Inhibition of Complement Activation by a Secreted *Staphylococcus aureus* Protein

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*Staphylococcus aureus* can cause a variety of acute and chronic diseases. The ability of *S. aureus* to cause persistent infections has been linked to its ability to evade or inactivate host immune responses. We have identified a secreted 19-kDa protein produced by *S. aureus* that binds to the complement protein C3. N-terminal sequencing of this protein identified it as the extracellular fibrinogen-binding protein (Efb). In this study, we demonstrate that Efb can bind to the α-chain of C3 and inhibit both the classical and alternative pathways of complement activation. In addition, we show that Efb can inhibit complement-mediated opsonophagocytosis in a dose-dependent manner and that Efb inhibits complement activity by blocking deposition of C3 or by preventing further complement activation beyond C3b. These data suggest that Efb is a virulence factor involved in facilitating persistent *S. aureus* infections by interfering with complement activity in vivo.
septicemia and septic arthritis, mice that were complement depleted by treatment with cobra venom factor presented with significantly increased disease severity, suggesting a role for complement in controlling hematogenously acquired *S. aureus* infections [23].

Considering the ability of *S. aureus* to cause persistent infections, it is likely that *S. aureus* can counter complement activity. C3 plays a central role in all 3 complement pathways and is therefore a potential target for inactivation by the pathogen. We initiated a search for an *S. aureus* C3-binding protein and have identified a secreted 19-kDa protein that can bind to C3 in a Western ligand blot assay. N-terminal sequencing identified this protein as the *S. aureus* extracellular fibrinogen-bind- ing protein (Efb) [5, 24–27]. Efb is a constitutively secreted protein that not only binds fibrinogen but also can interfere with platelet aggregation and is hypothesized to play a role in delaying wound healing [27–30]. The present report supports a role for Efb as an inhibitor of complement activation and suggests that Efb may play a role as a virulence factor in persistent *S. aureus* infections.

**MATERIALS AND METHODS**

**Cloning of the efb and sa1755 genes from *S. aureus* strain Newman.** The *efb* and the *sa1755* genes, excluding the 5′ sequence encoding the signal peptide, were amplified by use of polymerase chain reaction (PCR), with *S. aureus* strain Newman DNA as a template. The following oligonucleotide primers were used: 5′-CGG GCA TCC CCA AGA GAA AAG AAA CCA GTG AGT A-3′ (forward primer) and 5′-AAC TGC AGC TAG TAT GCA TAT TCA TTA-3′ (reverse primer) and 5′-CGG GCA TCC CCG TTT CCT ACA AAT GAA GAA-3′ (forward primer) and 5′-AAC TGC AGA GTT TTA TTT AAC TAA TCC TGG-3′ (reverse primer) and 5′-CGG GCA TCC CCG TTT CCT ACA AAT GAA GAA-3′ (forward primer) and 5′-AAC TGC AGA GTT TTA TTT AAC TAA TCC TGG-3′ (reverse primer) (IDT), for *efb* and *sa1755*, respectively. The resulting PCR amplifications were subsequently cloned by use of the TA Expression Kit into the pCRT7/NT-TOPO expression vector (both from Invitrogen) and were designated as pCRT7/NT-Efb or pCRT7/NT-Sa1755, respectively. Nucleotide sequencing of *efb* and *sa1755* were performed by automated sequencing (Molecular Genetics Core Facility, University of Texas–Houston Medical School).

**Expression and purification of recombinant proteins.** The *S. aureus* proteins Efb, SA1755, and major histocompatibility complex analog protein (Map) 19 [12] and the *Staphylococcus epidermidis* fibrinogen-binding protein (SdrG) [31] were expressed as recombinant N-terminal His-tagged proteins, which allowed for purification using metal ion–chelating chromatography, as described elsewhere [12, 32]. Map19 and SdrG were expressed by use of the pQE30 (QIAGEN) expression vector in *Escherichia coli* (JM101; Stratagene), and Efb and SA1755 were expressed by use of the pCRT7/NT-TOPO (Invitrogen) expression vector in *E. coli* (BL21) harboring the corresponding plasmids. Proteins were expressed and purified as described elsewhere [32, 33]. Protein concentrations were determined by use of the bicinchoninic acid (BCA) protein assay (Pierce), and proteins were stored at −20°C until use.

