

Fibrinogen-binding proteins of Gram-positive bacteria

Jose Rivera¹, Ganesh Vannakambadi¹, Magnus Höök¹, Pietro Speziale²

¹Institute of Biosciences and Technology, Center for Extracellular and Matrix Biology, Houston, Texas, USA; ²Department of Biochemistry, University of Pavia, Pavia, Italy

Summary

Fibrinogen (Fg), the major clotting protein in blood plasma, plays key roles in blood coagulation and thrombosis. In addition, this 340 kD glycoprotein is a stress inducible protein; its synthesis is dramatically upregulated during inflammation or under exposure to stress such systemic infections. This regulation of Fg expression indicates that Fg also participates in the host defense system against infections. In fact, a number of reported studies have demonstrated the involvement of both the intrinsic and ex-

trinsic pathways of coagulation; the thrombotic and the fibrinolytic systems in the pathophysiology of infectious diseases. It is, therefore, perhaps not surprising that many pathogenic bacteria can interact with Fg and manipulate its biology. This review focuses on the major Fg-binding proteins (Fgbps) from Gram-positive bacteria with an emphasis on those that are known to have an effect on coagulation and thrombosis.

Keywords

Bacterial infection, infectious diseases, bacteria, fibrinogen / fibrin, protein function / activity

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Introduction

Infective endocarditis (IE) affects nearly 15,000 individuals a year. IE is a persistent health concern due to a high mortality (almost 40%) (4) and because the incidence of IE cases has not declined in the last 30 years perhaps due to emerging new high-risk factors including drug use, vascular prostheses, nosocomial disease and dialysis (5). About 75% of the bacteria implicated in IE are streptococci and staphylococci (4). *Staphylococcus aureus* is now the most common cause of IE (5, 6). In IE pathogenesis, the interaction between bacteria and platelets is believed to play a central role in virulence. For the establishment of thrombi on healthy or damaged heart valves, bacteria must first interact with resting platelets followed by bacterial-induced platelet activation and subsequent aggregation leading to infected endocarditis. Bacterial surface proteins can interact with platelet receptors directly (reviewed in [7]). One example is *Streptococcus sanguis* that interacts with the platelet glycoprotein Ib (8). The interaction can also be indirect and involves a host protein such as Fg that forms a link between a bacterial component and a platelet receptor (7, 9). The direct or indirect interaction of bacterial proteins with platelets can activate the platelets, leading to thrombus development (7).

In recent years a number of Fgbps in Gram-positives have been described and characterized (Table 1). These Fgbps can be divided into two categories: bacterial surface adhesins termed MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) and secreted adhesins termed SERAMs (secretable expanded repertoire adhesive molecules). We are beginning to understand the details of the microbial Fg interactions. The emerging picture suggests that microbial proteins not only bind Fg but can also influence or manipulate its biology.

Structural organization of Fg

Fg is a heterogeneous dimeric glycoprotein composed of three pairs of non-identical peptide chains named A α , B β and γ (Fig. 1). The three peptide chains are connected by 29 disulfide bonds (1, 10). Based on electron microscopy studies and plasmin digestion products, the fibrinogen structure has been defined as two D domains flanking a central E domain (10, 11). Fg is synthesized primarily in the liver under normal conditions, but is also synthesized under inflammatory and stress conditions in the lung and intestine epithelium (2, 12). The determining step in the coagulation pathways involves the α -thrombin catalyzed conver-

Correspondence to:
Dr. Jose J. Rivera
CEMB, Institute of Biosciences and Technology
2121 W. Holcombe
Houston, Texas 77030, USA
Tel.: +1 713 677 7588, Fax: +1 713 677 7576
E-mail: jriversa@ibt.tamhsc.edu

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Table 1: Fibrinogen-binding proteins.

Protein	Binding site in Fg (Ref.)	Binding mechanism
<i>S. aureus</i> FnbpA (fibronectin binding protein A)	C-terminus γ -chain (24)	Predicted "Dock, Lock and Latch"(DLL)
<i>S. aureus</i> ClfA (clumping factor A)	C-terminus γ -chain (25)	DLL (unpublished data)
<i>S. epidermidis</i> SdrG (serine-aspartate repeat protein)	N-terminus β -chain (29)	DLL (31)
Group A,C, and G streptococci M protein	D fragment (57, 58, 60, 61)	ND
Group A streptococci SOF (serum opacity factor)	β -chain (70)	ND
FbsA (fibrinogen binding protein <i>S. agalactiae</i>)	D fragment (77)	ND
<i>S. aureus</i> Eap (Extracellular adherence protein)	α -chain (81)	ND
<i>S. aureus</i> Efb (extracellular fibrinogen binding)	α -chain (92, 93)	ND
<i>S. aureus</i> Coagulase protein	ND	Coagulase-prothrombin complex forms active site (102, 103)

ND, not determined.

sion of Fg to fibrin, releasing the fibrin peptides A and B from the N-termini of the A α and B β chains. Fibrin monomers polymerize spontaneously and form fibrin clots at sites of injury (1).

