Immune Evasion by Staphylococci

Abstract

Staphylococci are a common cause of hospital-acquired infections and are increasingly linked to community-acquired infections. There are two primary human pathogens in the genus; *Staphylococcus aureus* and *Staphylococcus epidermidis*. The latter is generally non-pathogenic, although it can cause disease in immunocompromised individuals. In contrast, *S. aureus* infection is often much more serious and can, in some cases, be life-threatening. *S. aureus* possesses a variety of virulence factors that enable it to evade the host immune system, the majority of which are not found in *S. epidermidis*. *S. aureus* produces a variety of proteins that perform various functions in evasion of the immune system, but also has certain structural features that enable it to thwart the host immune response. Understanding the pathogenesis of this organism is critical to developing effective and sustainable treatments for *S. aureus* infections. By elucidating the mechanisms by which the organism causes disease, it may be possible to identify new drug targets either for preventative or curative treatments. In addition, there has been interest in exploiting this knowledge for use in the treatment of autoimmune disorders. Autoimmune disorders are characterised by an excessive or unwanted immune response, which is harmful to the patient rather than protective. Therefore, the proteins produced by *S. aureus*, which have the effect of interfering with or inhibiting the immune response, may have use as therapeutic products for these types of illnesses. This review will focus on the evasion of the innate immune response in humans by *S. aureus*, namely evasion of the complement system, phagocytes and antimicrobial peptides, as well as briefly outlining some of the promising applications of this research in the treatment of *S. aureus* infection and auto-immune disorders.
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Introduction

Staphylococci are a common cause of nosocomial (hospital-acquired) infections and are increasingly linked to community-acquired infections (Carleton et al., 2004). *Staphylococcus aureus* and *Staphylococcus epidermidis* are the primary staphylococcal pathogens in humans. *S. epidermidis* is generally non-pathogenic, although it can cause disease in immunocompromised individuals, including the elderly and those with indwelling medical devices (Foster, 2005). On the other hand, *S. aureus* infection is often much more serious and can lead to life-threatening diseases. This may be due in part to the fact that *S. aureus* has a variety of virulence factors which enable it to evade the host immune system that are not present in *S. epidermidis* (Foster, 2005). For this reason, *S. aureus* will be the primary focus of this review.

*S. aureus* has a variety of virulence factors, but in this review we will focus only on those virulence factors that are involved directly in evasion of the immune system. The majority of immune evasion strategies used by *S. aureus* relate to evasion of the innate immune system. The innate immune system consists of three primary components; complement, phagocytes and antimicrobial peptides. The various strategies that *S. aureus* uses to evade these three components will be discussed. It should be noted that there is a degree of overlap between these three subheadings, e.g. certain staphylococcal proteins have multiple immune evasion mechanisms. *S. aureus* primarily uses an array of secreted, cell wall-anchored and membrane-bound proteins to defend against the host immune response. However, it also has some structural features which contribute to its ability to evade destruction by the immune system. These mechanisms will be outlined and their potential implications for treatment of *S. aureus* infection will be discussed.

1.1 *Staphylococcal infection*

Staphylococci are a genus of coccoid, Gram-positive, facultative aerobes (Casey et al., 2007). They are commonly found in commensal relationships with animals and humans. They are primarily found on the skin of healthy individuals, particularly in moist skin areas, such as the nostrils. However, they are also found in lower numbers
in the respiratory tract, the gastrointestinal tract and the urogenital tract (Zecconi and Scali, 2013).

*Staphylococcus epidermidis* and *Staphylococcus aureus* are the two species in the genus that most frequently cause disease in humans. Of these two, *S. aureus* (Figure 1) is more clinically significant, i.e. it is more frequently associated with disease. *S. epidermidis* is generally nonpathogenic, although it can cause disease in patients with severely compromised immune systems (Foster, 2005).

