The immunopathogenesis of Staphylococcal skin infections – a review

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**Highlights**

- *Staphylococcus aureus* and *Staphylococcus pseudintermedius* are common pathogens of humans and dogs respectively.
- The mammalian immune system has evolved multiple strategies to combat pathogenic bacteria.
- The interplay between these bacteria and the host immune system can develop into a titanic battle involving sophisticated attack and defence mechanisms.
- The final outcome is unpredictable, with either side capable of emerging victorious. The result can range from clearance of the infection to death of the host.
The immunopathogenesis of Staphylococcal skin infections – a review

Abstract: Staphylococcus aureus and S. pseudintermedius are the major causes of bacterial skin disease in humans and dogs. These organisms can exist as commensals on the skin, but they can also cause severe or even devastating infections. The immune system has evolved mechanisms to deal with pathogenic microorganisms and has strategies to combat bacteria of this type. What emerges is a delicate “peace” between the opposing sides, but this balance can be disrupted leading to a full blown “war”. In the ferocious battle that ensues, both sides attempt to get the upper hand, using strategies that are comparable to those used by modern day armies. In this review article, the complex interactions between the immune system and the organisms are described using such military analogies. The process is described in a sequential manner, starting with the invasion itself, and progressing to the eventual battlezone in which there are heavy casualties on both sides. By the end, the appearance of a simple pustule on the skin surface will take on a whole new meaning.

1. Introduction
Bacterial skin infections are common in domestic animals and represent an important reason for dogs to be presented to veterinarians for treatment. A variety of lesions can be seen with the dermatosis commonly referred to as canine pyoderma, including papules, pustules, crusted papules and epidermal collarettes (Figure 1). The inflammatory events that underlie this condition comprise two major aspects of skin immunity, namely the development of grossly visible erythema (which is a manifestation of inflammation, and more specifically vasodilatation) and the infiltration of neutrophils at a microscopic or gross level. Both of these changes reflect a complex series of immunological events that are initiated by the presence of pathogenic bacteria on the skin surface. In the case of canine pyoderma, the inciting bacteria are already present on the skin and take advantage of an opportunity that allows them to proliferate and gain entry. What follows is a titanic battle between the invaders and the host, a battle in which both sides try to outwit each other with sophisticated attack and defence mechanisms. As with any invasion, the enemy will attempt to thrust forward, build up its numbers and establish a base on the newly captured territory. To repel and defeat such an invasion requires a range of highly coordinated defences, including physical barriers, an army of highly trained and well equipped soldiers, and some lethal weaponry. As in any battle, the outcome is never certain, and either side can get the upper hand. In this review article, we will attempt to explain the complicated interactions between staphylococci and the host immune system using such an analogy. Our aim is to simplify and demystify an area of veterinary dermatology that can appear overwhelmingly complex. In order to achieve this, we will compare each element of the immune system to a commonly understood concept.
of a traditional battle, with all the technology that entails. The various components of this bacterial “enemy” and the immune system “army”, are summarized in Tables 1 and 2, and will be expanded upon throughout this article. Table 3 provides a list of abbreviations used throughout this paper.

2. The invaders
For the purpose of this article, the bacterial invaders will be restricted to a single genus of organisms – *Staphylococcus*. This genus represents the major cause of bacterial skin infections in both dogs and humans. *Staphylococcus pseudintermedius* is a Gram-positive bacterium that lives as a commensal organism on the skin of dogs [1]. It is an opportunistic pathogen and is the major cause of canine pyoderma [1]. In dogs, it can be cultured at high frequency from the nares, oropharynx and anal ring of normal and infected dogs [2-6], but molecular analysis of the cutaneous microbiome has shown it to be present in all dogs at most sites in low numbers [7]. From these sites, it can be spread to other regions of the body such as the hair shafts, sites of infection, other dogs in the household or in-contact humans [3, 5, 8]. It does not cause disease unless the resistance of the host is lowered and the skin barrier altered by predisposing factors such as atopic dermatitis, medical and surgical procedures and/or immunosuppressive disorders [1]. Much of our understanding of the immunology of staphylococcal skin infections is based on work done in humans with *Staphylococcus aureus*, although it is likely that similar mechanisms occur in dogs infected with *Staphylococcus pseudintermedius*.

3. The invasion commences – establishment of a beachhead
In military terms, a beachhead is an attempt by a military unit to seize a landing beach following an invasion by sea. The aim is to hold the area until reinforcements arrive, thus allowing the invasion to proceed inshore. If the soldiers encounter physical barriers or walls, they might use grappling hooks or other devices to help them get over. In relation to infections, the analogous situation is bacterial adherence. In order to establish an infection, bacteria have to initially adhere to the tissues in which they wish to grow and penetrate [9]. To promote colonization of human and animal skin, *S. aureus* expresses various factors that facilitate skin surface binding and survival [10-12]. To bind to host surface components such as fibrinogen, fibronectin and cytokeratins, which are derived from epidermal keratinocytes, *S. aureus* utilises microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), such as fibronectin-binding protein A (Fnbp A) and Fnbp B, fibronogen-binding proteins/clumping factors (ClfA and ClfB), iron-regulated surface determinant A and cell wall teichoic acid (Figure 2) [13-17]. In dogs, similar mechanisms are likely to be involved [18-20], but some degree of species specificity is seen with *S. pseudintermedius* and *S. aureus* with their respective hosts [21]. Isolates of staphylococci from cases of canine pyoderma have also been shown to produce a mucoid substance known as slime that can aid adherence [12]. Using these mechanisms,
Staphylococci are well placed to take advantage of disruptions in the skin barrier function that allow increased amounts of proteins such as fibronection to appear on the skin surface. Conditions in which this occurs, such as atopic dermatitis, are known to result in increased adherence and colonization rates of staphylococci on the stratum corneum in both humans and dogs [14, 18, 22-24].

4. The stratum corneum - the first line of defence
Although it is a dead structure, the stratum corneum provides a robust physical barrier against potential invaders. Just like the walls of a castle, it is difficult to penetrate. In addition, the continuously desquamating corneocytes make it difficult for bacteria to set up a permanent base because they are endlessly cast off into the environment. Furthermore, the intracellular emulsion that sits between the corneocytes contains a number of substances that have inherent antibacterial properties such as free fatty acids [25], sodium chloride, and other molecules such as complement, transferrin and antibodies (Figure 2). Despite this, the stratum corneum is not completely impenetrable. First, it is regularly punctuated by the orifices of hair follicles, which in dogs, may represent a portal of entry [26]. Second, staphylococci have evolved many mechanisms that allow them to attach to, and then survive on, the stratum corneum.

5. Additional defences – the complement cascade and other serum based proteins
One of the first active defence mechanisms that invading bacteria will encounter is a number of innate immune response proteins that circulate in the blood and permeate into intercellular fluid and onto body surfaces, especially at sites of tissue damage or inflammation. Two major examples of such proteins are the complement components and transferrin. Both the third component of complement (C3) and transferrin are present in high concentrations in the blood and can easily be observed on a serum electrophoresis profile [27]. However, both of these proteins can also be found in the stratum corneum [28-30].

The complement system is like a mini army in itself and offers a first line of defence against infection by quickly triggering inflammatory responses and alerting the immune system to impending danger [31, 32]. Made up of multiple different molecules (named C1 to C9), its various components have the ability to recognize an enemy, call for reinforcements, surround it, kill the invaders and help with the clean-up operation. When bacteria manage to penetrate the defences put forward by the stratum corneum and epidermis, they will encounter complement proteins that have extravasated from the local vasculature. Complement proteins normally circulate in an inactive form, but a constant low level of complement activation (tick-over) ensures occasional probing of healthy cells for signs of infection [31]. When faced with microbial intruders, the complement “army” is rapidly mobilized, a process that can be
achieved by three different activation mechanisms [33]. The classical pathway of activation involves the binding of C1 molecules to antibodies on the surface of the bacteria (Figure 3). In the case of staphylococci, antibodies produced against commensal species are able to cross-react with pathogenic species. Hence, anti-staphylococcal antibodies are likely to be present from an early age. Once the C1 molecules have attached, the C4 and C2 molecules bind and are cleaved into two subunits (a and b). The C4b and C2a fragments combine together to produce an enzyme that is able to catalyse the conversion of C3 (the most plentiful component in the blood) into C3a and C3b. The activation of complement can also be achieved by direct binding of C3b to the surface of bacteria, with downstream events being promoted by two proteins known as Factors B and D [34]. The final activation pathway involves the binding of mannose binding lectin to mannose residues on the bacterial surface. This can activate C4 and C2 in a similar way to that described for the classical pathway. Up until this stage, these mechanisms of activation all converge on a common pathway – the cleavage of C3 into C3a and C3b [33]. C3a, along with C2b and C4a are known as anaphylotoxins [35]. They can increase blood vessel permeability, allowing influx of humoral factors to the site of infection. They can also degranulate mast cells and act as potent chemoattractants for neutrophils. These aspects of host defence will be discussed in more detail later. C3b is an important opsonin [33]. Opsonisation is a process similar to prisoner capture. The bacteria are identified as foreign, surrounded and held captive until they are dealt with by other soldiers. In this case, those “soldiers” would be neutrophils and macrophages. As well as being an opsonin, C3b can bind to the C4bC2a complex to produce an enzyme called C5 convertase. This enzyme cleaves C5 into C5a and C5b. C5a is another anaphylotoxin, whereas C5b initiates a process that leads to the formation of the membrane attack complex (MAC). The membrane attack complex is composed of complement components C 5(b), 6, 7, 8 and 9, which arrange themselves into a cylindrical shaped structure that can insert itself into the bacterial wall [31]. When this concept is scaled up, it is analogous to the bacteria being riddled with machine gun bullets.

