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REVIEW *in* MICROBIOLOGY

Extracellular fibrinogen-binding protein (Efb), a key immune evasion protein of *Staphylococcus aureus* and a potential therapeutic target

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Due to the continued growth of antibiotic-resistant *Staphylococcus aureus* strains, it is necessary to explore alternative targets for future therapeutic applications. For this reason, is important to understand the staphylococcal immune evasion mechanisms with a special focus on extracellular fibrinogen-binding protein (Efb) and Efb related proteins. Therefore, it was conducted a literature review to compile relevant information on this protein. It was found that Efb has three binding sites with biological relevance that could be used as therapeutic targets with specificity for fibrinogen, platelets, and complements. First, the fibrinogen-binding motifs also found in coagulase block neutrophil αMβ2 adherence to fibrinogen and attract fibrinogen to the bacterial surface, forming capsule-like structures that block phagocytosis. Second, Efb is a potent anti-thrombotic agent, probably related to its P-selectin binding capacity. Efb P-selectin binding blocks the interaction of P-selectin with the PSGL-1 receptor, thereby impairs the mechanisms of platelet-mediated leukocyte recruitment to the site of vascular injury. Third, the Efb complement binding domain, also found in other staphylococcal complement inhibitory proteins like Ecb, Sbi, and SCIN, is responsible for the evasion of the complement-mediated immune response. Efb reduces the formation of C3 convertase and the interaction with neutrophils, affect B-cells activation, and maturation. Efb binding sites have a clear implication on the virulence of *Staphylococcus aureus* in mastitis, wound infection, staphylococcal pneumonia, and infections related to implanted devices, and contributes to staphylococcal persistence in host tissues and abscess formation in the kidneys. Given the biological relevance of Efb binding sites in staphylococcal infections, they are promising vaccine targets. Additionally, due to the inhibitory effect of Efb on platelets and complements, Efb can be a potential therapeutic agent to treat diseases associated with thrombosis and abnormal complement activity.

Keywords: Efb, fibrinogen-binding, platelet-binding, complement-binding, evasion immune system, *Staphylococcus aureus*.

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Introduction

Staphylococcus aureus has the ability to bind different types of host tissues causing a wide spectrum of diseases from mild wound infections to serious, life-threatening conditions such as osteomyelitis and endocarditis (1). The continuous growth of methicillin and multidrug-resistant S. aureus strains has become a serious problem in the treatment of *S. aureus* infections (2). Therefore, alternative treatment approaches are urgently needed. *S. aureus* escapes from the host immune responses using different proteins such as V8 (3), superantigens (4), protein A (5), hemolysins, and hyaluronidase (6,7). The extracellular fibrinogen-binding protein (Efb) (8–11) contributes to the evasion of the host defenses by disrupting multiple biological processes of the host. The primary objective of this review is to determine the biological effects of the different binding domains of Efb in staphylococcal infections and their potential as therapeutic targets.

Sources of information

An extensive literature review was done in the databases of PubMed related to *Staphylococcus aureus* Efb in combination with fibrinogen-binding, complement-binding, and platelet-binding. Articles of proteins related to Efb such as coagulase and complement inhibitory proteins were studied.

Efb background

Efb is produced by *S. aureus* strains, but not by other staphylococcal species like *Staphylococcus epidermidis*, *Staphylococcus hyicus*, *Staphylococcus lugdunensis*, or *Staphylococcus intermedius*. All *S. aureus* clinical isolates possess the *efb* gene and produce the Efb protein (12). Efb contains 165 amino acids, including a 29-amino-acid signal peptide that is cleaved as the protein is secreted and converted into a mature protein of 136 residues. This protein does not contain the LPXTG anchor motif that covalently attached cell surface protein to the bacterial cell wall. As the inactivation of the *efb* gene has no effect on bacterial attachment to immobilized fibrinogen (11) suggests that this protein is not involved in the direct binding of staphylococcal cells to fibrinogen. However, the biological function of Efb is related to its binding to fibrinogen, platelets, and components of the complement system.

Fibrinogen-binding

There are at least two fibrinogen-binding sites involved in the interaction between Efb and fibrinogen (9,10,13). Efb has two similar sequences of 22 amino acids at the N-terminal region that are responsible for fibrinogen-binding. Peptides containing the first (residues 30-67) or second repeat (residues 68-97) bind strongly to soluble fibrinogen and inhibit the binding of Efb to fibrinogen (9,10,13). Also, several residues in the second repeat of Efb (K68, H74, Y76, I78, F81, D83, T85, F86, Y88, and R91) seem to be crucial for fibrinogen-binding (13). A single Efb molecule can bind two different fibrinogen molecules simultaneously, and the combination of equimolar amounts of these two proteins led to the formation of a complex (9). The amino acid sequence revealed an EF-domain with Ca2+-binding motifs(10) which explains why calcium or zinc enhances the precipitation of the Efb-fibrinogen complex.

Coagulase is a protein similar to Efb regarding fibrinogen-binding. It possesses both prothrombin and coagulase activity at the N-terminal portion (residues 27 to 310) (14). However, the C-terminal part of coagulase (residues 484 to 636) contains several tandem repeats of around 27-residues which bind fibrinogen. These repeats contain fibrinogen-binding motifs found in the repeat regions of Efb (13,15,16) (Fig. 1), which recognize the same site on fibrinogen. Also, Efb repeat regions can compete with the corresponding repeat regions of coagulase for binding to fibrinogen (16). A peptide corresponding to residues 68 to 98 of Efb blocks coagulase binding to fibrinogen indicating that they bind to the same or overlapping site on fibrinogen (13). Furthermore, antibodies against Efb recognize coagulase and inhibit coagulase function in plasma (17). This indicates that Efb and coagulase have a common motif and similar functions related to fibrinogen binding. Also, five residues in the repeat regions of coagulase are crucial for coagulase binding to fibrinogen (N508, Y510, V512, Y522, and R525)(13).

Staphylococcus aureus produces several proteins in addition to Efb and coagulase that specifically recognize fibrinogen (18) including clumping factors A and B (19,20), the extracellular adherence protein (Eap) (21), Map (22), and von Willebrand factor-binding proteins (23). None of these proteins have the fibrinogen-binding motifs of Efb or

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coagulase (24). Interestingly, a part of coagulase fibrinogen-binding sites was identified in the nucleocapsid phosphoprotein (N-protein) of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (GenBank: QOQ16111.1). The region corresponding to the amino acid residues 261-271 of the N-protein has some similarity to the repeats of coagulase (GenBank: AKJ16095.1). The fibrinogen-binding capacity of the N-protein must be tested for potential biological function. It is tempting to think that the N-protein mimics the function of Efb and coagulase for fibrinogen-binding to facilitate the evasion of host defense by SARS-CoV-2 (25).

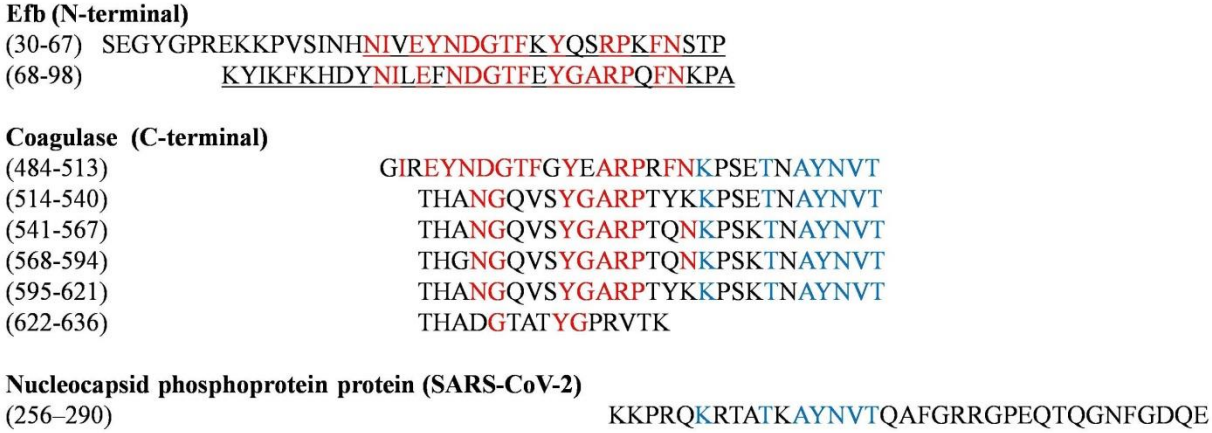

Fig. 1. Sequence similarities of proteins with Efb fibrinogen- binding motifs.
The N-terminal haft of Efb (GenBank: ADJ67155.1) is responsible for fibrinogen and P-selectin binding. This contains two nearly identical regions (underlined) that have high similarity with the fibrinogen-binding tandem repeats of coagulase (red)(GenBank: AKJ16095.1). The N- protein of SARS-CoV-2 (GenBank: QOQ16111) (residues 261–271) shares some sequence similarity with the repeat regions of coagulase (blue).

The Biological effect of the fibrinogen-binding sites.