![SEM of Staphylococcus aureus](image)

Figure 1. SEM of *Staphylococcus aureus*. (Source: Centers for Disease Control and Prevention, Public Health Image Library)

*S. aureus* is a common healthcare-associated pathogen and often causes infection in immunocompromised individuals. The emergence of antibiotic-resistant strains such as MRSA (Methicillin Resistant *Staphylococcus aureus*) represents a significant threat to human health in hospital environments; MRSA is now estimated to cause 44% of all nosocomial infections in Europe every year (Gould et al, 2012). Infection with *S. aureus* can cause diseases of the skin, soft tissue, respiratory tract and endovascular system. Diseases caused by *S. aureus* infection can range from the superficial (e.g. boils) to the deeply invasive (e.g. meningitis) and can be life-threatening. The bacterium has a plethora of virulence factors that enable it to cause
infection, and a subset of these virulence factors relate directly to evasion of the host immune response.

1.2 Immune mechanisms encountered by staphylococci

Immunity is defined as the ability of the body to resist disease. The immune system in humans consists of innate and adaptive immunity. The innate immune system comprises the non-specific aspect of the immune system and is the first line of defence against invading microorganisms. It consists of various cells and molecules, including phagocytes, complement, natural killer cells and dendritic cells. The innate immune system is immediately activated upon infection with a pathogen, as many of the components of innate immunity are present and fully functional prior to infection. Components of innate immunity then stimulate the adaptive immune response (Medzhitov and Janeway, 2000).

Adaptive immunity is the specific response of the immune system to a pathogen. While the cells and molecules of the innate immune system are designed to defend against a wide range of pathogens, the cells and molecules of adaptive immunity are specific to a particular pathogen, with a particular antigen. The main components of the adaptive immune system are B and T lymphocytes. B cells produce antibodies that are specific to a particular antigen, while effector T cells carry out a range of functions in destroying pathogens (Medzhitov and Janeway, 2000). The majority of staphylococcal immune evasion mechanisms relate to the innate immune response. The adaptive immunity appears to be ineffective in providing immunological memory in the case of staphylococcal infection and is generally not sufficient to prevent recurring infection (Foster, 2005). The innate immune system has three primary components that combat invading microbes – complement, phagocytes and antimicrobial peptides.

1.2.1 Complement

The complement system consists of a series of proteins that are activated in sequence in response to an invading pathogen (Dunkelberger and Song, 2010). Complement proteins circulate in the blood as inactive enzyme precursors until they are activated. Once the first protein in the sequence has been activated, it becomes capable of
activating the next protein in the sequence by proteolytic cleavage. This protein in
turn activates the next protein in the sequence in the same manner, and so on
(Dunkelberger and Song, 2010). Complement serves two primary functions –
opsonisation and direct lysis of pathogens. In the case of Gram-positive bacteria like
staphylococci, their thick cell wall confers resistance to direct complement attack
(Rooijakkers, et al., 2005c). Therefore opsonisation is the main function of the
complement system in the case of S. aureus. Opsonisation is the labelling of
pathogens for destruction by phagocytes. Phagocytes have receptors that enable them
to recognise specific molecules (chemoattractants) that cause migration of phagocytes
to the site of infection. This process is called chemotaxis.