**Western ligand blotting.** *E. coli* strain JM101, *Staphylococcus carnosus* strain TM300, and *S. aureus* strain Newman were grown under constant shaking conditions overnight at 37°C in 5 mL of Lennox broth (Sigma-Aldrich), as described elsewhere [12, 34]. Bacteria were washed in PBS, and total protein was quantified by use of BCA (Pierce). Bacteria (20 μg), *S. aureus* supernatants from overnight cultures (20 μL), recombinant proteins (4 μg each), C3b or C3 (2 μg) (Advanced Research Technologies), human fibrinogen (2 μg) [31], or human serum (20 μL of a 1:20 dilution in PBS) (Diamedix) were subjected to SDS-PAGE and examined by staining with 0.05% Coomassie brilliant blue or were electrotransferred to a 0.45-μm Immobilon-P polyvinylidene fluoride (PVDF) membrane (Millipore), as described elsewhere [34].

Membranes subjected to Western blot analysis were blocked overnight at 4°C in 5% nonfat dry milk in TBST (0.15 mol/L NaCl, 20 mmol/L Tris-HCl, and 0.05% Tween 20 [Sigma-Aldrich] [pH 7.4]), were probed accordingly, and then were developed with 10 mL of 1-Step nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate solution (Pierce). All incubations were performed in 15 mL of 1% TBST for 1 h at room temperature, with shaking, and membranes were washed in TBST between all steps. Labeling with digoxigenin was performed as described elsewhere, in accordance with the manufacturer’s instructions [34]. N-terminal sequencing was performed at the Protein Chemistry Laboratory of Texas A&M University, College Station.

**ELISA-type binding assays.** Immulon-1 microtiter plate wells (Dynatech Laboratories) were coated with 0.25 μg of either C3b or fibrinogen in 50 μL of PBS for 2 h. The plates were washed and blocked with 200 μL of Super Block (Pierce) for 1 h. Recombinant proteins (0–20 μmol/L [at a final volume of 100 μL/well]) were added to the wells and incubated for 1 h. In the next step, 100 μL of anti-His antibodies (1:5000) were added and incubated for 1 h, followed by addition of 100 μL of goat anti–mouse alkaline phosphatase (AP)–conjugated antibodies (1:5000). Next, 100 μL of a 1 mg/mL Sigma 104 phosphatase substrate (Sigma) dissolved in 1 mol/L diethanolamine and 0.5 mmol/L MgCl2 (pH 9.8) was added, and the plates were allowed to develop for 1 h. Plates were read at 405 nm by use of a microplate reader (Molecular Devices). Plates were washed between all steps, with PBS–0.05% Tween 20, and all incubations took place at 37°C. All dilutions were made using Super Block, unless otherwise specified.

**Complement-activity assays.** The EZ Complement CH50 clinical diagnostic assay kit (Diamedix) was used to evaluate the effects of recombinant Efb on activation of the classical
pathway and was used as described by the manufacturer. In brief, human serum (5 μL of complement reference serum) was incubated in the presence of various doses of Efb (0.13–13 μmol/L) or in the presence of SdrG (13 or 20 μmol/L), at a final volume of 20 μL, at 37°C for 1 h, before a 1-h incubation at room temperature with antibody-coated sheep red blood cells (RBCs) (3 mL). Control high and low reference serum samples (5 μL) were incubated with 15 μL of PBS in the same fashion as were test serum samples, before incubations with antibody-coated sheep RBCs. After incubation, cells were centrifuged (800 g for 10 min), and the absorbance of the supernatants (150 μL) was measured at 405 nm by use of a microplate reader, as described above, to determine the percentage lysis of each sample. The data are expressed as percentage lysis of the standard reference serum, and the values were derived by use of the following equation: (absorbance of sample/absorbance of reference) × CH50 value of reference (Diamedix).

Activation of the alternative pathway was measured by incubating the recombinant proteins Efb or SdrG (at the concentrations described above) with 10 μL of standard reference serum (Diamedix) for 1 h at 37°C. To each recombinant protein/serum mixture, 10 μL of gelatin-veronal buffer (GVB; Advanced Research Technologies) [35], 10 μL of Mg2+/EGTA (ethylene glycol-bis[β-aminoethyl ether]-N,N,N′,N′-tetra acetic acid; 100 mmol/L), and 30 μL of a 5 × 10⁴ cells/mL rabbit RBC stock (Advanced Research Technologies) were added, and the mixtures were incubated for 30 min at 37°C. Reactions were stopped by adding 1 mL of ice-cold GVB.