Both Efb and coagulase inhibit the attachment of neutrophils and monocytes to fibrinogen by interfering with fibrinogen-binding to αMβ2 (26,27). The fibrinogen-binding sites of Efb are implicated in this inhibition because the N-terminal half of Efb, but not its C-terminal half, blocks the attachment. The fibrinogen-binding regions of Efb (residues 68 to 98) and coagulase (residues 506 to 532) effectively inhibit phagocytosis of neutrophils. Also, the inhibitory effect is caused by Efb binding to fibrinogen rather than binding to a component on neutrophil surface. Interesting, these interactions are unaffected by peptides containing only one fibrinogen-binding site, suggesting that more than one fibrinogen-binding site is necessary to obtain full inhibition. Furthermore, the fibrinogen-binding sites are essential for Efb or coagulase to produce capsule-like structures formed by fibrinogen around *S. aureus* (13,28). Also, Efb and coagulase are important for staphylococcal survival in the bloodstream, bacteremia, abscess formation, and persistence in host tissues (29).

Platelet-binding

Platelet aggregation is essentially mediated by fibrinogen, which binds to GPIIb/IIIa on activated platelets. There are two ways of interaction between Efb and platelets: one is dependent on the fibrinogen-GPIIb/IIIa interaction and the other is independent of fibrinogen [20] which is mediated by a receptor expressed on the surface of platelets that seems not to be GPIIb/IIIa (Fig. 2).

Fibrinogen dependent binding to platelets.

The expression of the fibrinogen receptor GPIIb/IIIa on the platelet surface is necessary for the aggregation of platelets, and only activated platelets are able to bind fibrinogen (30). Also, activated platelets adhere to each other by fibrinogen, which is a dimer that can bind two GPIIb/IIIa receptors, forming a bridge between platelets. Interestingly, one single platelet can display up to 45000 fibrinogen-binding sites when they are activated (31). Efb is an effective inhibitor of the aggregation of adenosine diphosphate activated platelets (16,32), even when increasing concentrations of Efb increase the level of

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fibrinogen-binding to activated platelets (16). This interaction could be neutralized with anti-GPIIb/IIIa antibodies, however, the interaction between Efb and washed thrombin-activated platelets is unchanged (32,33). It was speculated that the inhibitory effect may be caused by Efb fibrinogen-binding that may retain fibrinogen in a non-functional manner which results in the obstruction of normal platelet function or by Efb binding to a protein different from fibrinogen (16).

fibrinogen-dependent Efb binding and not the interaction between Efb and washed thrombin-activated platelets (32,33). This indicates that Efb triggers a new type of fibrinogen-binding to platelets that seems not to be implicated the GPIIb/IIIa receptor (32) or GPIb (34). A subsequent study revealed that the component recognized by Efb in platelets was P-selectin (33), an interaction that is independent of fibrinogen. P-selectin is a member of the selectin family of cell adhesion receptors that mediate the

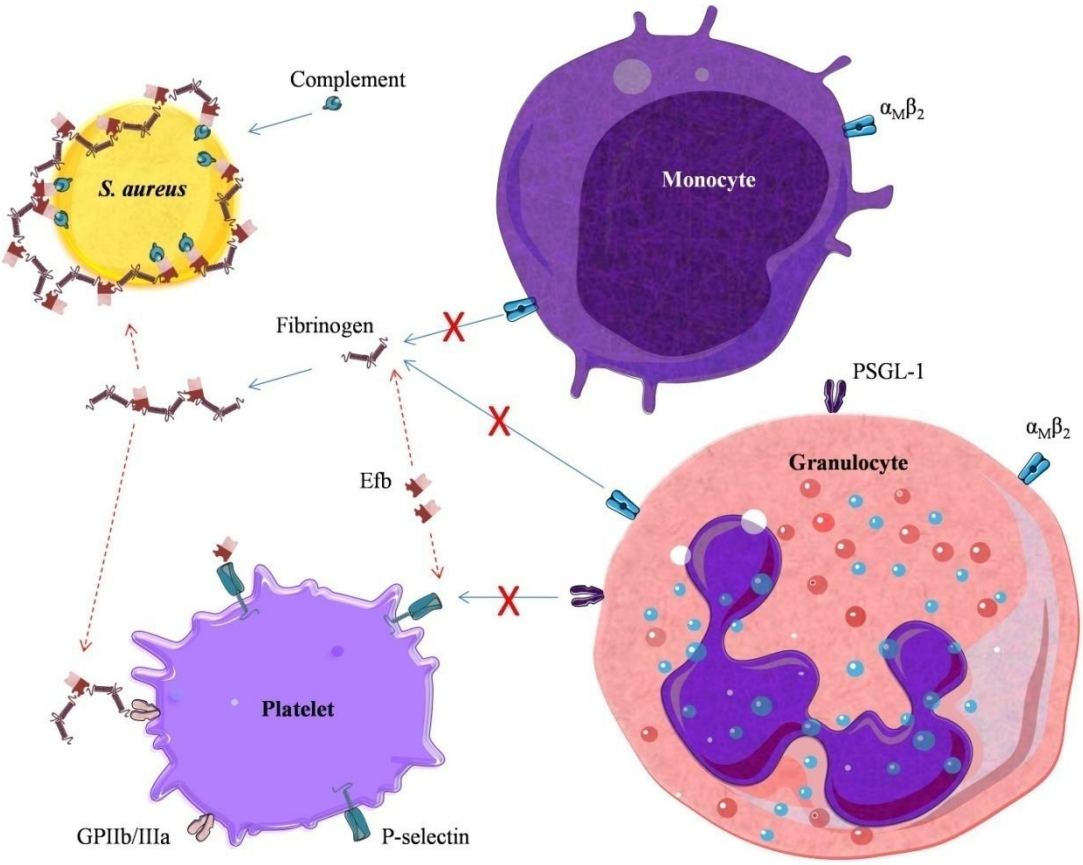

Fig. 2. Schematic representation of the interaction between Efb, fibrinogen, and platelets.
A single Efb molecule is capable of attaching at least two fibrinogen molecules simultaneously, leading to the precipitation of the Efb-fibrinogen complex. Binding to fibrinogen is essential for Efb or coagulase to produce capsule-like structures around bacteria that prevent phagocytosis. The interaction of Efb and platelets can be dependent or independent of fibrinogen. Efb stimulates fibrinogen binding to activated platelets, mediated by the integrin receptor GPIIb/IIIa. Efb recognized P-selectin on activated platelets, independently of fibrinogen, thus blocking the binding of P-selectin to the PSGL-1 receptor on granulocytes and affecting platelet aggregation. Both Efb and coagulase inhibit the attachment of fibrinogen to αMβ2 on neutrophils and monocytic cells.

Fibrinogen independent binding to platelets.
Efb binds to washed activated platelets depleted of fibrinogen, but not to those that are not activated. Also, antibodies against GPIIb/IIIa can only neutralize the

adhesion of neutrophils to platelets (35) and endothelial cells (36). P-selectin is the major component of the platelet alpha-granule membrane which is released when platelets are stimulated with thrombin (37) and can stabilize the initial platelet aggregation formed by

GPIIb/IIIa-fibrinogen interactions (38). Surprisingly, the expression of P-selectin was significantly decreased by the two repeats of Efb, probably associated with the inhibition of platelet aggregation or another effect caused by Efb binding. Also, the platelet binding site of Efb is located in the region with two repeats within the N-terminal domain of Efb (residues 37-98) and binds directly to platelets (16,32,33). Furthermore, an Efb fragment corresponding to residues 29-103 and 20 aa-peptide located within Efb-N between Lys68 and Glu87, interfere with the binding of P-selectin to the P-selectin glycoprotein ligand-1 (PSGL-1), thereby blocking the interaction of platelets with thrombin-stimulated leukocytes (33,39). This has a negative effect on platelet recruitment of leukocytes to sites of vascular damage (40). This occurs without exogenous fibrinogen, indicating that the mechanism of binding is independent of integrin GPIIb/IIIa. Finally, the inhibitory effect of Efb on platelet aggregation was studied in vivo which showed an increased survival of *S. aureus* in a mouse model of acute infection (41).

Other staphylococcal proteins that interact with platelets.

Efb is also an antagonist to several staphylococcal proteins that trigger platelet aggregation, such as clumping factor (42), fibronectin-binding protein A (43), and alpha-toxin (44). They induce platelet aggregation in the presence of fibrinogen and depending on the platelet receptor GPIIb/IIIa (42)(43). This is thought to contribute to the development of infective endocarditis (45,46) and liver injury during staphylococcal sepsis (44). For example, patients with coagulase-positive endocarditis showed increased aggregation of adenosine diphosphate stimulated platelet mediated by direct interaction between clumping factor and the adenosine diphosphate receptor or by indirect platelet pre-activation. (47). In contrast to these proteins, the bacterial lipoteichoic acid has an antiplatelet activity that may cause the bleedings in gram-positive septicemia (48). All these proteins with different effects on platelet aggregation are probably regulated differently and some of them have synergistic effects on hemostasis.

Efb as an antiplatelet agent in vivo.

The two repeats regions from the N-terminal portion of Efb significantly prolong the bleeding time in mice (49), caused by the direct impact of Efb on platelets and not on the coagulation cascade. In this model, acute thrombosis was induced by the intravenous injection of a combination of the powerful platelet activator collagen and the potent vasoconstrictor epinephrine (50). Moreover, this model has been useful for screening antithrombotic agents that act primarily by inhibiting platelet aggregation, leading to microcirculation in the lungs and ending with animal death. Also, this thrombotic effect could be prevented by administering the two repeats of Efb to the animals immediately before the thrombogenic stimulus (51). One in vivo study also showed that the inhibitory effect of the terminal portion of Efb on platelet aggregation also improves bacterial survival in acute infections in mice (41).

