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Fibrinogen Is at the Interface of Host Defense and Pathogen Virulence in *Staphylococcus aureus* Infection

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Abstract

Fibrinogen not only plays a pivotal role in hemostasis but also serves key roles in antimicrobial host defense. As a rapidly assembled provisional matrix protein, fibrin(ogen) can function as an early line of host protection by limiting bacterial growth, suppressing dissemination of microbes to distant sites, and mediating host bacterial killing. Fibrinogen-mediated host antimicrobial activity occurs predominantly through two general mechanisms, namely, fibrin matrices functioning as a protective barrier and fibrin(ogen) directly or indirectly driving host protective immune function. The potential of fibrin to limit bacterial infection and disease has been countered by numerous bacterial species evolving and maintaining virulence factors that engage hemostatic system components within vertebrate hosts. Bacterial factors have been isolated that simply bind fibrinogen or fibrin, promote fibrin polymer formation, or promote fibrin dissolution. *Staphylococcus aureus* is an opportunistic gram-positive bacterium, the causative agent of a wide range of human infectious diseases, and a prime example of a pathogen exquisitely sensitive to host fibrinogen. Indeed, current data suggest fibrinogen serves as a context-dependent determinant of host defense or pathogen virulence in *Staphylococcus* infection whose ultimate contribution is dictated by the expression of *S. aureus* virulence factors, the path of infection, and the tissue microenvironment.

Keywords

fibrinogen; infection; *Staphylococcus aureus*; host defense

Bacterial species differ widely in their modes of transmission, sites of colonization, capacities for intracellular and extracellular growth, complement of virulence factors, host preferences, and strategies for evading immune surveillance. However, despite the disparate functional properties and niches of pathogens, bacterial infection frequently results in perturbation of the host hemostatic system. Indeed, systemic inflammation associated with

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Conflict of Interests

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bacterial infection has a significant impact on hemostatic activity at multiple levels. Several key procoagulant hemostatic system components, including von Willebrand factor, factor VIII, and, notably, fibrinogen^{1–3} are acute-phase proteins whose plasma concentration sharply increases following acute inflammatory events. Plasminogen activator inhibitor-1, a key fibrinolytic pathway inhibitor, is also a well-established acute-phase protein.^{4,5} Significantly increased coagulation and fibrinolytic activities also typically accompany both acute and chronic bacterial infections.^{6–10} High levels of thrombin–antithrombin complexes and fibrin degradation products have been documented in patients with bacterial infections and in animal models of infectious diseases.^{11–14} Collectively, the expression and activity changes of coagulation system components following infection favor the rapid generation of fibrin matrices. An uncontrolled coagulation response following infection can lead to disseminated intravascular coagulation (DIC), a consumptive coagulopathy. DIC promotes ischemia, inflammation, hemorrhage, and shock, and is a primary cause of septic death.^{15–17} However, localized procoagulant function leading to fibrin deposition within infected tissues can be an important feature of host antimicrobial defense.

The potential of fibrin to mediate host protective functions and limit bacteria-mediated pathologies is punctuated by the fact that numerous species have evolved and maintained the capacity to engage hemostatic system components (e.g., prothrombin, fibrinogen, and plasminogen) within vertebrate hosts. The binding and/or activation of hemostatic factors by bacterial agents has been documented in over a dozen gram-negative and gram-positive human pathogens, including *Escherichia coli*, *Helicobacter pylori*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Borrelia burgdorferi*, *Streptococcus* species, *Yersinia pestis*, *Staphylococcus aureus*, and others.^{18–25} That a remarkable number of bacterial species target host coagulation and fibrinolytic factors suggests a point of commonality whereby the suppression or alteration of host coagulation function is beneficial for bacterial inoculation, proliferation, dissemination, or a combination of processes to ensure bacterial survival. Bacterial factors have been isolated that simply bind fibrinogen or fibrin, promote fibrin polymer formation, or promote fibrin dissolution. In some cases, the same bacterium produces factors that promote the formation of fibrin matrices and factors that promote fibrin breakdown. Over the last decade, significant progress has been made both in defining the role of fibrinogen in host defense and in understanding the function of fibrin(ogen)-directed bacterial virulence factors.

Fibrinogen as a Mediator of Antimicrobial Host Defense

Beyond playing a fundamental role in controlling hemorrhage and promoting vessel repair following vascular injury, fibrinogen has the inherent capacity to combat invading bacterial pathogens. As a rapidly assembled provisional matrix protein, fibrinogen can serve as an early line of defense for host protection by limiting bacterial growth, suppressing dissemination of microbes to distant sites, and mediating host bacterial killing. Current studies suggest that fibrinogen-mediated host defense largely occurs through two general mechanisms. First, soluble fibrinogen or fibrin matrices can physically entrap bacteria or encapsulate bacterial foci within infected tissue, which can limit growth and dissemination. Second, fibrin can support the recruitment and activation of host immune cells, which in turn mediate the elimination of invading microbes. Notably, these mechanisms are not mutually

exclusive, but rather, based on the pathogen and microenvironment, can function in concert for host defense.