Although many bacteria might be neutralized by the onslaught of immune responses described above, pathogenic bacteria such as staphylococci are well equipped to overcome the defences put forward by the complement army as they have evolved elaborate evasion mechanisms [36]. S. aureus synthesises capsular polysaccharides that impede antibody recognition [37-39], and interfere with opsonisation and C3b recognition by its receptors [40]. Furthermore, like a bullet proof vest, the peptidoglycan-rich structure of its Gram-positive cell wall renders the bacterium resistant to MAC formation [41, 42]. Staphylococci are also able to target the initial, amplifying and proinflammatory steps of complement activation using mechanisms such as impairment of the classical pathway [43-49], use of proteolytic enzymes to counteract complement [50], suppression of complement amplification by
interference at the stage of C3 conversion [50-57], and inhibition of C5 to disrupt the downstream inflammatory responses [42, 58-60].

Another major serum protein with anti-bacterial effects is transferrin, an iron binding protein that can deprive bacteria of an important cofactor for growth and reproduction [61]. Transferrin provides the major antibacterial effect in serum [62] However, although transferrin has been shown to inhibit the growth of Malassezia pachydermatitis in vitro [63], studies have shown that pathogenic Staphylococcus aureus contains transferrin binding proteins on its surface that enable it to capture the molecule and extract the iron stores for its own use [62].

Taken together, these studies indicate that serum based proteins such as complement and transferrin are unlikely to pose much of a threat to virulent strains of pathogenic staphylococci intent on invading the host and establishing an infection.

6. The attack on the living epidermis – recognition of the enemy and raising the alarm
Once the stratum corneum has been colonized, the organisms will need to penetrate the epidermis in order to establish an infection [11]. Little is known about the transition from asymptomatic colonization to an invasive infection [64], despite the fact that persistently colonized humans have nearly triple the risk of developing S. aureus bacteremia and are most often infected by their own colonizing strains [65-68]. One mechanism that may play a role is the entry of staphylococci into host cells [69]. FnBP-mediated adhesion of staphylococci to the surface of mammalian cells can promote subsequent internalization [70-72]. S. aureus can therefore direct its uptake into cells that are not normally phagocytic, and host cells can be damaged from within by bacterial cytotoxins [73]. This mechanism of bacterial invasion may be one step in the development of an active infection, and be associated with the initiation of cutaneous host defences.

In addition to its role as a physical barrier, keratinocytes in the epidermis are equipped with mechanisms to detect surface components of Staphylococci and initiate early cutaneous innate immune responses (Figure 4). To detect the presence of the bacteria, keratinocytes need a recognition system to alert them to the presence of the danger. Just as an enemy can be recognized by its soldiers’ uniforms, the keratinocytes can recognize the bacterial invaders due to the presence of certain molecules in their cell wall – molecules such as lipopeptides, lipoteichoic acid and the peptidoglycan breakdown product muramyl dipeptide [74]. In order to recognize these molecules, keratinocytes don’t use binoculars or fingerprint recognition software. Instead, they use a microscopic equivalent known as pattern recognition receptors (PRRs). Important examples of such receptors
include Toll-like receptors (TLRs) and a cytoplasmic receptor known as nucleotide-binding oligomerization domain containing 2 (NOD2) [74-76].

Of all the known human TLRs (1-10), TLR2 has been the most implicated in host defence against *S. aureus* [77]. TLR2 is expressed on the surface of numerous cell types in the skin, including keratinocytes, Langerhans cells, monocytes/macrophages, dendritic cells, mast cells, endothelial cells, fibroblasts, and adipocytes [75]. TLR2 heterodimerizes with TLR1 and TLR6 to recognize tri- and di-acyl lipopeptides, respectively [78-80]. TLR2 also recognizes *S. aureus* peptidoglycan (PGN), with CD14 and CD36 acting as TLR2 co-receptors [81, 82].

NOD2 belongs to a family of innate immune system PRRs known as nucleotide-binding, oligomerization domain (NOD)-like receptors (NLRs) [83]. It is an intracellular cytoplasmic receptor that can recognize the *S. aureus* peptidoglycan breakdown product muramyl dipeptide [83]. As mentioned earlier, although *S. aureus* is classically considered an extracellular pathogen, it is now clear that *S. aureus* can invade the cytoplasm of many different cell types [69]. In this location, the NOD2 receptors are able to interact with muramyl dipeptide and trigger signal transduction mechanisms [69, 74].

Both TLR2 and NOD2 derived signals within keratinocytes lead to activation of nuclear factor-κB (NF-κB) and mitogen-activated protein kinases (MAPK) [76, 83]. These transcription factors activate the keratinocyte, leading to the production and secretion of some important proteins in the early stages of an immune response [84, 85]. One such protein is responsible for declaring war on the enemy. Keratinocytes store biologically active interleukin (IL)-1α [86] which is released following interaction with the organisms. They are also able to produce the inducible form of the cytokine, IL-1β [87]. These crucial signaling molecules raise the alarm and call for mobilization of the army. It is like an early warning or radar system that warns the rest of the body that an attack is underway. The initiation of IL-1-mediated cutaneous immune responses against *S. aureus* shares a similar signaling cascade to TLRs [75]. IL-1 activates the IL-1 receptor on other keratinocytes, as well as many other inflammatory cells. Subsequent transduction via the myeloid differentiation primary response protein 88 (MyD88) and interleukin-1 receptor-associated kinase 4 (IRAK4) signaling pathway triggers the activation of NF-κB. The end result is the further production of cytokines (such as TNF-α, IL-6, IL-7 and IL-18), chemokines and mediators that prepare the host for the battle ahead [74, 88, 89]. Essentially, in conjunction with the inflammatory mediators produced by mast cells, this represents the start of the inflammatory cascade that results in the grossly visible erythema seen at the sites of the lesions.
7. Antimicrobial peptides – an arsenal of antibacterial weapons

A second major outcome of keratinocyte activation via TLRs and NOD2 receptors is the stimulation and release of one of the body’s first lines of defence - a class of weapons known as antimicrobial peptides (Figure 4). Antimicrobial peptides (AMPs) are small, predominantly cationic proteins that have direct bacteriostatic or bactericidal activity against S. aureus and S. pseudintermedius. The two major groups are defensins and cathelicidins, but other examples exist. AMPs attach to Staphylococci due to their high affinity for anionic molecules on the microbes’ surface and lead to membrane damage, disruption of the ion gradient and subsequent bacterial death [90]. As weapons, they are like daggers or bayonets that are thrust into the organism’s body. At least 16 defensins have been described in man, with α and β subtypes being recognized [91]. β-defensins are secreted by epithelial surfaces, including from keratinocytes [92]. In humans, β-defensin 2 (hBD2) and hBD3 are the most important in relation to defence against staphylococci [92-94]. At least 30 cathelicidin family members have been described in mammalian species, but only one is produced in humans (LL-37) [95]. LL-37 is also produced by human keratinocytes and plays a role in the defence against skin pathogens [96]. Another antimicrobial molecule produced by human keratinocytes with anti-staphylococcal activity is RNase 7 [97]. Activation of TLR2 on keratinocytes by harmless commensal organisms such as Staphylococcal epidermidis can promote production of AMPs, which in turn can provide protection against Staphylococcus aureus [98, 99].

In dogs, a number of β-defensins (cBD1, 2, 3, 103, 107) and a single cathelicidin (cCath) are present in the skin [100-102]. Conflicting data is present in the literature as to how the levels of these AMPs change in canine atopic dermatitis, a disorder in which staphylococcal infections are common. Levels of cBD1, cBD3 and cCath have been reported to be elevated [103] or the same [104] in atopic compared to healthy dogs, and some publications report a decrease in cBD103 in the skin of atopic dogs [100] whereas others report an increase [104]. Despite these inconsistent findings, it is clear that AMPs are produced by canine keratinocytes both in vitro [105] and in vivo [101-103], and these molecules have demonstrable antibacterial efficacy against Staphylococcus pseudintermedius [102, 106, 107].

Hence, any bacteria trying to invade through the epidermis will have to cross a swamp of antibacterial peptides, and there are likely to be many casualties. However, staphylococci are not totally defenceless when faced with an attack by AMPs. Strains of S. aureus that produced the metalloproteinase aureolysin were able to cleave LL-37, instantly annihilating its antibacterial activity [108] and production of the surface protein iron-regulated surface protein A (isdA) was able to impart
resistance to human \( \beta \)-defensin 2 [25].