Cell wall-anchored Fg-binding MSCRAMMs

The staphylococcal Fg-binding MSCRAMMs ClfA, FnbpA, and SdrG

S. aureus and *Staphylococcus epidermidis* are Gram-positive commensal organisms. Primarily *S. aureus* but also *S. epidermidis* have emerged as major nosocomial pathogens causing a number of different types of infections from superficial skin infections to life-threatening conditions, including endocarditis and pneumonia. Staphylococci are responsible for more than half of all nosocomial blood infections reported in the US (13–15). In addition, an increasing number of invasive infections caused by community-acquired methicillin-resistant *S. aureus* (CA-MRSA) have been recently documented in previously healthy young patients (16, 17). The molecular pathogenesis of staphylococcal infections is not completely understood, but adherence of pathogenic bacteria to the ECM appears critical in the initial stages of most types of staphylococcal infections (18). The attachment of bacteria to the host ECM is mediated by MSCRAMMs (18). Three staphylococcal MSCRAMMs, clumping factor A (ClfA) and fibronectin-binding protein A (FnbpA) from *S. aureus* and serine-aspartate repeat protein G (SdrG) from *S. epidermidis* specifically interact with Fg and are important virulence factors in staphylococcal infections (19, 20).

Domain organization and biochemical characterization

The Fgbps ClfA, FnbpA, and SdrG belong to a family of structurally related cell wall attached proteins found in most Gram-positive bacteria (Fig. 2). The N-terminus of these proteins contains a signal sequence of approximately 40 residues followed by an A domain that is exposed on the bacterial surface and contains the ligand-binding region. The C-terminus contains the LPXTG motif required for cell surface anchoring, a hydrophobic transmembrane domain, and a positively-charged cytoplasmic end (21–24). Interspersed between the A domain and the cell anchoring domain are repeat regions that vary among the different proteins in staphylococci. The A domain is composed of three subdomains, N1-N3. The Fg-binding domain of ClfA, FnbpA and SdrG is within the N2 and N3 subdomains, a region composed of approximately 320 residues. In ClfA, the N2-N3 subdomains are found in residues 229–545, in SdrG in residues 273–597 and in FnbpA in residues 194–511. Although these N2 and N3 subdomains from the three proteins only share about 20% amino acid sequence identity, they have a highly conserved secondary structure. ClfA and FnbpA both bind to the 17 residues at the C-terminus (residues 395–411) of the Fg γ -chain (24, 25). This same region in Fg contains residues important for binding to the platelet $\alpha_{IIb}\beta_3$ integrin receptor (26–28). In fact, ClfA inhibits both platelet aggregation and the binding of platelets to immobilized Fg (25). In contrast, the SdrG Fg-binding region is localized to the N-terminus of the Fg B β -chain. This binding site overlaps with the thrombin cleavage site and SdrG has been shown to inhibit fibrin clotting (29).

A common model of Fg recognition by SdrG, ClfA and FnbpA

The crystal structures of the Fg-binding domains of ClfA and SdrG show remarkable structural similarities. The N2-N3 subdomains of both proteins contain two similar IgG-like structures (30, 31). Based on binding studies with truncated FnbpA recombinant proteins and molecular modeling predictions, the ligand binding domain of FnbpA is predicted to form a structure similar to that of ClfA_{221–559}. It is likely that ClfA and FnbpA bind Fg by a similar mechanism. In support of this hypothesis, FnbpA mutants involving residues at equivalent positions to critical residues in ClfA that affect its interaction with Fg also showed impaired Fg binding (32). These studies suggest a similarity in the SdrG, ClfA and FnbpA mechanism of Fg-binding.