Complement-binding

Besides Efb, there are at least three proteins produced by *S. aureus* implicated in the evasion of the complement-mediated immune response: the extracellular complement binding protein (Ecb) (52), the staphylococcal binder of immunoglobulins (Sbi) (53,54), and the staphylococcal complement inhibitor (SCIN) (55). They share a common domain of three helices at the C3 binding region (55)(Fig. 3). In addition, the extracellular adherence protein (Eap) binds fibrinogen (21) and blocks the classical and lectin pathway of complement activation (56) but it has not the three-helix domain. The mechanisms of actions of the different *Staphylococcus aureus* complement inhibitory proteins are shown in Fig. 4.

The N-terminal half of the Efb (residues 35-120) binds to fibrinogen, whereas the other half of the protein (residues 98-165) recognizes the alpha-chain of C3 and its thioester-containing fragments (C3b, iC3b, C3d) (57,58). The Efb complement inhibitory domain (residues 98-165) is characterized by three alpha-helices (58–62) and contains two residues (R131 and N138) that are crucial to Efb affinity with the C3d domain of C3 and its inhibitory activity (57). The binding between Efb and C3d is dominated by electrostatic forces, and the region implicated in the binding is localized close to a highly conserved acidic pocket of C3d (61). Efb affects both the classical and alternative pathways of complement activation, phagocytosis, and cell-surface deposition of C3b (57).

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Ecb (known as Ehp) shares 44% amino acid identity with the complement inhibitory domain located in the C-terminal half of the Efb (52). Specific mutations of Ecb plasminogen simultaneously, which is then converted to plasmin by staphylokinase or urokinase-type plasminogen activator, causing subsequent degradation of surface-

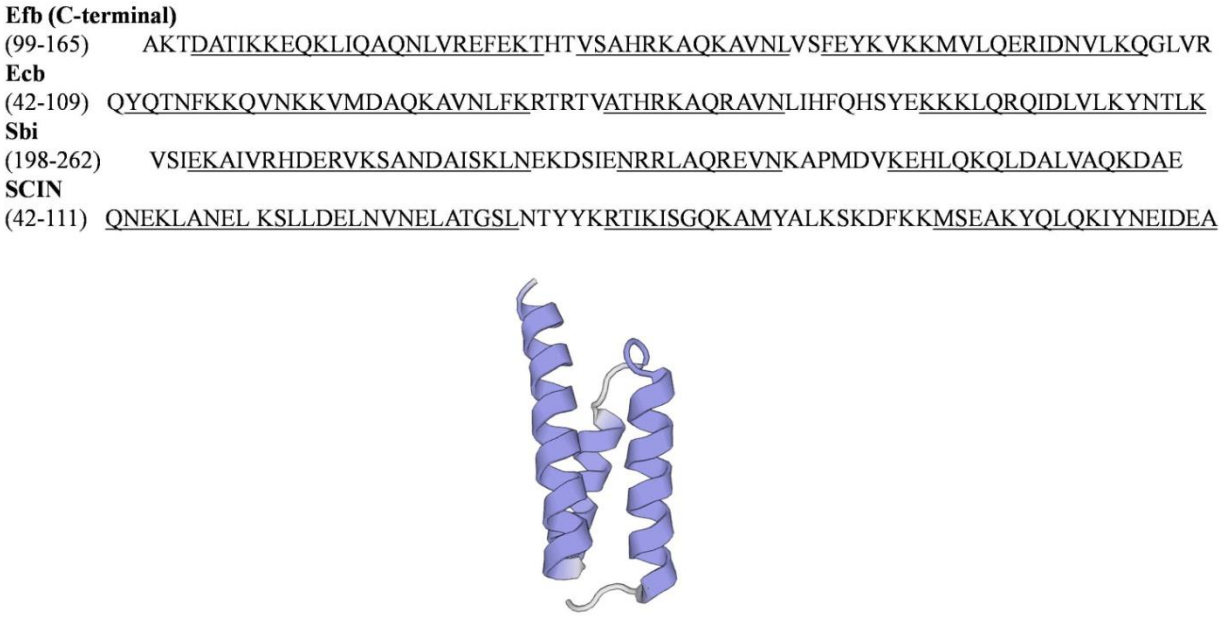

Fig. 3. Sequence and structural similarities of proteins with Efb C3-binding domains.
The C3 binding site is located at the C-terminal half of the Efb (GenBank: ADJ67155.1) (residues 98-165) which is composed of three alpha-helices (underlined), a typical structure of Staphylococcus aureus complement inhibitory proteins including Ecb (GenBank: ADL22954.1), Sbi (GenBank: EHO91427.1), and SCIN (GenBank: MBC3017125.1). The 3D structure prediction and modeling were done using SWISS-MODEL [92].

residues R75 and N82, corresponding to the two Efb residues (R131 and N138) (63) that are essential for C3 binding, did not completely abolish its binding to C3, suggesting a second binding site in Ecb (52). Two binding domains (residues 198-266) (64) with the two crucial residues R231 and N238 for binding to C3d(63) have also been identified in Sbi(65). SCIN was the first bacterial protein identified to target C3 convertases (66). The SCIN family consists of three members (SCIN-A, SCIN-B, and SCIN-C) that inhibit the complement activation and a putative inactive protein (ORF-D) (55).

Blocking or reduction of C3b deposition.

Efb binds to a loop region in the thioester-containing C3d domain of human native C3, impeding the conformation of the C3 molecule into a functional C3b opsonin (58). This was demonstrated by studying the deposition of C3b on the surface of rabbit red blood cells (RBCs) which was undetectable if human serum was pre-incubated with Efb (57). In addition, Efb and Sbi can interact with C3/C3b and bound C3/C3b (67).

Prevention of phagocytosis

The mechanism employed Efb to block phagocytosis is independent of complement inhibition. Fibrinogen is attracted to the surface of staphylococcal cells to form a capsule-like shield that not only allows bacteria to escape phagocytosis but also prevents C3b binding to its receptor; this essentially camouflages opsonins under the protection of fibrinogen (68). The dual interaction of Efb with C3b on the bacterial surface and fibrinogen effectively blocks neutrophil-mediated phagocytosis of *S. aureus*. However, the regions responsible for the two types of binding were not able to disrupt phagocytosis when they were in different protein fragments, indicating that the two binding sites are required in the same molecule to obtain full inhibition. The fibrinogen shield interferes with the recognition of C3b on bacterial surfaces by complement receptor (CR) 1 and IgG by Fc receptors. This blocks phagocytosis of *S. aureus* by human neutrophils

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which was demonstrated in vivo by a human whole blood model and a mouse peritonitis model (68).

Ecb builds aggregation with different types of C3 through interaction with their C3d domain. This results in the inhibition of C5 convertase and subsequent inhibition of neutrophil migration (69) and neutrophil adhesion to fibrinogen (70). The impact in vivo of Ecb on the C5a-dependent neutrophil recruitment to the site of infection (71) was demonstrated using a mouse model of immune complex peritonitis (72). In addition, the Ecb blocks the recognition of C3b by the neutrophil CR1 (73) and enhanced by the complement factor H which causes reduction of neutrophil phagocytosis of *S. aureus*, thereby protecting it from the host's immune system. This occurs because Ecb, C3b, and factor H form a complex that inhibits the activity of factor H. Normally, factor H interacts with C3b which with factor I cleaves C3b into iC3b (74). iC3b is covalently bound to the surface of the target cell and is therefore strategically positioned to work as phagocyte recognition molecules. However, the tripartite Ecb:C3d:factor H complex does not turn the deposited C3b on the surface of *S. aureus* into iC3b. Therefore, the consequence of the Ecb tripartite is the inhibition of complement activation, opsonization, and generation of the chemotactic components (75).

Sbi acts as a potent complement inhibitor that hinders the hemolytic activity of human serum by forming a tripartite complex with factor H and C3b. This was demonstrated in vivo in a hemolytic assay with rabbit erythrocytes which only blocked the alternative pathway

