Staphylococcus aureus: structure and functions

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Introduction

1. 1 Staphylococcus aureus

Staphylococcus aureus is an opportunistic yet versatile pathogen that can infect almost all types of tissue in the human body. 33-50% of healthy individuals were reported to be S. aureus carriers. The diseases resulting from S. aureus infection range from superficial infections; invasive infections such as endocarditis to the life threatening septic shock and toxic shock. The presence of foreign material greatly increases the risk of infection by providing a base for attachment and biofilm formation. S. aureus appears as clustered gram positive cocci under the microscope. Criteria used to identify this organism include the production of golden pigment on nutrient agar; being positive for coagulase; mannitol fermentation and the production of deoxyribonuclease. The virulence of the infecting strain and the nature of the host immune response are important determinants for the outcome of severe S. aureus infection .

1. 1. 1 S. aureus genome and regulation of gene expression

S. aureus has a 2. 8-2. 9 Mbp circular genome. House-keeping genes and genes associated with central metabolism and some virulence determinants (e. g. protein A) are highly conserved among strains and make up the majority of S. aureus genome. The rest more variable regions mainly consists of mobile elements such as pathogenicity islands (SaPI, 7 identified), genomic islands (νSaα and νSaβ), Staphylococcal chromosomal cassettes (SCC) and bacteriophages. S. aureus expresses an array of adhesins, immunomodulatory molecules; anti-inflammatory proteins and as many as 30 toxins to invade, evade and cause host tissue damage. Many of the

virulence factors discussed below are encoded by genes located in the variable region. Their expression is under the control of a complicated and interconnected regulatory network .

Four main gene regulators have been identified, including the two component regulatory system (agr, sae); the transcription factors (SarA and its homologous); the nutrient regulated CodY regulator and alternative transcription factor σB. agr is an auto-inducible quorum-sensing system, promoting expression of extracellular virulence factors and down-regulating cell surface proteins. σB has been shown to participate in the general stress response, and may be involved in antibiotic resistance; pigmentation; biofilm and micro-colony formation (referenced in). Many surface proteins/adhesin have been shown to be positively influenced by σB, while the expression of most exoproteins and toxins were down-regulated. Activity ofthe agr system is influenced by other gene regulators and may be regulated by cell density. The transcription of agr components were found to be promoted by SarA and gene products of the sar locus and repressed by CodY. To add up the complexity, transcription of the Sar locus was controlled by three promoters that can be differently activated during in vitro growth. One of these promoters was demonstrated to be σB dependent .

It is generally regarded that exponential growth in vitro is correlated with the colonization phase of infection. Correspondingly, genes encoding surface proteins were found to be expressed earlier, starting during the transition from stationary to exponential growth while the expression of secreted proteins started at late-exponential growth phase. The in vivo situation of infection is likely to be more dynamic. Different stimuli trigger combined and

coordinated action of the regulatory network, reflecting the particular state of bacterial growth, host defense and environmental nutrition. For example, phagocytosis by neutrophil triggered differential expression of 21. 8-39. 1% of S. aureus genes “ at any time” following the event. The suppressive effect of CodY on S. aureus virulence factors lead to the possible suggestion that S. aureus may maintain its virulence factor to low level to ‘ co-exist’ harmlessly with the host until a invading opportunity arise. In any case, it would be interesting to see how host immunological activity ‘ feedback’ on S. aureus regulation of its virulence factors, especially immune evasion molecules.

1. 1. 2 Antibiotic resistance

The rapid spreading of antibiotic resistant S. aureus strains through human communities presents a major challenge for conventional treatment. In 2005, it was reported that methicillin-resistant S. aureus (MRSA) infection caused more death than AIDS in USA. S. aureus demonstrated amazing ability to “ co-evolve” with the development of antibiotics. The first generation penicillin uses β-lactam to bind penicillin-binding proteins (PBP, bacterial transpeptidase) and inhibits bacterial cell wall synthesis. S. aureus resists this class of antibiotics by producing β-lactamases (aka penicillinase) that cleaves β-lactam. Second generation of penicillin, such as methicillin was developed. The structure of their β-lactam has been modified to resist β-lactamases. S. aureus soon acquired the mecA operon that encodes a modified PBP (PBP 2a), which showed reduced susceptibility for β-lactam and thus granted resistance to all β-lactam containing antibiotics. mecA is located on the staphylococcal chromosome cassette mec (SCCmec), a mobile genetic element that allows horizontal transfer of mecA between