Complement is initially activated by one of three pathways (Figure 2). Two of these
pathways (the mannose-binding lectin pathway and the alternative pathway) are
components of the innate immune response and one (the classical pathway) is part of
the adaptive immune response. Although the pathways are activated in different ways,
they share one common step – the production of a C3 convertase that cleaves the
complement protein C3 into the fragments C3a and C3b. C3a acts as a
chemoattractant, while C3b acts as an opsonin. Several other important proteins and
protein fragments are released throughout the complement cascade which are
involved in recruiting phagocytes to the site of infection or in directly destroying the
pathogen (Rooijakkers et al., 2005c). S. aureus produces a variety of proteins that
interfere with this process.
Figure 2. Three systems of complement activation. A. The classical pathway: the C1 complex (containing proteins C1q, C1r and C1s) binds to antibodies bound to the bacterial surface. This leads to autoactivation of C1r, which in turn activates C1s, which cleaves C4 and C2 to form the C3 convertase, C4bC2a. Cleavage of C3 produces C3a and C3b. C3a is a chemoattractant, while C3b binds to the C3 convertase to form the C5 convertase, C3bC4bC2a. Cleavage of C5 produces C5a and C5b. C5a is a chemoattractant and C5b forms the membrane attack complex, which is involved in direct killing of pathogens. B. The lectin pathway: mannose-binding lectin (MBL) or ficolin binds to carbohydrate groups on the bacterial cell surface. MBL and ficolin are associated with a variety of MBL-associated serine proteases; MASP1, MASP2, MASP3 and sMAP (small MBL-associated protein). MASP2 activates C4 and C2 to form the C3 convertase, C4bC2a. MASP1 is also capable of directly cleaving C3. The pathway then proceeds as in the classical pathway. C. The alternative pathway: low-level cleavage of C3 by the hydrolysed form of C3 in combination with activated factor B (Bb) gives C3a and C3b. C3b binds factor B, followed by cleavage of B to Bb by factor D, resulting in the formation of the C3 convertase C3bBb. The C3 convertase cleaves C3 to C3b and C3a, resulting in an amplification of the pathway wherein the C3b produced by the C3 convertase feeds back into the pathway to form more C3 convertases. Source: Foster, 2005.

1.2.2 Phagocytes

Phagocytes are cells that engulf and destroy pathogens (Figure 3). Phagocytes recognise a variety of chemoattractants including complement fragments C5a and C3a, and formylated peptides released by bacteria. Neutrophils are the primary
Phagocytes involved in combatting *S. aureus* infection and can also recognise immunoglobulin G (IgG) and complement proteins bound to the bacterial surface. Phagocytes contain a variety of substances which they use to kill pathogens once they have been ingested, including lysozyme, antimicrobial peptides and reactive oxygen species (Foster, 2005).

![Phagocytosis diagram](image)

**Figure 3.** Phagocytosis. The phagocyte migrates to the site of infection by chemotaxis. It then adheres to the bacterial cell, which may or may not be opsonised. The bacterium is taken up into the cell by endocytosis. This results in the formation of a phagosome, a membrane-bound vesicle containing the bacterium. Fusion of the phagosome with lysosomes results in the formation of the phagolysosome. The lysosome contains toxic substances, such as lysozyme, which act to kill the bacterial cell. Reactive oxygen species (e.g. OH\(^{-}\), H\(_2\)O\(_2\), and O\(_2\)\(^{-}\)) are also produced in the phagolysosome as a result of the respiratory burst, requiring the cell to take up O\(_2\). In macrophages, the destroyed bacterial cell is then egested from the cell, while the antigen is processed and presented to lymphocytes. In neutrophils, the phagocyte itself will die after a period of active phagocytosis.