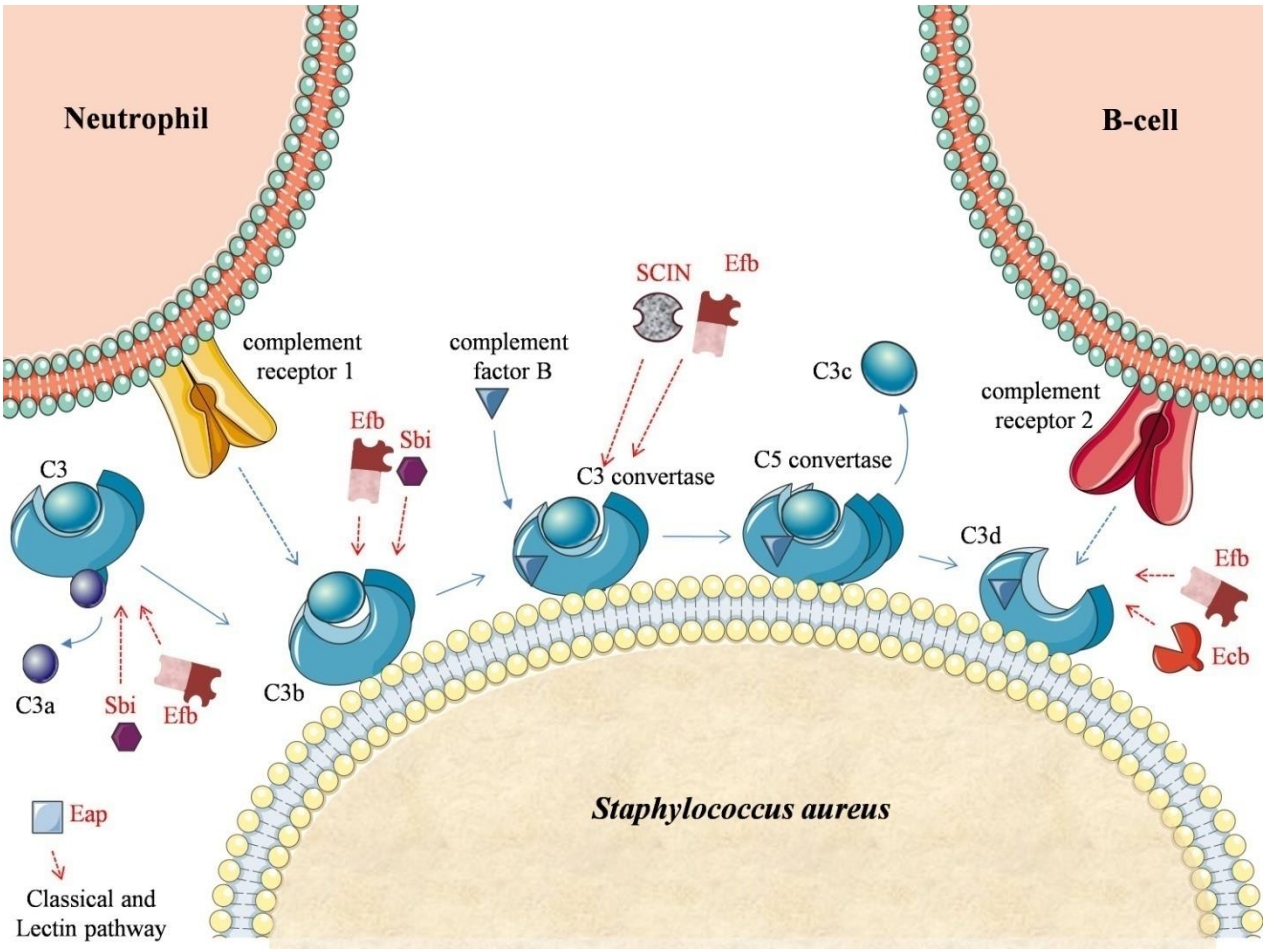

Fig. 4. Schematic representation of the interaction between S. aureus complement inhibitory proteins with elements of the complement system.
Efb binds C3 or C3b impairing the binding of complement factor B to C3b thereby the formation of C3 convertase. Also binding to C3b decreases C3b interaction with CR1 of neutrophils and C5 convertase conformation. Efb and Ecb bind C3d interfering with the interaction of C3d with the CR2 of B cells. Sbi blocks convertase activity and induces degradation of C3 and C3b. SCIN impairs the conversion of C3b into iC3b and blocks recognition of C3b by complement receptors. Eap blocks the classical and lectin pathway-dependent activation of C3 and the deposition of C3b.

while the classical and the lectin pathways were not affected (76).

SCIN competes with factor H to bind C3b which impairs the action factor H and factor I to degrade surface-bound C3b into iC3b. The binding site is located within the C3c domain of C3b since SCIN can bind C3b and iC3b, but not C3d, and it was blocked with anti-C3c antibodies (77). SCIN induces the formation of convertase dimers that block the recognition of C3b by complement receptors expressed on the surface of lymphoid and phagocytic cells (78).

Effect on the adaptive immune response.
Since Efb and Ecb have contact with a site in C3d that is essential for CR2 binding, they block the interaction between C3d and CR2, which plays a crucial role in the activation and maturation of B cells (79). Therefore, Efb may have an effect on the complement-mediated activation of the adaptive immune response. The impact of Efb or Ecb on other CR2-expressing cells such as follicular dendritic cells, and immature T lymphocytes (80,81) is unknown.

Reducion of convertase activities.
Efb induces conformational changes in the alpha-chain of C3 or C3b, impairing the binding of complement factor B to surface-bound C3b, thereby reducing the formation of the major C3 convertase (58). This permits *S. aureus* to evade the immune response since C3 convertase plays a key role in complement activation and complement attack (82). Further studies showed that Efb and Ecb bind to the complement fragment C3d in C3b-containing convertases (e.g. C3bBb, C4b2aC3b, and C3b2Bb) deposited on bacterial surfaces, inactivating their activities thereby hindering the cleavage of C3 and C5. Consequently, it impedes the formation of C5a and its capacity to induce neutrophil migration (72).

SCIN acts as a bridge between C3b (55,66) and complement factor B stabilizing the assembled convertase in a catalytically inactive state. This complex still interacts with C3, but without generating or depositing C3b (83,84). SCIN binds to C3bBb and C4b2a so that the convertase is not able to cleave C3 (66).

The biological effect of Efb

In staphylococcal infection.
Efb has many biological functions in staphylococcus infection. That Efb plays an important role in the pathogenesis of *S. aureus* is supported by the fact of its high incidence of Efb among *S. aureus* isolates from clinical samples (12) and that Efb is expressed in vivo (85). Most important, Efb is implicated in staphylococcal pathogenesis in wound infection, determined by using an Efb mutant in an animal model. Also, the number of animals infected with the Efb mutant that had clinical signs of staphylococcal infection was lower than the number of animals infected with parenteral strains with similar signs of infection (11). This finding was supported by experiments from a mouse mastitis model that resembles wound infection caused by *S. aureus*, since the mammary glands were traumatized during the procedure (86). In addition, Efb vaccination protects against *S. aureus* wound infection associated with foreign bodies in animal models (17). In another study, it was shown that Efb and Ecb enhanced the virulence of *S. aureus* in a lung infection model in mice by blocking neutrophil recruitment to the site of infection, thereby contributing to staphylococcal persistence in host tissues and abscess formation in the kidneys (70). Furthermore, the role of Efb was tested in vivo by comparing a negative Efb mutant to the wild type strain in the peritoneal cavity of mice. The defective Efb mutant was phagocytosed by neutrophils to a significantly greater extent than the wild type strain (68).

Antibody response against Efb.
The antibody responses against Efb were analyzed in serum samples of patients with staphylococcal septicemia. Their levels of antibodies against Efb were significantly lower (10%) than in samples of healthy individuals (49%). However, these antibodies increased during the convalescent phase of patients with arthritis, osteitis, and abscesses (85). This indicated that these antibodies were produced later within the development of the infection. The elevated antibody response to Efb indicates that Efb is expressed and secreted in vivo and provides protection against *S. aureus* infection. Furthermore, to understand how humans respond to *S. aureus* exposure, the antibody level of antibodies against Efb was studied in young children. Also, children that have been nasally colonized with *S. aureus* in the first two years of life had significantly higher levels of IgG against Efb than non-colonized children (87). Understanding the determinants of carriage and how humans respond to *S. aureus* exposure is important to the development of novel antistaphylococcal measures.

Efb vaccination.
In a mouse mastitis model of *S. aureus* infection, mice vaccinated with Efb and coagulase presented a reduced rate of mastitis compared to the control group vaccinated with collagen-binding protein. Also, the number of bacteria recovered from the glands of the infected mice was significantly lower after immunization with the two fibrinogen-binding proteins. Furthermore, no pathological

changes in the mammary glands were observed in animals vaccinated with the two fibrinogen-binding proteins compared to an unvaccinated group (86). In another study, mice vaccinated with Efb were protected against wound infections related to implanted biomaterials (17). Probably, these antibodies suppress the delay of Efb-related wound healing. Interestingly, human donors possess neutralizing anti-Efb antibodies that attenuate bacterial survival (88). The fact that the antibody response to Efb was significantly lower in patients with staphylococcal infection compared to the control group suggests that Efb is a potential vaccine candidate against staphylococcal infection (85). It is also possible that passive immunization using neutralizing antibodies against Efb may protect against staphylococcal infection in patients with high susceptibility to infection, such as in immunocompromised patients undergoing surgery. It was demonstrated that antibodies against Efb could neutralize the function of Efb both in vitro and in vivo by blocking its interaction with C3. This has a protective effect against *S. aureus* survival in a whole blood model that closely mimics human bacteremia (88).

Therapeutic applications of Efb.

Efb can be a potential therapeutic agent to treat disorders related to the immune system, complement pathways, and thrombosis. For instance, ankylosing spondylitis is a rare type of arthritis with complement system activation within the spinal bone marrow, followed by notable alterations of the number of neutrophils and macrophages in the bone marrow and spleen. Treatment with the C3-binding domain of Efb delayed the evolution of this disease in mice. Efb also impeded the course of proteoglycan-induced ankylosing spondylitis mice by reducing osteoblast differentiation and complement inhibition (89). Furthermore, Efb can be helpful in treating some inflammatory diseases due to its anti-thrombotic activity and its ability to block the platelet-leukocyte interaction. The binding between platelets and leukocytes plays a crucial role in inflammation, which in turn may be involved in atherosclerosis (90,91) and in many other acute and chronic diseases (92).