strains. MRSA infection shows increasing prevalence in all continents, primarily in healthcare-environments (HA-MRSA). Alarmingly, these strains have been shown to exhibit resistance to other types of commonly used non-lactam antibiotics such as ciprofloxacin and erythromycin. Vancomycin, a peptidoglycan polymerization inhibitor, is now considered as the last-line antibiotics for MRSA treatment. However, strains of vancomycin-intermediate S. aureus (VISA) have emerged. Notably, these strains have developed thicker cell walls that require higher concentrations of vancomycin to be effective (minimal inhibitory concentration MIC over 4ug/ml). A vancomycin resistant S. aureus (VRSA) strain has been reported recently (MIC > 32 µg/ml). This complete resistance is most likely due to the horizontal transfer of the vancomycin resistant gene (VanA) from Enterococcus faecalis. Development of effective new antibiotics against S. aureus has met with only limited success, urging the development of alternative therapies based on better understanding of the pathogenicityof S. aureus .

1. 2 Adhesin / ECM interaction as key to S. aureus colonization/invasion

Colonization is always the first step in bacterial infection and is an important component in pathogenesis. The wide range of tissues that S. aureus can infect reflects its ability to adhere and therefore colonize many different biological niches. S. aureus is now recognized as an invasive organism, targeting non-professional phagocytes such as keratinocytes, epithelial cells, endothelial cells and osteoblasts. The tasks of initial colonization and invasion are assisted by S. aureus surface anchored and secreted adhesive molecules known as MSCRAMM, microbial surface components recognizing adhesive matrix molecules and SERAM, secretable expanded repertoire

adhesive molecules. The majority of MSCRAMM and SERAM bind extracellular matrix (ECM) proteins such as fibrinogen and fibronectin. In general, the interaction with ECM proteins is regarded as providing a ‘ foothold’ for S. aureus to establish and to exacerbate infection. However, it has been reported that in some animal models loss of fibronectin binding proteins (FnBPs) led to an increase in bacterial virulence, indicating a role of the ECM protein in limiting bacterial dissemination or enhancing bacterial clearance. Due to overlap in target specificity; functional redundancy and the difference in experiment settings, the exact contribution of each isolated staphylococcal adhensin protein remains ambiguous. Nevertheless, fibronectin (Fn) and fibrinogen (Fg) are the most popular targets of known S. aureus adhesins.

The interaction between FnBPs and Fn is thought to play a key role in S. aureus internalization by non-phagocytic cells, as FnBP-coated latex beads and non-invasive bacteria expressing FnBPs were readily taken into the human cells (293 cells). Internalization provides several benefits for the bacterium: firstly, protection from host serum defense mechanisms and antibiotics ; secondly, access to nutrient-rich environments after escape from phagocytic vessels; thirdly, facilitating the crossing of the endothelial/epithelial layer and spreading of the infection; and lastly, establishing intracellular bacterial reservoirs for recurrent infection. S. aureus produces two related FnBPs (namely A and B) that contain almost identical Fn-binding domains in the close proximity to their cell-wall-spanning domain. The Fn-binding repeats of FnBPA were crystallized in complex with the N-terminal F1 modules of Fn. FnBPA peptide is inserted along the triple-

stranded β sheets of the Fn F1 module to form an antiparallel tandem β zipper. Since the C-terminus of Fn interacts with α5β1 integrin that are expressed on most host cell surfaces, S. aureus can attach to the host cell through a Fn bridge. The attachment was shown to trigger the accumulation of actin and focal-contact-associated proteins (e. g. tensin) at the bacteria contact site and initiate internalization in a protein tyrosin kinase FAK-dependent manner. It was predicted that one FnBP can bind six to nine Fn molecules. This multivalent interaction was suggested to be important in mediating internalization .

