Leukocidins: staphylococcal bi-component pore-forming toxins find their receptors

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Abstract | *Staphylococcus aureus* is a major bacterial pathogen that causes disease worldwide. The emergence of strains that are resistant to commonly used antibiotics and the failure of vaccine development have resulted in a renewed interest in the pathophysiology of this bacterium. Staphylococcal leukocidins are a family of bi-component pore-forming toxins that are important virulence factors. During the past five years, cellular receptors have been identified for all of the bi-component leukocidins. The identification of the leukocidin receptors explains the cellular tropism and species specificity that is exhibited by these toxins, which has important biological consequences. In this Review, we summarize the recent discoveries that have reignited interest in these toxins and provide an outlook for future research.

Endocarditis
Inflammation of the inner layer of the heart that is most often of infectious origin. *Staphylococcus aureus* is a major cause of endocarditis.

Virulence factors
Molecules produced by pathogens that contribute to the pathogenicity of the organism and that enable colonization, immune evasion and suppression, entry into and release from host cells, and the acquisition of nutrients.

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doi:10.1038/nrmicro.2017.27
Published online 19 Apr 2017

Staphylococcus aureus is one of the most important bacterial pathogens that has affected human health to date[1]. The organism colonizes approximately 30% of the human population[1], but once it invades deeper tissues the clinical manifestations of *S. aureus* range from mild skin and soft tissue infections (SSTI) to more debilitating infections, such as sepsis, endocarditis and pneumonia[1]. Severe infections with *S. aureus* have a poor prognosis[2], which is complicated by resistance to commonly used antibiotics[3]. As no vaccines are currently approved for *S. aureus*, there is substantial interest in further understanding the pathophysiology of this bacterium. Virulence factors are crucial for the success of *S. aureus* in the human host[4], as these factors control many aspects of its commensal and pathogenic lifestyles[5–9].

An important group of staphylococcal virulence factors are bi-component leukocidins, which are pore-forming toxins (PFTs) that kill immune cells (also known as leukocytes)[7]. Among leukocytes, phagocytes are required for the containment of *S. aureus* infection by the host[9] and are considered to be the major target of leukocidins[7]. Leukocidins can also target natural killer cells, dendritic cells and T lymphocytes[10] (TABLE 1), which suggests that these toxins can disrupt both innate and adaptive immune responses. In addition to their leukocidal activity, some leukocidins are able to lyse erythrocytes[11] (TABLE 1). For historical reasons, these bi-component toxins are referred to collectively as leukocidins or leukotoxins[12]. Nevertheless, *S. aureus* secretes other toxins that are also able to target phagocytes, lymphocytes and erythrocytes, including α-toxin, β-toxin and small cytotoxic peptides known as phenol-soluble modulins (PSMs)[8,13].

Bi-component leukocidins are a collection of PFTs produced by many staphylococci. PFTs are generally secreted as inactive monomeric subunits that multimerize on binding to the membrane of a target host cell, which results in the formation of a pore that spans the phospholipid bilayer and induces cell death (FIG. 1). On the basis of the secondary structure of the membrane-spanning domains, PFTs are classified into α-helical PFTs or β-barrel PFTs[14]. β-Barrel PFTs are further classified into cholesterol-dependent cytolysins or haemolysins; the staphylococcal bi-component leukocidins belong to the haemolysin class of toxins. *S. aureus* isolates that are associated with human infections can produce up to five different leukocidins: Panton–Valentine leukocidin (PVL or LukSF–PV), γ-haemolysin AB and γ-haemolysin CB (HlgAB and HlgCB), leukocidin ED (LukED) and leukocidin AB (LukAB; also known as LukGH)[7] (TABLE 1).

The other leukocidins that are known to be produced by *S. aureus* are leukocidin MF′ (LukMF′)[15] and leukocidin PQ (LukPQ)[16]; however, these toxins are associated with zoonotic infections and are rarely found in human isolates of *S. aureus* (BOX 1). Although the first description of leukocidin activity in *S. aureus* culture supernatants was published around 1895, (the discoveries that lead to the identification

Table 1 | **Leukocidins produced by human *S. aureus* isolates and their respective myeloid and erythroid receptors**

Leukocidin			Receptors		Specificity*	Species (activity)‡
Name	**Cognate pairs**	**Non-cognate pairing§**	**Myeloid receptors**	**Erythroid receptors**	**Target cells**	**Human, rabbit, mouse**
PVL	LukS–PV (S type) and LukF–PV (F type)	• LukD • HlgB	• C5aR1 • C5aR2		• Neutrophils • Monocytes • Macrophages	• Human (high) • Rabbit (medium) • Mouse (none)
LukED	LukE (S type) and LukD (F type)	• LukF–PV • HlgB	• CCR5 • CXCR1 • CXCR2	DARC‖	• Neutrophils • Monocytes • Macrophages • Dendritic cells • T cells • Erythrocytes • NK cells	• Human (high) • Mouse (high)
HlgAB¶	HlgA (S type) and HlgB (F type)	• LukF–PV • LukD	• CCR2 • CXCR1 • CXCR2	DARC‖	• Neutrophils • Monocytes • Macrophages • Erythrocytes	• Human (high) • Mouse (medium)
HlgCB	HlgC (S type) and HlgB (F type)	• LukF–PV • LukD	• C5aR1 • C5aR2		• Neutrophils • Monocytes • Macrophages	• Human (high) • Rabbit (medium) • Mouse (low)
LukAB (also known as LukGH)	LukA (LukH; S type) and LukB (LukG; F type)	None	CD11b		• Neutrophils • Monocytes • Macrophages • Dendritic cells	• Human (high) • Rabbit (medium) • Mouse (low)