Discussion

The multiple binding capacities of Efb permit the induction of different mechanisms that allow *S. aureus* to escape the immune system. Efb has fibrinogen-binding sites that induce the formation of a capsule-like structure around the bacteria built of fibrinogen that masks bacterial cells from being recognized by opsonizing and neutralizing antibodies and prevents attack by complements and other components of the immune system. This information is critical to future vaccination attempts, since opsonizing antibodies may not function in the presence of Efb. Furthermore, Efb and coagulase fibrinogen-binding sites inhibit fibrinogen binding to different receptors on cells involved in the immune response, thus preventing phagocytosis. The Efb fibrinogen-binding motif recently described in the N-protein of SARS-CoV-2 makes us speculate that SARS-CoV-2 could escape the immune system using fibrinogen. If that is the case, asymptomatic patients with SARS-CoV-2 infection may have neutralizing antibodies against fibrinogen-binding epitopes, so that the virus has less ability to escape the immune response. In addition, Efb has a P-selectin binding site that converts Efb into a powerful antithrombotic agent, which alters the interaction between platelets and leukocytes and affects platelet aggregation, leukocyte recruitment to vascular injury, inflammation, and other processes of the immune system. Efb has a C3-binding domain that blocks the classical and alternative pathways of complement activation and cell-surface deposition of C3. These mechanisms are reinforced by other complement inhibitory proteins like Ecb, Sbi, and SCIN or they act when Efb is not available. The abundance of C3 in human plasma suggests that it is unlikely that a single inhibitory mechanism is fully effective. The fact that the virulence of Efb in *S. aureus* infections has been demonstrated in various animal models makes us consider Efb and its binding sites a good vaccine candidate.

Conclusion

Staphylococcus aureus survives in the host by producing different proteins, including Efb, that has specific motifs and domains that interfere with hemostasis, wound healing, and the host immune response. These motifs and domains are potential vaccine candidates against *S. aureus* infections. Efb binding sites for fibrinogen, P-selectin, and components of the complement system are potential targets for active or passive immunization and represent potential therapeutic agents to treat disorders related to thrombosis, inflammation, and abnormal complement activity.

Declarations

Ethical approval

Not required.

Author contributions

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Declaration of competing interest
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References

1. Lowy FD. Staphylococcus aureus Infections. N Engl J Med [Internet]. 1998 Aug 20;339(8):520–32. Available from: http://www.nejm.org/doi/abs/10.1056/NEJM199808203390806. https://doi.org/10.1056/NEJM199808203390806
2. Guo Y, Song G, Sun M, Wang J, Wang Y. Prevalence and Therapies of Antibiotic-Resistance in Staphylococcus aureus. Front Cell Infect Microbiol [Internet]. 2020 Mar 17;10. Available from: https://www.frontiersin.org/article/10.3389/fcimb.2020.00107/full. https://doi.org/10.3389/fcimb.2020.00107
3. Rousseaux J, Rousseaux-Prévost R, Bazin H, Biserte G. Proteolysis of rat IgG subclasses by Staphylococcus aureus V8 proteinase. Biochim Biophys Acta - Protein Struct Mol Enzymol [Internet]. 1983 Oct;748(2):205–12. Available from: https://linkinghub.elsevier.com/retrieve/pii/0167483883902960. https://doi.org/10.1016/0167-4838(83)90296-0
4. Krakauer T, Stiles BG. The staphylococcal enterotoxin (SE) family. Virulence [Internet]. 2013 Nov 15;4(8):759–73. Available from: http://www.tandfonline.com/doi/abs/10.4161/viru.23905. https://doi.org/10.4161/viru.23905
5. Kim HK, Thammavongsa V, Schneewind O, Missiakas D. Recurrent infections and immune evasion strategies of Staphylococcus aureus. Curr Opin Microbiol [Internet]. 2012 Feb;15(1):92–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1369527411001822. https://doi.org/10.1016/j.mib.2011.10.012
6. Wiseman GM. The hemolysins of Staphylococcus aureus. Bacteriol Rev [Internet]. 1975 Dec 1;39(4):317. Available from: http://mmbr.asm.org/content/39/4/317.abstract
7. Hynes WL, Walton SL. Hyaluronidases of Gram-positive bacteria. FEMS Microbiol Lett [Internet]. 2000 Feb;183(2):201–7. Available from: https://academic.oup.com/femsle/article-lookup/doi/10.1111/j.1574-6968.2000.tb08958.x. https://doi.org/10.1111/j.1574-6968.2000.tb08958.x
8. Bodén MK, Flock JI. Fibrinogen-binding protein/clumping factor from Staphylococcus aureus. Infect Immun [Internet]. 1989;57(8):2358–63. Available from: https://iai.asm.org/content/57/8/2358. https://doi.org/10.1128/IAI.57.8.2358-2363.1989
9. Palma M, Wade D, Flock M, Flock J-I. Multiple Binding Sites in the Interaction between an Extracellular Fibrinogen-binding Protein from Staphylococcus aureus and Fibrinogen. J Biol Chem [Internet]. 1998 May 22;273(21):13177–81. Available from: http://www.jbc.org/lookup/doi/10.1074/jbc.273.21.13177. https://doi.org/10.1074/jbc.273.21.13177
10. Wade D, Palma M, Löfving-Arvholm I, Sällberg M, Silberring J, Flock J-I. Identification of functional domains in Efb, a fibrinogen binding protein of Staphylococcus aureus. Biochem Biophys Res Commun. 1998;248(3). . https://doi.org/10.1006/bbrc.1998.9028
11. Palma M, Nozohoor S, Schennings T, Heimdahl A, Flock J-II. Lack of the extracellular 19-kilodalton fibrinogen-binding protein from Staphylococcus aureus decreases virulence in experimental wound infection. Infect Immun [Internet]. 1996;64(12):5284–9. Available from: https://iai.asm.org/content/64/12/5284. https://doi.org/10.1128/IAI.64.12.5284-5289.1996
12. Bodén Wästfelt MK, Flock JI. Incidence of the highly conserved fib gene and expression of the fibrinogen-binding (Fib) protein among clinical isolates of Staphylococcus aureus. J Clin Microbiol [Internet]. 1995;33(9):2347–52. Available from: https://jcm.asm.org/content/33/9/2347. https://doi.org/10.1128/JCM.33.9.2347-2352.1995
13. Ko Y-P, Kang M, Ganesh VK, Ravirajan D, Li B, Höök M. Coagulase and Efb of Staphylococcus aureus Have a Common Fibrinogen Binding Motif. MBio [Internet]. 2016 Mar 2;7(1). Available from: https://mbio.asm.org/content/7/1/e01885-15. https://doi.org/10.1128/mBio.01885-15
14. KAWABATA S, MORITA T, IWANAGA S, IGARASHI H. Staphylocoagulase-Binding Region in Human Prothrombin12. J Biochem [Internet]. 1985 Jan;97(1):325–31. Available from: https://academic.oup.com/jb/article-lookup/doi/10.1093/oxfordjournals.jbchem.a135057. https://doi.org/10.1093/oxfordjournals.jbchem.a135057
15. Bodén MK, Flock J-I. Cloning and characterization of a gene for a 19kDa fibrinogen-binding protein from Staphylococcus aureus. Mol Microbiol [Internet]. 1994 May;12(4):599–606. Available from: http://doi.wiley.com/10.1111/j.1365-2958.1994.tb01046.x. https://doi.org/10.1111/j.1365-2958.1994.tb01046.x
16. Palma M, Shannon O, Quezada HC, Berg A, Flock J-I. Extracellular Fibrinogen-binding Protein, Efb, from Staphylococcus aureus Blocks Platelet Aggregation Due to Its Binding to the α-Chain. J Biol Chem [Internet]. 2001 Aug 24;276(34):31691–7. Available from: http://www.jbc.org/lookup/doi/10.1074/jbc.M104554200. https://doi.org/10.1074/jbc.M104554200
17. Shannon O, Uekotter A, Flock J-I. The Neutralizing Effects of Hyperimmune Antibodies Against Extracellular Fibrinogen-Binding Protein, Efb, from Staphylococcus aureus. Scand J Immunol [Internet]. 2006 Mar;63(3):184–90. Available from: http://doi.wiley.com/10.1111/j.1365-3083.2006.01735.x. https://doi.org/10.1111/j.1365-3083.2006.01735.x
18. Bodén MK, Flock J-I. Evidence for three different fibrinogen-binding proteins with unique properties from Staphylococcus aureus strain Newman. Microb Pathog [Internet]. 1992 Apr;12(4):289–98. Available from: https://linkinghub.elsevier.com/retrieve/pii/088240109290047R. https://doi.org/10.1016/0882-4010(92)90047-R
19. McDevitt D, Vaudaux P, Foster TJ. Genetic evidence that bound coagulase of Staphylococcus aureus is not clumping factor. Infect Immun [Internet]. 1992;60(4):1514–23. Available from: https://iai.asm.org/content/60/4/1514. https://doi.org/10.1128/IAI.60.4.1514-1523.1992
20. Ní Eidhin D, Perkins S, Francois P, Vaudaux P, Höök M, Foster TJ. Clumping factor B (ClfB), a new surface-located fibrinogen-binding adhesin of Staphylococcus aureus. Mol Microbiol [Internet]. 1998 Oct;30(2):245–57. Available from: http://doi.wiley.com/10.1046/j.1365-2958.1998.01050.x. https://doi.org/10.1046/j.1365-2958.1998.01050.x
21. Palma M, Haggar A, Flock J-I. Adherence of Staphylococcus aureus Is Enhanced by an Endogenous Secreted Protein with Broad Binding Activity. J Bacteriol [Internet]. 1999 May 1;181(9):2840–5.

