amino acid unit repeated four times (D1 to D4) with the repeat D4 being incomplete (shown for FnBPA in Fig. 7.2). A fifth repeat (Du) is located approximately 100 residues N-terminal to D1. Only the *fnbA/fnbB* double-knockout mutant of *S. aureus* 8325-4 showed strongly reduced

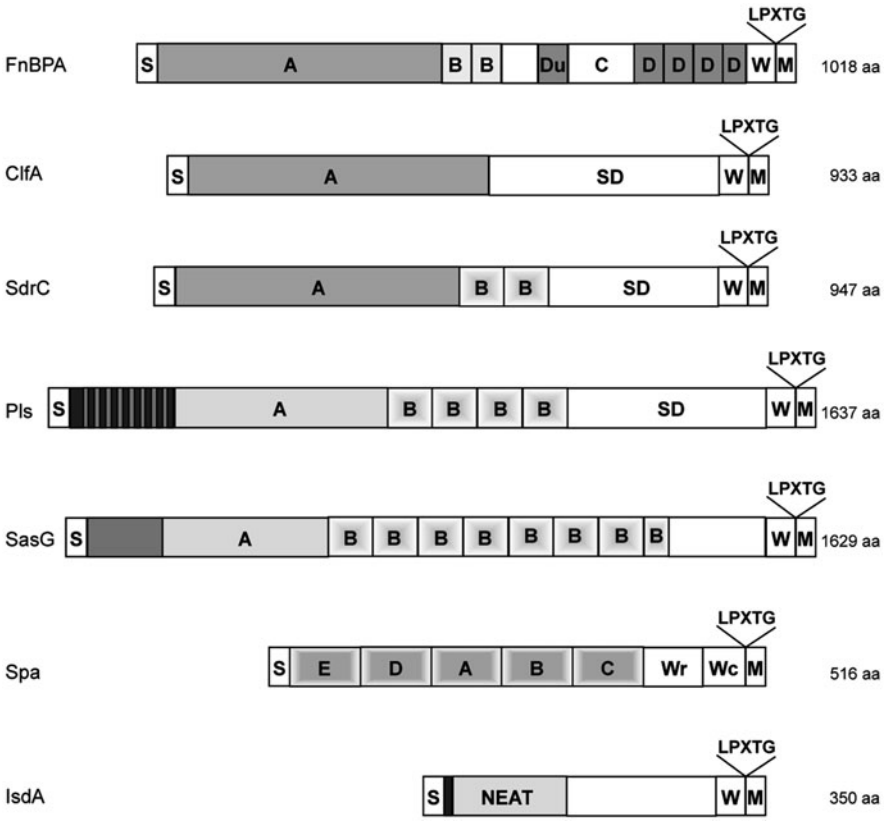

Fig. 7.2 Schematic model of selected *S. aureus* MSCRAMMs. The positions of the signal sequences (S), the ligand-binding A domains (A), the B repeats (B), the serine-aspartate (SD) dipeptide repeats (or region R), the wall (W) and membrane (M)-spanning regions including positively charged residues, and the LPXTG motifs are shown. The domains are defined in the text. The A domains of FnBPA, ClfA, and SdrC are homologous. Moreover, the A domains of Pls and SasG as well as the B repeats of SdrC, Pls, and SasG are homologous. The sizes of the proteins may vary among different *S. aureus* strains. FnBPA: D, Fn-binding D repeats. C, non-repeated region with unknown function. Spa: E, D, A, B, C, IgG-binding domains; Wr is composed of an octapeptide repeat, and Wc is a non-repeated region. IsdA: ligand-binding NEAT (near iron transporters) domain

Fn-binding activity (Greene et al., 1995). Complementation of the *fnbA/fnbB* double mutant with either plasmid-encoded *fnbA* or *fnbB* was able to fully restore the Fn-binding activity. This indicates that both proteins are expressed in *S. aureus* and contribute to the ability of strain 8325-4 to adhere to Fn-coated surfaces. The approximately 500-residue N-terminal domains (A domain) of FnBPA and FnBPB share only 40% sequence identity. The A domain of FnBPA exhibits substantial sequence identity to the A domains of the Fg-binding proteins ClfA and ClfB, and like them has been shown to contain Fg-binding activity (Wann et al., 2000). Recombinant FnBPA seems to bind to the same binding site in the γ-chain of Fg like ClfA and can compete with ClfA for binding to both immobilized and soluble Fg. Moreover, the A domains of both FnBPA and FnBPB can bind to immobilized elastin (Roche et al., 2004). Recently, the structural organization of FnBPA has been revised with the Fn-binding domain consisting of 11 tandem repeats. Each repeat is predicted to interact with Fn by a tandem β-zipper mechanism (Schwarz-Linek et al., 2003).

Most *S. aureus* strains carry both *fnb* genes, but there seems to be no difference in Fn-binding activity between the strains carrying one or two *fnb* genes (Greene et al., 1995). However, a study analysing a larger collection of isolates from infected patients indicated that *S. aureus* strains associated with invasive disease were more likely to encode both *fnb* genes (Peacock et al., 2000).

Besides being multifunctional adhesins, the FnBPs are efficient mediators of *S. aureus* internalization. Classically, staphylococci have been regarded as extracellular pathogens. However, it is now widely accepted that *S. aureus* can also be internalized by human host cells and replicate intracellularly. The FnBP-mediated mechanism of *S. aureus* internalization requires Fn as bridging molecule and the $\alpha_5\beta_1$ integrins as the host cell receptor resulting in signal transduction, tyrosine kinase activity, and cytosceletal rearrangements (Chavakis et al., 2005).