*Shown are the leukocytes for which there are experimental data in the literature. ‡On the basis of published susceptibility of tested primary cells. §Potential non-cognate pairing of the S component with an F component of another leukocidin, which results in functional mixed pores or inactive hybrid complexes, depending on the pair. ‖DARC renders erythrocytes susceptible to the haemolytic activity of LukED and HlgAB. ¶HlgAB targets both human and murine CCR2 and DARC, but only human CXCR1 and CXCR2. C5aR1, C5a anaphylatoxin chemotactic receptor 1; CCR2, CC-chemokine receptor 2; CXCR1, CXC chemokine receptor 1; DARC, Duffy antigen receptor for chemokines; HlgA, γ-haemolysin A; LukA, leukocidin A; NK cells, natural killer cells; PVL, Panton–Valentine leukocidin.

of the leukocidins were recently reviewed elsewhere; see REF. 7), the molecular mechanisms that cause pore formation have remained incompletely understood.

Bi-component leukocidins are thought to protect *S. aureus* from being killed by host phagocytes; however, the necessity of the apparently redundant range of phagocyte-targeting toxins is incompletely understood. The ability of the bi-component leukocidins to target and kill human leukocytes *in vitro* has been established and supported by more 100 years of research[12]; however, whether they target and kill human leukocytes *in vivo* to promote *S. aureus* infection remains controversial[3]. This controversy is a result of the poor understanding of the molecular mechanisms that underlie the differential cellular tropism and species specificity exhibited by these toxins (TABLE 1). Moreover, an incomplete awareness that animal leukocytes are not as susceptible to leukocidins as human leukocytes has hindered progress in understanding the contribution of the bi-component leukocidins to staphylococcal pathophysiology[12,17].

During the past five years, the receptors that are targeted by staphylococcal leukocidins have been identified, which has helped to explain the cellular tropism and species specificity of these toxins. The identification of these receptors has also enabled the role of each individual leukocidin to be assessed and has improved our understanding of the complex interplay between different leukocidins during infection. In addition to their cytotoxic potential, the discovery of the leukocidin receptors has revealed new insights into how leukocidins modulate immune cell functions. Similarly, the identification of an erythroid receptor for leukocidins has also provided new insights into the link between virulence and nutritional immunity. This Review provides an overview of the similarities and differences in the interactions between leukocidins and their respective receptors, and discusses the implications of receptor identification for the mechanisms of action of leukocidins, their diverse roles during pathogenesis *in vivo* and their potential as targets for therapeutic interventions.

Bi-component leukocidins
Toxins that consist of two protein subunits that hetero-oligomerize to form multimeric pores. The pores penetrate the lipid bilayer of the target cell.

Leukocytes
A generic term used to describe immune cells that are of haematopoietic origin.

Phagocytes
Immune cells that specifically engulf and degrade bacteria, fungi, parasites, dead host cells, and cellular and foreign debris by a process termed phagocytosis.

Phenol-soluble modulins
(PSMs). Small amphipathic peptides produced by staphylococci that exhibit cytotoxic activity against host cells.

Leukocidins and receptors

Leukocidin structures. The bi-component leukocidins are PFTs that share a highly conserved structure[18] (FIG. 1a). All of the subunits of the different leukocidins have an approximate molecular weight of 33 kDa. Leukocidins have two subunits that are classified as the host cell targeting S component (for slow migration in chromatography columns: LukS–PV, LukE, HlgA, HlgC and LukA), and the polymerization F component (for fast migration: LukF–PV, LukD, HlgB and LukB)[7] (TABLE 1). In contrast to the S components and F components of other leukocidins, which are secreted as two soluble and independent monomers, LukAB is pre-assembled as a soluble heterodimer[19].

Both the S component and the F component have three important domains — a cap, a rim and a stem (FIG. 1a). In the soluble form, the hydrophobic stem region is packed within the cap domain. The oligomerization of leukocidins is thought to induce a conformational change in each component that causes the extension and unfolding of the stem domain,