Available from: https://jb.asm.org/content/181/9/2840. https://doi.org/10.1128/JB.181.9.2840-2845.1999
22. McGavin MH, Krajewska-Pietrasik D, Rydén C, Höök M. Identification of a Staphylococcus aureus extracellular matrix-binding protein with broad specificity. Infect Immun [Internet]. 1993;61(6):2479–85. Available from: https://iai.asm.org/content/61/6/2479. https://doi.org/10.1128/IAI.61.6.2479-2485.1993
23. BJERKETORP J. The von Willebrand factor-binding protein (vWbp) of Staphylococcus aureus is a coagulase. FEMS Microbiol Lett [Internet]. 2004 May;234(2):309–14. Available from: http://doi.wiley.com/10.1016/j.femsle.2004.03.040. https://doi.org/10.1016/j.femsle.2004.03.040
24. Thomas S, Liu W, Arora S, Ganesh V, Ko Y-P, Höök M. The Complex Fibrinogen Interactions of the Staphylococcus aureus Coagulases. Front Cell Infect Microbiol [Internet]. 2019 Apr 16;9. Available from: https://www.frontiersin.org/article/10.3389/fcimb.2019.00106/full. https://doi.org/10.3389/fcimb.2019.00106
25. Sangith N. Unique fibrinogen-binding motifs in the nucleocapsid phosphoprotein of SARS CoV-2: Potential implications in host-pathogen interactions. Med Hypotheses [Internet]. 2020 Nov;144:110030. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0306987720313621. https://doi.org/10.1016/j.mehy.2020.110030
26. Ko Y-P, Liang X, Smith CW, Degen JL, Höök M. Binding of Efb from Staphylococcus aureus to Fibrinogen Blocks Neutrophil Adherence. J Biol Chem [Internet]. 2011 Mar 18;286(11):9865–74. Available from: http://www.jbc.org/lookup/doi/10.1074/jbc.M110.199687. https://doi.org/10.1074/jbc.M110.199687
27. Flick MJ, Du X, Witte DP, Jirousková M, Soloviev DA, Busuttil SJ, et al. Leukocyte engagement of fibrin(ogen) via the integrin receptor αMβ2/Mac-1 is critical for host inflammatory response in vivo. J Clin Invest [Internet]. 2004 Jun 1;113(11):1596–606. Available from: http://www.jci.org/articles/view/20741. https://doi.org/10.1172/JCI20741
28. Kuipers A, Stapels DAC, Weerwind LT, Ko Y-P, Ruyken M, Lee JC, et al. The Staphylococcus aureus polysaccharide capsule and Efb-dependent fibrinogen shield act in concert to protect against phagocytosis. Microbiology [Internet]. 2016 Jul 1;162(7):1185–94. Available from: https://www.microbiologyresearch.org/content/journal/micro/10.1099/mic.0.000293. https://doi.org/10.1099/mic.0.000293
29. Cheng AG, McAdow M, Kim HK, Bae T, Missiakas DM, Schneewind O. Contribution of Coagulases towards Staphylococcus aureus Disease and Protective Immunity. PLoS Pathog [Internet]. 2010 Aug 5;6(8):e1001036. Available from: https://dx.plos.org/10.1371/journal.ppat.1001036. https://doi.org/10.1371/journal.ppat.1001036
30. Shattil SJ, Hoxie JA, Cunningham M, Brass LF. Changes in the platelet membrane glycoprotein IIb.IIIa complex during platelet activation. J Biol Chem [Internet]. 1985 Sep 15;260(20):11107–14. Available from: http://www.jbc.org/content/260/20/11107.abstract
31. Bennett JS, Vilaire G. Exposure of platelet fibrinogen receptors by ADP and epinephrine. J Clin Invest [Internet]. 1979 Nov 1;64(5):1393–401. Available from: http://www.jci.org/articles/view/109597. https://doi.org/10.1172/JCI109597
32. Shannon O, Flock J-I. Extracellular fibrinogen binding protein, Efb, from Staphylococcus aureus binds to platelets and inhibits platelet aggregation. Thromb Haemost [Internet]. 2004 Dec 6;91(04):779–89. Available from: http://www.thieme-connect.de/DOI/DOI?10.1160/TH03-05-0287. https://doi.org/10.1160/TH03-05-0287
33. Posner MG, Upadhyay A, Abubaker AA, Fortunato TM, Vara D, Canobbio I, et al. Extracellular Fibrinogen-binding Protein (Efb) from Staphylococcus aureus Inhibits the Formation of Platelet-Leukocyte Complexes. J Biol Chem [Internet]. 2016 Feb 5;291(6):2764–76. Available from: http://www.jbc.org/lookup/doi/10.1074/jbc.M115.678359. https://doi.org/10.1074/jbc.M115.678359
34. Heilmann C, Herrmann M, Kehrel BE, Peters G. Platelet-Binding Domains in 2 Fibrinogen-Binding Proteins of Staphylococcus aureus Identified by Phage Display. J Infect Dis [Internet]. 2002 Jul;186(1):32–9. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1086/341081. https://doi.org/10.1086/341081
35. Larsen E, Celi A, Gilbert GE, Furie BC, Erban JK, Bonfanti R, et al. PADGEM protein: A receptor that mediates the interaction of activated platelets with neutrophils and monocytes. Cell [Internet]. 1989 Oct;59(2):305–12. Available from: https://linkinghub.elsevier.com/retrieve/pii/0092867489902924. https://doi.org/10.1016/0092-8674(89)90292-4
36. Geng J-G, Bevilacquat MP, Moore KL, McIntyre TM, Prescott SM, Kim JM, et al. Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. Nature [Internet]. 1990 Feb;343(6260):757–60. Available from: http://www.nature.com/articles/343757a0. https://doi.org/10.1038/343757a0
37. Stenberg PE, McEver RP, Shuman MA, Jacques Y V, Bainton DF. A platelet alpha-granule membrane protein (GMP-140) is expressed on the plasma membrane after activation. J Cell Biol [Internet]. 1985 Sep 1;101(3):880–6. Available from: https://rupress.org/jcb/article/101/3/880/28308/A-platelet-alphagranule-membrane-protein-GMP140-is. https://doi.org/10.1083/jcb.101.3.880
38. Théorêt J-F, Yacoub D, Hachem A, Gillis M-A, Merhi Y. P-selectin ligation induces platelet activation and enhances microaggregate and thrombus formation. Thromb Res [Internet]. 2011 Sep;128(3):243–50. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0049384811001812. https://doi.org/10.1016/j.thromres.2011.04.018
39. Wallis S, Wolska N, Englert H, Posner M, Upadhyay A, Renné T, et al. A peptide from the staphylococcal protein Efb binds P-selectin and inhibits the interaction of platelets with leukocytes. J Thromb Haemost [Internet]. 2021 Dec 16; Available from: https://onlinelibrary.wiley.com/doi/10.1111/jth.15613. https://doi.org/10.1111/jth.15613
40. Ed Rainger G, Chimen M, Harrison MJ, Yates CM, Harrison P, Watson SP, et al. The role of platelets in the recruitment of leukocytes during vascular disease. Platelets [Internet]. 2015 Aug 18;26(6):507–20. Available from: http://www.tandfonline.com/doi/full/10.3109/09537104.2015.1064881. https://doi.org/10.3109/09537104.2015.1064881
41. Zhang X, Liu Y, Gao Y, Dong J, Mu C, Lu Q, et al. Inhibiting platelets aggregation could aggravate the acute infection caused by Staphylococcus aureus. Platelets [Internet]. 2011 May 25;22(3):228–36. Available from: http://www.tandfonline.com/doi/full/10.3109/09537104.2010.543962. https://doi.org/10.3109/09537104.2010.543962
42. O´Brien L, Kerrigan SW, Kaw G, Hogan M, Penadés J, Litt D, et al. Multiple mechanisms for the activation of human platelet aggregation by Staphylococcus aureus: roles for the clumping factors ClfA and ClfB, the serine-aspartate repeat protein SdrE and protein A. Mol Microbiol [Internet]. 2002 May 9;44(4):1033–44. Available from: http://doi.wiley.com/10.1046/j.1365-2958.2002.02935.x. https://doi.org/10.1046/j.1365-2958.2002.02935.x