1.2.3 Antimicrobial peptides

Antimicrobial peptides are a group of molecules that can recognise and kill pathogens. They generally do this by disrupting the bacterial membrane, causing lysis.
of the cell, although variations on this method have been observed. These molecules are found in high concentrations on the skin, on mucous surfaces and in neutrophils and platelets (Peschel and Collins, 2001). The majority of antimicrobial peptides are positively charged, which allows them to bind to the negatively charged bacterial cell membrane. Therefore, they are referred to as cationic antimicrobial peptides (CAMPs). A variety of CAMPs are produced within human neutrophils and platelets, however, \textit{S. aureus} has developed various ways to evade these molecules. In particular, \textit{S. aureus} has been found to thwart the action of defensins, cathelicidins and thrombocidins (Peschel, 2002). Defensins are short cationic peptides that bind to the membranes of invading cells and form pores, causing efflux of the cell contents and, ultimately, cell death. Two classes of defensins are produced in humans; \(\alpha\)-defensins and \(\beta\)-defensins. \(\alpha\)-defensins are found primarily in neutrophils while \(\beta\)-defensins are produced by a wide range of leukocytes and epithelial cells (Pescel and Collins, 2001). Cathelicidins serve many functions in antimicrobial defence, including leukocyte recruitment and direct killing of antimicrobial cells. Only one cathelicidin is found in humans, cathelicidin LL-37, which is produced in neutrophils and epithelial cells (Zanetti, 2004). Thrombocidins are produced by platelets and are thought to damage the cell membrane of pathogens (Peschel, 2002). Lysozyme is a bacteriolytic enzyme with a similar function to CAMPs. Lysozyme cleaves peptidoglycan, a unique component of the Gram-positive cell wall, and irreversibly damages the cell wall (Peschel, 2002). \textit{S. aureus} has developed mechanisms of evading destruction by all of these molecules.

2. Complement evasion

Complement proteins act as opsonins, chemoattractants and effector molecules in innate and adaptive immunity (Rooijakkers \textit{et al.}, 2005c). \textit{S. aureus} has developed a variety of mechanisms to evade and interfere with the complement cascade (Table 1). Most of these mechanisms take the form of proteins that interfere with complement (Figure 4).
2.1 Staphylokinase

Staphylokinase is a secreted protein that interferes with the innate immune response in several ways, and acts specifically against complement at two different stages. It does this by converting plasminogen to plasmin, which has the ability to cleave and inactivate both immunoglobulin G (IgG) and C3b. S. aureus cells have surface-bound plasminogen receptors that bind plasminogen, which is then converted to the active form of plasmin by staphylokinase. Activated plasmin then removes the opsonins IgG and C3b which are bound to the surface of the pathogen, disrupting the complement cascade at the beginning of the classical pathway (by preventing opsonisation by IgG) and/or the C3 convertase stage of all pathways. This results in impaired opsonisation and phagocytosis (Rooijakkers et al., 2005a; Rooijakkers et al., 2005c).

<table>
<thead>
<tr>
<th>Protein name</th>
<th>Protein abbreviation</th>
<th>Function</th>
<th>Immune evasion result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylokinase</td>
<td>Sak</td>
<td>Converts plasminogen to plasmin</td>
<td>Cleaves complement factors, inhibits complement cascade, inhibits opsonisation</td>
<td>Rooijakkers et al., 2005a</td>
</tr>
<tr>
<td>Protein A</td>
<td>SpA</td>
<td>Binds IgG</td>
<td>Inhibits classical pathway, inhibits Fc-receptor mediated phagocytosis</td>
<td>Atkins et al., 2008; Falugi et al., 2013</td>
</tr>
<tr>
<td>Staphylococcal binder of immunoglobulins</td>
<td>Sbi</td>
<td>Binds IgG</td>
<td>Inhibits classical pathway, inhibits Fc-receptor mediated phagocytosis</td>
<td>Atkins et al., 2008; Smith et al., 2011</td>
</tr>
<tr>
<td>Staphylococcal superantigen-like protein 10</td>
<td>SSL-10</td>
<td>Binds IgG</td>
<td>Inhibits classical pathway, inhibits Fc-receptor mediated phagocytosis</td>
<td>Itoh et al., 2010</td>
</tr>
<tr>
<td>Staphylococcal complement inhibitor</td>
<td>SCIN</td>
<td>Binds C3 convertases</td>
<td>Inhibits complement cascade, prevents production of opsonins</td>
<td>Rooijakkers et al., 2005b</td>
</tr>
<tr>
<td>Extracellular fibrinogen binding protein</td>
<td>Efb</td>
<td>Binds to C3</td>
<td>Inhibits complement cascade, inhibits opsonisation by C3b</td>
<td>Lee et al., 2004a; Lee et al., 2004b</td>
</tr>
<tr>
<td>Extracellular complement binding protein</td>
<td>Ecb</td>
<td>Binds to C3</td>
<td>Homolog of Efb, inhibits complement cascade, inhibits opsonisation by C3b</td>
<td>Jongerius et al., 2010</td>
</tr>
<tr>
<td>Staphylococcal superantigen-like protein 7</td>
<td>SSL-7</td>
<td>Binds to C5</td>
<td>Inhibits opsonisation by C5a</td>
<td>Langley et al., 2005</td>
</tr>
</tbody>
</table>
2.2 Protein A