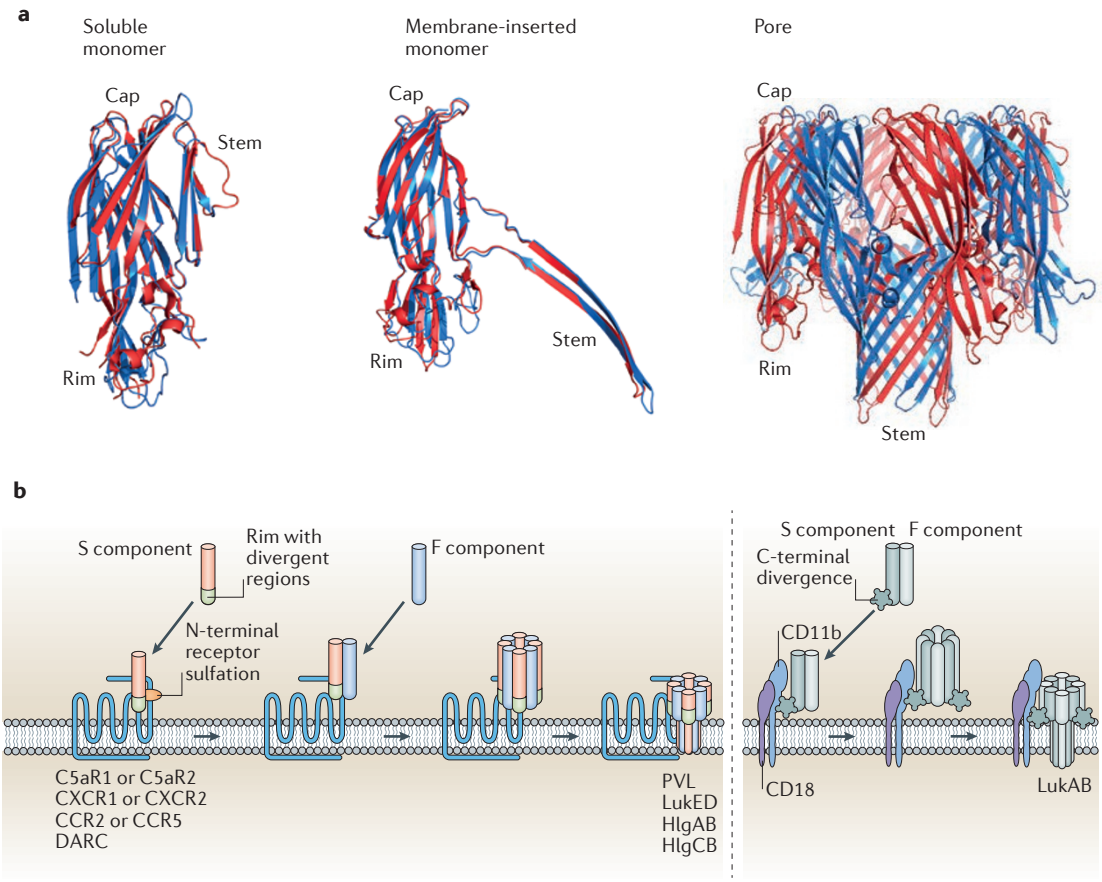


Figure 1 | **Pore formation by leukocidins. a** | Crystal structures of single leukocidin protein components and multimeric β-barrel leukocidin pores show high structural similarity. In the soluble form, hydrophobic residues in the β-barrel stem of the S component and the F component are covered by the cap. The rim domain of the S component, which is responsible for the initial binding to the host target cell, is involved in receptor recognition. Hetero-oligomerization of the S component with the F component induces a conformational change that results in insertion of the hydrophobic stem into the membrane of the target cell. The resulting octameric β-barrel pore consists of alternating four S components and four F components. γ-Haemolysin A (HlgA) is shown in red and HlgB is shown in blue. Structural information was acquired from the RCSB Protein Data Bank, with accession numbers 2QK7 (unbound HlgA), 1LKF (unbound HlgB) and 3B07 (single HlgA and HlgB from HlgAB octamer). The major structural domains were coloured using PyMOL software. **b** | Binding and pore formation of different leukocidins to their respective receptor targets. Differences in the events between leukocidins that target chemokine receptors (Panton–Valentine leukocidin (PVL), leukocidin ED (LukED), HlgAB, HlgCB; left side) versus the leukocidin that targets CD11b (LukAB; right side) are highlighted. For PVL, LukED, HlgAB and HlgCB, the initial binding event of the S component to its specific receptor enables the secondary binding of the polymerizing F component, hetero-oligomerization and pore formation. In the rim domain of the S component (green), divergent region 1 (DR1) of LukE determines the recognition of CC-chemokine receptor 5 (CCR5), whereas DR4 of LukE determines the recognition of CXC chemokine receptor 1 (CXCR1) and CXCR2. The bottom loops in the rim domain of LukS–PV are essential for targeting C5a anaphylatoxin chemotactic receptor 1 (C5aR1). The interaction of C5aR1 and C5aR2 with LukS–PV and HlgC is multifactorial and involves the amino termini and extracellular loops of the receptors. Sulfated tyrosine residues (orange) in the N termini of the receptors C5aR1 and Duffy antigen receptor for chemokines (DARC) are essential for their interaction with PVL (for C5aR1) and HlgAB and LukED (for DARC). Uniquely, LukAB is secreted as a pre-assembled dimer. The dimerization of LukAB results in the leukocidin having a high affinity for the I-domain of its receptor CD11b. Receptor recognition by LukAB is mediated by a divergent carboxy-terminal extension of LukA (grey spike). The actual number of receptors per pore is unknown. Images in part **a** courtesy of B. W. Bardoel, University Medical Center Utrecht, The Netherlands.

which penetrates the plasma membrane of target cells[18]. During oligomerization, a ring-shaped pre-pore is formed that enables the multimeric β-strand domains of the stem regions to be inserted into the cell membrane, which results in the formation of a pore that is 1–2 nm in diameter[18,20] (FIG. 1a). Structural studies of HlgAB and HlgCB have revealed that these leukocidins form an octameric pore that has alternating HlgA or HlgC and HlgB subunits[18,20] (FIG. 1a). Similarly, LukAB was recently found to form a hetero-octameric pore[21]. On the basis of the crystal structure of the LukAB heterodimer, the sites of interaction between LukA and LukB help to explain why this toxin is pre-assembled in solution[21,22]. Three salt bridges between the interfaces