There have been contrasting results regarding the importance of the FnBPs in virulence (Clarke and Foster, 2006). Previous results suggested that the FnBPs do not play a significant role in infection, i.e. in the induction and/or propagation of endocarditis or in the development of septic arthritis by *S. aureus*. In a rat model of pneumonia, an *fnbA*/*fnbB* mutant even showed increased virulence compared to its wild-type strain. However, expression of *fnbA* or *fnbB* in the non-pathogenic *Staphylococcus carnosus* significantly increased adherence to intact endothelium in vivo and *Lactococcus lactis* cells expressing *fnbA* revealed a considerably higher infectivity in an experimental rat endocarditis model in comparison with *L. lactis* cells harbouring an empty vector. The latter findings were further supported by the observation that FnBPA and FnBPB mediate adherence to platelets and induce platelet activation (Chavakis et al., 2005). Moreover, the FnBPs were found to play an important role in the induction of systemic inflammation (Clarke and Foster, 2006). Most recently, increased efficiency of host cell invasion and virulence in a murine sepsis model mediated by FnBPA has been associated with an increased number of the 11 Fn-binding tandem repeats (Edwards et al., 2010).

7.2.1.2 Fibrinogen-Binding Proteins (ClfA and ClfB) and the Sdr Protein Family

Fibrinogen-Binding Proteins: ClfA and ClfB

S. aureus produces several proteins that can specifically bind to Fg. Two of them are the covalently-linked Fg-binding MSCRAMMs, clumping factors A and B (ClfA and ClfB), which are encoded by *clfA* and *clfB*, respectively (McDevitt et al., 1997, Ni Eidhin et al., 1998). The Fg-receptor was first recognized as a factor that mediates cell clumping in the presence of human plasma and therefore was named clumping factor. *clfA* mutants not only failed to form clumps in soluble Fg, but also adhered poorly to surface-immobilized Fg. Additionally to the common features of all MSCRAMMs, ClfA and ClfB are characterized by the region R that consists of repeating serine-aspartate (SD) dipeptides and is located between the wall-spanning domain and the ligand-binding domain A (shown for ClfA in Fig. 7.2) Thus, ClfA and ClfB are members of an MSCRAMM subfamily, the SD repeat-containing (Sdr) protein family.

The predicted 92 kDa ClfA has significant sequence similarity with the FnBPs, especially in the N-terminal region A. The Fg-binding activity of ClfA was mapped to a 218-residue domain within the ~500-residue region A (332–550). In a later study, the adjacent residues Glu^{526} and Val^{527} were identified as being important for the Fg-binding activity (Hartford et al., 2001). Despite similar functions and structural organization, the A domains of ClfA and ClfB are only 26% identical. ClfA exclusively binds to the Fg γ-chain with the binding site for ClfA located within the C-terminus of the γ-chain. In contrast, ClfB binds to the α- and β-chains of Fg (McDevitt et al., 1997, Ni Eidhin et al., 1998). A recombinantly expressed truncated ClfA protein comprising residues 221–550 inhibits ADP-induced, Fg-dependent platelet aggregation in a concentration-dependent manner and moreover competes for platelet adhesion to immobilized Fg under shear stress indicating that the platelet receptor and ClfA binding sites on Fg overlap. A later study indicated that ClfA also mediates direct binding to platelets by interacting with a 118 kDa platelet membrane receptor (Siboo et al., 2001). Furthermore, ClfA as well as ClfB are able to aggregate platelets, which is thought to play a role in the establishment of experimental endocarditis (O'Brien et al., 2002a). Platelet aggregation mediated by ClfA and ClfB occurs in a Fg-dependent manner. However, ClfA as well as ClfB can cause platelet aggregation in a Fg-independent manner that requires IgG and complement deposition (Loughman et al., 2005, Miajlovic et al., 2007).

ClfB binds also to human desquamated nasal epithelial cells and to cytokeratin 10, which was shown to be present on the surface of these cells, suggesting that ClfB may be an important factor in *S. aureus* nasal colonization (O'Brien et al., 2002b). A remarkable difference between ClfA and ClfB is their expression pattern, with ClfB being only detectable on cells grown to the early exponential phase, but absent from cells from late exponential phase or stationary phase cultures.

The binding of ClfA as well as ClfB to Fg is regulated by the divalent cations Ca^{2+} and Mn^{2+} (Clarke and Foster, 2006). Both cations inhibit ClfA-mediated clumping of *S. aureus* in the presence of soluble Fg as well as the interaction of a

recombinant ClfA subdomain with a peptide resembling the C-terminal Fg γ-chain. In agreement with this, ClfA harbours a putative EF-hand motif within the A region (310–321) that mediates both regulation by Ca^{2+} and ligand binding.

The ClfA region A is composed of three domains (N1, N2, and N3). The crystal structure of the Fg-binding segment (residues 221–559), which contains two of the domains (N2N3) demonstrates that each domain adopts an IgG-like fold (Deivanayagam et al., 2002).

Consistent with its platelet-binding and -aggregation function, ClfA has been shown to be a virulence factor in a rabbit and rat infective endocarditis model as well as in a mouse model of septic arthritis. Moreover, active and passive immunization studies suggested ClfA as a suitable vaccine compound and novel anti-staphylococcal agents have been proposed based on a structural model of the ClfA-Fg interaction (Clarke and Foster, 2006, Ganesh et al., 2008).

Sdr protein Family: SdrC, SdrD, and SdrE

Other members of the *S. aureus* Sdr-protein family are SdrC, SdrD, and SdrE (Clarke and Foster, 2006). The Sdr proteins are predicted to have a similar structural organization like ClfA and ClfB. Additionally, they contain two (SdrC), five (SdrD), or three (SdrE) B repeats with 110–129 residues per repeat, which interconnect the regions A and R (shown for SdrC in Fig. 7.2). Like ClfA and ClfB, SdrE induces platelet aggregation while SdrC and SdrD play a role in adherence to human desquamated nasal epithelial cells (O'Brien et al., 2002a, Corrigan et al., 2009). Moreover, by using the phage display technique, the N2N3 domain of SdrC was recently found to bind to β-neurexin (Barbu et al., 2010).