Protein A is a membrane-bound protein which binds to the Fc region of surface-bound IgG in the classical pathway (Atkins et al., 2008). The Fc region of IgG is the region that is normally recognised by C1q, the first protein in the classical pathway. Protein A prevents binding of C1q and the initiation of the classical pathway, and also inhibits Fc-receptor mediated phagocytosis (Falugi et al., 2013). Staphylococcal binder of immunoglobulins (Sbi) and staphylococcal superantigen-like protein 10 (SSL10) also bind IgG in this way (Smith et al., 2011; Itoh et al., 2010).

2.3 Staphylococcal complement inhibitor

Staphylococcal complement inhibitor (SCIN) is a secreted protein that binds to the C3 convertases in all three complement activation pathways (C4b2a in the classical and mannose-binding lectin pathways and C3bBb in the alternative pathway). Binding of SCIN to the C3 convertases inactivates them, thereby blocking the complement pathway at this point. The opsonin C3b and the chemoattractant C3a are not produced, resulting in reduced phagocytosis. In addition, binding of SCIN stabilises these complexes, which are usually unstable. The C3 convertases normally dissociate after a short time, leaving C4b and C3b bound to the bacterial cell membrane to form more C3 convertases. By preventing this dissociation, SCIN prevents the formation of more convertases and the amplification of the complement cascade (Rooijakkers et al., 2005b).

2.4 Extracellular fibrinogen binding protein

Extracellular fibrinogen binding protein (Efb) and its homolog Ecb bind to C3 and prevent it from binding to the bacterial cell surface. This prevents opsonisation and the formation of the C3 and C5 convertases, thereby further reducing phagocytosis (Lee et al., 2004a; Lee et al., 2004b; Jongerius et al., 2010).
3. Phagocyte evasion

Phagocytes play a key role in innate immune defence against bacterial pathogens. *S. aureus* is resistant to direct complement attack, therefore the innate immune defence is largely dependent on the action of phagocytic cells, particularly neutrophils (Rooijakkers *et al.*, 2005c). *S. aureus* has developed three main mechanisms of evading neutrophil attack – interference with neutrophil chemotaxis, resistance to the respiratory burst and the production of pore-forming leukotoxins which destroy leukocytes (Table 2).
Table 2. Neutrophil evasion strategies of *S. aureus*