The bindings of Fg by the clumping factors (ClfA and ClfB) and FnBPA are mediated by a shared protein structure called the A domain and by a “ dock, lock and latch” mechanism. Interestingly, Clf A is expressed during stationary phase and binds to the γ chain of Fg. ClfB is expressed at the exponential growth phase and binds to the C-terminus of Fg Aα chain. This portion of Fg has been reported to be involved in the coagulation process and wound healing .

Fn and Fg interactions have been blamed particularly in S. aureus wound infection and infective endocarditis in humans. Fg binding alone could initiate experimental endocarditis in mice and has been correlated with valve colonization. However, cell invasion and persistence of the infection only occurred when the Fn-binding ability was enabled (via FnBPs). Both interactions were statistically correlated to disease severity. Binding to Fg and Fn simultaneously significantly accelerated the rate of internalization into cultured endothelial cells. In this regard FnBPA that is capable of binding both proteins may play an important role in establishing endocarditis .

Platelets accumulation on the heart valve is another critical factor for the development of infective endocarditis. S. aureus-induced platelet aggregation is a complicated and multifactorial process and was suggested to be dependent on Fg or fibrin. FnBPA, ClfA, ClfB and SdrE (serine-aspartate repeat protein) were shown to be able to cause platelet aggregation independently of other S. aureus surface proteins. ClfA displayed the strongest aggregation effect among the last three. SdrE required the presence of plasma to cause aggregation, although the mediator was not identified. ClfA was proposed to interact with platelet indirectly through an Fg bridge or directly with platelet surface protein p118. Other studies have argued that ClfA-specific IgG also participated in platelet activation by cross-linking ClfA to platelet FcγRIIa receptor .

1. 3 S. aureus toxins–direct damage of host cells

S. aureus produces a range of cytotoxins, including the β barrel pore forming toxin (e. g. α hemolysin); the two component pore forming leukocidins and the exfoliative toxin. Besides the effects of reducing viable phagocytes and weakening host immune system, S. aureus cytotoxins are currently believed to contribute to bacterial dissemination. Lysis of host cells might also provide nutrients for proliferating bacteria, especially iron from hemolysis .

These toxins have different prevalence in different diseases. Exfoliative toxin (ET) operates at the epidermal layer of the skin and causes staphylococcal scalded-skin syndrome. ET-A acts as serine protease and specifically cleaves Desmoglian-1 (Dsg-1). Dsg-1 is a cell-cell adhesion molecule expressed on epidermal keratinocytes. Cleavage of Dsg-1 disrupts the superficial layer of epidermis and helps bacterial invasion. α-hemolysin (Hla) is strongly

implicated in S. aureus lung infections. Active and passive immunization of Hla strongly protected mice against S. aureus pneumonia. Hla is released as a water soluble monomer and oligomerises on the host cell membrane to insert the hydrophobic stem domains. Seven Hla monomers are required to form a pore which eventually leads to cell lysis. At sub-lethal concentrations of Hla, host cells produce pro-inflammatory cytokines like IL-8; IL-6; vasoregulators (PGI2, PGE2 and thromboxane), which could have detrimental systemic effect. The expression of twenty S. aureus virulence factors were disrupted individually or in combination and the virulence of the resultant deletion mutants were compared in a mice pneumonia model. Deletion of argA, which encodes a component of the arg gene regulation system, resulted in almost complete loss of virulence. Only the Hla deletion mutant produced comparable effects, indicating the importance of Hla in causing lung tissue damage. Human neutrophils can resist Hla lysis but these cells are targeted by Leukocidins. Leukocidins are thought to act in the similar way as Hla. Among the leukocidins, Panton-valentine leukocidin (PVL) is found in all CA-MRSA (community acquired-MRSA) isolates and strongly associated with CA-MRSA infection. However in mouse models of abscess, sepsis, and pneumonia, the severity of diseases caused by PVL- deletion strains were not significantly different from that caused by the wt strains. More surprisingly, PVL-expressing S. aureus strains did not lyse human neutrophils better than that without PVL. However, it is not clear how these experimental conditions resemble the amount and time of PVL production during infection in human. Another significant problem is that PVL might be human specific and might not work in mice. Nevertheless, a new class of leukocidal molecules, the α type phenol-soluble modulin (PSMα), was

produced at a considerably high level by CA-MRSA. They were shown to contribute to the increased neutrophil lysis, skin lesion formation and mortality rate of experimental animals caused by CA-MRSA infection .