Fibrinogen and Barrier Function

The concept that local fibrin clot formation can serve as a critical barrier for host defense is well evidenced in group A streptococci (GAS) infection. GAS produce streptokinase, a highly specific activator of human plasminogen, showing little to no activity toward plasminogen from other mammalian species including mouse.^{26–28} A study of transgenic mice expressing human plasminogen revealed that these animals had significantly diminished survival following skin infection with GAS. A primary mechanism was linked to fibrinolysis mediated by streptokinase in promoting escape from the initial site of infection and dissemination to distant organs.²⁹ Indeed, skin infection or direct intravenous infection with GAS in mice depleted of fibrinogen resulted in high host mortality regardless of the presence or absence of host human plasminogen.²⁹ In a separate study, genetically modified mice carrying mutations resulting in diminished thrombin generation also displayed significantly increased mortality following GAS skin infection.²¹ Fibrinogen was implicated as the key thrombin target as a similar significant reduction in host survival following GAS skin infection of fibrinogen-deficient mice was observed. Further, the concept that fibrin is critical for host–pathogen barrier function following GAS infection was supported by a trend of increased systemic spread of bacteria to distant organs in fibrinogen-deficient animals.²¹ That all pathogenic GAS isolates express streptokinase suggests that penetration of fibrin matrices is a common virulence mechanism across *Streptococcus* species.^{26–28}

Protective fibrinogen barrier function was also illustrated in rodent infection models of *Listeria monocytogenes*, an intracellular gram-positive bacterium. Rats administered the anticoagulant heparin displayed significantly increased bacteremia and host lethality following *L. monocytogenes* infection.³⁰ Similarly, mice treated with the anticoagulant warfarin or imposition or genetically imposed fibrinogen deficiency resulted in 100% host lethality, increased anemia, and increased bacterial burden in the livers of *Listeria*-infected animals.²⁰ In this report, Mullarky and colleagues speculated that it was fibrin barrier function that either restricted movement of infected cells or restricted cell–cell pathogen transfer, and not a fibrin immune modulatory mechanism mediating the protective effects. However, no supportive studies defining the precise fibrin protective function in *Listeria* infection have been presented.

Fibrinogen and Immune Modulation

The role of fibrinogen in antimicrobial host defense extends far beyond simply serving as a physical barrier to pathogen spread. Rather, fibrinogen is a highly integrated component of the host immune system. The engagement of fibrin by leukocytes can be crucial for activation of immune cell effector functions (e.g., degranulation, oxidative response, phagocytosis, expression of inflammatory cytokines). Fibrin(ogen) has ideal functional attributes for serving a fundamental role in leukocyte activation. First, fibrin is found within the extracellular matrix at virtually any site of tissue damage, regardless of the underlying insult, but is distinctly absent within normal tissues. Second, fibrin provides a nondiffusible and spatially defined modulator of inflammatory cell function. Thus, fibrin provides a

universal cue marking the precise location of any challenge to locally regulate leukocyte function at sites where a pathogen has already initiated local tissue disruption.

The role of fibrin in the regulation of leukocyte function is supported by multiple in vitro studies. Fibrin(ogen) can direct a multitude of cellular activities, including mitogenic, chemotactic, and immunoregulatory functions^{31–36} through both integrin (e.g., $\alpha_v\beta_3$, $\alpha_1\beta_5$, $\alpha_M\beta_2$ ^{37–43}) and nonintegrin (e.g., intercellular adhesion molecule 1 [ICAM-1], cadherins^{44–50}) receptors found on endothelial cells, macrophages, neutrophils, and other cells. Furthermore, the pharmacological depletion or genetic elimination of fibrinogen in mice has been shown to improve the survival of animals challenged with high concentrations of lipopolysaccharide, as well as impede the development of numerous inflammatory diseases as shown in animal model studies of arthritis, colitis, and neuroinflammatory disease.^{51–55}

Studies of *Yersinia* infection in mice were some of the first to document a protective role for fibrin matrices in promoting host immune cell activity to clear an invading pathogen. *Y. pestis* is a gram-negative bacterium and the causative agent of plague. Like streptococcal and staphylococcal species, *Y. pestis* produces a plasminogen activator called Pla that is a major virulence factor. Subcutaneous infection of mice with *Y. pestis* expressing Pla (i.e., *Pla*+) increased pathogen virulence a million-fold compared with a Pla-deleted (i.e., *Pla*–) strain.⁵⁶ Plasmin-mediated fibrin degradation and subsequent dampening of host inflammatory cell activity were identified as the molecular mechanism mediating this dramatic difference in virulence. Wild-type (WT) mice challenged intravenously with *Pla*+ bacteria had high mortality with large bacterial foci within the liver and spleen with little to no evidence of infiltrating host inflammatory cells. Elimination of bacterial Pla or host plasminogen resulted in excellent host survival, and sites of *Y. pestis* colonization were uniformly filled with fibrin deposits and significant numbers of host inflammatory neutrophils with few bacteria.⁵⁷ Local fibrin deposits were essential for the enhanced host inflammatory cell accumulation and activity as the host survival profile was dramatically diminished if fibrinogen was simultaneously eliminated.⁵⁷ Similarly, in a mouse model of *Yersinia enterocolitica* peritoneal infection, fibrinogen-deficient mice displayed significantly higher mortality, higher bacterial burdens in liver and spleen, and reduced cytokine production and peritoneal neutrophil recruitment compared with infected WT mice.⁵⁸ Collectively, these findings highlight the potency of fibrin matrices in supporting immune function.