Sdr protein Family: Pls

Pls "plasmin sensitive" contains short N-terminal repeats and four B repeats (Fig. 7.2). It has a surprisingly divergent function. On the one hand, Pls binds to cellular lipids and glycolipids and promotes bacterial cell-cell interaction as well as adherence to nasal epithelial cells (Huesca et al., 2002, Roche et al., 2003b). On the other hand, Pls prevents binding of *S. aureus* to immunoglobulin (Ig) G, soluble and immobilized Fn, as well as internalization by human host cells probably by steric hindrance (Hussain et al., 2009).

Sdr protein Family: Bbp

The 97 kDa bone sialoprotein-binding protein Bbp has similarity with SdrC, SdrD, and SdrE (Tung et al., 2000). Bbp was identified from *S. aureus* strains associated with bone and joint infections and found to specifically interact with bone sialoprotein, which is a glycoprotein of bone and dentine extracellular matrix. Further studies indicated that Bbp is immunogenic and expressed during infection suggesting an important role in the pathogenesis of osteomyelitis.

7.2.1.3 Protein A (Spa)

Protein A (Spa) was the first *S. aureus* protein identified and has been used as the model system to study sortase-mediated cell wall anchoring (Schneewind et al., 1993). Protein A contains five N-terminally located approximately 60-residue long tandem repeats (designated E, D, A, B, and C), which bind to the Fc portion of immunoglobulins (Ig) G, leading to reduced antibody-mediated opsonisation. Consequently, the inhibition of phagocytosis has been proposed to be the basic contribution of protein A to virulence. However, it is now clear that its function in pathogenesis is more complex than previously thought. Protein A also binds to both soluble and immobilized vWF, a large multimeric glycoprotein that mediates platelet adhesion at sites of endothelial damage, which is thought to play a role in endovascular infection (Chavakis et al., 2005). Spa has also been identified to directly interact with platelets via binding to the platelet receptor gC1qR/p33 (Nguyen et al., 2000). Moreover, protein A can bind to TNFR1, which is a receptor for tumour-necrosis factor-α (TNF-α) widely distributed on the airway epithelium and stimulate an inflammatory response in airway epithelial cells. Thus, the protein A-TNFR1 signalling pathway is thought to have a central function in the pathogenesis of staphylococcal pneumonia (Gomez et al., 2004).

7.2.1.4 Iron-Regulated Surface Determinants (IsdA, IsdB, IsdC, IsdH)

The iron-regulated surface determinants include IsdA, IsdB, IsdC, and IsdH. The transcription of *isdA*, *isdB*, *isdC*, and *isdH* is regulated by environmental iron and the iron-responsive regulator Fur (Clarke and Foster, 2006). Each of the Isd proteins contains one to three NEAT (near iron transporters) domains and bind to one or more iron-containing proteins, such as transferrin, haemin, or haemoglobin. Thus, these proteins have been suggested to have a role in iron acquisition, but this has not yet been proven. The NEAT domains in IsdA and IsdH are responsible for the binding to their ligands. IsdA binds to a wide range of host products, among them Fg, Fn, and human desquamated nasal epithelial cells (Clarke and Foster, 2006, Corrigan et al., 2009). However, physiologically relevant binding of IsdA to Fg and Fn can only be observed when *S. aureus* is grown under iron-limited conditions, such as in vitro in serum or in vivo, which favours the expression of IsdA. Moreover recently, IsdA was found to bind to proteins of the cornified envelope of human desquamated epithelial cells, such as involucrin, loricrin, cytokeratin 10, which are thought to be the predominant ligands in the ecological niche of *S. aureus*, further delineating the importance of IsdA in nasal colonization (Clarke et al., 2009). IsdH binds to haptoglobin and haptoglobin-haemoglobin complexes (Dryla et al., 2003). Differences in the ligand-binding specificity of IsdA and IsdH might be due to the pronounced sequence differences among their NEAT domains. Immunization with purified IsdA and IsdH resulted in reduced nasal colonization by *S. aureus* in the cotton rat model.

IsdB was found to be involved in platelet adhesion and aggregation: when *S. aureus* was grown under iron-limited conditions, mutants deficient in IsdB, but

not IsdA or IsdH were unable to adhere to or aggregate platelets. The platelet integrin GPIIb/IIIa was identified as the platelet receptor for IsdB and the direct interaction of these proteins could be demonstrated by surface plasmon resonance (Miajlovic et al., 2010). Immunization with IsdB protects against staphylococcal sepsis in different animal models.

7.2.1.5 Biofilm-Associated Proteins

In recent years, several *S. aureus* surface-anchored proteins have been associated with the formation of biofilm, especially with the accumulation phase and intercellular adherence. However, the first protein reported to be involved in the accumulative growth during biofilm formation was the 220 kDa accumulation-associated protein Aap from *S. epidermidis*. Aap is highly homologous to the *S. aureus* surface protein SasG (Fig. 7.2), which also mediates biofilm accumulation (Heilmann and Götz, 2010). The function of Aap in the accumulation process was speculated to be the anchoring of PIA (polysaccharide intercellular adhesin) to the cell surface. However recently, it was shown that Aap is able to mediate intercellular adhesion and biofilm accumulation in a completely PIA-independent background. A repeat domain B, which becomes active only after proteolytic cleavage of the N-terminal A-domain, mediates intercellular adhesion. Recently, the B repeats of Aap (also known as G5 domains) were found to be zinc-dependent adhesion modules and a "zinc zipper" mechanism was suggested for G5 domain-based intercellular adhesion in Aap- or SasG-mediated biofilm accumulation. A zinc-dependent dimerisation of recombinant B repeats was also observed with SasG (Geoghegan et al., 2010). SasG not only mediates bacterial intercellular adhesion, but also promotes binding to nasal epithelial cells (Roche et al., 2003b). Moreover as with Pls, which has sequence similarities with SasG and Aap, the expression of SasG masked the ability of the *S. aureus* cells to adhere to Fg, Fn, cytokeratin 10, and IgG. SasG-mediated binding to nasal epithelial cells was proposed to compensate for masking the ability of ClfB to bind to cytokeratin 10. While intercellular adhesion is mediated by the SasG B repeats with at least five of the eight repeats being required, binding to the nasal epithelial cells is carried out by the N-terminal region A. The functions of SasG and Aap might be explained by their fibrillar structure, which was recently observed by transmission electron microscopy (Corrigan et al., 2007).