8. Mast cells – land mines buried in the skin
Mast cells are often thought of as mediators of allergic inflammation. However, they did not evolve to fulfil that role, and they provide a protective function not only against parasites, but also bacteria [109]. Studies have shown that mast cell deficient mice are critically disabled in their ability to fight serious bacterial infections, with high mortality being seen in experimental models of peritonitis and pneumonia [110, 111]. However, what role are mast cells likely to play in the defence against skin-invading bacteria such as staphylococci? Mast cells residing in the dermis can be considered analogous to land mines, with activation resulting in an explosive degranulation (Figure 5). The detonating mechanism could be either anaphylotoxins derived from complement activation or binding of staphylococcal antigens to IgE antibodies on the mast cell surface. When the mast cells explode, the release of pre-formed inflammatory mediators, followed by the subsequent production and secretion of lipid mediators and cytokines, will initiate a cascade of inflammatory events that will step up the host’s immune response, and prepare the ground for the battle ahead [109, 112]. First, the release of granule based mediators such as histamine will have an immediate effect on the local vasculature, causing vasodilatation and increasing permeability [113]. This function, which is augmented by complement derived anaphylotoxins, will enhance the influx of humoral and cellular elements. Another granule based mediator, tryptase, is an important chemoattractant for neutrophils. Second, occurring some minutes after the initial degranulation event, the mast cell will commence synthesis of the lipid derived mediators prostaglandins, leukotrienes and thromboxanes. The function of these molecules, derived from metabolism of arachidonic acid by cyclooxygenase and lipoxygenase enzymes, overlaps those of histamine and tryptase, resulting in vasodilatation, vascular permeability and recruitment of neutrophils. Finally, after a number of hours, the mast cell will start to release a plethora of cytokines. Many of these are geared towards immune protection against parasites, but tumour necrosis factor \( \alpha \) (TNF-\( \alpha \)) is a critical chemoattractant for neutrophils [111]. Hence, although mast cells are unable to kill staphylococci themselves, they play a major role in the initiation of immune responses that contribute to the overall battle.

9. The call for reinforcements – the influx of neutrophils and macrophages
As the invasion continues, and the organisms have overcome the initial barrier defences and the sustained attacks mediated by serum-derived proteins and antimicrobial peptides, the host needs to mobilise the main army if there is to be any hope of a reversal in fortune. In relation to the immune system, blood born neutrophils can be regarded as the infantry soldiers of the army. Macrophages, which accompany the neutrophils in smaller numbers, can be thought of as captains. They still fight
on the front line, but they play a larger role in coordination of the battle. Just as soldiers are trained and equipped in a military academy, followed by mobilization to a war zone, neutrophils and monocytes develop and mature in the bone marrow before being transported in the blood to sites of inflammation and infection. Transportation of soldiers to the front line requires a high level of coordination, and precise communication is required to ensure they arrive in the correct place. A similar level of coordination is involved in neutrophil trafficking.

Neutrophils in circulation are informed about sites of infection by chemoattractant molecules. Numerous mediators, cytokines and chemokines are capable of fulfilling this role, including the complement anaphylotoxins (C3a, C2b, C4a and C5a) [35], tryptase [114], TNF-α [111], prostaglandin D2 and leukotriene B4 [115, 116] (derived from mast cells), interleukin-1 (derived from keratinocytes and macrophages) [74], and numerous chemokines including interleukin-8 (derived from keratinocytes and leucocytes) [117].

Neutrophils arriving in the bloodstream at a site of infection need to be “disembarked” in order to face the bacteria directly. In military scenarios, this would involve troop carriers, landing craft and parachute deployments. The immune system achieves it by using a complex system of adhesion molecules and chemoattractants that stop the neutrophils from circulating and allow them to leave the blood vessels (Figure 6) [118]. These adhesion molecules are up-regulated following signals from keratinocytes, mast cells and macrophages such as TNF-α and IL-1 [119, 120]. The process involves four stages: rolling, adhesion, intraluminal crawling and paracellular and transcellular migration [121]. Rolling is mediated by the presence of P- and E-selectins on endothelial cells of blood vessels [121, 122]. These selectins interact with ligands on the neutrophil membrane, in particularly P-selectin glycoprotein ligand 1 [121, 123]. This ligand requires the addition of sialyl Lewis X tetrasaccharide (sLex) in order to become functional. The binding of the ligand to the selectins has a high on-off rate, which accounts for the rolling phenomenon. Once slowed, the neutrophils are arrested by the interaction between integrins on the neutrophil surface and immunoglobulin superfamily members on the endothelium. Intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) are the main immunoglobulin superfamily members that are upregulated on the vascular endothelium following activation by inflammatory cytokines such as IL-1 and TNF-α [121]. On the neutrophil surface, the β1-integrin Very late antigen-4 (VLA4) and the β2-integrin Lymphocyte function associated antigen-1 (LFA1) have been the most studied examples. Adhesion between integrins and immunoglobulin superfamily members is often imagined to be a simple binding interaction, like a form of molecular velcro. However, the process is bewildering complex. Following binding, the integrin can activate intracellular signal transduction mechanisms involving G proteins and the inositol
triphosphate pathway. After some complex interactions with the cytoskeleton, the integrin can actually change its molecular confirmation, resulting in an increased affinity. This process, known as “outside-in” signalling can be augmented by similar mechanisms that are triggered by cytokines and don’t involve ligand binding (known as “inside-out” signalling) [121]. This increase in affinity of binding results in a phenomenon referred to as adhesion strengthening [121] and results in the neutrophil being halted on the blood vessel wall. The complexity of the process is illustrated by the estimation that at least 900 proteins and 6000 protein-protein interactions are involved [121].

Once the neutrophil has been arrested on the blood vessel wall, it needs to commence its journey to the battle ground. Like a soldier crawling under a barbed wire fence, the neutrophil has its own barriers to penetrate, namely the endothelial cell layer, the basement membrane and the pericyte layer. The process is again complex, involving many protein-protein interactions. First, the neutrophil crawls across the surface of the blood vessel in order to find a suitable exit point. This activity is modulated by interactions between ICAM-1 and a receptor known as macrophage antigen-1 (MAC-1) [124]. It then starts to extend membrane protrusions into the intercellular junctions. This is facilitated by activation of the endothelial cell’s cytoskeleton and separation of vascular endothelium cadherins, resulting in contraction of the cell and creation of an opening. The neutrophil’s passage between the endothelial cells, known as paracellular migration, is controlled by multiple adhesion molecules including ICAMs and platelet/endothelial-cell adhesion molecule 1 (PECAM1) [121]. Neutrophils can also traverse the endothelium by passing through the endothelial cell itself, a process known as transcellular migration. This involves the formation of a passageway in the cell’s cytoplasm through which the neutrophil can pass. When it reaches the basement membrane and pericyte sheath, the neutrophil may use enzymes such as matrix metalloproteinases (MMPs) and neutrophil elastase in order to create a hole [121]. After this incredible journey, the neutrophil finds itself in the dermis where further migration along chemotactic concentration gradients will take it to the surface or follicular epidermis (Figure 7). The pro-inflammatory cytokines IL-1α, IL-1β, TNFα and IL-6 induce the expression of neutrophil attracting chemokines such as CXC-chemokine ligand 1 (CXCL1; also known as Gro-α in humans and KC in mice), CXCL2 (also known as Macrophage inhibitory protein 2), CXCL5 (also known as epithelial cell-derived neutrophil-activating peptide 78), and CXCL8 (also known as IL-8) [121]. Once there, the army of neutrophils will start to amass, resulting in the accumulation of cells we refer to as pus.

Macrophages also play an important role in the defence against pathogenic staphylococci and they join the neutrophils on the front line of the battle. Monocytes leaving the bloodstream are referred to as macrophages when they enter sites of inflammation within tissues. Macrophages have to be
activated before they can join in the battle, analogous to a soldier receiving his orders. In the past, it was thought that activated macrophages represented a single population of cells. However, it is now known that there are three distinct activation pathways that can result in different functions [125]. The classic pathway of activation involves two key signals – interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α). In many infections, the macrophage is activated not only by exogenous TNF-α from cells such as mast cells, but also by ligation of Toll-like receptors that induce endogenous production of TNF-α [125]. These “Type 1” activated macrophages produce IL-12 and are primed for phagocytosis and intracellular killing of pathogens. Macrophages can also be activated via their Fcγ receptors, resulting in a Type 2 phenotype which produces IL-10 and has an anti-inflammatory function, and by IL-4 which results in an “alternative” macrophage which contributes to wound healing [125].