1. 4 Interaction of S. aureus with host immune system

1. 4. 1 Brief overview of host defense against S. aureus

Host defense against S. aureus mainly relies on the innate immune system, in particular, neutrophil mediated killing. The integrity of skin and mucosal layer is the first line of defense against invading bacteria. They also encounter antimicrobial substances released by epithelial cells and phagocytes (e. g. defensins; cathelicidins and lysosome). Defensins and cathelicidin are positively charged antimicrobial peptides that could permeate the bacterial membrane. Lysozyme is produced by many cell types and secreted into various tissues. It cleaves bacterial cell wall peptidoglycan at β 1-4 glycosidic linkage between N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG). Innate pattern recognition receptors (e. g. Toll-like receptor 2) and immunoglobulin also detect the presence of S. aureus. The latter is recognized by C1q and initiates the complement classical pathway (CP) or directly activates phagocytosis through Fc receptor expressed on neutrophils or monocytes. The human complement system is discussed in more detail below. One outcome of complement activation is the production of anaphylatoxin C3a and C5a. Together with formyl methionine peptides produced by bacteria, these chemoattractants are sensed by their receptors expressed on leukocytes and attract leukocytes to the sites of infection. Leukocyte recruitment is accomplished through highly coordinated interactions between adhesins on leukocytes and endothelial cells. Leukocytes change from rolling in the blood stream to firm adhesion to the

endothelium and then transmigrate through the endothelial layer. Subsequent migration of leukocytes to infection sites is mediated by integrin interacting with ECM proteins. Once they reach the infection site, the phagocytes recognize antibodies or complement fragments deposited on the bacterial surface through their respective receptors. Phagocytosis then occurs. The phagosome is fused to the lysosome to form the phagolysosome for enzyme-mediated and oxygen free radical mediated destruction .

1. 4. 2 The human complement system

The human complement system is a sequentially activated proteolytic cascade that involves more than 30 fluid phase and surface bound proteins. It is one of the key elements of the innate immune system that connects bacterial recognition, leukocyte chemotaxis; phagocytosis as well as adaptive immunity .

Three main activation pathways are utilized to recognize foreign and danger signals. The classical pathway (CP) senses antigen bound antibody by C1q. Bacterial carbohydrate ligand is recognized by mannose binding lectin (MBL) or ficolins and initiates the lectin pathway (LP). Upon ligand recognition, both pathways use their specific proteases to cleave C4 and then C2. The resultant C4b2a (the C3 convertase) converts C3 to C3a and C3b. The Alternative pathway (AP) starts by direct binding of C3b to the bacterial surface generated by spontaneous lysis of C3 to C3b called “ tick-over”. Factor B that associates with surface-bound C3b is subsequently cleaved to Bb and forms the alternative pathway C3 convertase C3bBb. The amplification loop of C3 activation is started. C3 cleavage is the central event and merging point in the three pathways. Accumulation of surface

bound C3b changes the substrate specificity of C3 convertase to C5. C5 cleavage produces C5a and C5b. The latter mediates the formation of the membrane attack complex C5b-9 (MAC), which inserts into the target cell membrane and ultimately causes lysis of target cells. C3a and C5a are powerful anaphylotoxin that trigger neutrophil homing. The overall picture of this complex system is depicted in Fig. 1. 1

MASP: mannose-binding lectin–associated serine proteases. Figure adapted from Walport et al 2001 (Ref.).

Three main outcomes of complement activation are: 1, chemotaxis of leukocytes via C5a and C3a; 2, C3b mediated phagocytosis via complement receptors on phagocytes; and 3, lysis of bacteria by MAC. It is also suggested by recent studies that the C3b presented on bacterial surface are critical factors for B cell activation and the production of specific antibodies. Although the MAC complex cannot function on gram positive bacteria like S. aureus, leukocytes mediated killing is critical for fighting against S. aureus infection .