Box 1 | **Leukocidins and non-human staphylococci**

Recent studies on bi-component leukocidins have focused on those produced by human isolates of *Staphylococcus aureus*. However, *S. aureus* can also infect different animals, including pigs, rabbits, cattle, horses and dogs[118]. These zoonotic strains are likely to encode bi-component leukocidins in their genomes that enable them to target and kill neutrophils from different species. For example, leukocidin MF′ (LukMF′) is a toxin that was found in *S. aureus* isolates from bovine infections[39,119]. LukMF′ has a remarkable cellular tropism for bovine phagocytes, a property that is mediated by the targeting of CC-chemokine receptor 1 (CCR1)[39], which is a chemokine receptor that is expressed in bovine neutrophils but not in human neutrophils. More recently, a novel bi-component leukocidin, LukPQ, was identified in *S. aureus* isolates from horses[16] and is highly cytotoxic in equine neutrophils. LukPQ is similar to LukED (91% amino acid identity between LukE and LukP)[16], and targets the equine CXC chemokine receptor A (CXCRA) and CXCR2 receptors on neutrophils.

The role of leukocidins in the pathogenesis of other staphylococcal species is exemplified by the leukocidin LukSF-I[15], which is encoded by *Staphylococcus pseudintermedius*, a pathogen that is primarily associated with infections in canines. LukSF-I has substantial amino acid sequence similarity with LukED and can target canine and human phagocytes[15]. However, in contrast to LukED, LukSF-I has low haemolytic activity.

Bi-component leukocidin-like toxins have also been found in *Staphylococcus argenteus*, *Staphylococcus schweitzeri* and *Staphylococcus delphini*. *S. argenteus* and *S. schweitzeri* encode a toxin that is similar to the human LukAB leukocidin, whereas *S. delphini* (a zoonotic pathogen that infects dolphins) encodes a toxin that is similar to LukSF-I from *S. pseudintermedius*. Species diversification in leukocidins provides strong support for the notion that the targeting of phagocytes by staphylococci is crucial for host adaptation.

of the cap and rim domains of LukA and LukB, which are not found in the other leukocidins, are required for the formation of LukAB dimers in solution. Hetero-octamers have been proposed as the preferred stable conformation for other leukocidins, such as PVL and LukED. Hexameric and heptameric pores have also been observed, but they have been hypothesized to represent intermediate structures during the formation of the stable pore[23,24]; however, this hypothesis remains to be tested experimentally.

For all of the bi-component leukocidins except LukAB, which binds to its target as a pre-assembled dimer, the initial binding of the S component to the host cell is followed by the recruitment of the F component[25–27] (FIG. 1b). During the multimerization of the S component and the F component, a pre-pore is formed that eventually spans the entire membrane. F components have also been shown to interact with the surface of neutrophils independently of the S component[28–30]; nevertheless, the cellular target, or targets, and the importance of this binding to pore formation by the bi-component leukocidins remain to be fully elucidated. Most leukocidins can form functional pores using both cognate (for example, LukED and HlgAB) and non-cognate combinations of subunits (for example, LukE–HlgB and HlgA–LukD)[31–33] (TABLE 1). This is true for PVL, LukED, HlgAB and HlgCB, but not for the pre-assembled LukAB dimer, which does not interact with any of the other leukocidin subunits owing to its structural divergence[21] (TABLE 1). The functional consequences of cognate and non-cognate interactions between the bi-component leukocidins are discussed below.

Leukocidin receptors. The distinct cellular and species specificities of the bi-component leukocidins (TABLE 1) have provided historical clues for the involvement of specific proteinaceous host receptors. In 2011, three receptors were described for LukED and PVL in different laboratories. For LukED, CC-chemokine receptor 5 (CCR5) was identified as a receptor[10]. Concurrently, C5a anaphylatoxin chemotactic receptor 1 (C5aR1) and C5aR2 were identified as the receptors for PVL[34] (TABLE 1). Subsequently, receptors were identified for all of the bi-component leukocidins: in addition to CCR5, LukED targets CXC chemokine receptor 1 (CXCR1) and CXCR2 (REF. 35); HlgCB, similarly to PVL, targets C5aR1 and C5aR2 (REF. 36); HlgAB targets CXCR1, CXCR2 and CCR2 (REF. 36); LukAB targets CD11b (also known as integrin αM)[37]; and LukED and HlgAB both target the Duffy antigen receptor for chemokines (DARC; also known as ACKR1)[38] (TABLE 1). The presence of the cognate leukocidin receptors on the surface of host target cells is required for leukocidin toxicity[10,34–38]. Moreover, the interspecies variations in the sequence and structure of leukocidin receptors are responsible for the divergent susceptibility of immune cells from different mammalian species to leukocidins[34,36,37,39] (BOX 2; TABLE 1).

The majority of the receptors that are targeted by bi-component leukocidins belong to the families of complement and chemokine receptors (BOX 1), of which most are class A rhodopsin-like G protein-coupled receptors (GPCRs) — C5aR1, C5aR2, CXCR1, CXCR2, CCR2, CCR5 and DARC[40]. These structurally and functionally related seven-transmembrane-spanning receptors are involved in transducing extracellular signals to the interior of the cell through cytosolic G proteins. By contrast, DARC is an atypical chemokine receptor that is not coupled to a G protein[41,42]. With the exception of DARC, these receptors are expressed in specific leukocytes at very high levels. Key cellular functions that are regulated by G proteins include cellular activation and migration[40]. CD11b is more distantly related to the family of GPCRs and a component of the Mac-1 integrin. The Mac-1 integrin is highly expressed on phagocytes and is involved in many crucial cellular functions, such as phagocytosis, cell-mediated killing and chemotaxis[43].