10. Facing up to the enemy – probing by receptors
Neutrophils and macrophages arriving at a site of infection need to be able to identify the enemy, and distinguish it from friendly forces (i.e. host cells and tissues). As with keratinocytes, the inflammatory cells do this by using an array of surface receptors that are able to recognize features on the surface of bacteria. Neutrophils carry a range of PRRs, the most notable being Toll-like receptors (TLRs). Currently, 10 human and 12 mouse TLRs have been identified [126]. Human neutrophils express mRNA for all 10 TLRs [127], each recognizing pattern associated molecular patterns (PAMPS) on bacteria, viruses or fungi [128]. One of the most studied TLRs is TLR2 which recognizes PAMPs from Gram-positive bacteria, including lipoproteins, lipopeptides, peptidoglycans, lipoteichoic acid and modulin [80, 81, 128, 129]. As stated earlier, TLR2 joins up with TLR1 and TLR 6 to increase the repertoire of PAMPS that it can identify [128]. Binding of PAMPs to TLRs activates internal signal transduction mechanisms involving MyD88, the IRAK family of serine/threonine kinases and the transcription factor NF-κB [76, 128]. Activation of TLRs has a range of effects on neutrophil function including enhancement of phagocytosis, production of IL-8, shedding of L-selectin and reduction of chemotaxis [130]. These effects make the neutrophil battle ready, preparing it for the action ahead.

In addition to TLRs, neutrophils carry a number of other surface receptors to aid recognition of pathogenic bacteria. Bacteria that are opsonized by complement proteins or immunoglobulins are primed for phagocytosis. Neutrophils express the complement receptors ClqR, CD35, CD11b/CD18 (CR3), and CD11c/CD18 (CR4), and the immunoglobulin receptors FcyR1, FcyR2, FcyR3 (to detect IgG) and FcαR (to detect IgA) [131].

11. Annihilation of the enemy – the main battle commences
The interaction between neutrophils and bacteria represents a fierce battle in which there will be heavy
casualties on both sides. The capture of staphylococci by opsonins, followed by their phagocytosis and subsequent killing is an example of close combat of a very violent kind. The first step in this process is opsonisation, which is achieved either by the complement protein C3b or by antibodies [132]. C3b is generated as part of the complement cascade as described earlier. Since most humans and animals are exposed to commensal staphylococci from early in life, common cell wall structures, and/or secreted proteins (e.g. clumping factor A and protein A) result in the generation of anti-staphylococcal antibodies, especially of the IgG and IgM isotypes [132-134]. This ongoing exposure to staphylococcal antigens provides sufficient amounts of opsonising and complement activating antibodies to facilitate recognition of the bacteria by Fc and complement receptors [131, 132].

Phagocytosis involves a number of steps (Figure 8). First, the opsonized bacteria are attached to the neutrophil via an appropriate receptor. Next, the neutrophil extends pseudopods around the organism whilst it is still exposed to the environment (a process called “zippering”). Finally, complete engulfment results in the formation of a phagosome, which is an outside-in compartment inside the cell [132]. This entire process involves a hugely complex interplay between large numbers of adhesion and signal transduction molecules, as well as substantial rearrangements of the actin cytoskeleton [135]. The additional plasma membrane required to line all the phagosomes is thought to be derived from “ironing out” of the neutrophil’s outer membrane [136]. Using these processes, a single neutrophil is capable of engulfing as many as 50 bacteria (Figure 9) [132].

At this stage, the bacteria have merely been taken prisoner, “hand-cuffed” by opsonins and “locked up” in the neutrophil phagosome. However, the next step involves no leniency, and the bacteria will be killed. The phagosomes are mobilized and fused with different granule types resulting in the liberation of granule contents that have potent antibacterial effects [137, 138]. Like the keratinocytes in the epidermis, neutrophil phagosomes contain defensins, but in this case, they are α-defensins. Human α-defensins are a closely related family of six peptides. α-Defensins 1–4, also called human neutrophil peptides (HNPs), are sequestered within intracellular granules and released when neutrophils are activated [139] (human defensins 5 and 6 are expressed by Paneth cells of the small intestine). α-Defensins comprise 40–50% of the content of neutrophilic azurophilic granules [139] and make up 5% of the total protein content of the cell [140]. The human cathelicidin LL-37 is also present in neutrophil granules [141]. Concurrently, a strong oxidative burst is initiated in the phagosome by NADPH-dependent oxidases upon triggering of specific cell surface receptors, leading to the generation of highly toxic reactive oxygen species (ROS) such as O2⁻, H2O2, and HClO⁻. These molecules are similar to household cleaning products such as hydrogen peroxide and bleach and were originally thought to provide the major killing activity in neutrophil phagosomes. However, it
is now thought their major function is to produce a charge in the phagosome membrane that induces a compensatory movement of K⁺ ions that makes the internal environment conducive to enzymatic killing [142-144]. Neutrophil granules contain a number of enzymes that contribute to bacterial killing including lysozyme, elastase, cathepsin G, azurocidin, proteinase-3, acid hydrolases and gelatinase [141, 144]. Lysozyme was discovered by Alexander Fleming in the 1920’s who showed it could lyse many species of bacteria [145]. Lysozyme is now known to split the β linkages between N-acetylmuramic acid and N-acetylglucosamine in the cell walls (peptidoglycan layers) of Gram-positive bacteria [146, 147]. Knockout mice that are deficient in neutrophil elastase are susceptible to Gram-negative infections, but not S. aureus, suggesting this enzyme is not critical in the defence against this organism [144]. Loss of cathepsin G alone does not lead to impaired defence against bacteria, but a loss of both enzymes confers a profound defect in bacterial killing [144]. Azurocidin is a serine protease, but a mutation in its gene sequence has left it devoid of proteolytic activity [141]. Despite this, it still has potent antibacterial activity. Unlike the above enzymes, it is primarily released following degranulation rather than being deposited in phagosomes [141].

Other factors contribute to the phagosome being a very hostile environment for bacteria. Neutrophil granules contain factors that sequester essential nutrients to inhibit bacterial growth and survival, such as lactoferrin, which sequesters iron and copper, transcobalamin II, which binds to and sequesters vitamin B12, and neutrophil gelatinase associated lipocalin, which binds to bacterial siderophores and prevents the bacteria from extracting iron [144]. If S. aureus bacteria enter the cytoplasm of neutrophils they encounter a protein complex called calprotectin (S100A8/S100A9), which inhibits S. aureus growth through chelation of Mn²⁺ and Zn²⁺ [148].

Type 1 activated macrophages also contribute to this fierce battle and fulfil many of the same roles outlined for neutrophils above, although they also fulfil higher level roles such as antigen presentation and secretion of many immunoregulatory cytokines [149]. Once activated by IFN-γ and TNF-α, they are able to phagocytose and kill staphylococci using many of the same mechanisms including lysosomal enzymes, reactive oxygen intermediates, reactive nitrogen intermediates and antimicrobial peptides [150]. In healthy individuals, these cells contribute to the development of staphylococcal abscesses, a mechanism that can wall off the infection and promote survival [150]. However, in certain immunosuppressive situations, such as following cutaneous burns, IL-10-producing Type 2 macrophages predominate. In mice, this was shown to inhibit abscess formation and promote dissemination of the infection, resulting in death [150]. Hence, the correct balance of activated macrophages appears to be crucial in containing staphylococcal infections to the skin. Macrophages also assist in the clean-up operation during the battle, removing neutrophils that have undergone
apoptosis to prevent release of enzymes and mediators that could cause tissue damage [151].

12. Suicide killing – the neutrophil extracellular traps
In addition to phagocytosis, neutrophils have evolved another mechanism to combat an attack by bacteria. In a recently discovered process that results in the cell’s death, neutrophils have been shown to cast out their DNA in response to infectious stimuli [152]. This network of extruded chromatin, to which is attached proteins from the neutrophil’s granules, is known as a neutrophil extracellular trap (NET) and is thought to be a central part of antimicrobial innate immunity (Figure 8). NETs have been observed in humans [153, 154], mice [155-157], rabbits [153], horses [158], cows [159] and fish [160]. This cell death process is distinct from apoptosis and necrosis and depends on the generation of reactive oxygen species (ROS) by NADPH oxidase [161]. Under NET-stimulating conditions, neutrophils undergo profound morphological changes in which the nuclei lose their shape, the chromatin homogenizes, the nuclear envelope and the granule membranes disintegrate, the NET components are mixed, and the cell membrane breaks releasing the material into the surroundings [161]. When the cell bursts open, this material unfolds into a fibrous structure occupying a volume that is several fold bigger than that of the cell it originated from [152]. Coated in antimicrobial peptides and antibacterial enzymes, this material provides ongoing bacterial killing in both time and space. This suicidal mechanism enhances the antimicrobial efficiency of neutrophils as the NETs can kill microorganisms that have no direct contact with the cell, and they are active even when the neutrophil has died [152]. Many clear benefits of NETs have been demonstrated in animal models of infection, inflammation or sepsis [162-165] and in human diseases where NET formation is disrupted or deficient [161, 166, 167].