<table>
<thead>
<tr>
<th>Factor Name</th>
<th>Abbreviation</th>
<th>Function</th>
<th>Immune evasion effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inhibition of neutrophil chemotaxis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemotaxis inhibitory protein of <em>S. aureus</em></td>
<td>CHIPS</td>
<td>Binds to C5a receptor and FPR</td>
<td>Prevents chemotaxis</td>
<td>Haas <em>et al.</em>, 2004; Haas <em>et al.</em>, 2005; Postma <em>et al.</em>, 2004</td>
</tr>
<tr>
<td>FPR-like inhibitory protein</td>
<td>FLIPr</td>
<td>Binds to FPR-like 1 receptor (FPRL1) and FPR</td>
<td>Inhibits chemotaxis</td>
<td>Prat <em>et al.</em>, 2006; Stemerding <em>et al.</em>, 2013</td>
</tr>
<tr>
<td>FLIPr-like</td>
<td>N/A</td>
<td>Binds to FPRL1 receptor and FPR</td>
<td>Inhibits chemotaxis</td>
<td>Prat <em>et al.</em>, 2009; Stemerding <em>et al.</em>, 2013</td>
</tr>
<tr>
<td>Extracellular adherence protein</td>
<td>Eap</td>
<td>Binds to ICAM-1 in endothelial cells, prevents binding of LFA-1</td>
<td>Prevents neutrophil adhesion and extravasation</td>
<td>Chavakis <em>et al.</em>, 2002; Haggar <em>et al.</em>, 2004</td>
</tr>
<tr>
<td><strong>Resistance to respiratory burst in phagosomes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphyloxanthin</td>
<td>N/A</td>
<td>Anti-oxidant</td>
<td>Scavenges free radicals and inhibits production of reactive oxygen species</td>
<td>Clauditz <em>et al.</em>, 2006; Liu <em>et al.</em>, 2005</td>
</tr>
<tr>
<td>Catalase</td>
<td>CatA</td>
<td>Degrades hydrogen peroxide</td>
<td>Inactivates reactive species</td>
<td>Cosgrove <em>et al.</em>, 2007</td>
</tr>
<tr>
<td>Alkylhydroxide reductase</td>
<td>AhpC</td>
<td>Catalase activity</td>
<td>Inactivates reactive species</td>
<td>Cosgrove <em>et al.</em>, 2007</td>
</tr>
<tr>
<td>Thioredoxin</td>
<td>N/A</td>
<td>Reducing agent</td>
<td>Inactivates reactive oxygen species</td>
<td>Chavakis <em>et al.</em>, 2007; Roos <em>et al.</em>, 2007</td>
</tr>
<tr>
<td>Superoxide dismutases</td>
<td>N/A</td>
<td>Catalyses dismutation of O$_2^-$</td>
<td>Inactivates superoxide radical</td>
<td>Karavolos <em>et al.</em>, 2003</td>
</tr>
<tr>
<td><strong>Leukotoxins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ð-haemolysin</td>
<td>Hld</td>
<td>Neutrophil binding</td>
<td>Leukolysis</td>
<td>Peacock <em>et al.</em>, 2002; Somerville <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>Panton-Valentine leukocidin</td>
<td>PVL</td>
<td>Pore-forming</td>
<td>Leukolysis</td>
<td>Aman <em>et al.</em>, 2010; Prevost <em>et al.</em>, 1995</td>
</tr>
<tr>
<td>Leukotoxin AB</td>
<td>LukAB</td>
<td>Pore-forming</td>
<td>Leukolysis, may facilitate release of bacterium from inside the phagosome</td>
<td>DuMont <em>et al.</em>, 2011; Ventura <em>et al.</em>, 2010</td>
</tr>
</tbody>
</table>
3.1 Inhibition of neutrophil chemotaxis

3.1.1 Chemotaxis Inhibitory Protein of \textit{S. aureus}

Binding of chemoattractants to receptors on the neutrophil surface stimulates chemotaxis. The chemotaxis inhibitory protein of \textit{S. aureus} (CHIPS) has two distinct binding domains – one which can bind to the neutrophil C5a receptor and one which can bind to the neutrophil formyl peptide receptor (FPR), thus preventing binding of the true ligand and inhibiting chemotaxis (Haas \textit{et al.}, 2004). Formyl peptide receptor-like 1 inhibitory protein (FLIPr) and its homolog FLIPr-like also bind to the FPR and to the formyl peptide receptor-like 1 protein, (a homolog of the FPR, which is also found on neutrophils) further inhibiting chemotaxis (Stemerding \textit{et al.}, 2013).