Leukocidins and receptor interactions. The identification of the leukocidin receptors has led to detailed molecular studies of leukocidin–receptor interactions. Targeting of the receptors by leukocidins is mediated by S components, which bind to their receptors with high affinity (all within a nanomolar range)[10,34–38]. The S components share 70–90% amino acid sequence identity[44] (BOX 2), and the alignment of leukocidin amino acid sequences has revealed divergent regions (DRs) in the rim of the S components[35] (FIG. 1a). For LukED, DR1 is involved in the targeting of CCR5 by LukE[45] (FIG. 1b), whereas DR4 determines the targeting of CXCR1 and CXCR2, but not of CCR5, by LukE[35] (FIG. 1b). The identification of different regions in one leukocidin that are involved in the recognition of different receptors enabled the generation of mutant LukED toxins that

Neutrophils
Abundant, short-lived and motile phagocytic cells of the innate immune system that are one of the first cell types to migrate to a site of infection.

Chemokine receptors
Cytokine receptors on the surface of mainly immune cells that interact with specific cytokines called chemokines and that belong to the family of G protein-coupled receptors.

G protein-coupled receptors
(Rhodopsin-like GPCRs).A family of receptors with a common structure comprising seven transmembrane helices that transduce extracellular signals through interactions with G proteins.

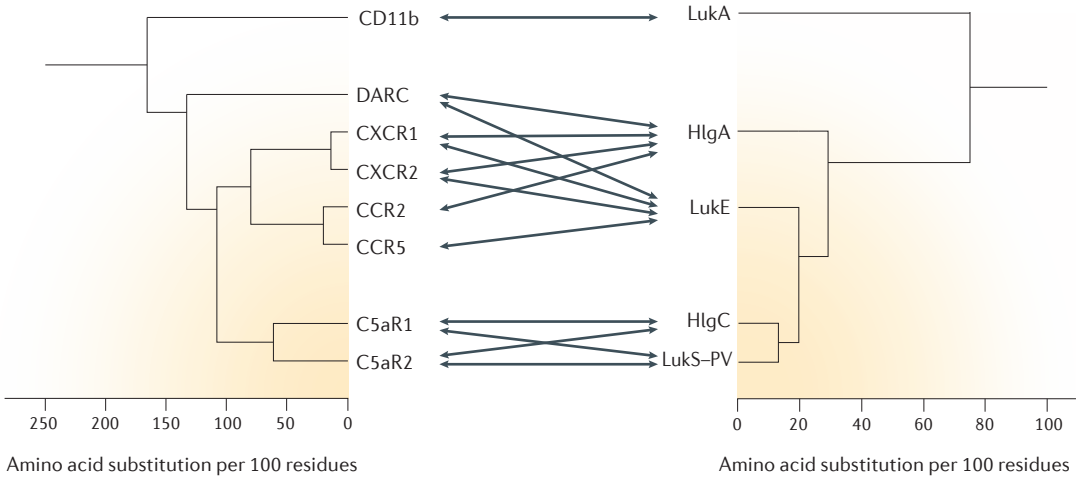
Mac-1 integrin
Macrophage 1 antigen (also known as complement receptor 3), which consists of CD11b (integrin α_M) and CD18 (integrin β_2).

Box 2 | **Leukocidin and receptor co-evolution**

The amino acid sequence identity between the leukocidin S components and F components is ~30%[110]. In their respective groups, the S components and F components share ~70% amino acid sequence identity[44]. Interestingly, leukocidin AB (LukAB) is more divergent from the other leukocidins, with an amino acid sequence identity of approximately 30% compared with other leukocidins[44]. The phylogenetic association between the different leukocidin S components from human isolates of *Staphylococcus aureus* is depicted below (see the figure). It is likely that the leukocidins have evolved from a common ancestor and by gene duplication[7].

Host–pathogen co-evolution is thought to be a powerful determinant in the biology of infections[120]. Genes that are involved in immune evasion have a major role in host adaptation of *S. aureus*[48]. Comparing the phylogenetic associations of the leukocidins and their cognate receptors results in an evolutionary 'mirror', as the divergence of the leukocidin S components is reflected in the relatedness of the receptors that are targeted by the leukocidins. The phylogenetic associations of the human receptors that are targeted by leukocidins from human *S. aureus* isolates and phylogenetic associations of the leukocidin S components from a human *S. aureus* isolate (USA300-FPR3575) are presented below (see the figure; arrows indicate specific interactions between leukocidin S components and human receptors). Amino acid sequence comparisons were generated by ClustalW alignment using Lasergene MegAlign software (DNASTAR).

During evolution, protein structures are more conserved than the amino acid sequences. The sequence variation that evolved within the stable structure of the β-barrel leukocidins resulted in high-affinity protein–protein interactions with diverse receptor targets and may have also enabled the emergence of species-specific mutations that enabled *S. aureus* to adapt to humans, who are their major mammalian hosts[48].