A mechanism of MSCRAMM-Fg binding termed "Dock, Lock and Latch" (DLL) has been proposed based on the crystal structures of the SdrG Fg-binding domain as an apo-protein and in complex with a Fg-derived synthetic peptide (31). In the DLL model, the SdrG apo-form of the protein adopts an open conformation with an exposed binding trench formed between the N2 and N3 subdomains. The ligand peptide enters and "docks" in the trench. A flexible extension of the N3 domain redirects toward N2, covers the ligand, and "locks" the ligand in place. Simultaneously, the C-terminal residues in the N3 extension interact with the N2 module and form an additional β -strand to complement one of the β -sheets in N2. The N3 extension functions as a "latch" when it interacts with the N2 β -sheet. This results in a stable SdrG-Fg complex (31). This model of Fg recognition can be extended to ClfA and FnbpA based on computer models that dock the Fg peptide within the binding pockets of both the apo-

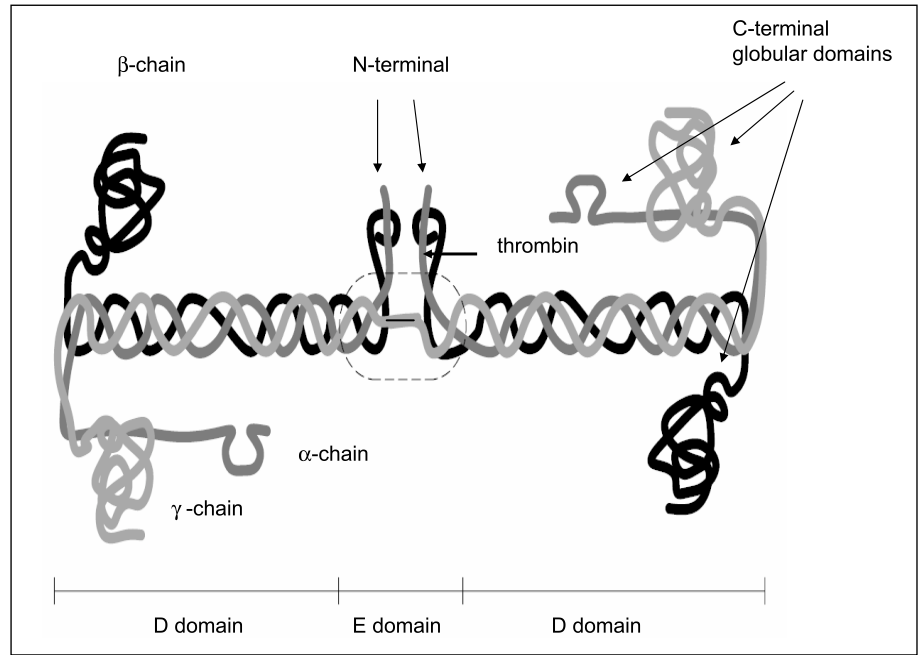


Figure 1: Diagram of fibrinogen. Fg is composed of three pairs of non-identical peptide chains named α , β and γ . The three peptide chains are connected by disulfide bonds. Fibrinogen structure has been defined as two D domains flanking a central E domain (10, 11). Diagram adapted from (1).

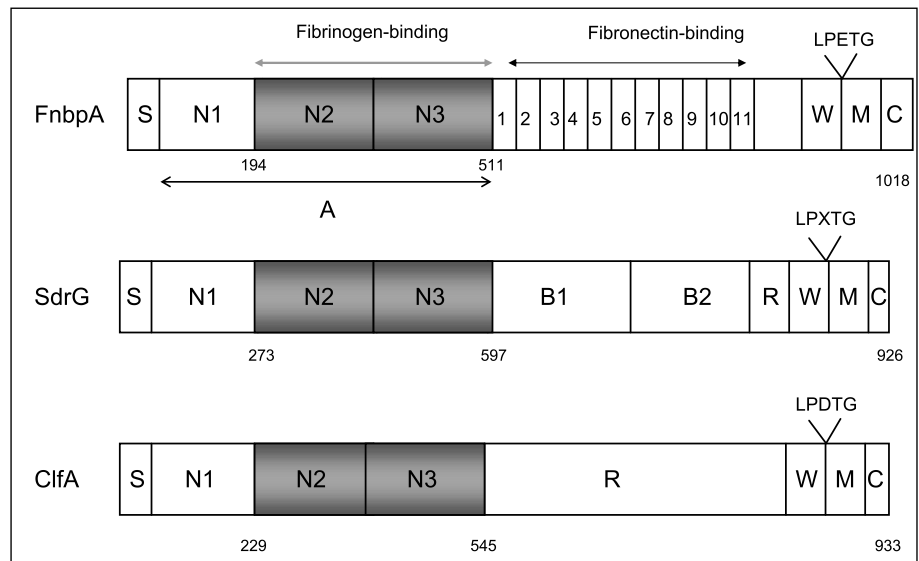


Figure 2: Schematic diagram of *S. aureus* ClfA and FnpA and *S. epidermidis* SdrG. These MSCRAMMs share a similar domain organization at the N-terminus A domain. The Fg-binding site is found within N2-N3 subdomains. These two subdomains fold into two IgG-like structures in SdrG and ClfA, and likely in FnbpA. The two IgG structures form a cavity in which the Fg ligand binds.