The 239 kDa biofilm-associated protein Bap was the first protein reported to be involved in *S. aureus* biofilm formation. Bap mediates *S. aureus* attachment to a polystyrene surface and intercellular adhesion leading to biofilm accumulation. Moreover, Bap was found to prevent adherence to Fg, Fn, and host tissue (sheep mammary glands) as well as cellular internalization (Cucarella et al., 2002). The clinical significance of Bap is not clear, because it is apparently present in only 5% of 350 bovine mastitis and absent from all human clinical *S. aureus* isolates tested so far. However, a gene encoding a Bap-homologous protein, the 258 kDa Bhp, is present in the human clinical strain *S. epidermidis* RP62A.

Recently, we reported that the *S. aureus* surface protein C (SasC) mediates attachment to polystyrene and biofilm accumulation, but not adherence to Fg,

thrombospondin-1, von Willebrand factor, or platelets. Intercellular adhesion is conferred by the N-terminal domain of SasC (Schroeder et al., 2009).

Furthermore, a function for the multifactorial virulence factor protein A in biofilm development was detected, when expressed at high levels due to a mutation in the accessory gene regulator *agr* (Merino et al., 2009). A role for Spa in the development of biofilm-associated infections was suggested in a murine model of subcutaneous catheter infection.

Additionally, FnBPA and FnBPB were found to be important for biofilm accumulation of methicillin-resistant *S. aureus* (MRSA) strains with the A domain of FnBPA, but not the Fn-binding domain, being responsible for biofilm accumulation. Accordingly, the FnBPs play a significant role in a catheter-associated murine infection model (O'Neill et al., 2008, Vergara-Irigaray et al., 2009).

7.2.1.6 Other Covalently-Linked Surface Proteins

Further MSCRAMMs of *S. aureus* include the collagen-binding protein Cna and the serine-rich adhesin for platelets (SraP, also called SasA) (Patti et al., 1995, Siboo et al., 2008). The collagen-binding site within the 133 kDa Cna is located in a region between residues Asp^{209} and Tyr^{233}. Based on crystal structures from the subdomains N1 and N2 of Cna, each of them adopting an IgG-like fold, and in complex with a synthetic collagen-like triple helical peptide, a "collagen hug" model was proposed for the interaction of the multidomain Cna with its extended rope-like ligand (Zong et al., 2005). In a rabbit model of soft contact lens-associated bacterial keratitis, Cna has been found to be a virulence factor (Rhem et al., 2000).

SraP is a glycoprotein with a calculated molecular mass of 227 kDa that is involved in adherence to platelets (Siboo et al., 2008). It contains an unusually long N-terminal signal peptide and two serine-rich repeat regions (srr1 and srr2) separated by a nonrepeat region. A recombinant fragment of SraP consisting of the N-terminally located Srr1 and the nonrepeat region was shown to directly bind to platelets, a trait that is thought to be an important pathogenicity factor in the development of infective endocarditis. Consistently, in a rabbit model of endocarditis, an *sraP* mutant strain had significant lower bacterial counts within vegetations than the wild-type strain. Recently, the accessory Sec system that is encoded downstream of the *sraP* structural gene has been found to be required for the export of SraP.

7.2.2 Non Covalently-Linked Surface-Associated Proteins

7.2.2.1 Autolysin/Adhesins

Another class of staphylococcal adhesins is represented by the autolysin/adhesins first described by us and others (Hirschhausen et al., 2010). These non-covalently bound proteins are associated with the surface by ionic or hydrophobic interactions and have both enzymatic (peptidoglycan-hydrolytic) and adhesive functions. In general, peptidoglycan hydrolases or autolysins are thought to play important roles in

cell-wall turnover, cell division, cell separation, and antibiotic-induced lysis of bacteria. Using transposon mutagenesis, the 148 kDa autolysin AtlE of *S. epidermidis* was identified as a surface-associated component, which mediates attachment to polystyrene, biofilm formation, and adherence to Vn. In a rat central venous catheter infection model, the *atlE* mutant was attenuated compared to the wild type. The homologous 137 kDa autolysin Atl from *S. aureus* also mediates attachment to polystyrene and biofilm formation (Biswas et al., 2006).

AtlE and Atl show the same structural organization and are proteolytically cleaved into two bacteriolytically active domains, an N-terminal amidase and a C-terminal glucosaminidase. The bacteriolytically active domains are interconnected by three direct repeated sequences (R1, R2, and R3), which are involved in binding to peptidoglycan. Each repeat consists of approximately 170 residues with two glycine-tryptophane (GW)-dipeptide motifs. GW-containing repeats have been previously characterized from surface proteins of *Listeria monocytogenes* that mediate adherence to and uptake by eukaryotic cells.

Recently, we found that Atl also binds to Fg, Fn, Vn, and human endothelial cells (Hirschhausen et al., 2010). Moreover, Atl/AtlE also functions in staphylococcal internalization by endothelial cells by using the 70 kDa heat shock cognate protein Hsc70 as the host cell receptor. While this novel Atl- or AtlE-mediated internalization mechanism may represent a "backup"-mechanism in *S. aureus* internalization, it may also be the major or even sole mechanism involved in the internalization of coagulase-negative staphylococci.

Further multifunctional autolysin/adhesins include the Aaa from *S. aureus* and the homologous Aae from *S. epidermidis*. Aaa and Aae both have bacteriolytic activities and bind to Fg, Fn, and Vn in a dose-dependent and saturable fashion and with high affinity (Heilmann et al., 2005).