1. 4. 3 S. aureus Immune evasion molecules

1. 4. 3. 1 Resistance to antimicrobial substances

S. aureus circumvents antimicrobial substances by three main strategies: 1, changing surface charges; 2, modifying the substrates of antimicrobial substances and 3, inactivating antimicrobial substances. S. aureus employs two enzymes to change the highly negatively charged teichoic acid in its cell wall (WTA). DltABCD (Dlt operon) adds D-alanine to WTA and the multiple peptide resistance factor F (Mprf) participates in the modification of membrane phosphatidylglycerol with L-lysine. Both modifications increase

bacterial surface charges and thus reduce the attraction to the cationic defensins. dlt or MprF defective strains were killed more efficiently by neutrophil oxygen-independent killing. The former was much less able to cause arthritis and mortality in mice sepsis and arthritis models .

S. aureus avoids lysozyme cell wall cleavage by modifying its cell wall peptidoglycan. O-acetyltransferase (OatA) was proposed to mediate this response. OatA catalyses the acetylation of muramic acid, which results in the addition of an acetyl group on S. aureus peptidoglycan. An oatA- strain was sensitive to lysozyme, while complementary expression of the enzyme restored its resistance .

Two enzymes have been reported to directly inactivate antimicrobial peptides. Aureolysin (a metalloproteinase) could cleave and therefore inactivate cathelicidin LL-37. Staphylokinase (SAK), a secreted plasminogen binding protein, was reported to form a complex with α defensin and almost completely blocked its antimicrobial activity. This activity was independent of plasminogen binding. S. aureus strains that produce SAK had a higher survival rate in vitro and higher virulence in a mouse arthritis model .

1. 4. 3. 2 Prevent phagocytosis and opsonision

1. 4. 3. 2. 1 General anti-opsonin molecules

S. aureus clinical isolates produce a capsular polysaccharide outer cell wall. Serotypes 5 and 8 of the capsular polysaccharide are associated with increased virulence. The capsule is anti-opsonic via blocking of surface deposition of opsonins and their receptor/ligand interaction.

Immunoglobulins are targeted by protein A (SpA) and its homologues Sbi (S. aureus IgG-binding protein), SSL7 (Staphylococcal superantigen-like protein

7) and SAK. SpAis a 42 kDa surface anchored molecule. It has four to five IgG binding units that could interact with IgG Fc portion. This interaction presents IgG to leukocytes Fc receptor in the wrong orientation and therefore prevents recognition. In the present of IgG, strains expressing high level of SpA were shown to be more resistant to leukocyte phagocytosis than SpA-poor strains, especially when the sole source of opsonin was purified IgG. Opposite results occurred with IgG-deficient serum, indicating SpA confers protection against neutrophil through IgG interaction. Sbi contains two IgG binding sites at the N-terminal domain. The significance of the Sbi/IgG interaction remains to be fully established. SAK forms a complex with plasminogen and converts plasminogen to plasmin. Plasmin cleaves IgG and C3b deposited on S. aureus resulting in reduced recognition by phagocytes and likely impaired initiation of C1q mediated CP activation .

1. 4. 3. 3 Complement inhibition

S. aureus is now recognized as ‘ a master of complement evasion’. Unlike other bacteria which use host factors to ‘ disguise’ the bacteria or inhibit complement activation, S. aureus produces a group of proteins to attack the key elements of the complement cascades. The list of anti-complement virulence factors is growing. So far, five complement inhibitors have been demonstrated and well characterized: SCIN; Efb; Ehp; SSL7 and Sbi. Interestingly, SCIN, Efb, Ehp and Sbi all use a triple α helix bundle as their functional domain while their modes of action are markedly different (Fig. 1. 2). SSL7 belongs to the SSL protein family. Its function will be discussed in context with other members of the family in a later section.