ClfA crystal structure and the putative FnbpA structure. Amino acid substitutions of residues located in the ligand binding groove on both ClfA and FnbpA also support the model (30, 33). Preliminary analysis of a crystal structure of ClfA in complex with the Fg ligand peptide confirms that the binding site is located between the N2 and N3 subdomains (Rivera et al., unpublished data).

Role in virulence

Essentially, all *S. aureus* clinical isolates carry the *clfA* gene (34). ClfA was shown to be a virulence factor in studies by Josefsson et al. using a septic arthritic mouse model. The degree of cartilage and bone destruction was more pronounced in mice injected

with wild-type bacteria than those injected with a *clfA* mutant bacteria (19). In a rabbit model of IE, a *clfA* deletion strain had reduced platelet binding ability and animals inoculated with this strain were less likely to develop endocarditis (35, 36). In a rat model of IE, animals infected with a *clfA* deletion mutant strain produced 50% less IE than animals infected with the wild-type parent strain. When the mutant strain was complemented with the wild-type *clfA* gene the strain produced wild-type levels of infectivity. The *clfA* deletion mutants were not able to bind Fg and bound significantly at lower levels to platelet-fibrin clots (20). When the *clfA* and *fnbA* genes were each constitutively expressed on the surface of the non-pathogenic *Lactococcus lactis*, 100-fold fewer bacteria were required to infect 80% of the rats

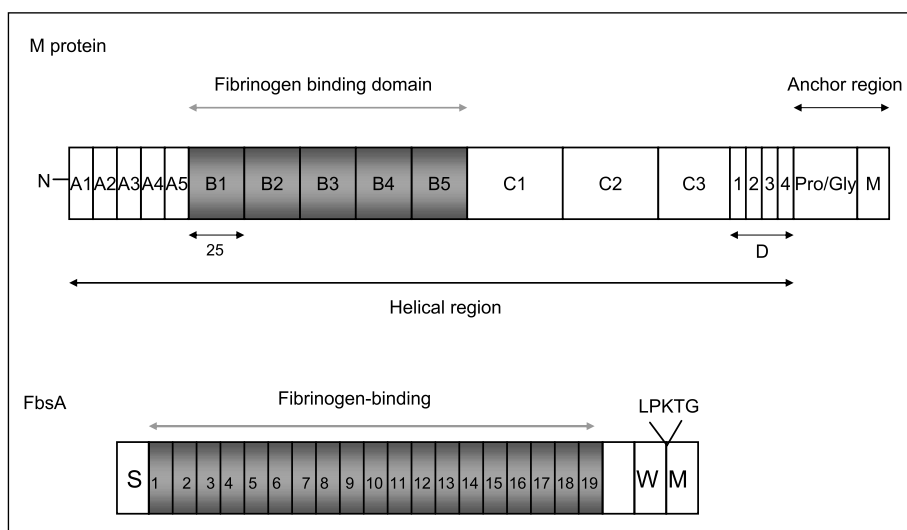


Figure 3: A schematic comparison of M protein from group A streptococcus and FbsA from group B streptococcus. The Fg-binding domains are represented by the shaded boxes. The Fg-binding domains are unrelated in sequence and presumably in structure (M protein adapted from [48]; FbsA from strain GBS 6313 adapted from [76]).

compared with *L. lactis* strains lacking both genes, indicating a role in virulence for both proteins (37, 38). However, recent evidence shows that FnbpA and ClfA may play different roles in endovascular infections. FnbpA may be more important in the early stages of *S. aureus* valve colonization. When expressed on *L. lactis*, FnbpA but not ClfA were able to infect and activate cultured endothelial cells. ClfA may play a more prominent role in damaged heart valves when Fg is deposited on endovascular lesions (38–40).