C5aR1, C5a anaphylatoxin chemotactic receptor 1; CCR2, CC-chemokine receptor 2; CXCR1, CXC chemokine receptor 1; DARC, Duffy antigen receptor for chemokines; HlgA, γ-haemolysin A; PV, Panton–Valentine.

only target CCR5 or CXCR1 and CXCR2, which subsequently enabled the relative importance of the different leukocidin–receptor interactions to be assessed *in vivo*[35]. For PVL, a cluster of amino acids in the bottom loops of the rim domain is essential for the recognition of C5aR1 by LukS–PV[46] (FIG. 1b). It is likely that the amino acids that are conserved in LukS–PV and HlgC determine the specificity of these toxins for C5aR1 and C5aR2, which are their shared receptors. The molecular mechanism by which HlgAB targets different receptors is not understood. For LukAB, the LukA subunit has unique amino-terminal and carboxy-terminal extensions. Remarkably, one conserved amino acid within the C-terminal extension, a glutamic acid at position 323 in LukA, was found to be involved in the interaction between LukAB and CD11b[19] (FIG. 1b). The salt bridge that mediates the dimerization of LukA–LukB in solution also seems to be essential for this leukocidin to efficiently bind to its receptor[21].

The molecular determinants in the receptor for the leukocidin–receptor interaction have also been investigated. For PVL and its receptor C5aR1, and for HlgAB and LukED and their shared erythroid receptor DARC, the sulfation of tyrosine residues in the N-terminal region of the receptor seems to be essential for leukocidin–receptor interactions[34,38] (FIG. 1b). Sulfated N-terminal tyrosine residues possibly define a conserved interaction site for leukocidins. By taking advantage of the differential species-specific engagement of PVL and HlgCB with their shared receptors C5aR1 and C5aR2, it was shown that different regions of the receptors are involved in binding and pore formation by these leukocidins[47]. The human specificity that is exhibited by PVL is determined by the second extracellular loop of C5aR1. In contrast to PVL, the specificity of HlgCB for C5aR1 is determined by the first and third extracellular loops of the receptor[47]. Differences in the interaction between PVL and HlgCB with C5aR1 have also been hypothesized, based on the use of specific C5aR1 antagonists *in vitro*. Although the toxicity of PVL can be inhibited by C5aR inhibitors, many of the inhibitors cannot neutralize the toxicity of HlgCB[47]. The specificity of LukAB for human CD11b is determined through its interaction with the receptor I-domain[37], which is the binding site for many

Outgrowth
The multiplication of bacteria during an infection.

Peritonitis
Inflammation of the peritoneum (the lining of the inner abdominal wall) that is most often of infectious origin.

Core genome
Genes that are present in all *Staphylococcus aureus* strains.

Accessory genome
Genes that are present in subsets of *Staphylococcus aureus* strains only.

of the CD11b ligands (FIG. 1b). With the exception of the conserved interaction of HlgAB and LukED with the sulfated N-terminal tyrosine residues of DARC, both toxins differentially interact with this receptor[38]. An N-terminal receptor residue that is involved in LukED-mediated, but not HlgAB-mediated, toxicity is also involved in the binding of the natural ligand interleukin-8 (IL-8; also known as CXCL8), which supports the notion that IL-8 competes for receptor binding with LukE but not HlgA[38]. Competition between S components and natural ligands for receptor binding has been shown for PVL, HlgAB, HlgCB and LukED[10,34–36,38].

Although the interactions between leukocidin subunits and their respective receptors have been investigated, the number of receptors that contribute to the formation of the leukocidin pore is unknown. Similarly, how the leukocidins transition from a receptor-bound state to form octameric pores remains to be elucidated. Nevertheless, recent studies have revealed similarities and differences in the interactions between bi-component leukocidins and their respective receptors. These differences challenge the presumed functional redundancy of this family of toxins and provide an explanation for their cellular tropism and species specificity (BOX 3).

Leukocidin genomic organization, regulation and expression. *S. aureus* has considerable variation in its gene content between strains, both in the core genome and the accessory genome[48]. The *hlgACB* and *lukAB* loci are located in the core genome and are present in more than 99.5% of human isolates of *S. aureus*. By contrast, genes that encode other leukocidins, such as PVL and LukED, are not as widely distributed[49,50]. PVL is located in the temperate phage ΦSa2 (in the accessory genome)[51] and is found in less than 2% of all clinical isolates; however, the majority of community-acquired methicillin-resistant *S. aureus* (CA-MRSA) isolates in the United States contain the genes that encode PVL[52]. LukED is encoded in the stable *S. aureus* pathogenicity island vSaβ[53], which is present in about 70% of all clinical isolates. For PVL, HlgCB, LukED and LukAB, the S component and the F component are found in an operon and are co-transcribed from a single promoter. By contrast, *hlgA* is transcribed as a single gene that is adjacent to the *hlgCB* locus[54].

The expression of bi-component leukocidins is complex and only partially understood. However, it is clear that *S. aureus* combines 'self-sensing' and 'host sensing' to regulate the expression of these toxins. Globally, the quorum sensing accessory gene regulator (Agr) system[55], which is a self-sensing system, regulates the expression of leukocidin genes by controlling the production of the repressor of toxins (Rot), which is a helix–turn–helix (HTH)-type transcriptional regulator[56,57]. In addition, several transcriptional regulators that control the Agr–Rot system, such as SarA, have been found to indirectly regulate the expression of certain leukocidins[56]. Interestingly, when *S. aureus* comes into contact with neutrophils or with human blood the expression of leukocidins is induced[58–62]. In this context, the host-sensing SaeRS two-component regulatory system, which consists of a histidine protein kinase (SaeS) and a response regulator (SaeR), is responsible for the observed enhanced expression of leukocidins[58–62]. Interestingly, the upregulation of *lukAB* expression following contact with neutrophils (a response that is mediated by the SaeRS system)[58,60,61] contributes to the ability of *S. aureus* to target and kill neutrophils[63]. Recent studies also suggest that *S. aureus* differentially regulates the expression of leukocidins by sensing metabolic shifts through a metabolite-sensing HTH-type transcriptional regulator known as RpiRc, which provides a link between metabolism and virulence[64]. Although our current understanding of leukocidin gene expression is incomplete, it is clear that the expression of leukocidins is regulated in response to environmental and metabolic cues.

