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Effects of *Staphylococcus aureus* hemolysin toxins on blood cells and association with skin and soft tissue infections

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Abstract

Staphylococcus aureus (*S. aureus*) is gram positive, catalase positive cocci which belongs to the family of Staphylococcaceae and is long known as clinical and foodborne pathogen. The emergence of multidrug resistance strain of S. aureus which is methicillin resistant *S. aureus* (MRSA) challenges the health care system because it can cause wide variety of hospital and community acquired skin and soft tissue infections which are difficult to treat. The virulence of *S. aureus* is because of different factors which includes toxins, enzymes and superantigens. *S. aureus* produce variety of exotoxins, enterotoxins and exfoliative toxins which contributes to the virulence of S. aureus. Hemolysin toxins produce by *S. aureus* strains are associated with different skin and soft tissue infections (SSTIs) and can cause the lysis of RBCs. Hemolysins are regulated by accessory gene regulator (agr) and is required for the enhanced expression of virulence factors secreted by *S. aureus*. Hemolysins have leucolytic activity and can help in iron scavenging from host. The most important toxin is alpha hemolysin which can induce the apoptosis and cause the lysis of epithelial cells, erythrocytes and keratinocytes. Human immune cells are affected by beta hemolysin and gamma hemolysin is a biocomponent toxin. Delta hemolysin is low molecular weight exotoxin which belongs to the class of phenol soluble modulins.

Keywords: MRSA, Exotoxins, Hemolysins, SSTIs

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1. INTRODUCTION

Staphylococcus aureus is known as opportunistic pathogen that is involved in the pathogenesis of various infections due to its ability to evade immune system. These infections include normal skin lesions to deep skin abscesses and life-threatening condition known as sepsis[1]. *Staphylococcus aureus* is Gram positive bacteria and the presence of a carotenoid pigment satphyloxanthin protects them from reactive oxygen species. Due to this carotenoid pigment these bacteria produce golden color colonies and have antioxidant properties. Macrophages and neutrophils control *Staphylococcus aureus* infection by phagocytosis but *S. aureus* possess different mechanisms to evade the immune response like avoid phagocytosis, survival inside the phagocyte and production of different toxins[2,3].

Normally *S. aureus* colonize the skin and mucous membrane but methicillin-resistant *S. aureus* (MRSA) which was limited to immune-compromised persons in hospital setting have now emerged to cause disease in healthy populations. In 20-30% population *S. aureus* is present asymptomatically most often in nostrils[4].

The most important protective barrier against bacterial infection is skin and epidermal cells has receptors that recognize pathogens and antimicrobial peptides are produced by skin as early immune response. Macrophages, dendritic cells NK cells in skin and other commensal microorganisms prevents pathogen growth by acting as a life of defense. Despite of these protective barriers 76% of all skin and soft tissue infections (SSTIs) are because of *S. aureus* [5].

Hospital acquired infections because of *S. aureus* and methicillin-resistant *S. aureus* (MRSA) strains challenges health care system. Community acquired MRSA (CA-MRSA) with increase virulence can affect the healthy individuals of community. Since 1990 CA-MRSA is emerged as global concern because it is the causative agent of skin and soft tissues infections. CA-MRSA is generally more susceptible than MRSA to non beta-lactam antibiotics but is more virulent than MRSA and a causative agent for life-threatening and rapidly progressive diseases[6].

S. aureus is renowned for wide range of virulence factors which includes hemolysins, secreted enzymes, exotoxins, and capsules. These virulence factors contribute to the pathogenesis of *S. aureus* and helps in the evasion host defenses. Alpha-hemolysin toxin that is encoded by genome is different from other mobile genetic elements encoded toxins. These toxins contribute to the development of different diseases like toxic shock syndrome (TSS), deep skin infections and necrotizing pneumonia. These toxins degrade inter-cellular connections or modulate immune response to damage the host cell membrane[7].

S. aureus strains are versatile because mobile genetic elements encode various virulence factors, and these are transferred by Horizontal gene transfer (HGT) between different *S. aureus* strains. It is revealed by genome analysis that genes sets that encode different virulence factors are present on pathogenicity islands and these genes are translocated by conjugation, transduction and direct uptake of DNA from environment[8]. The aim of this review is to describe the role of hemolysins producing *S. aureus* and its association with different skin and soft tissue infections.