Vaccine potential

S. aureus is well adapted to avoid the host immune system. A previous *S. aureus* infection does not preclude a subsequent *S. aureus* infection. This may be due to factors expressed by the pathogen that allow it to evade host defense systems. In addition, *S. aureus* produces superantigens that inhibit a strong immune response leading to immunosuppression (reviewed in [41]). However, surface proteins generate strong immune responses and have shown potential as vaccine targets. In one study, mice injected with a recombinant ClfA (residues 40–559) containing the Fg-binding domain and subsequently infected with wild-type *S. aureus* showed significantly less pronounced arthritic parameters (19). Immunization of mice with rabbit polyclonal antibodies and monoclonal antibodies developed against the Fg-binding domain of ClfA protected mice from septic death (42). The humanized monoclonal antibody Aurexis[®] has a high affinity for ClfA and inhibits ClfA binding to Fg. Aurexis protected mice against *S. aureus* in a septicemia model and rabbits in an IE model. Aurexis in combination with antibiotic therapy is currently in clinical trials for treatment of bacteremia (18).

Effect on coagulation and thrombosis

The interaction between ClfA and Fg is responsible for the clumping of *S. aureus* in plasma in a process that resembles Fg-induced platelet aggregation (9, 25). Furthermore, *S. aureus* interact with, activate and induce aggregation of platelets (reviewed in [7]). *S. aureus*-induced platelet aggregation requires Fg. The *S. aureus*-induced platelet activation is inhibited by cro-tavirin, a snake venom $\alpha_{IIb}\beta_3$ integrin antagonist. Since cro-tavi-

rin does not alter *S. aureus* binding to Fg, cro-tavirin may block access to the $\alpha_{IIb}\beta_3$ integrin and prevent activation (43). Addition of cro-tavirin significantly reduced the levels of platelet-derived growth factor and thromboxane B₂, indicators of platelet activation (43). It is believed that the *S. aureus*-induced activation and aggregation of platelets is a contributing factor to the pathogenesis of IE. In fact, *S. aureus* are found within platelet-fibrin thrombi in heart valves where they presumably avoid neutrophils (5, 41). The interaction between *S. aureus* and platelets is proposed to be mediated through a Fg bridge between the $\alpha_{IIb}\beta_3$ integrin on the platelet surface and ClfA. ClfA expressed on *S. aureus* or on the non-pathogenic *L. lactis* induced platelet aggregation (9). Host antibodies against ClfA are also required for platelet activation to occur. These antibodies are thought to form a second bridge between the bacteria and the platelet. In this case, the IgG Fab binds ClfA and the IgG Fc domain binds to the platelet Fc γ RIIa receptor leading to intracellular signaling and subsequent platelet activation and aggregation (33, 41).

Group A streptococci (GAS) M proteins and serum opacity factor (SOF) protein

The M proteins of GAS (*Streptococcus pyogenes*) constitute a family of cell wall-anchored proteins containing a conserved C-terminus and a semi-variable N-terminal region with a surface exposed hypervariable N-terminal end (44, 45). Essentially all GAS isolates express M proteins (46) and there are over 100 M-protein variants (47). The M protein is a major virulence factor in streptococcal infections. It is also important for optimal growth and survival in blood and for the bacteria's antiphagocytic properties (48, 49). The different serologically distinct M variants share common structural features. The M protein is a filamentous, α -helical, coiled-coil dimer attached to the cell surface through the C-terminus (48). Members of the M-protein family contain characteristic cell wall sorting features, including a proline-glycine rich domain that spans the peptidoglycan, a hydrophobic region that traverses the membrane, and a charged tail extending into the cytoplasm (Fig. 3) (48, 50). Although covalently bound to the cell wall, some active M-protein fragments are released through the action of a bacterial proteinase (51) or pro-

teases produced by polymorphonuclear neutrophils (PMNs) (52).

The M-protein family can interact with different host serum factors including Fg, fibronectin, immunoglobulins, plasminogen, albumin, factor H, collagen IV and the complement regulator C4BP (53–57). Some members of GAS bind to the Fg D fragment with high affinity (5 nM) and it was shown that this binding could be blocked by addition of anti-M sera (56). Subsequently, M protein was determined to be the Fg high-affinity receptor for GAS (58, 59). Similarly to group A, group C and G streptococci also bind Fg through M proteins (60, 61). The Fg-binding domain in group A M protein was localized to the B-repeat region near the N-terminus of the protein (Fig. 3). The B-repeat region is composed of a variable number of repeats depending on the strain. There is some sequence variability between the B regions of different M proteins, but the α -helical coiled-coil structure is conserved (48, 62). M proteins are antiphagocytic, and there is evidence that binding of Fg may play a role in allowing GAS to escape phagocytosis by neutrophils (45). In one study, bacteria with a mutant M5 protein containing a B-repeat deletion was less resistant to phagocytosis (63). There is also evidence that M protein induces vascular leakage which may contribute to toxic shock syndrome. Herwald et al. have shown that an M-protein/Fg complex binds and activates PMNs likely through interactions with β_2 integrins on PMNs. The activated PMNs release the inflammatory activator, heparin-binding protein, inducing a strong inflammatory response (52).