7.2.2.2 SERAMs (Eap/Map/P70 and Emp)

Further examples of non-covalently associated surface proteins of *S. aureus* are secreted proteins that bind back to the bacterial cell surface by so far unknown mechanisms and have a broad binding spectrum; therefore they were termed SERAMs (Secretable expanded repertoire adhesive molecules) (Chavakis et al., 2005). Among the SERAMs are the 60–72 kDa extracellular adherence protein Eap (also designated as Map, "MHC class II analogous protein" or P70) and the 40 kDa extracellular matrix and plasma binding protein Emp, both of which bind to various components of the extracellular matrix, such as Fg, Fn, or Vn. Map/Eap contains six repeated domains of 110 residues each, with a subdomain of 31 residues sharing significant homology to a segment in the peptide binding groove of the β-chain of the major histocompatibility complex (MHC) class II proteins from different mammalian species. *S. aureus* strains that do not express Eap were less able to colonize and invade host tissue and mutants defective in *emp* showed reduced attachment to immobilized Fg and Fn (Chavakis et al., 2005).

The SERAMs are thought to play a role in endovascular infection, like infective endocarditis. The pathogenesis of infective endocarditis is characterized by a serious

of events, such as endocardial damage, exposure of the subendothelial matrix, deposition of activated platelets and extracellular matrix and plasma proteins that serve as binding foci for adhering bacteria. Thus, Eap and Emp can mediate binding to these vegetations as well as to the surface of endothelial cells most likely via plasma proteins as bridging molecules. Moreover, binding of Eap to endothelial cells via Fn may enhance the staphylococcal uptake by the eukaryotic cell. Additional functions of Eap include the direct interaction with the host adhesive protein intercellular adhesion molecule 1 (ICAM-1), inhibition of neutrophil binding to and transmigration through the endothelium and the decrease in phagocytic activity (Chavakis et al., 2005). Thus, Eap is a potent anti-inflammatory factor. More recently, another function for Eap as a potent angiostatic agent was described (Sobke et al., 2006).

No homologues of the *S. aureus* extracellular matrix and plasma binding protein Emp and the extracellular adherence protein Eap could be detected in *S. epidermidis*.

7.2.2.3 Membrane-Spanning Proteins (Ebh and Ebps)

Further non-covalently anchored cell surface proteins include the giant 1.1 mDa Fn-binding protein Ebh (extracellular matrix-binding protein homologue) of *S. aureus* and the homologous Embp of *S. epidermidis* (Clarke and Foster, 2006, Williams et al., 2002), whose genes are by far the largest of the *S. aureus* and *S. epidermidis* genomes, respectively. The Fn-binding sites of Ebh and Embp seem to be unrelated to those of the *S. aureus* FnBPs. Ebh appears to be anchored to the cell surface by a C-terminally located membrane-spanning domain that is followed by a repeat region containing positively charged residues, which is predicted to be located intracellularly. Furthermore, Embp was recently demonstrated to mediate biofilm accumulation (Christner et al., 2010).

EbpS is another protein with a membrane anchor that binds elastin, which is a major component of the extracellular matrix (Downer et al., 2002).

7.3 Non-proteinaceous Staphylococcal Adhesins

7.3.1 Polysaccharide Intercellular Adhesin (PIA)

S. epidermidis transposon mutants not able to accumulate in multilayered cell clusters lack a specific polysaccharide antigen referred to as polysaccharide intercellular adhesin (PIA) (Götz, 2002, Heilmann and Götz, 2010), also designated as poly-N-acetylglucosamine (PNAG). Purification and structural analysis of PIA revealed that it is a linear β-1,6-linked N-acetylglucosaminoglycan with 15–20% of the N-acetylglucosaminyl residues being non-N-acetylated. Thus, the designation as PNAG is certainly not correct. Later, it was found that PIA is also produced by *S. aureus*. It has been published that the *N*-acetylglucosamine residues of PIA

from *S. aureus* are completely succinylated, which led to its designation as poly-*N*-succinyl β-1,6-glucosamine (PNSG). However, it is now clear that the succinyl groups were an artefact.

The partial deacetylation of 15–20% of the N-acetylglucosaminyl residues renders the polysaccharide positively charged, which determines its biological activity (Götz, 2002). Possibly, it functions as an intercellular adhesin by electrostatically attracting the negatively charged teichoic acid to the bacterial cell surface. The structure of PIA so far is unique. However, PIA-mediated biofilm formation might represent a common principle, because PIA-related structures have also been identified to play a role in the biofilm formation of pathogenic Gram-negative bacteria.

PIA is produced by the gene products encoded by the *icaADBC* operon. The *icaADBC* operon was first identified in *S. epidermidis* and is also present in *S. aureus* and other staphylococcal species. The *N*-acetylglucosaminyltransferase activity is carried out by IcaA, which requires IcaD for full activity. With its transmembrane helices, IcaC very likely is an integral membrane protein that putatively transports the *N*-acetylglucosamine oligomers across the membrane. IcaB is mainly found in the culture supernatant and deacetylates PIA (Heilmann and Götz, 2010).

The importance of PIA as a pathogenicity factor has been confirmed in various foreign-body animal infection models with different *S. epidermidis icaADBC* mutants. However in *S. aureus*, conflicting results were obtained: PIA production did not increase the capacity to induce persistent infections in a tissue cage model (Kristian et al., 2004). A study investigating the pathogenic properties of *S. epidermidis* strains obtained from polymer-associated septicemic disease compared with saprophytic skin and mucosal isolates demonstrated a strong correlation of biofilm formation and presence of the *ica* gene cluster essentially associated with disease isolates (Ziebuhr et al., 1997).