13. The battle isn’t won – the staphylococcal counterattack
Phagocytosis is a very effective mechanism for bacterial clearance, but staphylococci are not just mere bystanders waiting to be decimated by the ruthless onslaught of weaponry brought forward by the neutrophils. Staphylococci have evolved many mechanisms to help them resist the might of the innate immune system, allowing them to sabotage various key defences (Figure 10). First, staphylococci can inhibit neutrophil recruitment to sites of infection by secreting various molecules such as staphylococcal superantigen-like protein-5 and protein-11 [131]. These proteins block the interaction between P-selectin glycoprotein ligand 1 on the neutrophil surface and P-selectin on the endothelial lining, thereby blocking neutrophil rolling [131]. Another protein, extracellular adherence protein, can inhibit the binding between ICAM-1 and Mac-1 or LFA-1, thus affecting firm adhesion [168]. Staphylococci also employ several strategies to resist opsonisation [132]. Many strains of S. aureus produce a capsule that covers the immunogenic and antigenic proteins on the bacterial surface.
Staphylococci can also acquire a shield of fibrinogen using an extracellular fibrinogen-binding protein (Efb) that links to C3b on the bacterial surface and prevents binding by phagocyte complement receptors [132]. *S. aureus* can also secrete molecules that prevent IgG binding, thus reducing opsonisation by immunoglobulins. The best example of this is Protein A, a predominantly cell bound protein but one which can also be found in the supernatant. Protein A can bind the Fc and Fab regions of immunoglobulin G and inhibit the antibody's ability to opsonise the bacteria [132]. *S. aureus* can also secrete Staphylokinase, an enzyme that can convert plasminogen into plasmin. Plasmin can cleave IgG and C3b that is bound to the bacteria’s surface, again inhibiting opsonisation [169]. Staphylococci can also secrete molecules that can interfere with the complement cascade including staphylococcal complement inhibitor SCIN, extracellular complement-binding protein Ecb, and staphylococcal superantigen-like protein SSL7 [132, 170]. These proteins can interfere with convertase enzymes, preventing development of the complement components and, again, inhibiting opsonisation.

Even if *S. aureus* fails in its bid to avoid opsonisation and it gets phagocytosed, it has strategies to facilitate survival within the neutrophil. The bacteria can produce toxins such as phenol-soluble modulins and leukocidin AB that lyse the neutrophil from within [132]. This process, known as programmed necrosis, involves activation of specific receptors that divert the neutrophil away from an apoptotic pathway and prevents its removal by macrophages [171]. *S. aureus* also evades the ROS mediated killing mechanism using scavengers like catalase, superoxide dismutase and the golden carotenoid pigment [132]. Using such strategies, it is now considered that survival of virulent staphylococci within neutrophils can actually contribute to the spread of infection within the host, acting like a Trojan horse to promote dissemination [172, 173]. Furthermore, whilst in this safe haven, *S. aureus* can persist in a semi-dormant state known as small colony variants which renders them intrinsically resistant to antibiotic therapy [174].

Staphylococci have also evolved mechanisms to deal with the threat of NETs. Like wire cutters that a soldier might use to escape from barbed wire, the staphylococci can secrete endonucleases that can liberate them from the traps [132]. *S. aureus* can also produce two enzymes, nuclease and adenosine synthase, that can convert NETs to deoxyadenosine. This process also triggers the caspase-3-mediated death of immune cells, resulting in the exclusion of macrophages from staphylococcal abscesses [175].

Hence, during this titanic battle that is taking place within a humble pustule, there is no guarantee that the immune system will come out on top. Staphylococci have proven to be a worthy adversary and in
many cases, they will prevail.

14. Bring in the special forces – the adaptive immune response to staphylococci
As described above, the armed forces of the innate immune system are not always sufficient to prevent or control a staphylococcal skin infection. However, the immune system also has an adaptive arm, which is critical for the clearance of many types of infection [176]. The adaptive immune system is essentially represented by T and B lymphocytes. Compared to the infantry soldiers represented by neutrophils and macrophages, T and B lymphocytes are more analogous to elite forces that use special weapons and tactics (SWAT). These cells are highly sophisticated, and use a much more complicated system of enemy recognition than is found in the innate immune system. The essence of the adaptive immune response lies in antigen specificity, which results in the generation of clones of antigen-specific T and B lymphocytes. The T cells play a fundamental role in coordinating the whole immune response, whereas the B cells are the source of antibodies, an important weapon in the fight against infections (Figure 11).

The starting point in the generation of a T cell response is recognition of antigens by professional antigen presenting cells. In the skin, such cells include the epidermal based Langerhans cells and dermal dendritic cells [177]. These cells are like specialized reconnaissance soldiers, monitoring the body surfaces for intruders. When an invasion is detected, these cells will phagocytose foreign antigens and break them down into small peptides. The peptides are deposited in the grooves of major histocompatibility complex 2 (MHC 2) molecules and transported to the cell's surface [178]. MHC 2 expression has been demonstrated in canine Langerhans cells, dermal dendritic cells and in macrophages [179]. The MHC 2 molecule functions as an antigen presentation system that brings the peptide antigen into contact with a T cell receptor that sits on the surface of a helper T lymphocyte [178]. The T cell is activated by the presence of the antigen, along with a variety of co-stimulatory molecules. In helper T cells, the CD4 molecule acts a bridge between the T cell and the MHC 2 molecule, but full activation requires additional signals comprising adhesion molecules and cytokine signaling [176]. Langerhans cells activated by staphylococci also produce cytokines and costimulators that enhance T cell activation and promote differentiation into effector CD4+ T helper cells [180, 181].

Once activated, clones of helper T cells can rapidly deploy to the skin. These cells express the cutaneous lymphocyte antigen (CLA), a member of the sialy Lewis-X glycoprotein family [182]. The ligand for CLA is likely to be E selectin, the adhesion molecule mentioned earlier that is critical for neutrophil trafficking. Once in the dermis, these lymphocytes can produce cytokines that influence
other aspects of the immune system. Like an army command centre that is issuing orders, T cells produce a wide array of cytokines with a diverse range of effects. Which cytokines are produced depends on further differentiation of the cells into Type 1 (TH1) or Type 2 (TH2) helper lymphocytes. In the case of staphylococcal infections, both types are likely to be active, with TH1 cells promoting macrophage activation, and TH2 cells promoting B cell activation and IgE antibody responses. TH1 lymphocytes produce IL-2 (promotes T cell proliferation), IFN-\(\gamma\) (activates macrophages and promotes TH1 responses) and TNF-\(\alpha\) (activates macrophages) [183, 184]. Th2 lymphocytes produce IL-3 (stimulates haematopoiesis and mast cell differentiation), IL-4 (promotes TH2 differentiation and IgE formation), IL-5 (promotes eosinophil development and survival), IL-13 (promotes B cell proliferation and IgE production) and IL-14 (promotes B cell differentiation) [184, 185].

A more recently classified type of T helper cell is known as a TH17 cell, so named because it produces IL-17 [185, 186]. However, these lymphocytes also produce the pro-inflammatory cytokines IL-21, IL-22 and IL-26 [185]. IL-17 is a key cytokine in the defence against S. aureus as it stimulates neutrophil recruitment to the site of infection and promotes abscess formation [183, 185]. Along with TH17 cells, IL-17 is also produced by other cells in the skin, including \(\gamma\delta\) T cells, mast cells and neutrophils [186]. These cells can be activated following stimulation of TLR2, or by cytokines such as IL-1\(\alpha/\beta\), IL-6 and IL-23 [186, 187]. IL-17 receptors are abundantly expressed on keratinocytes, and activation results in production of defensins and cathelicidin to aid with bacterial killing [74, 186, 188, 189].

One of the functions of helper T cells is to stimulate the proliferation of B lymphocytes. B lymphocytes can also be stimulated by complement breakdown products that are produced during the innate immune response [74, 180]. B lymphocytes recognise large, 3-dimensional antigens using cell bound antibodies as receptors. A clone of antigen-specific B cells will proliferate and differentiate into plasma cells, upon which large amounts of immunoglobulins will start to be produced. Antibodies of the IgM isotype are produced initially, but class switching to IgG and IgA results in these being the predominant immunoglobulins that are produced [176]. Antibodies have three major effects in the fight against staphylococcal infections. First, they can act as neutralising antibodies, binding to the pathogen to prevent its attachment to host target surfaces, such as epithelial layers or the extracellular matrix [190]. They also block the binding of toxins and other virulence factors and neutralize their harmful effects [190]. Second, they can act as opsonizing antibodies, coating the organism to allow its detection by Fc receptors on phagocytic cells. Third, they can bind to pathogens and activate the classical complement pathway. These latter two functions represent a bridge between the innate and adaptive immune responses.
In humans, antibody responses have been demonstrated to huge numbers of proteins from *S. aureus*, including toxins, haemolysins, virulence factors, enzymes, cell wall proteins and molecules involved in metabolism, as well as non-protein antigens such as teichoic acid and peptidoglycan [190]. However, it is still uncertain as to whether or not these antibodies actually confer any protection to the host. There is conflicting evidence about the role of antibodies in preventing or controlling colonization of mucosal surfaces. Some studies have shown that antibody levels are higher in non-carriers, suggesting that IgG or IgA could have some role in preventing attachment of staphylococci to body surfaces [16, 191]. However, other studies have demonstrated increased antibody responses to certain antigens in human carriers [192]. Anti-staphylococcal antibodies are also boosted during active infections, but it is still uncertain if they alter the course of the disease or offer any protection [190]. Attempts to make a vaccine that will protect against *S. aureus* colonization or infection have so far met with failure, despite promising results having been initially seen in mice [190].