The M protein-Fg interaction is also involved in platelet thrombus formation. In one study where GAS was immobilized on a glass surface, flowing human blood over the surface induced platelet thrombus formation (64). This prothrombic activity was mediated by the Fg-binding B-repeats. When the Fg-binding M1 and M5 were immobilized on a glass surface in the presence of flowing human blood, platelet adhesion was induced but not in a recombinant M5 Δ B lacking the Fg-binding B-repeats (64). Platelet aggregation on immobilized recombinant M5 was blocked by antibodies that inhibit Fg binding to the platelet integrin $\alpha_{IIb}\beta_3$, indicating Fg in blood likely mediates M protein-induced platelet adhesion (64).

GAS causes skin and soft tissue infections and is a common cause of pharyngitis in children (47). It also causes necrotizing fasciitis, pneumonia, bacteremia and toxic shock syndrome. The M protein has been a main target for streptococcal vaccine development. However, this strategy is complicated by the more than 100 M-protein serotypes (48). In addition, some antibodies developed against M protein cross-react with skeletal and cardiac muscle (65). A therapeutic approach that involves blocking the interaction between Fg and PMNs has shown some promise in a mouse model. A peptide that interferes with Fg and the β_2 integrin on PMNs protected mice against pulmonary lesions caused by injection of M1 protein or bacteria expressing M1 protein (52). In another approach, a multi-epitope vaccine using conserved and serotype-specific epitopes covalently linked to a lipid core peptide that acts as an adjuvant was used in a mouse model of GAS infection. The vaccine, containing three peptides from the M protein variable N-terminal region of different serotypes and a conserved C-terminal repeat region peptide, protected mice against a lethal dose of GAS (66). Another multi-epitope

vaccine containing 26 epitopes from the variable N-terminal region of 26 different serotypes is being tested as a vaccine and has shown promise in phase I clinical trials (67).

The SOF from GAS is an approximately 112 kDa cell wall attached protein named for its ability to turn human serum opaque by cleaving a serum apolipoprotein (68, 69). SOF binds fibronectin (Fn) and Fg (68–70). The *sof* gene was found in 55% of 93 isolates tested (71). Structurally, SOF has three main domains. The N-terminus contains the opacity activity, followed by a four tandem Fn-binding repeat domain (residues 820–956), a Fg-binding domain (residues 844–1047) and a C-terminus containing an LPASGD motif and other characteristic features for cell wall anchoring (68). SOF binds the β -chain of Fg (70). The Fg-binding domain overlaps with the Fn-binding domain, and in ELISA-type inhibition assays, Fg inhibits SOF binding to immobilized Fn in a concentration dependent manner. SOF is a virulence factor in a mouse model (72). However, the role that SOF Fg-binding plays in virulence has not been determined.

Group B streptococcus FbsA

Group B Streptococcus (GBS) (*Streptococcus agalactiae*) is a prevalent cause of sepsis, pneumonia and meningitis in newborns. In adults it causes endocarditis, arthritis, soft tissue infections and invasive infections in elderly and immunocompromised individuals (73, 74). The FbsA protein (Fg-binding protein from *S. agalactiae*) is a constitutively expressed cell surface protein containing a secretion signal peptide, a C-terminus cell wall attachment LPKTG motif, a hydrophobic membrane spanning region and a region containing a strain-specific variable number of 16 amino acid tandem repeats (70, 71). Deletion of the *fbsA* gene in different clinical isolates results in a loss of Fg binding, indicating FbsA is the Fg receptor of *S. agalactiae* (75, 76). The Fg-binding domain has been localized to the tandem repeat region (Fig. 3). A single repeat is able to bind Fg but the affinity increases as the number of repeats increase (76, 77). FbsA binds the Fg D-fragment, but the mechanism of binding has not been determined. The FbsA binding site in Fg is different from that of SdrG, CifA and FnbpA (77). The FbsA repeat region is different in sequence and predicted structure to the M-protein repeats. The FbsA Fg-binding motif has no homology to other characterized Fg-binding motifs from Gram-positive organisms (76). This suggests FbsA probably binds Fg by a unique mechanism. Since a *fbsA* deletion mutant grows poorly in human blood, FbsA may protect the bacteria against opsonophagocytosis indicating a role in pathogenesis (76). Different clinical isolates have been shown to adhere and invade human lung epithelial cell lines. Deletion of *fbsA* abolished the ability of clinical isolates to adhere and invade lung epithelial cells. The ability of the strains to adhere and invade also correlated with the ability to bind Fg. Strains that showed strong binding to Fg were more capable of adhering and invading human epithelial cells. FbsA can bind epithelial cells directly and can block bacteria from adhering and invading epithelial cells (75). There is evidence that the FbsA protein is an important factor in IE caused by *S. agalactiae*. Similar to other Fg-binding Gram-positive bacteria, *S. agalactiae* binds platelets and induces platelet aggregation (78, 79). The platelet aggregation activity requires FbsA and is mediated by Fg. A *fbsA* deletion mutant lacked the ability to aggregate platelets. The ability