1. 4. 3. 3. 1 SCIN

SCIN (staphylococcal complement inhibitor) is a human-specific 9. 8 kDa secreted protein with a trip α helix structure (Fig. 1. 2) and is produced by 90% of S. aureus strains. SCIN was shown to inhibit all three complement activation pathways. In the presence of SCIN, the deposition of C3b and MAC were prevented, so was the C2b and Ba release. However the amount of surface bound C3 convertases (both forms) were increased, suggesting SCIN had a stabilizing effect on this complex. SCIN bound to surface-immobilized C3b directly and the binding site was predicted to involve the C3b MG7 and MG8 domain and the area nearby. This was confirmed by the co-crystallized SCIN-C3bBb complex. In vitro binding studies suggested the complex was likely to form in a 1: 1 or 2: 2 ratio. Indeed in the crystal structure, the complex appeared as a SCIN2C3b2Bb2 heterodimer that was bridged by the two SCIN molecules cross-linking the two C3b fragments. However this cross-linkage was not critical in SCIN complement inhibition as monomeric interaction also resulted in stable and inhibited C3 convertase. Native C3 could still associate with the SCIN-C3 convertase complex, but the production of C3b was largely inhibited, suggesting the convertase was likely trapped in a dysfunctional yet stable state by SCIN. Moreover, SCIN exerted a partial competition for factor B binding to C3b and a complete competition for factor H/C3b binding. The significance of the inhibition on factor H was not clear although reducing iC3b (inactive product of the cleavage of C3b) mediated downstream signaling could be beneficial for the bacterium. Physiologically, C3 convertase decays shortly after assembly. This disassociation is required for further cleavage of C2 and factor B. Thus SCIN functions by preventing C3 convertase decay; hindering conformational

activation of C3 convertase and possibly by blocking C3b/iC3b mediated signaling .

1. 4. 3. 3. 2 Efb

Efb (extracellular fibrinogen-binding protein) and the recently discovered Ehp (Efb-homologous protein aka Ecb) have been shown to inhibit C3b deposition in AP and C5 convertase activity in all three pathways. The efb gene is present in 85% of S. aureus isolates sequenced to date. Its inhibitory effects on complement mediated lysis and neutrophil activation in response to C5a have been demonstrated. The Efb complement binding domain (Efb-c) is also a triple α helix huddle, although the arrangement is different from SCIN (Fig. 1. 2). It binds both C3 and C3b via the C3d fragment. However, which of the C3 forms is the primary target of Efb is debated. Structural-biochemical analysis suggested the binding affinity of Efb-C to native C3 was higher than that to C3b. The binding was proposed to induce conformational changes in C3 and prevent proper cleavage of the molecule. This view was challenged by Jongerius et al based on the observation that C3 cleavage was not influenced by Efb-C in CP/LP. Instead, they suggested Efb-C attacked complement intermediates that contain C3b. Therefore, in CP/LP, C3 convertase (C4b2a) was not affected by Efb but C5 convertase (C4b2aC3b) was inhibited, as evidenced by the decreased C5a production. Ehp was found to contain two C3d binding sites and showed higher levels of inhibition compared to Efb, though the mechanism of action was proposed to be similar. Nevertheless, the C3d fragment carries another important task: mediating interaction with complement receptor 2 (CR2) on B cells and facilitating B cell activation. Both Efb-C and Ehp were shown to completely

inhibit C3d: CR2 interaction and its stimulatory effect on a B lymphoma cell line. The predicted binding site of CR2 on C3d was in close proximity to the residues that have been shown to be involved in Efb-C: C3d and Ehp: C3d binding. The exact competition mechanism remains to be confirmed .

1. 4. 3. 3. 3 Sbi

Sbi is another secreted S. aureus protein that targets C3 activation. Sbi binds C3b but has a stronger affinity to C3dg. The binding was shown to be improved when the C3a domain was also present. These observations suggested the C3dg and C3a portion of C3 were important for Sbi/C3 interaction. By comparing binding profiles of different domains of Sbi, the C3 binding site was proposed to be located in its fourth domain (Sib IV). Interestingly, Sbi IV alone inhibited AP activation in a dose-dependent manner. However when the third domain was also present, incubation of human serum with this recombinant protein induced activation of complement, as shown by the degradation of native C3. A distinctive C3 derivative was present in Sbi-III-IV treated serum, whose molecular weight and migration behavior on two dimensional SDS-PAGE correspond to a transacylation product of C3b and Sbi. The cleavage of C3 activates an internal thioester moiety in the C3b fragment, which allows C3b to form covalent bonds with hydroxyl groups in close proximity (e. g. bacterial surface). Thus it was proposed the Sbi III-IV provided a fluid-phase transacylation target for C3 cleavage and cause non-effective activation of the alternative pathway. Since mutational study confirmed the Sbi-IV was responsible for the complement inhibitiory effect, Sbi-III was proposed to be important in consumptive complement activation. Once again, Sbi IV adopts

the triple helix structure, arranged in a similar fashion as Efb-c and Ehp (Fig. 1. 2). Just like Efb and Ehp, the binding of C3dg to CR2 was inhibited by Sbi-III-IV, implying that Sbi may also influence adaptive immunity .