7.3.2 Wall Teichoic Acid (WTA) and Lipoteichoic Acid (LTA)

S. aureus teichoic acids are highly charged cell wall polymers, composed of alternating phosphate and ribitol (wall teichoic acid; WTA) or glycerol (lipoteichoic acid; LTA) groups, which are substituted with D-alanine and N-acetylglucosamine. While the WTA is covalently linked to the peptidoglycan, the LTA is anchored in the outer leaflet of the cytoplasmic membrane via a glycolipid. The *S. aureus* colonization of abiotic surfaces depends on the charge of its teichoic acid. A *dltA* mutant lacks D-alanine in its WTA rendering it higher negatively charged. The *dltA* mutant has a biofilm-negative phenotype due to a decreased initial attachment to polystyrene or glass, which is hydrophobic or negatively charged, respectively (Götz, 2002). WTA also mediates adherence to human nasal epithelial cells and is involved in nasal colonization, which is considered a major risk factor for serious *S. aureus* infections. Moreover, a WTA-deficient mutant (Δ*tagO*) showed decreased adherence to human endothelial cells especially under flow conditions and was attenuated in a rabbit model of infective endocarditis (Weidenmaier et al., 2005).

The glycolipid synthase YpfP is involved in the biosynthesis of LTA. An *S. aureus* SA113 *ypfP* mutant, which showed a markedly decreased production of LTA, revealed altered physicochemical properties and a reduced capacity to form a biofilm on a polystyrene surface (Fedtke et al., 2007). Thus, LTA and its biosynthetic enzymes were proposed as potential targets in the development of novel anti-biofilm measurements.

In *S. epidermidis*, the cell wall teichoic acid is involved in adherence to Fn (Hussain et al., 2001).

7.4 Conclusion

In conclusion, *S. aureus* harbours a variety of proteinaceous and non-proteinaceous adhesins that can mediate adherence to an abiotic surface or a multitude of host factors, such as extracellular matrix and plasma proteins and human host cells. Upon adherence, the bacteria may proliferate and accumulate into multilayered cell clusters, which requires intercellular adhesion and finally leads to the formation of a biofilm. Alternatively upon adherence, the bacteria may be internalized by the human host cell and "hide" within the host cell. Both, the formation of a biofilm as well as the intracellular environment seem to protect the bacteria against the host immune system and antibiotic therapy. Therefore, understanding *S. aureus* adhesive mechanisms represents a necessary first step to developing strategies to prevent or combat infections with this serious pathogen.

References

Barbu EM, Ganesh VK, Gurusiddappa S, Mackenzie RC, Foster TJ, Sudhof TC, Höök M (2010) β-Neurexin is a ligand for the *Staphylococcus aureus* MSCRAMM SdrC. PLoS Pathog 6:e1000726

Biswas R, Voggu L, Simon UK, Hentschel P, Thumm G, Götz F (2006) Activity of the major staphylococcal autolysin Atl. FEMS Microbiol Lett 259:260–268

Chavakis T, Wiechmann K, Preissner KT, Herrmann M (2005) *Staphylococcus aureus* interactions with the endothelium: the role of bacterial "secretable expanded repertoire adhesive molecules" (SERAM) in disturbing host defense systems. Thromb Haemost 94:278–285

Christner M, Franke G, Schommer N, Wendt U, Wegert K, Pehle P, Kroll G, Schulze C, Buck F, Mack D, Aepfelbacher M, Rohde H (2010) The giant extracellular matrix binding protein of *Staphylococcus epidermidis* mediates biofilm accumulation and attachment to fibronectin. Mol Microbiol 75:187–207

Clarke SR, Andre G, Walsh EJ, Dufrene YF, Foster TJ, Foster SJ (2009) Iron-regulated surface determinant protein A mediates adhesion of *Staphylococcus aureus* to human corneocyte envelope proteins. Infect Immun 77:2408–2416

Clarke SR, Foster SJ (2006) Surface adhesins of *Staphylococcus aureus*. Adv Microb Physiol 51:187–224

Corrigan RM, Miajlovic H, Foster TJ (2009) Surface proteins that promote adherence of *Staphylococcus aureus* to human desquamated nasal epithelial cells. BMC Microbiol 9:22

Corrigan RM, Rigby D, Handley P, Foster TJ (2007) The role of *Staphylococcus aureus* surface protein SasG in adherence and biofilm formation. Microbiology 153:2435–2446

Cucarella C, Tormo MA, Knecht E, Amorena B, Lasa I, Foster TJ, Penadés JR (2002) Expression of the biofilm-associated protein interferes with host protein receptors of *Staphylococcus aureus* and alters the infective process. Infect Immun 70:3180–3186

Deivanayagam CC, Wann ER, Chen W, Carson M, Rajashankar KR, Höök M, Narayana SV (2002) A novel variant of the immunoglobulin fold in surface adhesins of *Staphylococcus aureus*: crystal structure of the fibrinogen-binding MSCRAMM, clumping factor A. EMBO J 21: 6660–6672

Downer R, Roche F, Park PW, Mecham RP, Foster TJ (2002) The elastin-binding protein of *Staphylococcus aureus* (EbpS) is expressed at the cell surface as an integral membrane protein and not as a cell wall-associated protein. J Biol Chem 277:243–250

Dryla A, Gelbmann D, von Gabain A, Nagy E (2003) Identification of a novel iron regulated staphylococcal surface protein with haptoglobin-haemoglobin binding activity. Mol Microbiol 49:37–53

Edwards AM, Potts JR, Josefsson E, Massey RC (2010) *Staphylococcus aureus* host cell invasion and virulence in sepsis is facilitated by the multiple repeats within FnBPA. PLoS Pathog 6:e1000964

Fedtke I, Mader D, Kohler T, Moll H, Nicholson G, Biswas R, Henseler K, Götz F, Zähringer U, Peschel A (2007) A *Staphylococcus aureus ypfP* mutant with strongly reduced lipoteichoic acid (LTA) content: LTA governs bacterial surface properties and autolysin activity. Mol Microbiol 65:1078–1091