Fibrinogen as a Central Mediator of Host Defense in *S. aureus* Infection

S. aureus is a pervasive gram-positive bacterium that is the causative agent of numerous diseases ranging from minor skin infections to serious, life-threatening conditions such as endocarditis, pneumonia, and sepsis.⁵⁹ *S. aureus* infects an estimated 50 million people globally and causes the death of ~10,000 Americans each year.^{60,61} The emergence of antibiotic-resistant strains of *S. aureus* (i.e., methicillin-resistant *S. aureus* [MRSA] and vancomycin-resistant *S. aureus*) has renewed interest in better defining mechanisms of pathogen virulence and host defense.^{62–66} Considering that *S. aureus* has the capacity to both promote fibrin formation and drive fibrin breakdown and that fibrin has the capacity to promote host immune function, a fundamental question is whether, on balance, host

coagulation system activity predominantly supports host defense or *S. aureus* virulence. Current data suggest fibrinogen is a *context-dependent determinant of host defense or pathogen virulence* in *S. aureus* infection whose contribution is dictated by the precise expression of virulence factors, path of infection, and tissue microenvironment. Indeed, current findings from animal studies indicate that host fibrinogen is a critical antimicrobial factor following *S. aureus* peritonitis infection, while, conversely, fibrinogen promotes bacterial virulence following blood stream infection.

It has been known for over 30 years that fibrin can act to contain *S. aureus* (and other microorganisms) following peritoneal infection.^{67,68} Indeed, multiple fibrinogen-dependent host defense mechanisms have been proposed to limit *S. aureus* peritonitis, including entrapping *S. aureus* bacteria via fibrinogen-mediated bacterial clumping or aggregation,^{67,68} fibrin-dependent adhesion of bacteria–peritoneal cell aggregates to the mesothelial surface,⁶⁹ and fibrin-driven bacterial killing.¹⁸ Attempts to take advantage of the concept that fibrin can entrap *S. aureus* in large aggregates within the peritoneal cavity has been incorporated into novel treatment strategies. The antibiotic streptomycin has low aqueous solubility, but when encapsulated by fibrin matrices had enhanced efficacy in preventing host lethality in a rat model of experimental multidrug-resistant *S. aureus* peritonitis.⁷⁰ However, the contribution of fibrinogen to host defense against *S. aureus* peritonitis goes beyond simple clumping or entrapment of bacteria.

Studies in mice have documented that fibrinogen can mediate a powerful antimicrobial response to intraperitoneal *S. aureus* infection. Following challenge with a dose of 1×10^9 colony-forming units (CFUs) of *S. aureus*, WT mice clear approximately two orders of magnitude of the initial bacterial CFU burden (or >99% of the inoculum) in less than 1 hour, whereas fibrinogen-deficient mice clear essentially none of the bacterial CFU over that same period and beyond.^{20,71} This early failure of bacterial clearance translates to significant mortality for fibrinogen-deficient mice compared with a near-uniform absence of mortality for control, fibrinogen-sufficient animals. Notably, this pattern of fibrinogen-dependent bacterial clearance was observed following infection with various independent strains of *S. aureus* (e.g., *S. aureus* Rosenbach 502A, *S. aureus* Newman, *S. aureus* USA300), including those defined as MRSA.^{18,20,71} That the primary fibrinogen-mediated mode of action is bacterial killing as opposed to mechanisms of clumping/adhesion was recently demonstrated by a combination of scanning electron microscopy studies of peritoneal lavage fluid and fate-tracking studies with radiolabeled *S. aureus* genomic DNA (M. Flick, PhD, and J. Degen, PhD, unpublished results).

The precise mechanism(s) of fibrinogen-dependent bacterial clearance remain to be fully elucidated; however, significant functional aspects have been delineated using mice engineered to express mutant forms of fibrinogen. In this regard, impaired clearance of *S. aureus* from the peritoneal cavity was documented for mice carrying a mutant form of fibrinogen lacking the $\alpha_M\beta_2$ -binding motif but maintaining full clotting function.¹⁸ In these studies, however, the impaired bacterial clearance for the mutant mice (termed Fib $\gamma^{390-396A}$) was not as extensive as was documented for fibrinogen-deficient mice. Retrieval of bacterial CFUs from the peritoneal cavity of Fib $\gamma^{390-396A}$ mice 1 hour after infection was 10-fold higher relative to WT animals as opposed to ~100-fold higher (or

identical to the initial inoculum) in fibrinogen-deficient mice.¹⁸ Importantly, Fib $\gamma^{390-396A}$ mice do not have an inherent altered composition or number of resident peritoneal immune cells nor are differences in leukocyte trafficking to the peritoneal cavity observed in Fib $\gamma^{390-396A}$ mice (or fibrinogen-deficient mice) following proinflammatory peritoneal challenges (e.g., bacterial infection or thioglycollate challenge).^{18,54} These findings (1) suggest the importance of the fibrin(ogen) $\alpha_M\beta_2$ -binding motif in host defense against peritoneal *S. aureus* infection, (2) implicate an $\alpha_M\beta_2$ -expressing cell in the mechanism of fibrin(ogen)-mediated bacterial clearance, and (3) highlight that fibrin formation is, in itself, insufficient to support the implementation of full antimicrobial function.