Opsonophagocytic assay (OPA) and fluorescence-activated cell sorter (FACS) analysis. This method was based on previously reported techniques for evaluation of opsonophagocytic activity by use of antigen-coated fluorescent beads [36–41]. Clumping factor A (ClfA) target antigen (Inhibitex) [42, 43] was coated on 1-μm diameter Flouresbrite yellow green (YG) carboxylate microspheres (Polysciences), by use of the manufacturer’s Carbodiimide Kit. Protein adsorption to the beads was verified by measuring the concentration of free protein remaining in the supernatant after antigen coupling, by use of a BCA total protein assay (Pierce). A human promyelocytic leukemia cell line (HL-60) [37], cultured under conditions promoting granulocytic differentiation [38], was used as the phagocytic effector cell (Flow Applications). The cell concentration was adjusted with Hank’s balanced salt solution and 1% bovine serum albumin (OPA buffer). For each OPA, 1 × 10⁶ antigen-coated microspheres were opsonized with 0.3 μg/mL anti-ClfA antibody for 30 min at 37°C, in OPA buffer. A total of 25 μL of a 1:5 dilution of sterile rabbit complement (Flow Applications) was mixed with an equal volume of Efb, at various dilutions covering a range of concentrations. Complement and Efb were incubated together for 30 min at room temperature. The complement/Efb mixtures were then added to the antibody-coated beads and were incubated, with shaking, for an additional 15 min at 37°C. The opsonized microspheres were subsequently washed, resuspended in OPA buffer, and mixed with 4 × 10⁴ HL-60 cells. The microspheres and cells were incubated together, with shaking, for 30 min at 37°C. Ice-cold OPA buffer was then added, and the samples were kept on ice until analyzed on a FACSCalibur flow cytometer (BD Biosciences). Data were collected from 1500 viable cells, by use of the FL1 photomultiplier (transmittance at 530 nm), after excitation with a 488-nm argon-ion laser. Electronic gating was used to analyze
uptake of beads by phagocytic cells. Data are expressed as the percentage of HL-60 cells with positive fluorescence. To determine the mechanism of Efb-mediated complement inactivation, the alternative complement–activity assay, in conjunction with flow cytometry, was performed to assess the deposition of C3b on the surface of rabbit RBCs. The alternative complement–activity assay was performed as described above, by use of 25 µL of Efb (20 µmol/L), SdrG (20 µmol/L), or PBS mixed with 10 µL of standard reference serum (Diamedix) and incubated for 30 min at 37°C. Each protein mixture was added to 1.5 × 10⁷ rabbit RBCs (Advanced Research Technologies), 10 µL of GVB (Advanced Research Technologies), and 10 µL of Mg²⁺/EGTA (100 mmol/L [pH 7.4]), and the mixtures were incubated for 15 min at 37°C. To quench the reactions, 1 mL of ice-cold GVB was added to each RBC mixture. A total of 3 × 10⁶ cells were removed from each RBC mixture, to assess the deposition of C3 on the surface of the cells by use of flow cytometry. In brief, RBCs were incubated with a 1:50 dilution of a fluorescein isothiocyanate (FITC)–conjugated polyclonal anti-human C3 antibody for 30 min on ice. Samples were analyzed on a FACSCalibur flow cytometer (BD Biosciences). Viable cells were gated, and data were collected from 5000 events.

**Antibodies.** The following antibodies were used: monoclonal mouse (IgG2a) anti-His (Amersham Pharmacia), AP-conjugated goat anti-mouse (whole IgG; ICN/Cappel), AP-conjugated anti-digoxigenin (Fab fragment; Roche Diagnostic), AP-conjugated avidin (ICN/Cappel), biotin-labeled chicken polyclonal anti-human C3 (Accurate Chemical and Scientific), FITC-conjugated polyclonal sheep anti-human C3 (Accurate Chemical and Scientific), and anti-C1F (Aurexis; Inhibetix).