2. *Staphylococcus aureus* and SSTI

When bacteria evade the skin, it acts as a first line of defense and provide a protective barrier against pathogenic bacteria to prevent their entry into deeper sites of skin. When this protective barrier is breached by pathogens and microorganism penetrate deeper tissue then a series of events take place and immune cells localized at infection site. *S. aureus* is an important bacterium for skin and soft tissue infection is long known since its role was discovered in pathogenicity of pyogenic abscesses by Alexander Ogston[9]. Keratinocytes that provide a physical barrier to prevent pathogen entry can also generate pro-inflammatory response because they can detect invading pathogens due to presence of pathogen recognition receptors[10].

Among all hospital acquired infections (HAI), surgical site infection accounts for 31% that occurs at site of incision. The most common SSTI is cellulitis in which disruption of skin protective barrier and lymphedema are most important factors. Necrotizing fasciitis is the most noxious soft tissue infection which destroys muscle fascia[11]. Different type of SSTI due to *S. aureus* are described in Table 1. *S. aureus* produce wide variety of exotoxins, but hemolysins are most important one which can help bacteria to spread within host cells by killing different host cell populations including immune cells[12].

Table 1. Different skin and soft tissue infections due to *S. aureus*.

Impetigo	Infection of children, red sores around mouth and nose, highly contagious
Cellulitis	Swollen red skin, Bacteria penetrate through skin crack
Abscesses	The most common community associated infection
Folliculitis	Inflammation and infection in hair follicles
Necrotizing Fasciitis	Noxious soft tissues infection

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Pyomyositis	Skeletal muscle infection caused by bacteria

3. Hemolysins

These toxins are receptor mediated and can lyse red blood cells. α, β and γ are different classes of hemolysins and δ-hemolysin activity is receptor independent and known as phenol soluble modulin (PSM). The most studied alpha-hemolysin that destroy red blood cells and leukocytes is β-barrel pore forming cytotoxin[13].

3.1 Alpha Hemolysin

In late 1800s toxic activity of *S. aureus* was studied, and these toxic substances were lethal to guinea pigs and rabbits results in hemolysis and conjunctival epithelium inflammation[14]. The discovery of α-hemolysin was accidental in 1928 in Australian town where 12 children out of 20 were died because they were vaccinated with diphtheria toxoid. It was reported by Burnet that *S. aureus* exo-substances have toxic properties and led to the discovery of α-hemolysin[15].

3.2 Alpha-toxin nature

Alpha-toxin which belongs to the class of pore forming toxins *hla* gene encodes this toxin[16]. It is the causative agent of skin and soft tissue infections, sepsis and pneumonia. Alpha-toxin active against different cell types, but rabbit erythrocytes are mostly affected and human lymphocytes and monocytes are susceptible to α-hemolysin[17]. α-hemolysin is secreted by 95% of *S. aureus* strains and is water soluble with a molecular weight of 33KDa and have pro-inflammatory activities[18]. Accessory gene regulator (*agr*) regulates the expression of *hla* gene. Alpha- hemolysin is produced by *S. aureus* in varying amounts because *hla* gene is not mobile. α-hemolysin destroy variety of host cells by forming heptameric pores including RBC's, monocytes, lymphocytes, and epithelial cells. Alpha-hemolysin binds to its proteinaceous receptor ADAM10[19].

Tissue barrier function, cell differentiation and control of cellular activation is regulated by ADAM10. Small pore is formed when toxin binds to the cell which restricts the movement of macromolecules but allows the rapid efflux of K^+ ions and ATP across the cell membrane. Extracellular Ca^{2+} influx is increased in the cell after pore formation and increase concentration of Ca^{2+} is involved in hydrolysis of membrane phospholipids. The binding of alpha-toxin activates ADAM10 that results in the degradation of E-cadherin in the epithelial adherens junction. These mechanisms contribute to vascular leakage and disrupts the integrity of epithelial layer and helps in the invasion of *S. aureus*[20].

As epithelial cells are receptors for alpha toxin, the substrate for ADAM10 is E-cadherin that is cleaved by ADAM10 and because of this interaction between cadherin molecules is lost and epithelial tissue barrier is disturbed between adjacent cells[21]. In different studies it is observed that during infection ADAM10 and Alpha toxin complex is the reason for E-cadherin cleavage that results in injury of epithelial tissues. The strength of tissue barrier is maintained by structural proteins and ADAM10-α toxin complex dephosphorylates these proteins that binds the cells to the basement membrane[19].