to induce platelet aggregation was recovered when the deletion mutant was complemented with the wild-type gene or when the *fbfA* gene was expressed on the non-platelet aggregating surrogate strain *L. lactis* (80). In GBS, the ability to induce platelet aggregation and activation, as measured by thromboxane synthesis, differs between clinical isolates of septic patients and strains isolated from healthy individuals. Only strains isolated from septic patients were able to induce platelet aggregation and activation (79).

S. aureus secreted Fg-binding SERAMs

The *S. aureus* Fg-binding Eap (extracellular adherence protein) is an approximately 70 kDa protein expressed in almost all clinical isolates (81, 82). The protein lacks an LPXTG motif; it is not covalently anchored to the bacterial surface (83) and substantial amounts of Eap are released into culture supernatants (84, 85). Eap has a broad binding specificity; it is capable of interacting with several plasma proteins including the Fg α -chain, Fn and prothrombin. Structurally, Eap (also known as Map) contains a characteristic domain architecture composed of six repeats of 110 amino acid residues (81). Each domain in turn contains a 31 amino acid subdomain with high homology to the N-terminal β -chain of the major histocompatibility complex (MHC) class II proteins (86). Deletion of *eap* does not affect binding of *S. aureus* to Fg or Fn. This may be due to multiple Fg and Fn-binding proteins (81). Exogenous Eap readily binds the *S. aureus* surface through interactions with endogenous Eap or through interactions with other cell surface components. The role of this Eap interaction is not clear but may be involved in colonization and/or infection (82). Eap is an anti-inflammatory factor and can bind ICAM-1 (intercellular adhesion molecule-1) on endothelial cells, blocking the interaction between leukocytes and endothelial cells. By blocking leukocyte binding to ICAM-1, Eap inhibits the recruitment of neutrophils that normally leads to inflammation (87). Eap is also involved in bacterial adhesion to fibroblasts. An *eap* deletion mutant showed a lower adherence to fibroblasts and epithelial cells (88, 89) and was shown that antibodies against Eap lowered the adherence of the Newman strain to epithelial cells and fibroblasts (82). Eap may be involved in host cell internalization since it was found that in some strains anti-Eap antibodies reduced internalization (88).

There is evidence that Eap is a virulence factor in chronic but not in short-term infections (81). Compared to a wild-type strain, an *eap* deletion mutant displayed significantly lower levels of arthritis, osteomyelitis and abscess development in a mouse model of infection performed over a span of several weeks (90). In another study performed over five days, no significant differences in abscess development between *eap* mutant and wild-type strains were reported (87). The mechanism of Eap binding to its multiple ligands has not been elucidated. It has been speculated that Eap may act as a bridge between bacteria and the host through its multiple interactions (85). Eap may also play a role in the survival of *S. aureus* by modulating or suppressing the host immunity. In wild-type mice, an *eap* negative strain displayed significantly lower levels of arthritis and osteomyelitis compared to an *eap* positive strain. However, T cell-deficient mice infected with an *eap* deletion mutant displayed similar levels of arthritis

and osteomyelitis as mice infected with an *eap* positive strain. Mice injected with Eap showed a lower delayed-type hypersensitivity response (90).