**RESULTS**

**Secretion of a C3-binding protein.** To identify putative C3-binding proteins, whole-cell lysates from *E. coli*, *S. carnosus*, *S. aureus*, or *S. aureus* supernatants were subjected to SDS-PAGE and were stained with Coomassie brilliant blue (figure 1A) or were transferred to a PVDF membrane and probed with digoxigenin-labeled C3b (figure 1B). A 19-kDa band from *S. aureus* was the most prominent C3-binding component (figure 1B). Additional C3b-binding components, of lower molecular weight, which may represent degradation products of the 19-kDa protein, were also detected in these lanes. No C3b binding was detected in the lysates from *E. coli* or *S. carnosus* (figure 1B). C3b, which contains a hydrolyzed thioester bond, and not C3, was used as a probe, to demonstrate that binding to bacterial proteins was independent of the thioester conformation. Blots probed with digoxigenin-labeled C3 revealed similar staining, and blots probed with an AP-labeled secondary antibody alone revealed no color change after the addition of substrate (data not shown). All subsequent assays were performed with C3b or digoxigenin-labeled C3b.

**Identification of the *S. aureus* C3-binding protein.** The C3b-binding proteins identified by Western ligand blot analysis of whole *S. aureus* and *S. aureus* supernatants fractionated by SDS-PAGE were compared with protein bands of similar molecular weight identified on Coomassie brilliant blue–stained PVDF membranes supporting the same materials. Two candidate protein bands were selected from the stained PVDF membranes and submitted for N-terminal sequencing. The 2 candidate *S. aureus* C3b-binding proteins were identified as Efb (Entrez Protein accession number Q08691) and SA1755 (Entrez Protein accession number E89983). Recombinant forms of Efb and SA1755, with N-terminal His-tags, were cloned, expressed, and purified (figure 2A). The binding of these proteins to C3b and fibrinogen was examined by use of Western ligand blot analysis of membrane-bound C3b. Extracellular fibrinogen-binding protein (Efb) binds to membrane-bound C3b. Lane 1, C3b; lane 2, human fibrinogen; lane 3, human serum. Proteins and serum were subjected to SDS-PAGE and stained with Coomassie brilliant blue (A) or transferred to polyvinylidene fluoride membranes (B–C). Membranes were probed with either 80 µg of Efb (B) or biotin-labeled chicken anti-human C3 antibodies (1:2000) (C), followed by a secondary incubation with either mouse anti-His antibodies (1:5000) (B) or avidin–alkaline phosphatase (AP) (1:10,000) (C). After a third incubation with anti-mouse AP–conjugated antibodies (B), the blots were developed.

**Figure 3.** Western ligand blot analysis of membrane-bound C3b. Extracellular fibrinogen-binding protein (Efb) binds to membrane-bound C3b. Lane 1, C3b; lane 2, human fibrinogen; lane 3, human serum. Proteins and serum were subjected to SDS-PAGE and stained with Coomassie brilliant blue (A) or transferred to polyvinylidene fluoride membranes (B–C). Membranes were probed with either 80 µg of Efb (B) or biotin-labeled chicken anti-human C3 antibodies (1:2000) (C), followed by a secondary incubation with either mouse anti-His antibodies (1:5000) (B) or avidin–alkaline phosphatase (AP) (1:10,000) (C). After a third incubation with anti-mouse AP–conjugated antibodies (B), the blots were developed.

**Figure 4.** ELISA-type assays of C3b-coated microtiter wells. Extracellular fibrinogen-binding protein (Efb) binds to C3b-coated microtiter wells. Efb or *Staphylococcus epidermidis* fibrinogen-binding protein (SdrG) was used to probe C3b-coated microtiter wells. Binding was detected by use of a primary monoclonal mouse anti-His, followed by a secondary incubation with a goat anti–mouse alkaline phosphatase–conjugated antibody. The data are expressed as the mean absorbance (405 nm) ± SE of the mean of triplicate samples.
Figure 5. Effect of extracellular fibrinogen-binding protein (Efb) on classical and alternative complement activation. Efb inhibits complement-mediated lysis of red blood cells (RBCs). The effects of Efb (10 μmol/L) or *Staphylococcus epidermidis* fibrinogen-binding protein (SdrG; 20 μmol/L) on complement activation were examined by use of different assays measuring the classical (A) or alternative (B) pathways of complement activation. The data are expressed as percentage lysis of RBCs of the complement standard reference serum. These experiments were performed 3 times, and the data are representative of all results.