In considering fibrin(ogen)-dependent *S. aureus* clearance from the peritoneal cavity, a fundamental question is the requirement for thrombin-mediated fibrinopeptide (Fp) release and fibrin formation. Analyses of *S. aureus* peritonitis in mice with constitutively low prothrombin levels (i.e., ~10% of normal) strongly implicated polymer formation as a mechanistic feature of fibrin(ogen)-dependent antimicrobial activity.⁷² To directly establish a model system for distinguishing fibrinogen- and fibrin-dependent processes in vivo, Fib^{AEK} mice were recently generated that carry normal levels of circulating fibrinogen but lack the capacity for fibrin polymer formation due to a germline mutation in the A α chain thrombin cleavage site. Analyses of *S. aureus* peritonitis revealed that Fib^{AEK} mice were a perfect phenocopy of fibrinogen-deficient mice in terms of a complete failure to eliminate bacterial CFUs in the peritoneal cavity within 1 hour of infection.⁷¹ Intriguingly, infection dose influenced the ultimate outcome of host mortality as related to fibrinogen genotype. At lower infection doses, Fib^{AEK} mice had a survival profile similar to fibrinogen-deficient mice, which was significantly worse than that of control animals, consistent with an early failure of bacteria elimination. At higher infection doses, Fib^{AEK} mice had a survival profile superior to fibrinogen-deficient mice indicating that even soluble, nonthrombin cleaved fibrinogen retains some capacity to limit host mortality following *S. aureus* peritonitis.

Fibrinogen as a Target of Bacterial Virulence Factors

Although fibrinogen has the capacity to significantly limit bacterial infection by serving as an antimicrobial host defense factor, human pathogens have evolved successful ways to interact with host fibrinogen to tip the balance in favor of bacterial pathogenesis. *S. aureus* is a model pathogen that has evolved and maintained an extraordinary array of factors that engage fibrinogen, including multiple bacteria cell wall-associated and secreted fibrin(ogen)-binding proteins,⁷³⁻⁷⁸ two distinct staphylocoagulases⁷⁹ that form proteolytically active procoagulant complexes with host prothrombin, and staphylokinase (SAK) that forms a 1:1 proteolytically active complex with host plasminogen to specifically degrade fibrin matrices. Such virulence factors have equipped *S. aureus* to both evade fibrin(ogen)-driven antimicrobial functions and in fact “hijack” the host coagulation system to promote pathogen virulence, at least in certain infection contexts.

S. aureus Fibrinogen-Binding Proteins

S. aureus produces over a dozen of fibrinogen-binding virulence factors that contribute to the pathogenesis of *S. aureus* infection. The known fibrinogen-binding staphylococcal proteins

largely fall into two groups: a family of structurally related cell wall–anchored proteins termed microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) (Fig. 1)^{80,81} and a group of secreted smaller proteins termed secretable expanded repertoire adhesive molecules (SERAMs) (Fig. 2). These staphylococcal proteins interact with different sites on fibrinogen to distinctly alter fibrinogen function, including regions within the D-domain and the central E-domain (Fig. 3).

Fibrinogen-Binding *S. aureus* MSCRAMMs—The fibrinogen-binding MSCRAMMs share structural similarities and have a common ligand-binding mechanism.⁸² The core structure includes an amino-terminal A region, which is composed of three separately folded subdomains: N1, N2, and N3. The A region is followed by repeat regions (i.e., either serine–aspartate dipeptide SD repeats or fibronectin-binding repeats), a bacterial cell wall–spanning region, and a C-terminal sorting signal, which is characterized by an LPXTG (Leu-Pro-X-Thr-Gly); sortase cleavage motif.^{74,83} Fibrinogen binding occurs through the two immunoglobulin G folded N2 and N3 subdomains of the A region by variations of the multistep “dock, lock, and latch” binding mechanism.⁸⁴ Here, a short disordered segment of fibrinogen “docks” in a trench formed between the two subdomains. This interaction triggers conformational changes resulting in redirection of a flexible C-terminal extension of the N3 subdomain to cover the ligand binding and “lock” it in place. Subsequently, the C-terminal extension of the N3 subdomain forms a β -complementation to a strand of the N2 subdomain to serve as a “latch” that stabilizes the interaction.

Clumping factor A (ClfA) is named from its ability to induce fibrinogen-dependent clumping of *S. aureus* in suspension. The ClfA binding site in fibrinogen is mapped to the C-terminus of the fibrinogen γ -chain, residues 395–411^{77,85} (Fig. 3), which also binds platelet integrin $\alpha_{IIb}\beta_3$.^{86–88} Recombinant soluble ClfA inhibits platelet aggregation and the binding of platelets to immobilized fibrinogen.^{89,90} The crystal structure of ClfA N2N3 domains in complex with a synthetic fibrinogen peptide identified key residues in the fibrinogen γ -chain carboxy-terminal region critical for ClfA binding and documented that although a common segment is associated with binding both ClfA and $\alpha_{IIb}\beta_3$, different residues mediate binding to each receptor.^{91,92} Fibrinogen γ K406 and D410 are required for binding to $\alpha_{IIb}\beta_3$ but are dispensable for ClfA binding.⁹¹ These studies highlight the concept that it is possible to selectively antagonize the ClfA–fibrinogen interaction without interfering with fibrinogen-mediated platelet function.⁹¹ ClfA is a multifunctional virulence factor. The fibrinogen-binding activity of ClfA in part functions to inhibit complement activation and opsonophagocytosis.^{93,94} The potency of ClfA as a virulence factor has been demonstrated in several animal models of infectious diseases, including a mouse model of sepsis and septic arthritis, and rabbit and rat models of infective endocarditis.^{95–98}

