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STAPHYLOCOCCUS AUREUS SECRETES COAGULASE AND VON WILLEBRAND FACTOR BINDING PROTEIN TO MODIFY THE COAGULATION CASCADE AND ESTABLISH HOST INFECTIONS.

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ABSTRACT

Staphylococcus aureus isolates secrete coagulases, polypeptides capable of binding to and activating prothrombin, causing fibrinogen to be converted to fibrin, which then promotes clotting. While staphylococci are pathogens, they cause similar modifications of the coagulation cascade during host infection by their products, canonical coagulase (Coa) and von Willebrand factor binding protein (vWbp). In addition to leading to abscesses and bacterial persistence in host tissues, the staphylococcus binds to fibrinogen or fibrin, which is also responsible for causing lethal sepsis. There is evidence that the cell-bound staphylococci, produced by coagulase activity, can cause these infections to develop into thromboembolic lesions and to resist opsonophagocytic clearance by the body's immune system. Also, the thrombin-mediated coagulation products of staphylococci appear to display distinct differences from those of staphylococci, showing these latter products represent one of the most effective innate defense mechanisms against many invading pathogens. According to preclinical studies, inactivation or neutralization of coagulases may prevent the formation of staphylococcal infections, a strategy that could be used to combat hospital-acquired infections caused by drug-resistant Staphylococcus aureus isolates.

Keywords: Staphylococcus aureus infection, Neutrophils, Host defense, Proteinases.

INTRODUCTION

During inflammation, bacteria are trapped and immobilized in a clot, thus defending the body from invading pathogens. Coagulation is, however, also a target of bacterial immune evasion strategies, as has been observed for many other host defense mechanisms [4]. Mycosal. Human skin and nasal passages are frequently colonized by Staphylococcus aureus, which frequently causes soft tissue infections by invading skin breaches. As well as sepsis and invasive infections such as endocarditis, osteomyelitis, pneumonia, gastrointestinal toxemias and oestrogen overload, S. aureus also causes fatal gastrointestinal and reproductive infections [5]. Invasive infections of S. aureus are a common problem in health care institutions [6, 7, 8]. It is thought that the coagulase test [5] is the clinical method of distinguishing S. aureus from less pathogenic staphylococci. Staph aureus, when injected into calcium-chelated plasma or blood, results in rapid clotting. For more than a century, scientists have studied this phenomenon, which was first described in 1903 [4]. The developing abscess promotes staphylococcal replication and causes clotting factors to be depleted from the blood, making this an essential component of virulence in Staphylococcus aureus infections. This describes ways staphylococcal proteins evade innate immune responses to promote the spread of staphylococcal diseases through coopting host coagulation cascades.

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Physiological Coagulation/Fibrinolytic Cascade

There is a cascade of serine proteases known to govern blood coagulation and control extracellular fluid coagulation. Following tissues injuries, these proteases are active to limit blood loss, and they are closely controlled to prevent systemic coagulation. As a result of tissue injury, tissue factor is exposed to the plasma to interact with plasma factor VIIa (fVIIa). This complex converts fX to fXa [11]. A prothrombinase complex is formed when fXa and fVa cleave prothrombin into thrombin. In order for these reactions to occur, calcium and phospholipids are needed. Activated coagulation factors and intrinsic coagulation cascades increase clotting in a positive feedback loop. Platelets exert their effect on the coagulation process by localizing calcium and phospholipids, as well as prothrombinase complexes [10]. Thrombin converts fibrinogen into fibrin during the coagulation cascade.

Blood and extracellular fluids contain blood fibrinogen, a soluble glycoprotein of 340 kDa. There are 29 disulfide bonds linking the two A-* chains and the two B-* chains in this dimer of trimers. Throughout the central domain E of these 6 polypeptides, their N-termini overlap one another while their C-termini form symmetrical globular domains [13]. A thrombin-induced cleavage of the N-termini of both the *- and *-chains initiates fibrin formation. These peptides cause structural rearrangements between adjacent polypeptides, leading to elongation and lateral agglomeration of fibrin, which forms the mesh network of clots. Cross-linking of fXIII strengthens fibrin aggregates [16]. As part of fXIII, adjacent *- and *-chains are reinforced by cross-bridges of *-(*-glutamyl)lysine [16].