Cells can be lysed if the diameter of pore is 1-2nm because of the leakage of molecules like ATP that maintain different cellular processes. The name alpha hemolysins can be justified by the fact that osmotic swelling occurs in erythrocytes and hemoglobin is released from cells. Cell death have consequences like monocytes and macrophages that are involved in body defense system are killed by these toxins[17].

4. Beta Hemolysin

Glenny and Stevens first identified beta-hemolysin in 1935[22]. A large number of *S. aureus* strains secretes this toxin, and it belongs to the non-pore forming class of hemolysin. β-hemolysin which is highly active against sheep erythrocytes is extremely basic, and involve in the lysis of erythrocytes for its haem source[23].

Beta hemolysin has a 35KDa molecular weight and gene that encodes beta hemolysin is *hlb*. Clinical conditions like lung and eye infection are associated with this toxin[24]. The second name for this toxin is hot-cold toxin because after 37C overnight incubation it shows enhanced hemolytic activity at below 10C.

Based on the isoelectric pH sphingomyelinase has three forms neutral, basic, and alkaline and beta hemolysin is neutral sphingomyelinase. It hydrolyzes sphingomyelin the most abundant eukaryotic sphingolipid into phosphorylcholine and ceramide and its activity is Mg^{2+} dependent. Stimulation of second messenger system, mitogen activation protein kinases (MAPKs), cell shape changes, and induction of cell death are different mechanism in which ceramide play an important role[25].

Because of sphingomyelin content cells are sensitive to beta toxin like Leukocytes and Keratinocytes are more susceptible than sheep erythrocytes. Mostly cells are not lysed by beta hemolysin but cells become sensitive to other toxin like alpha-hemolysin and PVL[26]. Different strains isolated from bovine mastitis and skin chronic infection produce large quantities of beta toxin. Its cytotoxic activity is against keratinocytes, monocytes, leukocytes and it inhibits interleukin 8(IL-8) expression that contributes to the escape from phagocytosis[27].

Recent studies describe that there is an association between beta toxin gene (*hlb*) carriage and potential of *S. aureus* to cause respiratory tract infections because *hlb* gene is present in MRSA strains[28]. It is observed that beta hemolysin activity is not efficient as other hemolysins because when cells are present only in low temperature it lyses red blood cells. beta hemolysin acts on sphingomyelin and mostly it disrupt the plasma membrane and cause irregularity in fluidity of membrane[29].

5. Gamma Hemolysin

In 1938 Smith and price discovered Gamma hemolysin which can lyse wide range of erythrocytes of different species[30]. The genes that encode gamma hemolysin is present in 99.5% *S. aureus* strains. Gamma hemolysin is a bicomponent toxin secreted by *S. aureus* and Panton-Valenine Leukocidin (PVL) is another toxin which is also bicomponent. Two secreted proteins known as S and F components made these toxins. Every strain of *S. aureus* secretes Gamma hemolysin but 2-3% strains produce PVL. Because of agar inhibitory effect it is not identifiable on agar [31].

The gamma hemolysin locus which is the part of core genome of *S. aureus* contains 3 genes, *hlgA, hlgC* and *hlgB* each of which is transcribed by its own promotor. S subunits contains HlgA and HlgC which combines with F-subunit (HlgB) and form leukocidins HlgAB and HlgCb with unique toxic properties. HlgAB has cytolytic activity against human and rabbit leukocytes and lyse red blood cells effectively as compared to the HlgCB which has low activity against red blood cells. For *S. aureus* survival during sepsis and to evade the macrophages HlgAB is necessary[32].

This biocomponent toxin is water soluble monomer and important components for its activity are S and F. S component has cell type specificity and it binds to receptor on cell and induce conformational changes that allows the oligomerization with F component and form pores in the membrane that results in lysis of cell[33].

6. Phenol soluble modulins

There are three different peptides identified as phenol-soluble modulins (PSMs) which include PSMα, PSMβ and PSMδ. PSMδ is known delta toxin because it is identical to δ-toxin of *S. epidermidis*. It was discovered in 2007 that delta hemolysin of *S. aureus* belongs to the class of secreted peptides known as phenol soluble modulins[34]. The pathogenesis of *S. aureus* is because of delta toxin is well studied[35].