ClfB has significant overall sequence similarity to ClfA, but the fibrinogen-binding domains (i.e., the A regions) are only 26% identical.⁹⁹ ClfB does not bind the fibrinogen γ -chain, but rather binds to the α -chain of fibrinogen (Fig. 3).⁹⁹ Crystal structure analyses revealed a core-binding motif for ClfB in fibrinogen as GSSGXGXXG, which also exists in other proteins such as cytokeratin 10 and loricrin.^{100–103} Notably, ClfB binding to fibrinogen is species specific. ClfB binds human fibrinogen with high affinity but not mouse fibrinogen, as mouse fibrinogen lacks a GSSGXGXXG motif. Like ClfA, ClfB also supports platelet

aggregation; however, the mechanism for ClfB is fibrinogen-dependent and much slower than that mediated by ClfA.¹⁰⁴ Although the full functional consequences of ClfB remain to be defined, one key contribution is to *S. aureus* nasal colonization.^{101,105}

The fibronectin-binding proteins (i.e., FnBPA and FnBPB) are promiscuous virulence factors named due to their ability to bind host fibronectin via multiple fibronectin-binding repeats (see Fig. 1).¹⁰⁶ The FnBP proteins possess a fibrinogen-binding A region with critical N2–N3 subdomains and bind to the same region of fibrinogen γ chain as shown for ClfA (Fig. 3).⁸⁵ However, the fibrinogen-binding mechanism is distinct as it involves the “dock” and “lock” features without the requirement for “latch” strand residues.¹⁰⁷ FnBPA is important for initialization of *S. aureus* infection¹⁰⁸ and is a virulence factor for *S. aureus* infective endocarditis.⁹⁷ FnBPA also plays a role in biofilm formation, which in turn contributes to biofilm-associated *S. aureus* infection on indwelling medical devices.^{109,110} The ability of FnBPA to promote biofilm formation is linked to the fibrinogen-binding A domain, which mediates cell–cell adhesion through low-affinity homophilic bonds.¹¹¹

Bone sialoprotein-binding protein (Bbp) and the *S. aureus* surface protein SdrE are two additional key fibrinogen-binding MSCRAMMs.^{103,112} Bbp was initially identified from bacteria in bone and joint infections and is named due to an ability to interact with bone sialoprotein.¹¹³ Bbp binds fibrinogen through an A region N2 and N3 subdomains at fibrinogen residues 561–575 of the A α chain¹¹⁴ (Fig. 3). Notably, this region of the fibrinogen A α chain is highly variable between species, and thus Bbp binds human fibrinogen but not feline, canine, bovine, ovine, murine, or porcine fibrinogen.¹¹⁴ Intriguingly, Bbp has potent anticoagulant activity as binding of Bbp to fibrinogen has been shown to inhibit thrombin-induced fibrin formation, although the molecular mechanism is currently unknown.¹¹⁴ SdrE is an allelic variant of Bbp with ~67% amino acid sequence similarity in the fibrinogen-binding N2N3 region (P. Francis, MD, PhD, and M. Höök, PhD, unpublished results). SdrE binds to fibrinogen from a variety of species including human fibrinogen. In fact, the affinity of SdrE for fibrinogen from species other than human is much greater than for human fibrinogen (P. Francis and M. Höök, unpublished results). Functionally, Bbp and SdrE also bind complement regulator factor H and C4BP, resulting in inhibition of bacteria opsonization and killing by polymorphonuclear cells.^{115,116} The precise role of these factors in regard to fibrinogen binding and *S. aureus* virulence remains largely speculation.

Fibrinogen-Binding *S. aureus* SERAMs—SERAMs do not share a common domain organization and the mechanism(s) of fibrinogen binding used by these proteins remain largely unknown. Intrinsically disordered regions represent a significant part of each protein, and these disordered segments are important functionally.¹¹⁷ Each SERAM interacts with multiple ligands, with fibrinogen being common to each and all appear to contribute to *S. aureus* abscess formation.^{73,118,119}

Extracellular fibrinogen-binding protein (Efb) has two high-affinity fibrinogen-binding motifs in the disordered N-terminal portion of the molecule.¹²⁰ The exact residues mediating fibrinogen binding in this unusually extended motif have recently been identified.¹²¹ The fibrinogen D-fragment is sufficient for binding, but the binding motif appears to be distinct

from that of the ClfA/ $\alpha_{IIb}\beta_3$ -binding domains on the fibrinogen γ chain (Fig. 3).¹²⁰ Efb protects *S. aureus* by forming a protective “shield” containing fibrin (ogen) that prevents phagocytosis and innate immune cell recognition.¹²² This action requires Efb to bridge C3b bound to the bacterial surface and fibrinogen acquired from plasma.¹²² Efb prevents neutrophil binding to fibrinogen through a mechanism linked to the integrin receptor $\alpha_M\beta_2$ that binds fibrinogen.¹²⁰ Efb blockade of fibrinogen- $\alpha_M\beta_2$ interactions occurs through a noncompetitive mechanism and may be critical for limiting fibrin-mediated neutrophil activation and downstream antimicrobial responses.¹²⁰ Efb also binds to C3d and is a potent inhibitor of C3/C5 convertases, which prevents complement activation.^{123,124} In contrast to ClfA/B and FnBPA that promote platelet aggregation, Efb inhibits fibrinogen-mediated platelet aggregation, limits platelet antimicrobial function, and delays wound healing.^{125–128} Thus, through multiple fibrinogen-dependent and independent mechanisms, Efb counteracts host defense function.