In dogs, anti-staphylococcal antibodies have also been demonstrated to *S. pseudintermedius* [193-196]. Concentrations of anti-staphylococcal IgG are elevated in dogs with active staphylococcal infections [193, 194, 196], but western blotting studies have demonstrated that both healthy and infected dogs mount an IgG response against multiple antigens from the organism [195]. These findings, coupled with the fact that canine pyoderma frequently persists until intervention with antibiotics, suggest that these IgG molecules are not protective. Anti-staphylococcal IgA is also present in both healthy dogs, and those with staphylococcal infections, but the concentrations are similar in both groups, again suggesting a lack of efficacy in host defence [194].

A further twist in the interplay between staphylococci and the host immune response is the development of anti-staphylococcal IgE. These antibodies have been detected against cell wall components of *S. aureus* [197] and to superantigens produced by the organism [198]. Elevated concentrations of IgE specific for *S. aureus* have been demonstrated in patients with atopic dermatitis [199-201] and in those with recurrent staphylococcal abscesses [200, 202]. Furthermore, atopic patients with the highest concentrations of anti-staphylococcal IgE tend to have more severe symptoms [201]. Elevated levels of IgE against *S. pseudintermedius* have also been demonstrated in dogs with atopic dermatitis [193, 196]. Taken together, these findings indicate that in atopic individuals, antigens from staphylococci can act as allergens and contribute to the pathogenesis of the disease.

**15. Superantigens – the enemy’s secret weapon**

In what appears to be a cruel twist of nature, staphylococci are able to produce a range of molecules
known as superantigens. Both \textit{S. aureus} and \textit{S. pseudintermedius} are able to elaborate staphylococcal enterotoxins A-E, staphylococcal exfoliative toxin (ET) and staphylococcal toxic shock syndrome toxin (TSST)-1 \[203-205\]. These proteins can have a duel effect on the host. First, the molecule itself may have a direct toxic action on the host. For example, the condition known as staphylococcal scalded skin syndrome in humans is caused by the direct effect of staphylococcal exfoliative toxin on the granular layer of the epidermis, an effect that is independent of its superantigenic properties \[203\]. However, superantigens are most renowned for the effect they have on the immune system. Unlike conventional antigens, which are processed by antigen presenting cells and presented to specific T cell receptors via MHC 2 molecules, superantigens can bypass this entire process. These molecules are able to cross-link the MHC molecule and TCR directly, without involving the highly specific interaction that take place between the MHC groove and TCR binding site \[206\]. What is lost is the highly specific antigenic stimulation that normally activates T cells. Instead of a highly controlled activation of a clone of T cells which are specific for a very precise antigen, the end result is that large populations of T lymphocytes are activated in a non-specific manner. In humans, up to 20\% of the circulating pool of T cells can be activated in this manner \[206\]. Furthermore, the superantigens lead to the upregulation of cutaneous lymphoid antigen (CLA) which promotes homing of the lymphocytes to the skin \[203\]. The activated T lymphocytes cause a cytokine storm that can contribute to, or cause, a range of human skin diseases and other illnesses. These include toxic shock syndrome, Kawasaki disease, psoriasis and atopic dermatitis \[206, 207\]. As mentioned earlier, atopic individuals can mount IgE responses to staphylococcal superantigens, and these can exacerbate the underlying disease \[198, 203, 208, 209\]. Staphylococcal superantigens can also exacerbate cutaneous T cell lymphomas by stimulating proliferation of the neoplastic lymphocytes \[206\]. This phenomenon explains why some patients with this condition show a clinical improvement following antibiotic therapy \[206\]. There may also be a similar situation in dogs with mucocutaneous pyoderma, in which a lymphocyte rich inflammatory infiltrate can be resolved with antibiotic therapy.

The production of superantigens is a final blow to the efforts of the immune system in the fight against staphylococci. In addition to all the evasion strategies described above, under certain circumstances, the organisms can launch a barrage of secret weapons that are able to cause utter devastation in the host. The effect is a firestorm of non-targeted damage that in the worst case scenario can prove fatal.

16. Conclusions

The interaction between staphylococci and their mammalian hosts has a long evolutionary history. In many cases, the two can co-exist peacefully, but in humans and dogs, two species from this genus of bacteria have become war-mongerers. \textit{S. aureus} and \textit{S. pseudintermedius} have armed themselves
with a range of weaponry and defence mechanisms that we now refer to as virulence factors. Using this arsenal of weapons, these organisms have found ways to bypass the barriers imposed by epithelial surfaces, have created countermeasures against antimicrobial peptides and complement components, have developed evasion strategies against opsonisation and phagocytosis, and even figured out a way to survive the devastating attack brought on by neutrophils and macrophages. Using tactics reminiscent of counter-intelligence agents, they have even learnt how to infiltrate the host immune system and use its transportation systems to aid their spread around the body. Even the might of the adaptive immune system, with the highly coordinated responses orchestrated by the T lymphocyte population, and the massive repertoire of antibodies produced by B lymphocytes, is unable to halt the invasion of pathogenic strains. Staphylococci have even been able to benefit from the dysregulated immune state we refer to as allergy, with IgE antibodies directed against the organisms becoming pathogenic rather than protective. In a final twist of evolutionary fate, the production of superantigens can wreak havoc in the host, causing damage way in excess of what would occur following the infection itself. For all these reasons, it comes as no surprise that staphylococcal infections continue to be a major cause of morbidity in both humans and dogs. The current epidemic of antibiotic resistance that is spreading around the world was essentially inevitable, brought on by the necessity for widespread antibiotic use against an enemy that is simply too sophisticated for the immune system to cope with.
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Table and figure legends

Table 1 - Some components of *Staphylococcus aureus* and *pseudintermedius* that make the organisms pathogenic. The various factors are described as though they are weapons or tactics that are used by an enemy.

Table 2 - Components of the immune system that aid in the protection against pathogenic microorganisms. The components are described as though they are weapons or defences used by an army.

Figure 1 – A: Papulo-pustular eruption on a dog’s ventral abdomen caused by infection with *Staphylococcus pseudintermedius*. B: Staphylococcal rings on the abdominal skin of a dog. These lesions comprise erythematous annular macules surrounded by epidermal collarettes. They can be considered as expanded pustules in which the overlying epidermis has disappeared. In both images, the lesions have induced cutaneous inflammation and gross or microscopic infiltration of neutrophils.

Figure 2 – Interaction between staphylococci and the stratum corneum. Staphylococci produce a range of surface proteins and a material called slime that enables them to attach to corneocytes and proteins on the skin surface. Whilst in this environment, staphylococci encounter a number of antibacterial molecules including free fatty acids, complement, transferrin and antibodies.

Figure 3 – Activation of the complement cascade by staphylococci. In the classical pathway, antibodies binding to the surface of the bacteria are detected by C1, the first component of complement. Components C4 and C2 then bind to C1, resulting in them splitting into sub-units a and b. C4a and C2b break away and become anaphylotoxins. C4bC2a, also known as C3 convertase, remains bound to C1 and cleaves the third component of complement, C3, into C3a and C3b. C3a is another anaphylotoxin, whereas C3b has two functions. First, it is an important opsonin, coating microorganisms to prepare them for phagocytosis. Second, it combines with C4bC2a to create C5 convertase. C5 is split into C5a (another anaphylotoxin) whereas C5b combines with C6-9 to create the membrane attack complex. The complement cascade can also be activated by the alternate pathway, in which C3b binds to the surface of bacteria and creates alternative C3 and C5 convertases using additional factors (not shown).

Figure 4 – Activation of keratinocytes by staphylococci. Molecules on the surface of the organism such as lipopeptides and peptidoglycan are recognized by Toll-like receptors. These activate mitogen-activated protein (MAP) kinases and nuclear factor-κB, a transcription factor. Nuclear
factor-κB can also be activated by intracellular nucleotide-binding, oligomerization domain (NOD)-like receptors. NOD2 is activated by muramyl dipeptide, a breakdown product of peptidoglycan that may originate from staphylococci that have entered the keratinocyte. Activation of the keratinocyte results in the production and release of antimicrobial peptides and interleukin-1.

Figure 5 – Mast cell activation by staphylococci. Mast cells can be activated by complement-derived anaphylotoxins or by specific IgE. IgE antibodies to staphylococcal proteins have been demonstrated in both humans and dogs. Once activated, mast cells will degranulate releasing pre-formed inflammatory mediators, but this is followed by the synthesis and release of eicosanoids and cytokines. Mast cell derived mediators can initiate inflammation, resulting in the erythema seen at the sites of staphylococcal infection. They also play a key role in neutrophil recruitment.