Figure 6. Dose-dependent effect of extracellular fibrinogen-binding protein (Efb) on complement-mediated opsonophagocytosis. Determination of the MIC of Efb required for the inhibition of either the alternative or classical pathways was performed by testing Efb, at various doses (0.13–13 μmol/L), for complement-inhibitory activity. *Staphylococcus epidermidis* fibrinogen-binding protein (13 μmol/L) was used as a negative control and had no effect on complement-mediated lysis in either assay (data not shown). Spontaneous lysis for the alternative and classical assays was 18% and 19%, respectively, and is represented by the dashed line. Data are the percentage of lysis of red blood cells (RBCs) of the complement standard reference serum.
phagocytic activity of HL-60 cells that were cultured under conditions designed to generate terminally differentiated granulocytes. Fluorescent beads coupled to a target antigen (ClfA) were opsonized with antigen-specific antibody (anti-ClfA) and complement. The opsonized particles were then incubated with the granulocytes, and the percentage of fluorescently labeled cells was used as a measure of phagocytic activity. Phagocytic activity was inhibited in a concentration-dependent manner by Efb, whereas the control protein SdrG (at any concentration) had no effect on phagocytosis (figure 7). At the highest concentration of Efb used (62 μg/mL), a significant reduction in phagocytic activity, from 52% to 24%, was observed. Control reactions containing complement only or monoclonal anti-ClfA antibodies only, in the absence of complement, resulted in 20% and 8% fluorescent cells, respectively (figure 7).

**Analysis of cell-surface deposition of C3b.** We have demonstrated that Efb preincubated with complement inhibited both the classical and the alternative pathways, on the basis of the RBC lysis assays. This inhibition of complement activity could be facilitated by a number of possible mechanisms: (1) Efb could block deposition of C3b on the cell surface/activator surface, (2) Efb could interfere with the assembly of the convertases, or (3) Efb could enzymatically degrade C3b. Efb did not appear to degrade C3, since C3 incubated in the presence of Efb did not result in any discernible degradation products, after examination by SDS-PAGE (data not shown). To further define the mechanism of Efb-mediated inhibition of complement activation, the deposition of C3b on the cell surface of RBCs was assessed by use of flow cytometry with FITC-conjugated polyclonal antibodies to C3, after the activation of the alternative pathway. Deposition of C3b on RBC surfaces was undetectable if human serum was preincubated with Efb (20 μmol/L) (figure 8A). Conversely, deposition of C3b on RBC surfaces was detectable if serum was preincubated in the presence of SdrG (20 μmol/L) (figure 8B) or left untreated (figure 8C). This experiment did not rule out, however, the possibility that Efb-bound C3 is unrecognized by anti-C3 antibodies as a result of blocked antibody epitopes. This scenario is not likely, since anti-C3 antibodies used in an ELISA-type binding assay were not inhibited from binding plate-bound C3b in the presence of increasing concentrations of Efb (data not shown). The high signal intensity and the low number of cells staining positive for C3b can also be explained by the fact that the RBCs were examined by use of FACS analysis during conditions of continuous complement activation, and it is likely that many

![Figure 7](image1.png)

**Figure 7.** Effect of extracellular fibrinogen-binding protein (Efb) on complement-mediated opsonophagocytosis. Efb inhibits complement-mediated opsonophagocytosis. Terminally differentiated granulocytes were incubated with clumping factor A–coated fluorescent (fluorescein isothiocyanate) beads, using various conditions, and phagocytosis was measured by determining the percentage of HL60 cells positive after a 30-min incubation. The data presented here represent the mean percentage fluorescence of 2 separate experiments. SdrG, *Staphylococcus epidermidis* fibrinogen-binding protein.

![Figure 8](image2.png)

**Figure 8.** Effect of extracellular fibrinogen-binding protein (Efb) on cell-surface deposition of C3b. Efb blocks cell-surface deposition of C3. Cell-surface deposition of C3 on rabbit red blood cells (RBCs) was examined by use of fluorescence-activated cell sorter analysis using fluorescein isothiocyanate (FITC)–conjugated anti–human C3 antibodies, after RBCs were incubated with human serum preincubated with Efb (A), *Staphylococcus epidermidis* fibrinogen-binding protein (SdrG) (B), or human serum only (C). RBCs incubated in the absence of human serum were used as a background control and are represented as thin lines, and treatment groups (Efb, SdrG, or human serum only) are represented as thick lines.
cells were lysed during the FACS analysis. These data suggest that Efb inhibits complement activity by preventing C3b from binding to activator surfaces.