Ganesh VK, Rivera JJ, Smeds E, Ko YP, Bowden MG, Wann ER, Gurusiddappa S, Fitzgerald JR, Höök M (2008) A structural model of the *Staphylococcus aureus* ClfA-fibrinogen interaction opens new avenues for the design of anti-staphylococcal therapeutics. PLoS Pathog 4:e1000226

Geoghegan JA, Corrigan RM, Gruszka DT, Speziale P, O'Gara JP, Potts JR, Foster TJ (2010) The role of surface protein SasG in biofilm formation by *Staphylococcus aureus*. J Bacteriol 192:5663–5673

Gomez MI, Lee A, Reddy B, Muir A, Soong G, Pitt A, Cheung A, Prince A (2004) *Staphylococcus aureus* protein A induces airway epithelial inflammatory responses by activating TNFR1. Nat Med 10:842–848

Götz F (2002) *Staphylococcus* and biofilms. Mol Microbiol 43:1367–1378

Greene C, McDevitt D, Francois P, Vaudaux PE, Lew DP, Foster TJ (1995) Adhesion properties of mutants of *Staphylococcus aureus* defective in fibronectin-binding proteins and studies on the expression of *fnb* genes. Mol Microbiol 17:1143–1152

Hartford OM, Wann ER, Höök M, Foster TJ (2001) Identification of residues in the *Staphylococcus aureus* fibrinogen-binding MSCRAMM clumping factor A (ClfA) that are important for ligand binding. J Biol Chem 276:2466–2473

Heilmann C, Götz F (2010) Cell-cell communication and biofilm formation in gram-positive bacteria. In: Krämer R, Jung K (eds) Bacterial signaling. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp 7–17

Heilmann C, Hartleib J, Hussain M, Peters G (2005) The multifunctional *Staphylococcus aureus* autolysin Aaa mediates adherence to immobilized fibrinogen and fibronectin. Infect Immun 73:4793–4802

Hirschhausen N, Schlesier T, Schmidt MA, Götz F, Peters G, Heilmann C (2010) A novel staphylococcal internalization mechanism involves the major autolysin Atl and heat shock cognate protein Hsc70 as host cell receptor. Cell Microbiol 12:1746–1764

Huesca M, Peralta R, Sauder DN, Simor AE, McGavin MJ (2002) Adhesion and virulence properties of epidemic Canadian methicillin-resistant *Staphylococcus aureus* strain 1: identification of novel adhesion functions associated with plasmin-sensitive surface protein. J Infect Dis 185:1285–1296

Hussain M, Heilmann C, Peters G, Herrmann M (2001) Teichoic acid enhances adhesion of *Staphylococcus epidermidis* to immobilized fibronectin. Microb Pathog 31:261–270

Hussain M, Schafer D, Juuti KM, Peters G, Haslinger-Löffler B, Kuusela PI, Sinha B (2009) Expression of Pls (plasmin sensitive) in *Staphylococcus aureus* negative for pls reduces adherence and cellular invasion and acts by steric hindrance. J Infect Dis 200:107–117

Jönsson K, Signas C, Müller HP, Lindberg M (1991) Two different genes encode fibronectin binding proteins in *Staphylococcus aureus*. The complete nucleotide sequence and characterization of the second gene. Eur J Biochem 202:1041–1048

Kristian SA, Golda T, Ferracin F, Cramton SE, Neumeister B, Peschel A, Götz F, Landmann R (2004) The ability of biofilm formation does not influence virulence of *Staphylococcus aureus* and host response in a mouse tissue cage infection model. Microb Pathog 36:237–245

Lentino JR (2003) Prosthetic joint infections: bane of orthopedists, challenge for infectious disease specialists. Clin Infect Dis 36:1157–1161

Loughman A, Fitzgerald JR, Brennan MP, Higgins J, Downer R, Cox D, Foster TJ (2005) Roles for fibrinogen, immunoglobulin and complement in platelet activation promoted by *Staphylococcus aureus* clumping factor A. Mol Microbiol 57:804–818

Mazmanian SK, Skaar EP, Gaspar AH, Humayun M, Gornicki P, Jelenska J, Joachmiak A, Missiakas DM, Schneewind O (2003) Passage of heme-iron across the envelope of *Staphylococcus aureus*. Science 299:906–909

McDevitt D, Nanavaty T, House-Pompeo K, Bell E, Turner N, McIntire L, Foster T, Höök M (1997) Characterization of the interaction between the *Staphylococcus aureus* clumping factor (ClfA) and fibrinogen. Eur J Biochem 247:416–424

Merino N, Toledo-Arana A, Vergara-Irigaray M, Valle J, Solano C, Calvo E, Lopez JA, Foster TJ, Penadés JR, Lasa I (2009) Protein A-mediated multicellular behavior in *Staphylococcus aureus*. J Bacteriol 191:832–843

Miajlovic H, Loughman A, Brennan M, Cox D, Foster TJ (2007) Both complement- and fibrinogen-dependent mechanisms contribute to platelet aggregation mediated by *Staphylococcus aureus* clumping factor B. Infect Immun 75:3335–3343

Miajlovic H, Zapotoczna M, Geoghegan JA, Kerrigan SW, Speziale P, Foster TJ (2010) Direct interaction of iron-regulated surface determinant IsdB of *Staphylococcus aureus* with the GPIIb/IIIa receptor on platelets. Microbiology 156:920–928

Nguyen T, Ghebrehiwet B, Peerschke EIB (2000) *Staphylococcus aureus* Protein A recognizes platelet gC1qR/p33: a novel mechanism for staphylococcal interactions with platelets. Infect Immun 68:2061–2068

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

O'Brien L, Kerrigan SW, Kaw G, Hogan M, Penadés J, Litt D, Fitzgerald DJ, Foster TJ, Cox D (2002a) Multiple mechanisms for the activation of human platelet aggregation by *Staphylococcus aureus*: roles for the clumping factors ClfA and ClfB, the serine-aspartate repeat protein SdrE and protein A. Mol Microbiol 44:1033–1044