S. aureus genome encodes three types of PSMs, Psmα operon encodes PSMα, psmβ operon encodes PSMβ and coding sequence for RNAIII encode delta toxin. 97% of *S. aureus* strains produce delta hemolysin that is a 26 amino acid containing small peptide toxin and have molecular weight of 3000 datons[36].

By binding with FPR2 receptor phenol soluble modulins generate inflammatory response and form short lived pores and it has ability to spread on different surfaces and biofilm formation contributes to its pathogenesis[37]. PSMs can mediate cytokines secretion from neutrophils even at very low concentration and generate inflammatory response[35].

7. Virulence factors regulation in *S. aureus*

Expression of virulence genes of *S. aureus* is regulated by accessory gene regular (*Agr)* system. Pathogenesis of *S. aureus* is because of expression of these genes. In contrast to Agr system other regulatory systems which work either coordinately or independent are also found in *S. aureus* which includes Staphylococcal accessory regular A(SarA), exoprotein expression (Sae) and Staphylococcal alternative sigma factor B (SigB)[38].

7.1 Agr and Sae

Agr system is the prominent regulator in the production of *S. aureus* virulent factors. The regulation of toxins such as alpha, beta, gamma hemolysins and PVL, exfoliative is controlled by this system. Agr system is required for the enhanced expresseion of various enzymes that are secreted by *S. aureus* [39].

Agr locus is made up of two transcriptional units which are regulated by P2 and P3 promotors. RNAII which encodes the *agr* system core machinery is an operon contains *agrBDCA* genes and its transcription is controlled by P2. RNAIII is the regulatory RNA of *agr* system and its transcription is controlled by P3. AgrD which is the peptide precursor of autoinducer peptide is processed and cyclize by AgrB and SpsB into an octapeptide and AgrB is involved in its export[40].

AgrC and AgrA form two component signal transduction system of bacteria that are homologous to histidine protein kinase sensors. For upregulation of toxins such as hemolysins and enzyme translation of RNAIII is necessary which is carried by binding of autoinducer peptide (AIP) on receptors present on AgrC, which undergo phosphorylation of AgrA. AgrA triggers the P2 and P3 driven transcription after changes into its active form and after this step RNAIII translation occurs. AgrA can also enhance the expression of PSMs by transcription of two promotors. The region that encodes RNAIII also has coding region for *hld* gene which encodes delta hemolysin[41].

The second two component regulatory system that is involved in *S. aureus* expression of toxins is encoded by sae locus. The regulatory response is generated by two genes saeR and saeS and saeR/S system is involved in the pathogenesis of *S. aureus* encodes different toxins like hemolysins, PVL, enterotoxin and protease[42].

Exoprotein synthesis is mediated by AgrC-AgrA and SaeR-SaeS systems. AgrA activates RNAIII transcription and control the expression of exoproteins. AgrD encodes an autoinducing peptide through which AgrA is activated by interaction with AgrC as described in Fig.1. It is studied that transcription of *hla, hlb* and *coa* genes is carried by sae and its inactivation has no effect on *agr* system. The mechanism by which sae and agr are acting is unclear but both these system are involved in the expression of virulence factors(*hla, hlb, hld and coa*)[43].