Extracellular matrix-binding protein (Emp) and extracellular adherence protein (Eap) bind a broad spectrum of host matrix ligands. Emp engages fibrinogen, fibronectin, collagen, and vitronectin,¹²⁹ whereas Eap binds fibrinogen, fibronectin, collagen, vitronectin, and ICAM-1.¹³⁰ The structure of Emp is unresolved but is predicted by the disordered protein prediction program (disEMBL) to be intrinsically disordered (Y.-P. Ko and M. Hook, unpublished result), whereas Eap adopts an elongated but structured conformation in solution.¹³¹ Although Emp is secreted, it appears to function by noncovalently binding to the bacterial surface via a yet unknown mechanism that mediates adherence of *S. aureus* to extracellular matrix and host cells.¹²⁹ *S. aureus* mutants lacking Emp have a reduced ability to engage host matrix proteins¹²⁹ and are unable to form abscesses in animal models.¹¹⁹ Eap has a great degree of functional diversity with a well-established role in promoting *S. aureus* adherence to host matrix proteins.¹³² Eap also promotes bacterial invasion into eukaryotic cells,^{133,134} inhibits leukocyte recruitment,^{135–137} and impairs host wound healing and angiogenesis.¹³⁸ Eap inhibits complement activation by binding to C4b and preventing formation of C3 convertase,^{139,140} and Eap inhibits neutrophil serine proteases.¹³⁹ The precise relationship of fibrinogen to Eap-mediated *S. aureus* pathogenesis remains to be determined.

***S. aureus*-Driven Fibrin Polymerization**—*S. aureus* produces two factors that activate thrombin proteolytic activity, coagulase (Coa) and von Willebrand factor-binding protein (vWbp). These virulence factors activate prothrombin through insertion of the Ile¹-Val² N-terminus of the Coa D1D2 domain or Val¹-Val² N-terminus of the vWbp into the Ile¹⁶ pocket of prothrombin, inducing a conformational change and a functional serine protease.^{141,142} The resulting thrombin protease activity does not require proteolytic cleavage of prothrombin. The Coa (or vWbp)/thrombin complex has substrate specificity for fibrinogen over other thrombin targets.⁷⁸ Coa and vWbp fibrinogen binding occurs through distinct mechanisms. The primary fibrinogen-binding motif for Coa is in the carboxy-terminal domain (Fig. 3) and is composed of 27-residue-long tandem repeats, is a variant of the same motif found in Efb, and does not require the aid of prothrombin.^{77,143} Coa also has fibrinogen-binding activity in the amino-terminal domain, but the functional significance of this interaction requires further study.¹²¹ The vWbp protein appears to have multiple

fibrinogen-binding sites, but the primary fibrinogen-binding motif is located in the amino-terminal D1 and D2 domains of vWbp.¹⁴⁴ vWbp binding to fibrinogen is required to support high-affinity prothrombin binding and induce vWbp/prothrombin protease activity.¹⁴² These distinct mechanisms support the concept that the two coagulases are required for unique aspects of pathogen virulence. Indeed, in vitro studies suggest that Coa and vWbp play distinct roles in forming a fibrin(ogen) protective shield around *S. aureus*. Coa mediates formation of a fibrin(ogen)-containing inner pseudocapsule that envelopes the staphylococcal microcolony, whereas vWbp contributes to an extended outer dense microcolony-associated meshwork.⁷⁹ Both fibrin(ogen)-containing structures exert barrier function against neutrophil migration and protect staphylococci against phagocytosis.⁷⁹

***S. aureus*-Driven Fibrinolysis**—*S. aureus* has also evolved mechanisms for activating and exploiting the host fibrinolytic system. SAK is a secreted factor that binds with high affinity to plasmin present in trace amounts in plasma.¹⁴⁵ The resulting SAK–plasmin complex serves as a plasminogen activator by cleaving additional plasminogen into active plasmin. SAK-driven plasmin activity readily degrades fibrin, but also neutralizes the bactericidal effects of α -defensins secreted from polymorphonuclear cells.¹⁴⁶ SAK appears to be particularly important for the establishment of *S. aureus* skin infections,^{147,148} but other potential roles of SAK in staphylococcal virulence remain unresolved. However, high SAK production does negatively correlate with host mortality during staphylococcal bacteremia.^{149–151} SAK production by the bacteria increases significantly in the late-exponential phase of *S. aureus* growth.¹⁵² Current thinking suggests the upregulation of SAK tips the *S. aureus* coagulation balance toward fibrinolysis to promote dissemination to new host sites.

Fibrinogen as a Target of Virulence Factors in *S. aureus* Infection

Studies in animal model systems have provided powerful evidence on the potential of *S. aureus* virulence factors targeting host fibrinogen to promote pathogenicity. Coa and vWbp were shown to be potent virulence factors in animal models of infectious diseases.^{73,153–155} In a mouse model of *S. aureus* abscess formation, fibrin(ogen) colocalized with Coa and vWbp within abscess lesions to form a protective border preventing phagocytes from accessing and clearing bacteria.⁷³ *S. aureus* mutants lacking Coa and vWbp are unable to form abscesses⁷³ and inhibition of Coa and vWbp together with ClfA was effective in treating murine sepsis.⁷⁶ Similarly, Coa/vWbp-deficient *S. aureus* or pharmacological inhibition of thrombin activity reduced bacterial load in a mouse model of jugular vein catheter infection.¹⁵⁶ The mechanism by which Coa and vWbp promote *S. aureus* virulence appears to be linked to (pro)thrombin proteolytic activity. Mice constitutively expressing 10% of normal prothrombin levels were shown to have a superior survival profile compared with WT mice following intravenous *S. aureus* challenge.¹⁵⁷ Similarly, inhibition of Coa (or vWbp):prothrombin complex activity with the oral thrombin inhibitor dabigatran reduced bacterial survival and limited abscess development in mice following intradermal infection.¹⁵³