An effective way to counter coagulation is fibrinolysis. When activated, plasminogen transforms into plasmin. In addition to cleaving fibrin at specific sites, plasmamin releases many less abundant species, which results in the sequential breakdown of fibrin mesh into three major products of digestion [18]. In addition to tissue-type plasminogen activator and urokinase plasminogen activator, several proteins contribute to fibrinolysis by activating plasminogen. Upon plasmin cleavage, lysines on fibrin exposed from the C-terminal region accumulate in the presence of plasmin, further accelerating the process [19]. A protein called *2-antiplasmin, plasminogen activator inhibitor-1, reduces fibrinolysis by sequestering plasmin or its activators into inactive enzyme-inhibitor complexes [19]. A fXIII protein is incorporated into the growing fibrin clot to promote clot stabilization against lysis [19].

A number of factors influence fibrin morphology, strength, and resistance to lysis. We don't understand all of them. In addition to fibrinogen concentration, thrombin concentration affects polymerization kinetics [20]. As fibrinopeptide A and fibrinopeptide B are removed at different rates, this leads to changes in protofibril assembly, which affects lateral aggregation and fibrin branching [20]. It is suspected that fXIIa, independently of thrombin generation, increases the lag time for fibrin formation, fibrin fiber density, and clot stiffness during clot formation [21]. Further, mutations that cause the activation of fXIII to occur more quickly lead to fibrin clots that are resistant to lysis [22] and thinner in nature. Physicochemical factors such as pH and calcium concentration may influence fibrin fiber morphology as well as fibrinogen-binding proteins like fibronectin and albumin [24]. Fibrin networks are formed when platelets aggregate [24]. This study demonstrates that the 3-dimensional structure of clots depends on their specific environments.

Fibrin dissolution is largely dependent on the fibrin network's structure. As stated above, lytic enzymes affect clotting efficiency by incorporating into growing clots and regulating their activity. The slower lysis rate of thinner fibres and smaller pores is attributed to their resistance to lysis [20]. In addition, fibrinogen and thrombin released by activated platelets exhibit less turbidity and higher resistance to lysis when polyphosphate is added to them when clots are formed [25]. Thus, clot formation and hemostasis are impacted by the unique conditions under which they are formed as well as their susceptibility to lysis and eventually achieving homeostasis.

Staphylococcal Coagulases

Coa and vWbp are both substances produced by S. aureus that encourage coagulation. Prothrombin is activated in both of these ways by these proteins without proteolysis [26, 27]. In order to form an active site, one end of Coa and one end of vWbp must interact with the prosite of prothrombin. This can only occur in thrombin. There is no cleavage of prothrombin by fVa and fXa during Coa- and vWbp-mediated activation [26]. Among different strains of bacteria, the size of Coa can vary considerably, with about 670 amino acids in each [28]. The 282 amino acids at the N-terminus of Coa make up the D1-D2 helices, which are linked to the prothrombin *-chain at the C-terminus [26, 29]. The linker region, which occupies 153 residues, follows the D1-D2 domains [26]. A 27-residue peptide is found at the C-terminal end of the fibrinogen-binding domain of another staphylococcal protein that binds fibrinogen at the C-terminus [26, 30]. Coa mediates the clotting of soluble fibrinogen, plasma, or blood through its interaction with fibrinogen and prothrombin [9, 31, 32].

[33] The D1 and D2 domains of vWbp are homologous to Coa. VWBP contains a von Willebrand factor binding site [33] that is unique in sequence. In contrast to Coas affinity for fibrinogen, vWbp binds prothrombin and fibrinogen on the molecular level [9, 27, 33]. Researchers have relied on methods of purification to isolate coa and VWB coagulases before discovering their genes. Most likely, one or both of these proteins exhibits different functions in vivo than the other. Nevertheless, their distinct interactions with their

binding partners, as described above, remain their primary differences.