43. Kerrigan SW, Clarke N, Loughman A, Meade G, Foster TJ, Cox D. Molecular Basis for Staphylococcus aureus –Mediated Platelet Aggregate Formation Under Arterial Shear In Vitro. Arterioscler Thromb Vasc Biol [Internet]. 2008 Feb;28(2):335–40. Available from: https://www.ahajournals.org/doi/10.1161/ATVBAHA.107.152058. https://doi.org/10.1161/ATVBAHA.107.152058
44. Surewaard BGJ, Thanabalasuriar A, Zeng Z, Tkaczyk C, Cohen TS, Bardoel BW, et al. α-Toxin Induces Platelet Aggregation and Liver Injury during Staphylococcus aureus Sepsis. Cell Host Microbe [Internet]. 2018 Aug;24(2):271-284.e3. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1931312818303299. https://doi.org/10.1016/j.chom.2018.06.017
45. Sullam PM, Bayer AS, Foss WM, Cheung AL. Diminished platelet binding in vitro by Staphylococcus aureus is associated with reduced virulence in a rabbit model of infective endocarditis. Infect Immun [Internet]. 1996;64(12):4915–21. Available from: https://iai.asm.org/content/64/12/4915. https://doi.org/10.1128/IAI.64.12.4915-4921.1996
46. Moreillon P, Que Y-A. Infective endocarditis. Lancet [Internet]. 2004 Jan;363(9403):139–49. Available from: https://linkinghub.elsevier.com/retrieve/pii/S014067360315266X. https://doi.org/10.1016/S0140-6736(03)15266-X
47. Polzin A, Dannenberg L, Naguib D, Achilles A, Mourikis P, Zako S, et al. P4189Effects of coagulase reaction on aggregation in patients with endocarditis. Eur Heart J [Internet]. 2018 Aug 1;39(suppl_1). Available from: https://academic.oup.com/eurheartj/article/doi/10.1093/eurheartj/ehy563.P4189/5082892. https://doi.org/10.1093/eurheartj/ehy563.P4189
48. Sheu J-R, Lee C-R, Lin C-H, Hsiao G, Ko W-C, Chen Y-C, et al. Mechanisms Involved in the Antiplatelet Activity of Staphylococcus aureus Lipoteichoic Acid in Human Platelets. Thromb Haemost [Internet]. 2000 Dec 8;83(05):777–84. Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/s-0037-1613907. https://doi.org/10.1055/s-0037-1613907
49. Dejana E, Callioni A, Quintana A, de Gaetano G. Bleeding time in laboratory animals. II - A comparison of different assay conditions in rats. Thromb Res [Internet]. 1979 Jan;15(1–2):191–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/0049384879900641. https://doi.org/10.1016/0049-3848(79)90064-1
50. DiMinno G, Silver MJ. Mouse antithrombotic assay: a simple method for the evaluation of antithrombotic agents in vivo. Potentiation of antithrombotic activity by ethyl alcohol. J Pharmacol Exp Ther [Internet]. 1983 Apr 1;225(1):57. Available from: http://jpet.aspetjournals.org/content/225/1/57.abstract
51. Shannon O, Uekötter A, Flock J-I. Extracellular fibrinogen binding protein, Efb, from Staphylococcus aureus as an antiplatelet agent in vivo. Thromb Haemost [Internet]. 2005 Dec 11;93(05):927–31. Available from: http://www.thieme-connect.de/DOI/DOI?10.1160/TH04-08-0501. https://doi.org/10.1160/TH04-08-0501
52. Hammel M, Sfyroera G, Pyrpassopoulos S, Ricklin D, Ramyar KX, Pop M, et al. Characterization of Ehp, a Secreted Complement Inhibitory Protein from Staphylococcus aureus. J Biol Chem [Internet]. 2007 Oct 12;282(41):30051–61. Available from: http://www.jbc.org/lookup/doi/10.1074/jbc.M704247200. https://doi.org/10.1074/jbc.M704247200
53. Zhang L, Jacobsson K, Vasi J, Lindberg M, Frykberg L. A second IgG-binding protein in Staphylococcus aureus. Microbiology [Internet]. 1998 Apr 1;144(4):985–91. Available from: https://www.microbiologyresearch.org/content/journal/micro/10.1099/00221287-144-4-985. https://doi.org/10.1099/00221287-144-4-985
54. Burman JD, Leung E, Atkins KL, O'Seaghdha MN, Lango L, Bernadó P, et al. Interaction of Human Complement with Sbi, a Staphylococcal Immunoglobulin-binding Protein. J Biol Chem [Internet]. 2008 Jun 20;283(25):17579–93. Available from: http://www.jbc.org/lookup/doi/10.1074/jbc.M800265200. https://doi.org/10.1074/jbc.M800265200
55. Rooijakkers SHM, Milder FJ, Bardoel BW, Ruyken M, van Strijp JAG, Gros P. Staphylococcal Complement Inhibitor: Structure and Active Sites. J Immunol [Internet]. 2007 Sep 1;179(5):2989–98. Available from: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.179.5.2989. https://doi.org/10.4049/jimmunol.179.5.2989
56. Woehl JL, Stapels DAC, Garcia BL, Ramyar KX, Keightley A, Ruyken M, et al. The Extracellular Adherence Protein from Staphylococcus aureus Inhibits the Classical and Lectin Pathways of Complement by Blocking Formation of the C3 Proconvertase. J Immunol [Internet]. 2014 Dec 15;193(12):6161–71. Available from: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.1401600. https://doi.org/10.4049/jimmunol.1401600
57. Lee LYL, Höök M, Haviland D, Wetsel RA, Yonter EO, Syribeys P, et al. Inhibition of Complement Activation by a Secreted Staphylococcus aureus Protein. J Infect Dis [Internet]. 2004 Aug;190(3):571–9. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1086/422259. https://doi.org/10.1086/422259
58. Hammel M, Sfyroera G, Ricklin D, Magotti P, Lambris JD, Geisbrecht B V. A structural basis for complement inhibition by Staphylococcus aureus. Nat Immunol [Internet]. 2007 Apr 11;8(4):430–7. Available from: http://www.nature.com/articles/ni1450. https://doi.org/10.1038/ni1450
59. Wade D, Palma M, Flock J-I, Berndt KD, Silberring J, Galaktionov SG. Structural analysis of EFB, a fibrinogen binding protein of staphylococcus aureus. Protein Pept Lett. 1998;5(4).
60. Lee LYL, Liang X, Höök M, Brown EL. Identification and Characterization of the C3 Binding Domain of the Staphylococcus aureus Extracellular Fibrinogen-binding Protein (Efb). J Biol Chem [Internet]. 2004 Dec 3;279(49):50710–6. Available from: http://www.jbc.org/lookup/doi/10.1074/jbc.M408570200. https://doi.org/10.1074/jbc.M408570200
61. Haspel N, Ricklin D, Geisbrecht B V., Kavraki LE, Lambris JD. Electrostatic contributions drive the interaction between Staphylococcus aureus protein Efb-C and its complement target C3d. Protein Sci [Internet]. 2008 Nov;17(11):1894–906. Available from: http://doi.wiley.com/10.1110/ps.036624.108. https://doi.org/10.1110/ps.036624.108
62. Chen H, Schuster MC, Sfyroera G, Geisbrecht B V., Lambris JD. Solution insights into the structure of the Efb/C3 complement inhibitory complex as revealed by lysine acetylation and mass spectrometry. J Am Soc Mass Spectrom [Internet]. 2008 Jan;19(1):55–65. Available from: https://pubs.acs.org/doi/10.1021/jasms.8b03045. https://doi.org/10.1016/j.jasms.2007.10.009
63. Upadhyay A, Burman JD, Clark EA, Leung E, Isenman DE, van den Elsen JMH, et al. Structure-Function Analysis of the C3 Binding Region of Staphylococcus aureus Immune Subversion Protein Sbi. J Biol Chem [Internet]. 2008 Aug 8;283(32):22113–20. Available from: http://www.jbc.org/lookup/doi/10.1074/jbc.M802636200. https://doi.org/10.1074/jbc.M802636200
64. Zhang L, Jacobsson K, Ström K, Lindberg M, Frykberg L. Staphylococcus aureus expresses a cell surface protein that binds both IgG and β2-glycoprotein I. Microbiology [Internet]. 1999 Jan 1;145(1):177–83. Available from: https://www.microbiologyresearch.org/content/journal/micro/10.1099/13500872-145-1-177. https://doi.org/10.1099/13500872-145-1-177
65. Clark EA, Crennell S, Upadhyay A, Zozulya A V., Mackay JD, Svergun DI, et al. A structural basis for Staphylococcal complement subversion: X-ray structure of the complement-binding domain of