1. 4. 3. 4 Preventing phagocyte extravasation and chemotaxis

1. 4. 3. 4. 1 CHIPS; FLIPr and FLIPr-like

CHIPS (the chemotaxis inhibitory protein of S. aureus) was identified for its ability to prevent neutrophil chemotaxis to formylated peptide and C5a. CHIPS encodes a 121-amino acids (aa) secreted protein and presents in 62% of S. aureus clinical isolates. Postma et al demonstrated that CHIPS selectively and directly bound to C5a receptor (C5aR) and formylated peptide receptor (FPR) expressed on cell surface and competed with their respective ligands for binding. Receptor activation, as measured by the Ca2+ influx and murine neutrophil migration to the injury site, was inhibited. The two receptors were shown to interact with different areas on CHIPS. The N-terminal 6 residues of CHIPS, F1 and F3 in particular, were identified as the FPR binding sites. While the C5aR-binding domain was mapped to residues 31-121, which forms a packed structure similar to the β grasp domain. Arginine 44 and lysine 95 were shown to be critical for its antagonizing activity. C5aR binds CHIPS through its N-terminus residues 10-18. These residues were suggested to be either involved directly in C5a binding or were required to stabilize the interaction. Therefore, CHIPS may function by direct competing with C5a or by disrupting the formation of the stable ligand/receptor complex .

Two more S. aureus proteins were found to inhibit neutrophil chemotaxis. The secreted FPR-like 1 inhibitory protein (FLIPr) and its homologue FLIPr-like

bound FPR and FPR-like 1 receptor (FPRL1). The N-terminal 6 residues of both proteins were important in both interactions but the phenylalanine was not critical for FRP binding. The inhibition of FPRL-1 by both proteins occurred in the nanomolar range. FLIPr-like was shown to be a potent inhibitor for FPR with activity comparable to CHIPS. However, animal infection models with chips or flipr isogenic deletion strains has not been reported. The contribution of CHIPS in bacterial infection is yet to be determined .

1. 4. 3. 4. 2 Map

Map (MHC class II analog protein a. k. a. Eap) is a multifunctional 60-70 kDa secreted protein expressed by 97% of S. aureus isolates. It is an anti-inflammatory molecule that reduces leukocyte availability and function. Map can interact with a wide range of host ligands, including Fg, Fn and ICAM-1 (intercellular adhesion molecule-1). Map largely blocked ICAM-1 mediated firm adhesion and transmigration of neutrophil. In a peritonitis mice model, intraperitoneal injection of Map or pre-treatment of mice with Map showed 50-75% inhibition of neutrophil recruitment. Neutrophil infiltration in mice infected with a map- strain was 2-3 folds higher than that seen with a map+ strain, demonstrating Map inhibits ICAM-1 mediated neutrophil migration in vivo. Interaction of Map with ICAM-1 may modify signal transduction in leukocytes. NF-κB activation and tissue factor release in THP-1 cells were diminished by Map treatment. A substantial amount of IL-6 and TNF-α and a smaller amount of IL-4 were released from Map treated human CD14+ PBMC in vitro. Anti-ICAM-1 antibody blocked the action, suggesting the induction was also mediated by ICAM-1. It is not clear if cellular processes other than

cytokine production are also influenced, although Map's effect on PBMCs appears to be dose dependent .

In addition to the cytokine changes, T cell function was impaired by Map. Mice treated with Map showed significantly reduced T cell mediated delayed-type hypersensitivity (DTH) and T cell proliferation. Adoptive transfer of T cells from Map-treated mice to untreated recipients prevented the recipients from developing DTH when challenged with allergen. The Map