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

O'Neill E, Pozzi C, Houston P, Humphreys H, Robinson DA, Loughman A, Foster TJ, O'Gara JP (2008) A novel *Staphylococcus aureus* biofilm phenotype mediated by the fibronectin-binding proteins, FnBPA and FnBPB. J Bacteriol 190:3835–3850

Patti JM, House-Pompeo K, Boles JO, Garza N, Gurusiddappa S, Höök M (1995) Critical residues in the ligand-binding site of the *Staphylococcus aureus* collagen-binding adhesin (MSCRAMM. J Biol Chem 270:12005–12011

Peacock SJ, Day NP, Thomas MG, Berendt AR, Foster TJ (2000) Clinical isolates of *Staphylococcus aureus* exhibit diversity in *fnb* genes and adhesion to human fibronectin. J Infect 41:23–31

Rhem MN, Lech EM, Patti JM, McDevitt D, Höök M, Jones DB, Wilhelmus KR (2000) The collagen-binding adhesin is a virulence factor in *Staphylococcus aureus* keratitis. Infect Immun 68:3776–3779

Roche FM, Downer R, Keane F, Speziale P, Park PW, Foster TJ (2004) The N-terminal A domain of fibronectin-binding proteins A and B promotes adhesion of *Staphylococcus aureus* to elastin. J Biol Chem 279:38433–38440

Roche FM, Massey R, Peacock SJ, Day NP, Visai L, Speziale P, Lam A, Pallen M, Foster TJ (2003a) Characterization of novel LPXTG-containing proteins of *Staphylococcus aureus* identified from genome sequences. Microbiology 149:643–654

Roche FM, Meehan M, Foster TJ (2003b) The *Staphylococcus aureus* surface protein SasG and its homologues promote bacterial adherence to human desquamated nasal epithelial cells. Microbiology 149:2759–2767

Schneewind O, Mihaylova-Petkov D, Model P (1993) Cell wall sorting signals in surface proteins of gram-positive bacteria. EMBO J 12:4803–4811

Schroeder K, Jularic M, Horsburgh SM, Hirschhausen N, Neumann C, Bertling A, Schulte A, Foster S, Kehrel BE, Peters G, Heilmann C (2009) Molecular characterization of a novel *Staphylococcus aureus* surface protein (SasC) involved in cell aggregation and biofilm accumulation. PLoS One 4:e7567

Schwarz-Linek U, Werner JM, Pickford AR, Gurusiddappa S, Kim JH, Pilka ES, Briggs JA, Gough TS, Höök M, Campbell ID, Potts JR (2003) Pathogenic bacteria attach to human fibronectin through a tandem β-zipper. Nature 423:177–181

Siboo IR, Chaffin DO, Rubens CE, Sullam PM (2008) Characterization of the accessory Sec system of *Staphylococcus aureus*. J Bacteriol 190:6188–6196

Siboo IR, Cheung AL, Bayer AS, Sullam PM (2001) Clumping Factor A Mediates Binding of *Staphylococcus aureus* to Human Platelets. Infect Immun 69:3120–3127

Signas C, Raucci G, Jönsson K, Lindgren PE, Anantharamaiah GM, Höök M, Lindberg M (1989) Nucleotide sequence of the gene for a fibronectin-binding protein from *Staphylococcus aureus*: use of this peptide sequence in the synthesis of biologically active peptides. Proc Natl Acad Sci USA 86:699–703

Sobke AC, Selimovic D, Orlova V, Hassan M, Chavakis T, Athanasopoulos AN, Schubert U, Hussain M, Thiel G, Preissner KT, Herrmann M (2006) The extracellular adherence protein from *Staphylococcus aureus* abrogates angiogenic responses of endothelial cells by blocking Ras activation. FASEB J 20:2621–2623

Tung H, Guss B, Hellman U, Persson L, Rubin K, Ryden C (2000) A bone sialoprotein-binding protein from *Staphylococcus aureus*: a member of the staphylococcal Sdr family. Biochem J 345(Pt 3):611–619

Vergara-Irigaray M, Valle J, Merino N, Latasa C, García B, Ruiz De LosMozos I, Solano C, Toledo-Arana A, Penadés JR, Lasa I (2009) Relevant role of fibronectin-binding proteins in *Staphylococcus aureus* biofilm-associated foreign-body infections. Infect Immun 77:3978–3991

Wann ER, Gurusiddappa S, Höök M (2000) The fibronectin-binding MSCRAMM FnbpA of *Staphylococcus aureus* is a bifunctional protein that also binds to fibrinogen. J Biol Chem 275:13863–13871

Weidenmaier C, Peschel A, Xiong YQ, Kristian SA, Dietz K, Yeaman MR, Bayer AS (2005) Lack of wall teichoic acids in *Staphylococcus aureus* leads to reduced interactions with endothelial cells and to attenuated virulence in a rabbit model of endocarditis. J Infect Dis 191:1771–1777

Williams RJ, Henderson B, Sharp LJ, Nair SP (2002) Identification of a fibronectin-binding protein from *Staphylococcus epidermidis*. Infect Immun 70:6805–6810

Ziebuhr W (2001) *Staphylococcus aureus* and *Staphylococcus epidermidis*: emerging pathogens in nosocomial infections. Contrib Microbiol 8:102–107

Ziebuhr W, Heilmann C, Götz F, Meyer P, Wilms K, Straube E, Hacker J (1997) Detection of the intercellular adhesion gene cluster (*ica*) and phase variation in *Staphylococcus epidermidis* blood culture strains and mucosal isolates. Infect Immun 65:890–896

Zong Y, Xu Y, Liang X, Keene DR, Höök A, Gurusiddappa S, Höök M, Narayana SV (2005) A 'Collagen Hug' model for *Staphylococcus aureus* CNA binding to collagen. EMBO J 24: 4224–4236