Coagulases Are Virulence Factors for *S. aureus* Pathogenesis

Coagulases have been studied extensively in relation to disease. When microbiologists discovered that S. aureus isolates coagulate plasma or blood, microbiologists also discovered that virulence (the association of a clinical staphylococcus isolate with disease) is related to coagulation ability [34, 35]. Despite washing the bacteria prior to injection, mice that were injected with coagulase-negative staphylococci would die if they had been incubated with purified coagulase [36]. Coagulase injections were unaffected by a coagulase-negative staphylococcus challenge, however [36]. Based on the results of these experiments, coagulase might be pathogenic in conjunction with staphylococci that are close to coagulation products.

Although experiments that have investigated coagulase's role in Staphylococcus aureus infection have not yielded conclusive results. An intact chromosome of coa did not affect the virulence of S. aureus in mouse models of subcutaneous infection or mastitis [37], or in murine models of infective endocarditis [38, 39]. When Streptococcus gordonii was heterologously expressed with clfA, which encodes the fibrinogen-binding clumping factor In comparison to streptococcus gordonii, S. aureus adherence to fibrin platelet thrombi was greater, but not for coa in Streptococcus gordonii, which led to a higher rate of infective endocarditis in rats without these genes [40]. Alternatively, the analysis of several S. aureus isolates for coagulase activity and virulence was found to correlate with bacterial load in the lung following positive intravenous challenge [41]. Additionally, the number of viable bacteria found in the lung 7 days after infection was significantly higher for the wild-type strain than for the coa deletion mutant [41]. Chemical mutations have been used to isolate coagulase-negative strains of Staphylococcus aureus; however, the mutational lesions were not characterized [42, 43, 44]. Several important discoveries were made concerning coa and vwb and the coagulases they encode (33). When either coa or vwb was targeted for deletion, virulence was reduced moderately, however in mice with a combined coa-vwb deletion, abscesses or lethal sepsis were dramatically reduced.

By injecting rabbits with purified coagulase, researchers have examined coagulase's activity in vivo [45]. Blood drawn from these rabbits failed to clot when given a dose of 2–5 mg Coa, indicating the coagulation system had been activated and fibrinogen stores depleted. Rabbits died within 20 minutes of receiving a 20 mg Coa injection [45]. The kidneys, adrenal glands, and lung tissue were found to contain fibrin thrombi during necropsy [45]. The experiment suggests that coagulase can clot blood in vivo, despite such high doses of coagulase being unlikely physiological. A coagulase is able to cause abscesses, a characteristic of infections caused by S. aureus. Staphylococcal extracts or live staphylococci implanted into collodion bags and placed into mice stimulates the development of a fibrinous capsule and a polymorphonuclear infiltrate around the bag [47]. Encapsulation cannot be seen when the staphylococcal extracts have been treated with coagulase [47]. Staphylococcus aureus that is not a coa mutant induced abscesses in mice inoculated subcutaneously [48]. Immunohistochemical staining shows that abscesses formed during infection with S. aureus contain Coa and vWbp and are surrounded by prothrombin and fibrinogen [9]. An intravenous infection with S. aureus causes almost complete ablation of abscess formation in mice lacking both coagulases [9].

Infections are fought by coagulases acting as antiphagocytes [41, 49]. Staphylococcus aureus was found in peritoneal lavage fluid with clumps of staphylococcins surrounded by eosinophilic material as a result of intraperitoneal infection. Despite being recruited by these conditions, very few of the recruited neutrophils are capable of phagocytosing staphylococci. The results of this study led to the hypothesis that staphylococcus coagulation/clumping may be responsible for staphylococcal escape from phagocytosis.