Staphylococcus aureus protein Sbi in complex with ligand C3d. Mol Immunol [Internet]. 2011 Jan;48(4):452–62. Available from: https://linkinghub.elsevier.com/retrieve/pii/S016158901000578X. https://doi.org/10.1016/j.molimm.2010.09.017
66. Rooijakkers SHM, Ruyken M, Roos A, Daha MR, Presanis JS, Sim RB, et al. Immune evasion by a staphylococcal complement inhibitor that acts on C3 convertases. Nat Immunol [Internet]. 2005 Sep 7;6(9):920–7. Available from: http://www.nature.com/articles/ni1235. https://doi.org/10.1038/ni1235
67. Koch TK, Reuter M, Barthel D, Böhm S, van den Elsen J, Kraiczy P, et al. Staphylococcus aureus Proteins Sbi and Efb Recruit Human Plasmin to Degrade Complement C3 and C3b. PLoS One [Internet]. 2012 Oct 11;7(10):e47638. Available from: https://dx.plos.org/10.1371/journal.pone.0047638. https://doi.org/10.1371/journal.pone.0047638
68. Ko Y-P, Kuipers A, Freitag CM, Jongerius I, Medina E, van Rooijen WJ, et al. Phagocytosis Escape by a Staphylococcus aureus Protein That Connects Complement and Coagulation Proteins at the Bacterial Surface. PLoS Pathog [Internet]. 2013 Dec 12;9(12):e1003816. Available from: https://dx.plos.org/10.1371/journal.ppat.1003816. https://doi.org/10.1371/journal.ppat.1003816
69. Jongerius I, Garcia BL, Geisbrecht B V., van Strijp JAG, Rooijakkers SHM. Convertase Inhibitory Properties of Staphylococcal Extracellular Complement-binding Protein. J Biol Chem [Internet]. 2010 May 14;285(20):14973–9. Available from: http://www.jbc.org/lookup/doi/10.1074/jbc.M109.091975. https://doi.org/10.1074/jbc.M109.091975
70. Jongerius I, von Köckritz-Blickwede M, Horsburgh MJ, Ruyken M, Nizet V, Rooijakkers SHM. <i>Staphylococcus aureus</i> Virulence Is Enhanced by Secreted Factors That Block Innate Immune Defenses. J Innate Immun [Internet]. 2012;4(3):301–11. Available from: https://www.karger.com/Article/FullText/334604. https://doi.org/10.1159/000334604
71. Liese J, Rooijakkers SHM, van Strijp JAG, Novick RP, Dustin ML. Intravital two-photon microscopy of host-pathogen interactions in a mouse model of Staphylococcus aureus skin abscess formation. Cell Microbiol [Internet]. 2013 Jun;15(6):891–909. Available from: http://doi.wiley.com/10.1111/cmi.12085. https://doi.org/10.1111/cmi.12085
72. Jongerius I, Köhl J, Pandey MK, Ruyken M, van Kessel KPM, van Strijp JAG, et al. Staphylococcal complement evasion by various convertase-blocking molecules. J Exp Med [Internet]. 2007 Oct 1;204(10):2461–71. Available from: https://rupress.org/jem/article/204/10/2461/46661/Staphylococcal-complement-evasion-by-various. https://doi.org/10.1084/jem.20070818
73. Amdahl H, Haapasalo K, Tan L, Meri T, Kuusela PI, van Strijp JA, et al. Staphylococcal protein Ecb impairs complement receptor-1 mediated recognition of opsonized bacteria. PLoS One [Internet]. 2017 Mar 8;12(3):e0172675. Available from: https://dx.plos.org/10.1371/journal.pone.0172675. https://doi.org/10.1371/journal.pone.0172675
74. Gaither TA, Hammer CH, Frank MM. Studies of the molecular mechanisms of C3b inactivation and a simplified assay of beta 1H and the C3b inactivator (C3bINA). J Immunol [Internet]. 1979 Sep;123(3):1195–204. Available from: http://www.ncbi.nlm.nih.gov/pubmed/469245
75. Amdahl H, Jongerius I, Meri T, Pasanen T, Hyvärinen S, Haapasalo K, et al. Staphylococcal Ecb Protein and Host Complement Regulator Factor H Enhance Functions of Each Other in Bacterial Immune Evasion. J Immunol [Internet]. 2013 Aug 15;191(4):1775–84. Available from: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.1300638. https://doi.org/10.4049/jimmunol.1300638
76. Haupt K, Reuter M, van den Elsen J, Burman J, Hälbich S, Richter J, et al. The Staphylococcus aureus Protein Sbi Acts as a Complement Inhibitor and Forms a Tripartite Complex with Host Complement Factor H and C3b. PLoS Pathog [Internet]. 2008 Dec 26;4(12):e1000250. Available from: https://dx.plos.org/10.1371/journal.ppat.1000250. https://doi.org/10.1371/journal.ppat.1000250
77. Ricklin D, Tzekou A, Garcia BL, Hammel M, McWhorter WJ, Sfyroera G, et al. A Molecular Insight into Complement Evasion by the Staphylococcal Complement Inhibitor Protein Family. J Immunol [Internet]. 2009 Aug 15;183(4):2565–74. Available from: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.0901443. https://doi.org/10.4049/jimmunol.0901443
78. Jongerius I, Puister M, Wu J, Ruyken M, van Strijp JAG, Rooijakkers SHM. Staphylococcal Complement Inhibitor Modulates Phagocyte Responses by Dimerization of Convertases. J Immunol [Internet]. 2010 Jan 1;184(1):420–5. Available from: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.0902865. https://doi.org/10.4049/jimmunol.0902865
79. Ricklin D, Ricklin-Lichtsteiner SK, Markiewski MM, Geisbrecht B V., Lambris JD. Cutting Edge: Members of the Staphylococcus aureus Extracellular Fibrinogen-Binding Protein Family Inhibit the Interaction of C3d with Complement Receptor 2. J Immunol [Internet]. 2008 Dec 1;181(11):7463–7. Available from: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.181.11.7463. https://doi.org/10.4049/jimmunol.181.11.7463
80. Carroll MC. The complement system in regulation of adaptive immunity. Nat Immunol [Internet]. 2004 Oct 28;5(10):981–6. Available from: http://www.nature.com/articles/ni1113. https://doi.org/10.1038/ni1113
81. Tsoukas CD, Lambris JD. Expression of EBV/C3d receptors on T cells: biological significance. Immunol Today [Internet]. 1993 Feb;14(2):56–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/0167569993900059T. https://doi.org/10.1016/0167-5699(93)90059-T
82. Chen H, Ricklin D, Hammel M, Garcia BL, McWhorter WJ, Sfyroera G, et al. Allosteric inhibition of complement function by a staphylococcal immune evasion protein. Proc Natl Acad Sci [Internet]. 2010 Oct 12;107(41):17621–6. Available from: http://www.pnas.org/lookup/doi/10.1073/pnas.1003750107. https://doi.org/10.1073/pnas.1003750107
83. Garcia BL, Tzekou A, Ramyar KX, McWhorter WJ, Ricklin D, Lambris JD, et al. Crystallization of human complement component C3b in the presence of a staphylococcal complement-inhibitor protein (SCIN). Acta Crystallogr Sect F Struct Biol Cryst Commun [Internet]. 2009 May 1;65(5):482–5. Available from: http://scripts.iucr.org/cgi-bin/paper?S174430910901207X. https://doi.org/10.1107/S174430910901207X
84. Rooijakkers SHM, Wu J, Ruyken M, van Domselaar R, Planken KL, Tzekou A, et al. Structural and functional implications of the alternative complement pathway C3 convertase stabilized by a staphylococcal inhibitor. Nat Immunol [Internet]. 2009 Jul 7;10(7):721–7. Available from: http://www.nature.com/articles/ni.1756. https://doi.org/10.1038/ni.1756
85. Colque-Navarro P, Palma M, Söderquist B, Flock J-I, Möllby R. Antibody Responses in Patients with Staphylococcal Septicemia against Two Staphylococcus aureus Fibrinogen Binding Proteins: Clumping Factor and an Extracellular Fibrinogen Binding Protein. Clin Diagnostic Lab Immunol [Internet]. 2000 Jan 1;7(1):14–20. Available from: https://cvi.asm.org/content/7/1/14. https://doi.org/10.1128/CDLI.7.1.14-20.2000

86. Mamo W, Bodén M, Flock J-I. Vaccination with Staphylococcus aureus fibrinogen binding proteins (FgBPs) reduces colonisation of S. aureus in a mouse mastitis model. FEMS Immunol Med Microbiol [Internet]. 1994 Nov;10(1):47–53. Available from: https://academic.oup.com/femspd/article-lookup/doi/10.1111/j.1574-695X.1994.tb00010.x. https://doi.org/10.1111/j.1574-695X.1994.tb00010.x
87. Verkaik NJ, Lebon A, de Vogel CP, Hooijkaas H, Verbrugh HA, Jaddoe VWV, et al. Induction of antibodies by Staphylococcus aureus nasal colonization in young children. Clin Microbiol Infect [Internet]. 2010 Aug;16(8):1312–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1198743X14642373. https://doi.org/10.1111/j.1469-0691.2009.03073.x
88. Georgoutsou-Spyridonos M, Ricklin D, Pratsinis H, Perivolioti E, Pirmettis I, Garcia BL, et al. Attenuation of Staphylococcus aureus–Induced Bacteremia by Human Mini-Antibodies Targeting the Complement Inhibitory Protein Efb. J Immunol [Internet]. 2015 Oct 15;195(8):3946–58. Available from: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.1500966. https://doi.org/10.4049/jimmunol.1500966
89. Yang C, Ding P, Wang Q, Zhang L, Zhang X, Zhao J, et al. Inhibition of Complement Retards Ankylosing Spondylitis Progression. Sci Rep [Internet]. 2016 Dec 4;6(1):34643. Available from: http://www.nature.com/articles/srep34643. https://doi.org/10.1038/srep34643
90. Sarma J, Laan CA, Alam S, Jha A, Fox KAA, Dransfield I. Increased Platelet Binding to Circulating Monocytes in Acute Coronary Syndromes. Circulation [Internet]. 2002 May 7;105(18):2166–71. Available from: https://www.ahajournals.org/doi/10.1161/01.CIR.0000015700.27754.6F. https://doi.org/10.1161/01.CIR.0000015700.27754.6F
91. Huo Y, Schober A, Forlow SB, Smith DF, Hyman MC, Jung S, et al. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. Nat Med [Internet]. 2003 Jan 16;9(1):61–7. Available from: http://www.nature.com/articles/nm810. https://doi.org/10.1038/nm810
92. Dinerman JL, Mehta JL. Endothelial, platelet and leukocyte interactions in ischemic heart disease: Insights into potential mechanisms and their clinical relevance. J Am Coll Cardiol [Internet]. 1990 Jul;16(1):207–22. Available from: https://linkinghub.elsevier.com/retrieve/pii/0735109790904814. https://doi.org/10.1016/0735-1097(90)90481-4
93. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, et al. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res [Internet]. 2018 Jul 2;46(W1):W296–303. Available from: https://academic.oup.com/nar/article/46/W1/W296/5000024. https://doi.org/10.1093/nar/gky427