3.1.2 Extracellular adherence protein

The extracellular adherence protein (Eap) recognises many ligands, including the intercellular adhesion molecule (ICAM-1), which is found on the surface of the endothelial cells that form the lining of blood and lymph vessels (Chavakis \textit{et al.}, 2002). Neutrophils have lymphocyte-function-associated antigen (LFA-1) on their surface, which binds to ICAM-1, allowing the neutrophils to adhere to the endothelial cells and migrate to the site of infection (Chavakis \textit{et al.}, 2002). \textit{S. aureus} releases Eap, which binds to ICAM-1, blocking neutrophil adhesion and preventing extravasation of neutrophils (Chavakis \textit{et al.}, 2002; Haggar \textit{et al.}, 2004).

3.2 Resistance to the respiratory burst

The respiratory burst takes place inside phagocytes once a pathogen has been engulfed. During the respiratory burst, reactive oxygen species (such as hydrogen peroxide and superoxide) are produced inside the acidic environment of the phagolysosome (Robinson, 2008). These compounds destroy invading organisms by oxidising components of the bacterial cell, e.g. lipids and proteins. \textit{S. aureus} has multiple ways of defending against these toxic substances. Staphyloxanthin, a yellow carotenoid pigment found in \textit{S. aureus} has potent antioxidant activity, i.e. it inhibits the production and action of reactive oxygen species by scavenging (i.e. inactivating) free radicals (Clauditz \textit{et al.}, 2006). A variety of enzymes, including catalase, alkylhydroxide reductase, superoxide dismutases and thioredoxin are also produced by the bacterium to inactive reactive oxygen species (Table 2).
3.3 Leukotoxins

*Staphylococcus aureus* produces four bi-component, pore-forming leukotoxins which directly act to disable leukocytes (Yoong and Torres, 2013). These toxins consist of two subunits that form a monomer and assemble together with other bi-component monomers to form oligomeric pores in the membrane of leukocytes (Aman *et al.*, 2010). This leads to osmotic dysregulation of the leukocyte and lysis of the cell (Yoong and Torres, 2013). Toxins are often produced in different amounts in different areas of the body, indicating that the environment of the bacterium may dictate which toxins are produced and in what quantities (Yoong and Torres, 2013).

4. Antimicrobial peptide evasion

*Staphylococcus aureus* has multiple mechanisms of anti-microbial peptide evasion, comprising a mixture of secreted proteins and structural features of the bacterial cell.

4.1 Protein secretion

4.1.1 Proteolytic cleavage and production of extracellular CAMP-binding molecules

*Staphylococcus aureus* secretes extracellular proteins which either cleave cationic antimicrobial peptides (CAMPs) or chelate them, rendering them inactive in both cases (Kraus and Peschel, 2008). Aureolysin and serine-protease V8 cleave the cathelicidin LL-37 (Sieprawska-Lupa *et al.*, 2004). Staphylokinase, as previously discussed, activates plasminogen, but it also has a second binding domain for α-defensins (Jin *et al.*, 2004).

4.2 Structural features

4.2.1 Modification of cell surface net charge

*Staphylococcus aureus* is capable of modifying the charge at its cell surface to repel antimicrobial peptides (Figure 5). Antimicrobial peptides are generally cationic, while the bacterial cell surface generally has a net negative charge, due to the presence of molecules such as peptidoglycan, teichoic acids and phospholipids (Kraus and Peschel, 2008). This makes antimicrobial peptides highly selective for bacterial cells, because human cells are usually uncharged. *Staphylococcus aureus* can incorporate D-alanine into its teichoic acids
(Collins et al, 2002). This introduces a positively charged amino group into the peptidoglycan structure. *S. aureus* can also incorporate L-lysine into phosphatidylglycerol, a membrane phospholipid (Kristian et al, 2003). This causes the cell surface net charge to become positive, thus repelling the cationic antimicrobial peptides and preventing them from binding to the bacterium.