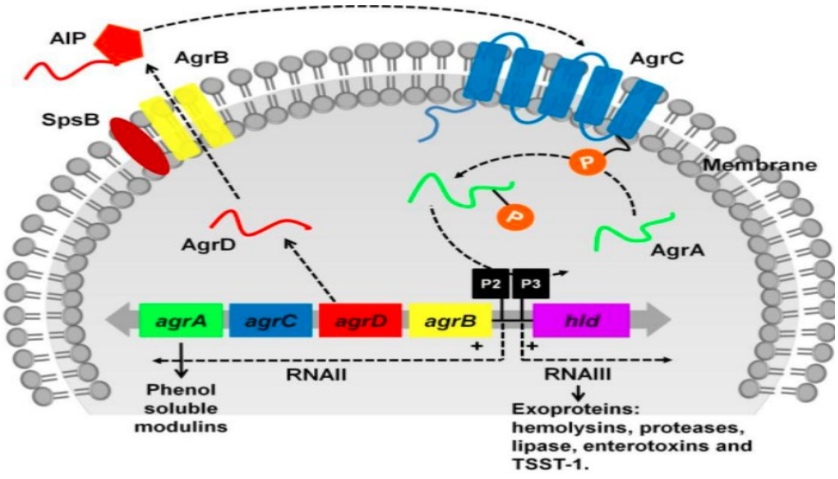

Fig.1. *Staphylococcus aureus* regulatory system

7.2 SarA and SigB

Since the discovery of this transcription regulator enough studies describe that *SarA* locus modulate more than 120 genes[44]. Different virulence factors like α and δ hemolysin, exotoxin of *S. aureus* and proteins like exoprotein A, fibrinogen all are upregulated by *SarA.* Biofilm formation ability of *S. aureus* is also corelated to *SarA* and without this bacteria are susceptible to different class of antimicrobials. P1, P2, P3 are three promotors that are present in *SarA-locus* that code for SarA protein. SarA can regulate the transcription of different toxins by binding to the promotor conserved sequence region and can also initiate the transcription of surface proteins[45].

In response to different stress conditions sigma factors mediate the regulation of proteins that respond to these stress conditions. *SigB* gene is present on operon and associated with three other genes *rsbU, rsbV, rsbW.* Under normal conditions transcription is not initiated and *SigB* is bounded with *rsb.* But when condition are stressed *SigA* mediate the expression of *SigB* and transcription of whole operon is initiated [46].

SigB can regulate the expression of 200 genes in which genes that are important is *S. aureus* virulence and antibiotic resistance are also included. *SigB* play an important role during lung infection because when bacteria colonize the lung, pulmonary surfactants are produced that can damage the bacteria[47].

8. Effect of toxins on cells

PFT receptors play important role in assembly of toxin and formation of pore and these can be easily identified on the basis of expression profile of receptor. There are several mechanisms which are used to determine toxic effects on cells. In intracellular signaling action of PFT is important in breakage of plasma membrane which leads to imbalance in osmotic pressure that causes cell death. PFT intoxicated cell increases intracellular Ca^{2+}. Entry of Ca^{2+} is due to through Hla pores which are formed in plasma membrane. More Ca^{2+} will store in endoplasmic reticulum but that which are present in lysosomes will act as acidic. Not only increase in Ca^{2+} Hla pore formation can also cause extracellular Na+ influx into the cell and K+ efflux. Entry and removal of Na+ and K+ is done by Na+/K+ ATPases[48].

Inflammatory signaling is harmful as well as beneficial during *S. aureus* infection. In one-way abnormal inflammation can cause tissue damage as well as lethality. On the other way it will fail to receive inflammatory signals which can damage the bacterial growth. PFT also stimulate necroptosis it is the process in which programmed cell death pathway cause inflammation. Necroptosis is more dependent on an oxidative burst which is caused by ion efflux which is specifically confirmed for the *S. aureus* PFTs independently[49].

Disruption of barrier is considered as best character of *S. aureus* infection. Hla has the capacity to direct damage the epithelial or endothelial cells. Monolayers will also disrupt by Hla in lung injury. They also have ability to develop smaller abscesses. Loss of monolyer is due to cell death and loss of intercellular adhesion. Hla can also induce upregulation of ADAM10 enzymatic activity which may cause cleavage of cadherins, which is an important component in adherent junction. ADAM10E384A has ability to bind with Hla which will allow pore-formation[50].

The expression profile of the receptors may have the capacity to target epithelial and endothelial cells for disruption of barrier function. PVL has capacity to create pores on the keratinocytes and induce cell death. Similarly, ADAM10 may also has the capacity to create leukocidin toxicity, furthermore its enzymatic activity may be important in activation of one other PFT, pneumolysin, which may result in E-cadherin cleavage. ADAM10 represent a common mechanism in which all PFTs can disrupt barrier function [51].

4. CONCLUSIONS

S. aureus is present as a commensal bacterium in 20-30% of individuals, but it is considered as an opportunistic pathogen because it can invade the skin through small cuts and can cause variety of SSTIs and blood stream infections. *S. aureus* produce a significant amount of virulence factors which helps it to evade the host immune system and can cause variety of infections. Biofilm formation ability and resistance to antibiotics is the major reason for *S. aureus* infections. *S. aureus* may have acquired these virulence factors through evolution so it can better adapt to variety of infections.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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