Staphylococcal Aggregation with Platelets

The release of platelet microbicidal proteins by platelets assists in the innate defense against staphylococcus aureus isolates [51]. There has also been a report that Staphylococci adhere to platelets, especially during infections of the endocardium and endovasculature [52]. In the presence of fibrinogen, Staphylococcus aureus ClfA and ClfB mediate platelet aggregation [53, 54]. For staphylococci and platelets to aggregate through ClfA/ClfB, fibrin rather than fibrinogen is the relevant form [55]. ADP or thrombin does not activate platelets to form aggregates of S. aureus-platelets; this occurs independent of the GPIIb/IIIa fibrinogen receptor [55]. Neither platelet activation nor the association between platelets and staphylococci is promoted by ancrod, an enzyme from viper venom that breaks down fibrinogen to produce soluble fragments. Despite this, staphylococci are associated with platelets when platelets are activated with ADP and fibrinogen is treated with Ancrod at the same time [55]. Staphylococcus aureus might promote platelet aggregation by working with fibrin, not fibrinogen [55]. During platelet aggregation, coagulases are not known to play a role. Nonetheless, we envision a model in which staphylococcal coagulation serves as a fibrin matrix that enables staphylococci to aggregate with platelets.

Soon after it was discovered that S. aureus clots blood, Much [56] described how staphylococci rapidly agglutinate into visible clumps when immersed in calcium-chelated plasma. Several strains of staphylococcus can

clump (agglutinate) in plasma when they express coagulase [58, 59], and fibrinogen as well as prothrombin are required [56, 57]. In more recent studies [60, 61, 62], staphylococci have been shown to clump in the presence of soluble fibrinogen alone, an activity that requires the clumping factor structural gene (clfA), but not the coagulase (coa) gene to function. Staphylococcal agglutination in plasma despite the lack of clfA, cwb and coa is still induced by clfA, and this leads to a network of fibrin cables with which staphylococci associate [63]. Earlier reports [64] showed fibrin cables visible between staphylococci clumped in fibrinogen, which might contain prothrombin. These results are consistent with those reported earlier in this study. Hematologic examination of As evidence of staphylococcal agglutination in vivo is provided by staphylococcus aggregates found in the heart tissue of septic mice infected with S. aureus [63]. Lacking coa, vwb, and clfA, these lesions do not occur in mice. The assumption is that S. aureus generates such thromboembolic lesions in order to disseminate throughout the entire host body when it infects it.

IgM B cell receptor VH3 type proteins [65] are found on S. aureus strains, which contain protein A, a surface polypeptide that binds to the Fc* portion of immunoglobulins. Additionally, Fc* binding site [69] of protein A binds to von Willebrand factor [68]. Staphylococci may assemble with platelets as a result of protein A binding to von Willebrand factor, although this is not yet certain [68].

Coagulation in Immunity and Pathogenesis

Contact-dependent intrinsic coagulation cascades are activated by the negatively charged surface of bacteria [10]. Among the coagulation cascade factors, transglutaminase (fXIII) is the most conserved in mammals [1]. Both the hemolymph of Drosophila melanogaster and the fibrin matrix of human plasma are cross-linked by the transglutaminase in S. aureus and Escherichia coli [1]. By cross-linking bacteria in clots, insects are less likely to become infected [1]. Additionally, a subcutaneous mouse model of Streptococcus pyogenes infections was found to be resistant to the bacteria using transglutaminase [2]. During infection, the coagulation cascade appears to play a significant role in the innate immune response.

The lectin pathway activates complement cascades on bacterial surfaces as well. When mannanbinding lectin-associated serine protease 2 (MASP-2) is activated, fibrin is deposited on the target surface, which converts prothrombin to thrombin more slowly than the prothrombinase complex [3]. In order to entrap bacteria, bacterial surfaces usually cause coagulation; why do staphylococci secrete two molecules (Coa and vWbp) that accomplish the same thing? It would be difficult to appreciate the advantage of the staphylocoagul-like polymerization products (vWbp*prothrombin and Coa*prothrombin) for S. aureus pathogenesis. Thrombin is also cleaved at the same site as fibrinopeptides by the Coa*prothrombin complex [70]. However, staphylocoagulase-generated fibrin is thought to have biochemical and physiological properties distinct from thrombin-produced fibrin. As far as blood clots are concerned, staphylococcal clots dissolve much more rapidly than physiological clots [70, 71, 72, 73, 74] and staphylocoagulase cleaves fibrinogen more slowly than thrombin [70, 71, 72, 73, 74]. Staphylocoagulase-produced clots undergo less damage than thrombin-produced ones when thromboelastography is conducted [75]. This study supports the hypothesis that staphylococcal coagulases cause fibrin clots that differ mechanically and morphologically from clots produced by endogenous thrombin activation.