The exploitation of host fibrinogen in the context of bacteremia challenge has also been well documented in mouse studies. Mice challenged intravenously with *S. aureus* expressing a

non-fibrinogen-binding mutant of ClfA (ClfA P₃₃₆S/Y₃₃₈A; ClfA^{PY}) have diminished systemic inflammation, less septic arthritis, and reduced septic death compared with mice challenged with WT *S. aureus*.¹⁵⁸ Furthermore, analyses of Fibγ⁵ gene-targeted mice expressing a mutant form of fibrinogen lacking the final five amino acids of the γ chain, and thus lacking the ClfA binding motif, support an essential role of ClfA–fibrinogen binding in driving virulence.¹⁵⁷ Fibγ⁵ mice exhibit a significant survival advantage over WT mice following intravenous *S. aureus* challenge.¹⁵⁷ The survival advantage of Fibγ⁵ mice was accompanied by reduced bacterial burdens in the tissues, a blunted proinflammatory cytokine response, and diminished tissue and organ damage. Reduced mortality in Fibγ⁵ mice was independent of platelet integrin α_{IIB}β₃-mediated engagement of fibrinogen as integrin α_{IIB}^{−/−} mice or platelet depletion did not confer a similar host survival advantage.¹⁵⁷ In line with these observations, induced expression of ClfA on nonpathogenic *Lactococcus lactis* renders this organism a potent killer in murine bacteremia.¹⁵⁹ The lethal effect of *L. lactis*: ClfA is dependent on its fibrinogen-binding activity as expression of non-fibrinogen-binding ClfAY₃₃₈A by *L. lactis* does not drive host mortality. Similarly, active or passive immunization with ClfA or ClfA-directed antibodies is protective against mice challenged with *S. aureus* septicemia.^{158,159}

Fibrin(ogen) in Host Defense and Pathogen Virulence: Unanswered Questions

Significant work remains to further elucidate the roles of fibrinogen in both antimicrobial function and bacterial virulence. That both thrombin-mediated polymer formation and the fibrin α_Mβ₂-binding motif support host defense is consistent with the concept that soluble fibrinogen is a poor ligand for α_Mβ₂, whereas fibrin polymer (or immobilized fibrinogen) is a strong ligand for this leukocyte integrin receptor.^{160,161} Recent independent studies have shown that factor XIII (FXIII) binds the fibrinogen γ^{390–396} region¹⁶² and that FXIII can influence bacterial virulence/host defense.¹⁶³ However, our preliminary studies indicate that FXIII catalytic A subunit-deficient mice clear *S. aureus* from the peritoneal cavity in a manner identical to WT mice, suggesting the phenotype observed in Fibγ^{390–396A} mice is not linked to FXIII (M. Flick, PhD, and J. Degen, PhD, unpublished results). A central unanswered question is the identity of the α_Mβ₂-expressing cell(s) participating in pathogen clearance.

The Fib^{AEK} mice studies suggest that Fp release and/or polymer formation is required to support *S. aureus* clearance from the peritoneal cavity. One possibility is that Fps themselves contribute to the *S. aureus* clearance as both FpA and FpB have antimicrobial activity against *S. aureus* and other pathogens.¹⁶⁴ Alternatively, fibrin formation may bridge bacteria with host immune cells. Similarly, the mechanisms by which soluble fibrinogen limits host mortality at high infection doses are also not yet defined. It is possible that at high levels of *S. aureus* soluble fibrinogen promotes bacterial aggregation and simply impedes dissemination out of the peritoneal cavity to distant organs.^{68,77} Alternatively, interaction of soluble fibrinogen with host platelets or inflammatory cells (i.e., neutrophils and macrophages) may support fibrinogen-mediated platelet interactions and platelet-linked bacterial killing mechanisms.^{165–169}

Significant work also remains in defining the contribution of fibrin(ogen)-targeted virulence factors to bacterial pathogenesis. The findings that fibrinogen promotes bacterial killing in one infection context (i.e., peritonitis) but bacterial virulence in another context (i.e., bacteremia) strongly suggest bacterial virulence factors have adapted to function in unique host microenvironments. Although, clear progress has been made to identify roles for ClfA, Efb, Coa, and vWbp, the contribution of other fibrinogen-binding virulence factors to pathogenicity remains to be determined. Understanding the interplay of the different virulence factors with respect to fibrinogen (e.g., fibrin-forming in relation to fibrinolytic virulence factors), and the role these factors play in different infection contexts, is of particular interest.