Figure 6 – Recruitment of neutrophils to sites of infection. Neutrophils in circulation are arrested by adhesion molecules that are upregulated on the vascular endothelium in response to inflammatory cytokines such as IL-1 and TNF-α. Initially, selectins bind to ligands on the neutrophil cell membrane resulting in rolling along the blood vessel wall. Firm adhesion and arrest of the neutrophil is achieved by interaction between integrins on the neutrophil and cellular adhesion molecules (CAMs) on the endothelium. After stopping, the neutrophil crawls along the endothelial surface until it finds an intercellular junction, a process that is mediated by MAC-1. Paracellular migration occurs when the neutrophil pushes pseudopodia between the endothelial cells which opens a gap that allows the cell to squeeze through. In order for this to happen, the vascular endothelium cadherins need to separate and the actin cytoskeleton contracts. As the neutrophil passes through, it seals against the vascular endothelial cells using adhesion molecules such as PECAM-1, LFA-1 and ICAM-2. Once in the dermis, the neutrophils are attracted to the epidermis or follicular epithelium by a variety of chemotactic factors. PSGL1 - P-selectin glycoprotein ligand 1; LFA-1 – Leukocyte function associated antigen-1; VLA-4 – Very late antigen-4; ICAM-1 – Intercellular adhesion molecule-1; VCAM-1 – Vascular cell adhesion molecule-1; MAC-1 – Macrophage antigen-1; VE cadherin – Vascular endothelium cadherin; PECAM-1 - platelet/endothelial-cell adhesion molecule 1; PG D2 – Prostaglandin D2; LT B4 – Leukotriene B4. CXCL refers to a group of chemokines in which the cysteine residues (C) are separated by another amino acid (X), with the L standing for ligand.

Figure 7 – A: Infiltration of neutrophils into the outer root sheath of a hair follicle. The neutrophils have travelled from blood vessels and across the dermis in response to chemotactic molecules. B: Accumulation of neutrophils in a hair follicle lumen, surrounding colonies of staphylococci.
Figure 8 – Killing of staphylococci by neutrophils. Neutrophils recognise bacteria using pattern recognition receptors such as Toll-like receptors (TLRs). They also detect bacteria that have been opsonised with antibody or complement using Fc receptors and complement receptors (CR). This initiates phagocytosis in which the organism is taken up into an invagination in the plasma membrane. The invagination is sealed off to produce a phagosome, into which various granules are deposited. These granules contain multiple antibacterial substances including defensins, cathelicidin, enzymes and iron-chelating molecules. Within the phagosome, there is also generation of reactive oxygen species which also contribute to bacterial killing. The neutrophil is also able to commit suicide and cast out its DNA, producing a NET (neutrophil extracellular trap). This network of chromatin is covered in antibacterial molecules and enzymes that greatly extend the range of the neutrophil’s weaponry.

Figure 9 – Phagocytosis of staphylococci by neutrophils. The neutrophil in the centre has engulfed approximately 50 organisms and enclosed them in phagosomes.

Figure 10 – Staphylococcal countermeasures used to combat neutrophil attacks. Staphylococci can produce proteins that interfere with many aspects of neutrophil function. Superantigen-like proteins-5 and 11, and extracellular adherence protein, block attachment of neutrophils to blood vessel walls. Protein A and extracellular fibrinogen-binding protein (Efb) can inhibit opsonisation by antibodies and complement respectively. Production of staphylokinase can lead to breakdown of C3b on the bacterial surface. A number of molecules are produced that can block the activity of C3 convertase including staphylococcal complement inhibitor (SCIN), extracellular complement-binding protein (Ecb), and staphylococcal superantigen-like protein (SSL7). Staphylococci also elaborate a number of molecules that can kill the neutrophil from within (phenol-soluble modulins and leukocidin AB), evade damage by reactive oxygen species (catalase, superoxide dismutase and the golden carotenoid pigment) or neutralise antimicrobial peptides (iron-regulated surface protein A (isdA) and aureolysin). Finally, staphylococci have evolved mechanisms to deal with neutrophil extracellular traps, using enzymes to break down the chromatin strands.

Figure 11 – Lymphocyte responses in staphylococcal infections. Staphylococcal antigens are picked up by antigen presenting cells such as Langerhans cells and dermal dendritic cells which break the proteins down into small peptides. These peptides are attached to the groove of a major histocompatibility complex 2 (MHC2) molecule and transported to the cell surface. They are presented to the T cell receptors (TCR) on the surface of CD4+ helper T lymphocytes. Co-stimulatory molecular interactions such as B7/CD28 and ICAM-1/LFA-1 ensure efficient activation of the T cell.
helper cells can differentiate into different phenotypes based on the cytokine profiles they preferentially produce. TH1 derived cytokines stimulate other T cells and macrophages, as well as helping B cells to produce antibodies. TH2 derived cytokines stimulate class-switching in B lymphocytes to increase production of IgE. TH17 derived cytokines are important in the recruitment of neutrophils to sites of infection.
Table 1 – Some components of *Staphylococcus aureus* and *pseudintermedius* that make the organisms pathogenic. The various factors are described as though they are weapons or tactics that are used by an enemy.

<table>
<thead>
<tr>
<th>General components</th>
<th>Specific functions</th>
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<tbody>
<tr>
<td><strong>Grappling hooks</strong></td>
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<tr>
<td>MSCRAMMS (adhesion proteins)</td>
<td>Promote adhesion to stratum corneum and dermis</td>
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<tr>
<td>Teichoic acid</td>
<td>Initiate internalisation of the organism into epidermal cells</td>
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<td>Lipoteichoic acid</td>
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<td>Fibronectin binding proteins</td>
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<td>Fibrinogen binding proteins</td>
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<td>Iron-regulated surface protein A</td>
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<tr>
<td>Collagen binding proteins</td>
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<td>Slime coating</td>
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<td><strong>Armour piercing weapons</strong></td>
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<tr>
<td>Protease</td>
<td>Allow movement through the epidermis and dermis</td>
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<td>Hyaluronidase</td>
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<td>Lipase</td>
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<tr>
<td><strong>Body armour</strong></td>
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<tr>
<td>Cell wall (including peptidoglycan)</td>
<td>Confers resistance to complement membrane attack complex</td>
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<td>Biofilms</td>
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<tr>
<td><strong>Camouflage</strong></td>
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<tr>
<td>Capsular polysaccharides</td>
<td>Inhibit antibody and C3b recognition to impair opsonisation</td>
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<tr>
<td>Extracellular fibrinogen-binding protein</td>
<td>Binds fibrinogen and stops recognition by complement receptors</td>
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<td><strong>Sabotage and anti-capture devices</strong></td>
<td><strong>Poisons</strong></td>
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<tr>
<td>Aureolysin (matrix metalloproteinase)</td>
<td><strong>Phenol-soluble modulin and Leukocidin AB</strong></td>
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<td>Iron-regulated surface protein A</td>
<td><strong>Staphylococcal enterotoxins</strong></td>
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<tr>
<td>Complement inhibitors (e.g. CHIPS, SCIN, Sbi)</td>
<td><strong>Toxic shock syndrome-1 toxin</strong></td>
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<td>Protein A</td>
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<tr>
<td>Staphylokinase</td>
<td></td>
</tr>
<tr>
<td>Extracellular fibrinogen-binding protein</td>
<td></td>
</tr>
<tr>
<td>Catalase, superoxide dismutase and the golden carotenoid pigment</td>
<td></td>
</tr>
<tr>
<td>Staphylococcal superantigen-like protein-5 and 11</td>
<td></td>
</tr>
<tr>
<td>Extracellular adherence protein</td>
<td></td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td><strong>Cleaves LL-37 (human cathelicidin)</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Confers protection against human β-defensin 2</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Inhibit C3 and C5 conversion, chemotaxis</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Binds IgG and inhibits complement activation</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Breaks down bound IgG and C3b to prevent oposonisation</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Prevents binding to complement receptors</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Evasion of reactive oxygen species</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Inhibit neutrophil rolling in blood vessels</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Inhibits adherence of neutrophils to blood vessel walls</strong></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Toxic to neutrophils</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Promote inflammation in the host</strong></td>
</tr>
<tr>
<td></td>
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</tbody>
</table>
Table 2 – Components of the immune system that aid in the protection against pathogenic microorganisms.