![Diagram](image)

**Figure 5.** Electrostatic repulsion of CAMPs by modification of cell surface net charge. Adapted from Peschel, 2002.

### 4.2.2 Alteration of membrane structure or fluidity

Modification of bacterial membrane structure has been shown to confer resistance to antimicrobial peptides (Bayer et al, 2000). Bacteria contain multidrug resistance exporters which can transport antimicrobial peptides out of the cell. One of these, the QacA pump, increases the resistance of *S. aureus* to certain antimicrobial peptides, but experiments suggest that this resistance is actually due to the effect of QacA on membrane structure rather than its function as an efflux pump (Bayer et al, 2006). *S. aureus* can also alter the fluidity of its membrane by introducing more long-chain unsaturated fatty acids. This change in fluidity causes increased resistance to antimicrobial peptides (Bayer et al, 2000).
4.2.3 Alteration of peptidoglycan structure

Lysozyme cleaves peptidoglycan, a unique component of the Gram-positive cell wall, between the N-acetylmuramic acid and N-acetylglucosamine residues. The oatA gene in \textit{S. aureus} causes O-acetylation of the N-acetylMuramic acid residue, preventing recognition by lysozyme and conferring absolute resistance (Bera \textit{et al}, 2005).

Conclusions

In this review, the primary mechanisms used by \textit{S. aureus} to evade the host immune system have been outlined. It is clear that \textit{S. aureus} possesses a diverse array of immune evasion mechanisms which contribute significantly to its virulence, particularly as a nosocomial pathogen which frequently infects individuals who are already immunocompromised. The emergence of MRSA has increased interest in a possible vaccine or preventative treatment against \textit{S. aureus} but attempts thus far have not been successful.

The apparent inability of the immune system to stimulate an effective and lasting adaptive immune response poses a difficulty for development of a prophylactic \textit{S. aureus} vaccine. However, knowledge of the immune evasion strategies of the bacterium will enable us to identify specific aspects of its pathogenesis that can be targeted for use in a therapeutic context. Kim \textit{et al} found that when mice were inoculated with a non-functioning variant of staphylococcal Protein A, antibodies were raised against wildtype \textit{S. aureus} and the mice became resistant to virulent strains of MRSA, indicating the potential use of Protein A as a possible vaccine antigen (Kim \textit{et al}, 2010). The \textit{S aureus} \(\alpha\)-haemolysin has also been identified as a possible antigen that could be used in vaccination (Wardenburg and Schneewind, 2008).

Beyond vaccination, the proteins used by \textit{S. aureus} to evade the immune system show promise as anti-inflammatory agents for use in autoimmune disorders. In particular, Eap has been identified as a potential anti-inflammatory agent that could be used against autoimmune disorders such as multiple sclerosis and rheumatoid arthritis (Chavakis \textit{et al}, 2007). Eap demonstrates potent anti-inflammatory activity by binding
antagonistically to ICAM-1 on endothelial cells, preventing the transmigration of leukocytes across the endothelium and into tissues (Chavakis et al. 2002). Studies on the effect of Eap on the mouse equivalent of multiple sclerosis, experimental autoimmune encephalomyelitis, indicated that administration of Eap prevented the development of the disease and even reversed the symptoms of the disease in mice that had already developed it (Xie et al., 2006). Similarly, CHIPS could potentially be used to treat sepsis, as blockade of the C5a receptor (a function of CHIPS) has been shown to reduce mortality in sepsis (Czermak et al., 1999; Guo and Ward, 2006). Furthermore, the staphylococcal protein FLIPr binds to FPRL1 (Table 2), which has been implicated in signalling pathways for Alzheimer’s disease, prion diseases and systemic amyloidosis (Chavakis et al., 2007). FLIPr and its homolog FLIPr-like may have potential uses in the treatment of these diseases, as they bind antagonistically to FPRL1 (Prat et al., 2006; Prat et al., 2009). Further research in these areas will be required to ascertain the therapeutic usefulness of these proteins, but it is possible that certain proteins of the pathogen, which are intended to cause infection, may in fact be used to our advantage.

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