**DISCUSSION**

*S. aureus* uses various immune-evasion strategies: protein A and Map can interfere with humoral [45] and memory T cell responses [12], respectively, and super antigens (bacterial toxins) can nonspecifically activate up to 20% of naive T cells, resulting in the release of large quantities of cytokines, generating a clinical condition resembling septic shock [1].

The present study has described another *S. aureus* microbial immunomodulatory molecule (Efb) that binds to C3 and interferes with complement activation and C3-mediated opsonization. A large number of human pathogens have evolved mechanisms of complement evasion [17, 46, 47]. However, the list of human pathogens that can generate proteins that directly or indirectly interact with C3 is limited and can be divided into 2 principal functional groups: (1) surface-expressed C3-binding proteins that promote pathogen internalization into host cells and (2) C3-binding proteins that interfere with complement function. For example, *Mycobacterium leprae*, *Leishmania major*, and *Legionella pneumophila* use C3 as a ligand to facilitate their internalization into mononuclear cells via complement receptors CR1 and CR3 [48–50]. *Trypanosoma cruzi* produce an anchored protein, gp160, that interferes with complement activation by binding C3b and inhibiting formation of C3 convertase [51]. *Pseudomonas aeruginosa* (alkaline protease) and herpes simplex virus type 1 and type 2 (glycoprotein gC-1 and -2) can all generate C3-binding proteins with complement-inhibitory properties, and they function by either degrading C3 or accelerating the decay of C3 convertases, respectively [52, 53]. *Streptococcus pneumoniae*, *Chlamydia trachomatis*, and *Mycobacterium tuberculosis* produce C3-binding proteins, but their complement-inhibitory properties have not been defined [54–56].

Efb is the first complement-regulatory microbial immunomodulatory molecule identified from *S. aureus*, and, in conjunction with other *S. aureus* immune-evasion mechanisms, may further explain the capacity of *S. aureus* to cause persistent infections. Efb is unique from other C3-binding proteins and represents a new functional group of proteins that involve preventing C3 activation by directly preventing its binding to activator surfaces.

Capsule formation by *S. aureus* appears to play a key role in the protection against complement-mediated phagocytosis. Previous studies have shown that encapsulated (CP+) *S. aureus* strains have decreased phagocytic killing and are more virulent than strains of unencapsulated (CP−) *S. aureus* [57]. The diminished phagocytic susceptibility of CP+ *S. aureus* was due, in part, to a decreased deposition of C3 fragments on the cell surface of *S. aureus*, in addition to the masking of surface-bound C3 by the capsule, resulting in diminished complement receptor–mediated recognition by neutrophils [24, 58, 59]. However, capsule production occurs primarily during the later stages of bacterial growth, compared with Efb, which is constitutively expressed [27, 59]. We hypothesize that the complement-inhibitory properties of Efb may serve to neutralize complement activation initiated by *S. aureus* during the early stages of infection, when capsule production has not been initiated. The following findings suggest that Efb may play an important role in *S. aureus* survival and virulence, at least, in part, by diminishing or preventing complement activation during infection: (1) the relatively low concentrations of Efb required to inhibit complement activation (3 μmol/L), (2) Efb’s highly conserved nature among Efb+ *S. aureus* strains [60], and (3) the higher percentage of invasive isolates containing the efb determinant, compared with carriage isolates [61].

Efb represents a novel member of a growing family of *S. aureus* immunomodulatory proteins. Efb had been previously characterized as a fibrinogen-binding protein [44]. The significance of dual binding to both C3 and fibrinogen by Efb needs to be further examined, although it is becoming clear that many microbial immunomodulatory proteins can also serve as adhesins. It is not unusual for bacterial proteins to have multiple biological activities [4, 10–12, 62, 63], and these multifunctional proteins may contribute to the bacteria’s ability to colonize, survive, and persist in vastly different environments (e.g., skin, blood, kidney, or bone) and cause a broad range of diseases. For example, the secreted *S. aureus* Map has been described as a microbial surface component recognizing adhesive matrix molecules [62, 64, 65] and as a microbial immunomodulatory molecule [4, 12, 62], and the streptococcal M protein, which also binds fibrinogen, can also interfere with the alternative pathway, by mechanisms not clearly defined [63].

The data presented here suggest that inhibition of complement activity by Efb is mediated by blocking deposition of C3 to activator surfaces. A more detailed investigation further defining the mechanism of complement inactivation by Efb and determining the role of Efb in vivo, by use of animal models of *S. aureus* infection, is ongoing.

**References**