Box 3 | **Animal models and leukocidin research**

The low cytotoxic activity of Panton–Valentine leukocidin (PVL), leukocidin AB (LukAB), γ-haemolysin CB (HlgCB) and HlgAB in murine polymorphonuclear neutrophils (PMNs) compared with human PMNs suggests that the reported activities of these toxins in murine models are underestimated, as murine phagocytes remain largely unaffected by these leukocidins. Currently, one of the best animal models to study the function of PVL is the rabbit[99,121–125]. PVL has been shown to enhance the early stages of bacteraemic spread to the kidneys in a bloodstream infection model[125] and to contribute to osteomyelitis[123]. In a model of necrotizing pneumonia[124], PVL has also been shown to be crucial for *Staphylococcus aureus*-mediated lung injury, pulmonary oedema, inflammation, increased levels of inflammatory cytokines and death. The increased lethality and pathologies observed with the PVL-producing strain in rabbits were associated with bacterial outgrowth, suggesting that this toxin also contributes to the growth of *S. aureus in vivo*. Importantly, purified PVL that was administered into the lungs recapitulated many of the observations with live *S. aureus*, which demonstrates that PVL alone can cause lung injury. PVL has been epidemiologically linked to skin and soft tissue infections (SSTI); however, the contribution of PVL to SSTI is controversial[98,121]. Nevertheless, it is known that administering purified PVL into the skin of rabbits results in inflammation[99] and necrosis[122]. Notably, data that are generated using rabbit models should be interpreted with caution, as many rabbits have been previously exposed to *S. aureus*, resulting in the presence of antibodies[126] that could mask the contribution of virulence factors to infection and pathogenesis.

More recently, the contribution of PVL to the pathogenesis of *S. aureus* has also been evaluated in humanized mice[113,114]. In these models, non-obese diabetic (NOD)/severe combined immune deficiency (SCID)/IL2Rγnull (NSG) mice were engrafted with primary human haematopoietic cells. Humanized mice were found to be more susceptible to *S. aureus*-mediated skin lesions[113] and pneumonia[114] than control mice, in a PVL-dependent manner. Together with the rabbit studies, these humanized mouse models reinforce the notion that PVL targets phagocytes during infection.

HlgAB and HlgCB have also been shown to contribute to infection. Initial studies that used an endophthalmitis rabbit model revealed that the HlgAB and HlgCB toxins are involved in inflammation of the eyelid and bacterial outgrowth[93]. Subsequent studies revealed that the presence of HlgAB and HlgCB mildly affected weight[92] and the survival of mice during bloodstream infections with *S. aureus*[62]. These data corroborate the observation that the receptor orthologues for HlgAB and HlgCB in murine neutrophils do not bind to these leukocidins[36]. By contrast, HlgAB is able to target both human and murine CC-chemokine receptor 2 (CCR2) in monocytes[36]. In a murine peritonitis model, HlgAB promoted bacteraemia *in vivo* in a CCR2-dependent manner[36].

Initially, bi-component leukocidins were only thought to target neutrophils, it is now clear that these toxins can also target natural killer cells, macrophages, dendritic cells and T lymphocytes. The innate and adaptive immunomodulatory activities of *S. aureus* could prevent the development of a functional and protective immune response. However, data to support this notion are limited, as a robust model to support the study of all of these toxins *in vivo* is currently lacking.

Self-sensing
Regulatory mechanisms by which bacteria coordinate information using stimuli provided by the bacterial population; for example, through the accessory gene regulator (Agr) quorum sensing system.

Host sensing
Regulatory mechanisms by which bacterial pathogens respond to stimuli provided by the host; for example, through the SaeRS system in *Staphylococcus aureus*.

Two-component regulatory system
Signal transduction modules that consist of a membrane receptor (sensor histidine kinase) and a transcription factor (response regulator) that enable bacteria to sense cues and coordinate a transcriptional response.

Pus
Exudate formed at the site of infection that mainly consists of debris from dead leukocytes.

Inflammasome
A multi-protein complex composed of inflammatory caspases, intracellular sensors (for example, nucleotide-binding domain, leucine-rich repeat containing protein or NLR), and the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) that is involved in pyroptosis and the processing of inflammatory cytokines.

Pyroptosis
A type of regulated necrosis that requires pore formation in the plasma membrane and results in cell swelling, ultimately causing massive leakage of cytosolic contents, which results in inflammation.

Necrosis
A non-apoptotic form of cell death that results in cell lysis and inflammation.

Cytokines
Small proteins that are involved in cell signalling that have immunomodulatory properties and act through receptors.

Gasdermin D
A host pore-forming protein that is responsible for cell lysis during pyroptosis.