A critical unanswered question is whether findings derived from in vitro analyses and, more notably, from animal model systems can relate to human patients with bacterial infections. Current data strongly support the notion that *S. aureus* factors targeting host coagulation system components significantly enhance pathogen virulence. Expression of coagulase by *S. aureus* has long been considered a good predictor of pathogenicity.¹⁷⁰ ClfA is produced by virtually all *S. aureus* clinical strains.¹⁷¹ Thus, targeting bacterial virulence factors holds great promise as a potential therapeutic strategy. Indeed, ClfA has shown potent efficacy as a vaccine component in active immunization and passive immunization studies in animal models of septic arthritis, sepsis, abscess formation, and endocarditis.^{96,98,158,159,172} Accordingly, ClfA has been considered a promising vaccine target candidate for patients and has been evaluated in several clinical trials. However, neither active immunization nor passive immunization has shown protection in these clinical trials, and the trials were stopped or suspended in phase 2 or 3 due to lack of efficacy.¹⁷³ A potential explanation for the success in animals but failure in human trials is, as outlined here, that *S. aureus* express numerous proteins that engage human fibrinogen but only a subset of these factors engage fibrinogen from mice (and other species used as model systems). More advanced animal models expressing “humanized” fibrinogen and/or other coagulation components will provide a greater means to better evaluate and dissect the spectrum of host–pathogen interactions as they relate to human disease.

The role of fibrinogen as an antimicrobial factor in patients with *S. aureus* remains largely unknown and certainly untested. To date, there are no studies suggesting patients with hypofibrinogenemia or even afibrinogenemia are at an increased risk of developing a bacterial infection. Notably, this absence of data is consistent with mouse models of fibrinogen deficiency. Over the 20 years since fibrinogen-deficient mice were first described, there has been no report indicating these mice develop spontaneous bacterial infections. However, as described here, in animal models fibrin matrices can be a powerful antimicrobial host defense factor limiting bacterial dissemination or mediating bacterial killing following an aggressive infection challenge. Whether fibrin functions in a similar manner in patients with an established infection remains to be determined. Previous studies documented that patients with afibrinogenemia have a diminished response in a delayed-type hypersensitivity reaction with bacterial antigens.¹⁷⁴ Further, hypofibrinogenemia has been noted in case reports as associated with severe bacterial infection,^{175,176} including *S. aureus* infection.¹⁷⁷ The consumption of fibrinogen that accompanies DIC significantly increases the chance of mortality in septic patients. Although the primary contributing factors to poor

outcome in septic DIC are increased risk of hemorrhage, loss of vascular integrity, and shock, it is possible that in certain cases the loss of fibrinogen also drives bacterial expansion and dissemination due to loss of fibrin-mediated antimicrobial function. Whether the loss of fibrinogen, be it congenital or acquired, indeed does promote bacterial growth and dissemination in patients with severe bacterial infections remains to be formally determined.

Conclusion

An increasingly persuasive body of evidence suggests that fibrinogen is positioned at the intersection of host defense and pathogen virulence. The capacity of fibrinogen to combat invading host bacterial pathogens has been countered by the evolution of bacterial virulence factors, which take advantage of the enzymatic and structural properties of coagulation system components to both limit host antimicrobial responses and in fact promote pathogen proliferation and dissemination. Thus, further defining the mechanisms of host coagulation factor and bacterial virulence factor interactions as well as developing strategies to exploit the potential of fibrinogen as an antimicrobial factor should point the way to novel therapeutic strategies for treating a broad spectrum of infectious diseases.

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Fig. 1.

Fibrinogen-binding *S. aureus* microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). Schematic presentation of the domain organization of the fibrinogen-binding *S. aureus* MSCRAMMs. The A region contains three separately folded domains, which are known as N1, N2, and N3 subdomains. N2 and N3 subdomains structurally form immunoglobulin G-like folds that bind fibrinogen by the “dock, lock and latch” mechanism. The *S. aureus* surface protein SdrE and bone sialoprotein-binding protein (Bbp) contain additional three B repeats that are located between the A region and the serine-aspartate repeat region. Fibronectin-binding protein A (FnBPA) and FnBPB have A regions that are structurally and functionally similar to the A region of the Clf or Sdr group and that is followed by tandemly repeated fibronectin-binding domains (11 in FnBPA, 10 in FnBPB).

Fig. 2.

Fibrinogen-binding *S. aureus* secreted expanded repertoire adhesive molecules (SERAMs). Schematic presentation of the domain organization of the fibrinogen-binding *S. aureus* SERAMs. The N-terminal intrinsically disordered region of extracellular fibrinogen-binding protein (Efb) harbors two fibrinogen-binding sites, whereas the C-terminal ordered region binds complement component C3. Extracellular matrix-binding protein (Emp) overall does not adopt a defined structure. Extracellular adherence protein (Eap) harbors six repeated domains (1–6) of approximately ~97 amino acids. Coagulase (Coa) and von Willebrand factor-binding protein (vWbp) are homologs that both contain D1D2 domains that activate prothrombin without proteolytic cleavage. The C-terminal part of Coa contains tandem repeats (R) of 27 amino acids and harbors predominant fibrinogen-binding sites. The numbers of repeats vary from five to eight among different strains of *S. aureus*. The C-terminal of vWbp contains von Willebrand factor-binding site of 26 amino acids.

Fig. 3.

Identification of the binding sites for *S. aureus* fibrinogen directed MSCRAMMs. Clumping factor A (ClfA), Fibronectin-binding protein A, and B (FnBPA and FnBPB) recognize the C-terminal of the fibrinogen γ chain, whereas clumping factor (ClfB) binds to C-terminal of the α chain. Bbp binds to C-terminus of the α chain. MSCRAMMs, microbial surface components recognizing adhesive matrix molecules.