The *S. aureus* Efb (extracellular fibrinogen-binding) is a 15.8 kDa extracellular secreted protein constitutively expressed by all *S. aureus* isolates (91, 92). The protein binds the Fg D fragment (111–197 of A α -chain). Efb contains an N-terminal and a C-terminal Fg-binding site (92, 93). The N-terminal Fg-binding site is within two almost identical 22 residue repeats (RR) (93). These repeats share high homology with the *S. aureus* coagulase 27-residue repeats that also bind the same region of Fg (92). Efb inhibits Fg-dependent platelet aggregation (92). In FACS analysis, the levels of Fg bound to platelets increased with incremental concentrations of Efb and a recombinant fusion containing the RR repeats and glutathione S-transferase (GST-RR) is a strong inhibitor of platelet aggregation (92). The inhibition mechanism has not been determined, but it has been proposed that Efb may block the RGD sequence on the Fg chain or cause a conformational change in Fg. Efb binds platelets through formation of a Fg bridge and directly to an uncharacterized platelet receptor in a mechanism that is independent of Fg (92, 94). The anti-thrombotic properties of Efb results in delayed wound healing. In mice, Efb inhibition of platelet aggregation resulted in extended bleeding and protected mice against thrombosis induced by collagen and epinephrine (94, 95).

Efb was found to be important for pathogenesis in a rat wound model and in a mouse model of severe wound infections (92, 96). Antibodies raised against Efb have a protective effect in a mouse mastitis model (92), and antibodies raised in sheep against Efb blocked binding of Efb to Fg, prevented inhibition of platelet aggregation and allowed wounds to heal faster (97). The Efb protein has shown promise as a vaccine. In mice vaccinated with Efb, 17% developed a severe infection compared to the 73% of non-vaccinated mice (97).

Coagulase is a secreted *S. aureus* protein involved in blood coagulation and is a primary example of how bacteria manipulate its host biology to its advantage. Almost all *S. aureus* strains carry the coagulase gene, but the levels of expression differ with different serotypes due to transcriptional and post-transcriptional modifications in coagulase negative strains (98). Structurally, coagulase is composed of three main regions: an N-terminal region containing the D1 and D2 segments, a highly conserved central region, and a C-terminal region containing a variable number of 27 amino acids tandem repeats (99). Coagulase binds Fg by two seemingly distinct mechanisms. The C-terminal repeats bind Fg directly (100) but the mechanism of binding and its role in virulence has not been characterized. The N-terminal region is also involved in Fg-binding but does not bind Fg directly. The N-terminal D1 and D2 domains form α -helices that interact with prothrombin and thrombin. Coagulase and prothrombin form a stoichiometric 1:1 complex. The complex activates prothrombin to a form that is capable of converting Fg to fibrin. Conversion of Fg to fibrin by the coagulase-prothrombin complex does not involve proteolytic cleavage of prothrombin by the clotting factor cascade (101). Instead, coagulase binds and activates prothrombin directly bypassing the classical proteolytic activation of prothrombin to thrombin. Structural and biochemical studies show that the coagulase-prothrombin complex gener-

ates a new Fg-binding domain not found in either molecule alone (102). The crystal structure of a fragment of coagulase (1–325) in complex with prothrombin has been solved, and a mechanism for coagulase induced prothrombin activation has been proposed. Coagulase_{1–325} forms a tight complex (apparent K_D 0.3 nM) with prothrombin. Coagulase_{1–325} binds the 148 loop and the (pro)exosite I of prothrombin (103, 104). The activation domains that are disordered and inactive in the free prothrombin are folded in an active conformation when bound to coagulase_{1–325} (103). Remarkably, N-terminal coagulase residues insert into a prothrombin activation cavity inducing a conformational change that exposes and activates the prothrombin catalytic site (103). The activity of the coagulase-prothrombin/thrombin complex is specific for Fg. It does not cleave other thrombin substrates such as platelets, factor V or factor VII (104, 105). No physiological inhibitors of the coagulase-prothrombin complex have been reported, and the complex is resistant to most anticoagulants (104). Coagulase is not required for *S. aureus* to establish an infection in a mouse endocarditis model (20, 106) but may contribute to IE since approximately 40% of neonatal and adult endocarditis is caused by coagulase positive strains (104, 107).

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Perspective

It is clear that Gram-positive bacteria express multiple Fg-binding proteins. Although many of these proteins employ distinct mechanisms of Fg-binding, the end result is to manipulate Fg's biology to enhance the microbe's survival in the host. Our knowledge of how these proteins interact with Fg has greatly increased in the last few years, and we will likely find additional Fg-binding MSCRAMMs and SERAMs as more pathogenic bacteria are studied. Progress has also been made in understanding the importance of these interactions within the disease process. Some of these Fgbps have shown promise as targets for vaccines or immunotherapeutics in animal studies. This is particularly important since new antibacterial strategies are critical to combat the new drug resistant bacteria that continue to emerge.

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