There are a number of thrombin substrates that can be proteolytically cleaved; however, staphylocoagulases have only been investigated for a few of these substrates. Thrombin-mediated activation of fiV and fVIII is inhibited by Coa binding to prothrombin [74]. The activation of fXIII by thrombin is clear, however data are inconsistent on whether fXIII is also activated by staphylocoagulase during staphylococcal coagulation; some authors found that fXIII was not activated by staphylocoagulase [71], while others found that fXIII was activated [73]. In addition to its platelet aggregation stimulating effects (via interactions with receptors GpIB, glycoprotein V, and protease-activated receptors which regulate platelet aggregation), thrombin has anticoagulant effects (sequestration of anti-thrombin; activation of protein C when bound to anti-thrombin) as well as reduced plasmin activity when bound to thrombin-activated fibrinolysis inhibitor [76]. The conversion of C5 to C5a by thrombin activates the complement cascade [77]. Phylocoagulases must be further examined to determine whether some of the many functions of thrombin have been preserved.

Conclusion

There is a staphylococcal infection epidemic in many countries. It has many virulence factors, immune evasion strategies, and toxins that make it highly versatile [5]. Its pathogenesis relies on Coa and vWbp, two of its secreted molecules. Coagulationases trigger fibrin polymerization by activating prothrombin nonenzymatically. By depositing fibrin and creating cross-links on bacteria, bacteria demonstrate innate immune defenses that reduce their infection risk. For Staphylococcus aureus, where the release of Coa and vWbp plays a key role in virulence, abscess formation, persistence of infection, staphylococcal sepsis, and endocarditis. In host tissues, staphylococci may use staphylocoagulase-generated fibrin networks to form pseudocapsules and escape opsonophagocytic clearance because of their unique structural, biochemical, and physiological attributes. Further, staphylococcal coagulation leads to thromboembolic events that contribute to bacterial spread throughout the body. Biological features of staphylococcal

agglutination will need to be examined in future studies, e.g., the clot formed by staphylocoagulase on which S. aureus adheres. Staphylococcal clots could provide essential nutrition as well as safeguard against host defenses. Staphylococcal abscess communities may be located near fibrin mesh-work scaffolds that help position bacteria and their virulent factors in an orderly fashion.

We can presume that the structural and dimension features of staphylococcal clots are dynamic: as the pathogen tries to invade deeper into host tissues, the clots need to be dissolved, yet host enzymes must resist dissolving the coagulase-assembled network to remove the invading microbes. Another nonenzymatic coagulation factor released by staphylococci is staphylokinase, which lyses fibrin clots by binding to plasmin and plasminogen. There has been a report that staphylokinaseplasmin(ogen) reduces the risk of mortality and morbidity from Staphylococcus aureus infections [80]. Staphylokinase's role in infection still needs to be elucidated, as well as how its activity relates to those of coagulases.

Taking into account their important roles in several pathophysiologic processes, the two S. aureus coagulase enzymes, Coa and vWbp, can serve as important targets for antimicrobial therapy and prevention [9]. Experimental animals can be protected from intraAn animal model of staphylococcal sepsis were reduced by neutralizing antibodies that block the association of Coa and vWbp with prothrombin, and small molecules that impede coagulase activity. Studying staphylococcal coagulases in the future could generate important biological insights and may contribute to solving the public health crisis caused by Staph aureus infections.

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