<table>
<thead>
<tr>
<th>The Defending Army – the innate and adaptive immune response</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General components</strong></td>
</tr>
<tr>
<td><strong>Physical barriers</strong></td>
</tr>
<tr>
<td>Stratum corneum</td>
</tr>
<tr>
<td>Epidermis</td>
</tr>
<tr>
<td>Dermis</td>
</tr>
<tr>
<td><strong>Infantry soldiers</strong></td>
</tr>
<tr>
<td>Neutrophils</td>
</tr>
<tr>
<td>Macrophages</td>
</tr>
<tr>
<td><strong>Reconnaissance/prisoner capture soldiers</strong></td>
</tr>
<tr>
<td>Antigen presenting cells</td>
</tr>
<tr>
<td>Dendritic cells</td>
</tr>
<tr>
<td><strong>Special weapons and tactics soldiers (SWAT)</strong></td>
</tr>
<tr>
<td>Lymphocytes</td>
</tr>
<tr>
<td><strong>Land mines</strong></td>
</tr>
<tr>
<td>Mast cells</td>
</tr>
<tr>
<td><strong>Bayonets and daggers</strong></td>
</tr>
<tr>
<td>Defensins</td>
</tr>
<tr>
<td>Cathelicidins</td>
</tr>
<tr>
<td><strong>Machine guns</strong></td>
</tr>
<tr>
<td>Complement membrane attack complex</td>
</tr>
<tr>
<td><strong>Chemical weapons</strong></td>
</tr>
<tr>
<td>Lysozyme</td>
</tr>
<tr>
<td>Nitric oxide</td>
</tr>
<tr>
<td>Hypochlorite</td>
</tr>
<tr>
<td>Peroxide</td>
</tr>
<tr>
<td><strong>Restraint devices (handcuffs, chains, ropes)</strong></td>
</tr>
<tr>
<td>Complement component C3b</td>
</tr>
<tr>
<td>Antibodies</td>
</tr>
<tr>
<td>Orders, signals and radio communications</td>
</tr>
<tr>
<td>----------------------------------------</td>
</tr>
<tr>
<td>Complement components C3a, C4a and C5a</td>
</tr>
<tr>
<td>IL-1, IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, IL-12</td>
</tr>
<tr>
<td>IFN-gamma</td>
</tr>
<tr>
<td>TNF-alpha</td>
</tr>
<tr>
<td>Prostaglandin D2</td>
</tr>
<tr>
<td>Leukotriene B4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Surveillance</th>
<th>Recognition of the enemy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toll like receptors</td>
<td></td>
</tr>
<tr>
<td>Mannose receptors</td>
<td></td>
</tr>
<tr>
<td>Cell bound antibodies</td>
<td></td>
</tr>
<tr>
<td>Fc receptors</td>
<td></td>
</tr>
<tr>
<td>Complement receptors</td>
<td></td>
</tr>
<tr>
<td>B cell receptors (antibodies)</td>
<td></td>
</tr>
<tr>
<td>T cell receptors</td>
<td></td>
</tr>
<tr>
<td>CD3, CD4 and DC8 molecules</td>
<td></td>
</tr>
<tr>
<td>Major histocompatibility complex</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Transport network and traffic control</th>
<th>Transport of inflammatory cells to sites of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circulatory system</td>
<td></td>
</tr>
<tr>
<td>Histamine</td>
<td></td>
</tr>
<tr>
<td>Serotonin</td>
<td></td>
</tr>
<tr>
<td>Platelet activating factor</td>
<td></td>
</tr>
<tr>
<td>Bradykinin</td>
<td></td>
</tr>
<tr>
<td>Integrons</td>
<td></td>
</tr>
<tr>
<td>Selectins</td>
<td></td>
</tr>
<tr>
<td>Prostaglandins</td>
<td></td>
</tr>
<tr>
<td>Leukotrienes</td>
<td></td>
</tr>
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</table>
Table 3 – Abbreviations used in this paper

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP</td>
<td>Antimicrobial peptide</td>
</tr>
<tr>
<td>C1 - C9</td>
<td>Complement components 1 - 9</td>
</tr>
<tr>
<td>cBD</td>
<td>Canine β-defensin</td>
</tr>
<tr>
<td>cCath</td>
<td>Canine cathelicidin</td>
</tr>
<tr>
<td>CLA</td>
<td>Cutaneous lymphocyte antigen</td>
</tr>
<tr>
<td>ClfA</td>
<td>Fibrinogen-binding protein/clumping factor A</td>
</tr>
<tr>
<td>ClfB</td>
<td>Fibrinogen-binding protein/clumping factor B</td>
</tr>
<tr>
<td>CXC</td>
<td>Chemokine with two cysteines separated by another amino acid (represented by X)</td>
</tr>
<tr>
<td>CXCL</td>
<td>CXC chemokine ligand</td>
</tr>
<tr>
<td>Ecb protein</td>
<td>Extracellular complement-binding protein</td>
</tr>
<tr>
<td>EfB protein</td>
<td>Extracellular fibrinogen-binding protein</td>
</tr>
<tr>
<td>ET</td>
<td>Exfoliative toxin</td>
</tr>
<tr>
<td>Fnbp A</td>
<td>Fibronectin-binding protein A</td>
</tr>
<tr>
<td>Fnbp B</td>
<td>Fibronectin-binding protein B</td>
</tr>
<tr>
<td>hBD</td>
<td>Human β-defensin</td>
</tr>
<tr>
<td>HNPs</td>
<td>Human neutrophil peptides</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Intercellular adhesion molecule 1</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Interferon-γ</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IRAK4</td>
<td>Interleukin-1 receptor-associated kinase 4</td>
</tr>
<tr>
<td>isdA</td>
<td>Iron-regulated surface protein A</td>
</tr>
<tr>
<td>LFA1</td>
<td>Lymphocyte function associated antigen-1</td>
</tr>
<tr>
<td>LL37</td>
<td>Human cathelicidin (37 amino acids starting with two leucines)</td>
</tr>
<tr>
<td>MAC</td>
<td>Membrane attack complex</td>
</tr>
<tr>
<td>MAC-1</td>
<td>Macrophage antigen-1</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>MMPs</td>
<td>Matrix metalloproteinase</td>
</tr>
<tr>
<td>MSCRAMMs</td>
<td>Microbial surface components recognizing adhesive matrix molecules</td>
</tr>
<tr>
<td>MyD88</td>
<td>Myeloid differentiation primary response protein 88</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NET</td>
<td>Neutrophil extracellular trap</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear factor-κB</td>
</tr>
<tr>
<td>NLR</td>
<td>NOD-like receptor</td>
</tr>
<tr>
<td>NOD</td>
<td>Nucleotide-binding, oligomerization domain</td>
</tr>
<tr>
<td>NOD2</td>
<td>Nucleotide-binding oligomerization domain containing 2</td>
</tr>
<tr>
<td>PAMPS</td>
<td>Pattern associated molecular patterns</td>
</tr>
<tr>
<td>PECAM1</td>
<td>Platelet/endothelial-cell adhesion molecule 1</td>
</tr>
<tr>
<td>PGN</td>
<td>Peptidoglycan</td>
</tr>
<tr>
<td>PRR</td>
<td>Pattern recognition receptor</td>
</tr>
<tr>
<td>PSGL</td>
<td>P-selectin glycoprotein ligand</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>S. aureus</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>S. pseudintermedius</td>
<td>Staphylococcus pseudintermedius</td>
</tr>
<tr>
<td>SCIN</td>
<td>Staphylococcal complement inhibitor</td>
</tr>
<tr>
<td>sLex</td>
<td>Sialyl Lewis X tetrasaccharide</td>
</tr>
<tr>
<td>SSL7</td>
<td>Staphylococcal superantigen-like protein 7</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>TSST</td>
<td>Toxic shock syndrome toxin</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Vascular cell adhesion molecule 1</td>
</tr>
<tr>
<td>VE cadherin</td>
<td>Vascular endothelium cadherin</td>
</tr>
<tr>
<td>VLA4</td>
<td>Very late antigen-4</td>
</tr>
</tbody>
</table>
Hill Immunopathogenesis of Staphylococcal infections

Figure 2
Hill Immunopathogenesis of Staphylococcal infections

Figure 3
Figure 5

Anaphylotoxins

Antigen/IgE

C4a
C2b
C3a
C5a

Mast cell

Seconds
Granule exocytosis
Histamine
Serotonin
Tryptase
Proteases

Minutes
Eicosanoid synthesis and secretion
Prostaglandins
Leukotrienes
PAF

Hours
Cytokine synthesis and secretion
TNF-α
IL-4
IL-5
IL-6
IL-13

Vasodilatation
Increased blood vessel permeability
Chemoattraction of neutrophils
Upregulation of inflammatory responses
Figure 6

Chemotaxis
- TNF-α
- IL-1
- IL-8
- CXCL1, 2, 5
- C3a, C4a, C5a
- PG D2
- LT B4
- Tryptase

Paracellular migration

Rolling
- PSGL1
- E- and P-Selectin

Adhesion
- LFA1
- VLA4
- ICAM-1
- VCAM-1

Crawling
- MAC-1
- ICAM-1

LFA-1 ICAM-2
PECAM1
VE cadherin
Figure 8

α-defensins
LL-37
Lysozyme
O₂⁻, H₂O₂, HOCl
Elastase
Cathepsin G
Azurocidin
Proteinase-3
Acid hydrolases
Gelatinase
Lactoferrin
Transcobalamin II
Calprotectin
α-defensins
LL-37
Enzymes
Phagocytosis
Neutrophil extracellular trap
Figure 10

- PSGL1
- Superantigen-like proteins-5, 11
- Selectin
- SCIN
- EcB
- SSL7
- C3 convertase
- Protein A
- LFA1/
- Mac-1
- Extracellular adherence protein
- ICAM-1
- Staphylococcus
- Capsule
- Efb
- Fibrinogen
- Phenol-soluble modulin
- Leukocidin AB
- Catalase
- Superoxide dismutase
- Golden carotenoid pigment
- Endonuclease
- Nuclease
- Adenosine synthase
- Neutrophil
- Defencin
- Cathelicidin
- Aureolysin
- isdA
- NET
Figure 11