A question that has concerned toxin researchers is whether the concentrations of toxin that are used in *in vitro* studies are biologically relevant during infections *in vivo*. In mammals, *S. aureus* is exposed to a range of environments and stresses that can influence gene expression in ways that are challenging to replicate *in vitro*. It is also unknown whether all leukocidins are expressed at the same time *in vivo*. Studies that have investigated the response of *S. aureus* following exposure to human blood or blood components have revealed the selective expression of leukocidin genes, in which the expression of HlgAB, HlgCB and LukAB is induced[62]. Another study found that LukAB seems to be the most upregulated leukocidin when *S. aureus* is exposed to human neutrophils[63]. These observations correlate with the contribution of these leukocidins to the survival of *S. aureus* (HlgAB and HlgCB)[62] or growth (LukAB)[63,65,66] in *ex vivo* models. Most studies that have tried to quantify leukocidin levels *in vivo* have focused on PVL. Pus samples from human infections contain up to 399 μg ml^{-1} PVL, a concentration that is about 1,000 fold higher than is required for PVL to kill a human neutrophil[67], which supports the notion that *S. aureus* produces sufficient amounts of these toxins to target immune cells. Recent studies that used *in vivo* imaging systems[65], mass spectometry[68] and RNA sequencing (RNA-seq)[69] have confirmed that leukocidins are produced during infection. These data are consistent with the observation that humans that are infected with *S. aureus* develop leukocidin-specific antibodies[70,71]. Thus, these toxins are likely to contribute to pathogenesis in a leukocyte-targeting-dependent manner. The various effects of leukocidins on cells at different concentrations are discussed in more detail in the following section.

Molecular mechanisms of action

Host cell death. Leukocidins can kill target cells at low concentrations (~1 nM) *in vitro*, and cell susceptibility is associated with receptor levels[10,34,35,37,38,47,72]. The formation of pores ultimately leads to the death of target cells through cell lysis, as it results in the leakage of divalent cations that are crucial for cell homeostasis (FIG. 2a). During the past decade it has become clear that numerous PFTs use cellular pathways to enhance their ability to lyse cells. One such pathway is the inflammasome pathway[73]. Leukocidins, such as HlgAB, HlgCB, PVL and LukAB, are able to activate the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome in macrophages and monocytes[74–77], which promotes their lytic and pro-inflammatory activities. Leukocidin-mediated inflammasome activation seems to be dependent on the presence of cognate cellular receptors, which are required for leukocidins to form pores, as was recently shown for LukAB in human monocytes[76]. The mechanism by which each leukocidin activates NLRP3 is not fully understood, but it is thought that toxin pores in the plasma membrane result in the leakage of potassium ions from the cytoplasm of target cells, resulting in the activation of NLRP3 (REF. 78) (FIG. 2a). The ability of leukocidins to form pores in the plasma membrane[33] provides an explanation as to how these toxins that target different receptors can all activate NLRP3. It is also possible that differences in leukocidin potency can be explained by differences in the signalling pathways that are used by the different cellular receptors (for example, GPCR versus integrin).

Following activation, NLRP3 forms a cytoplasmic protein complex with apoptosis-associated speck-like protein containing a CARD (ASC) and caspase 1 that is known as the NLRP3 inflammasome, which can trigger pyroptosis (a necrosis-like cell death pathway) and the increased production of pro-inflammatory cytokines by macrophages[74–76,79]. How leukocidin-mediated pores and the NLRP3 inflammasome work together to promote cell lysis is not fully understood. Recently, it has been shown that intracellular lipopolysaccharide (LPS)-induced pyroptotic cell death through caspase 4 (in mice) and caspase 4 and caspase 5 (in humans) mediates the cleavage of gasdermin D[80,81]. The cleavage of gasdermin D results in the release of the N-terminal membrane-targeting domain, which promotes the assembly of the protein into pores that disrupt the membranes of mammalian cells[82,83]. Moreover, the observation that caspase 1 can also cleave gasdermin D[80,84] suggests that gasdermin D-mediated pore formation could also be involved in leukocidin-mediated pyroptotic cell death. If proven correct, this would suggest that host cells respond to damage from PFTs by producing their own intracellular pore-forming protein to cause cell death (FIG. 2a). Detailed mechanistic studies of the pathways that are involved in the cell death of neutrophils — traditionally considered to be the major target of the leukocidins — are lacking and will be required to fully understand the role of leukocidins in promoting cell death.

Modulation of host cell signalling. In addition to killing cells, leukocidins can alter cell signalling pathways at sublytic concentrations (below 1 nM) in neutrophils and macrophages[75,76,79], alter the activation status of neutrophils by priming the cells[34,85], and trigger the formation of neutrophil extracellular traps[86] (FIG. 2b). Among the toxins, PVL and LukAB have been studied the most in the context of host cell signalling. Interestingly, PVL and LukAB can alter the cell in different ways. For example, PVL primes human neutrophils, which results in an enhanced production of superoxide in response to a secondary stimulus and enhanced phagocytic capacities[85], whereas LukAB does not prime human neutrophils[86]. As single subunits, LukE, LukS–PV, HlgC and HlgA functionally antagonize their respective receptors *in vitro*[10,34,36] (FIG. 2b). Owing to the clustering of S component and F component genes in a single operon, the significance of functional antagonism by a single S component during infection has not been studied. Nevertheless, studies on the non-lytic roles of leukocidins have highlighted the complexity of how each leukocidin can alter the function of their target cell. These effects are probably due to differential signalling of the targeted receptors. Future studies are required to further understand the effects that leukocidins have on leukocytes, and the contribution of these non-lytic effects to the pathophysiology of *S. aureus*.

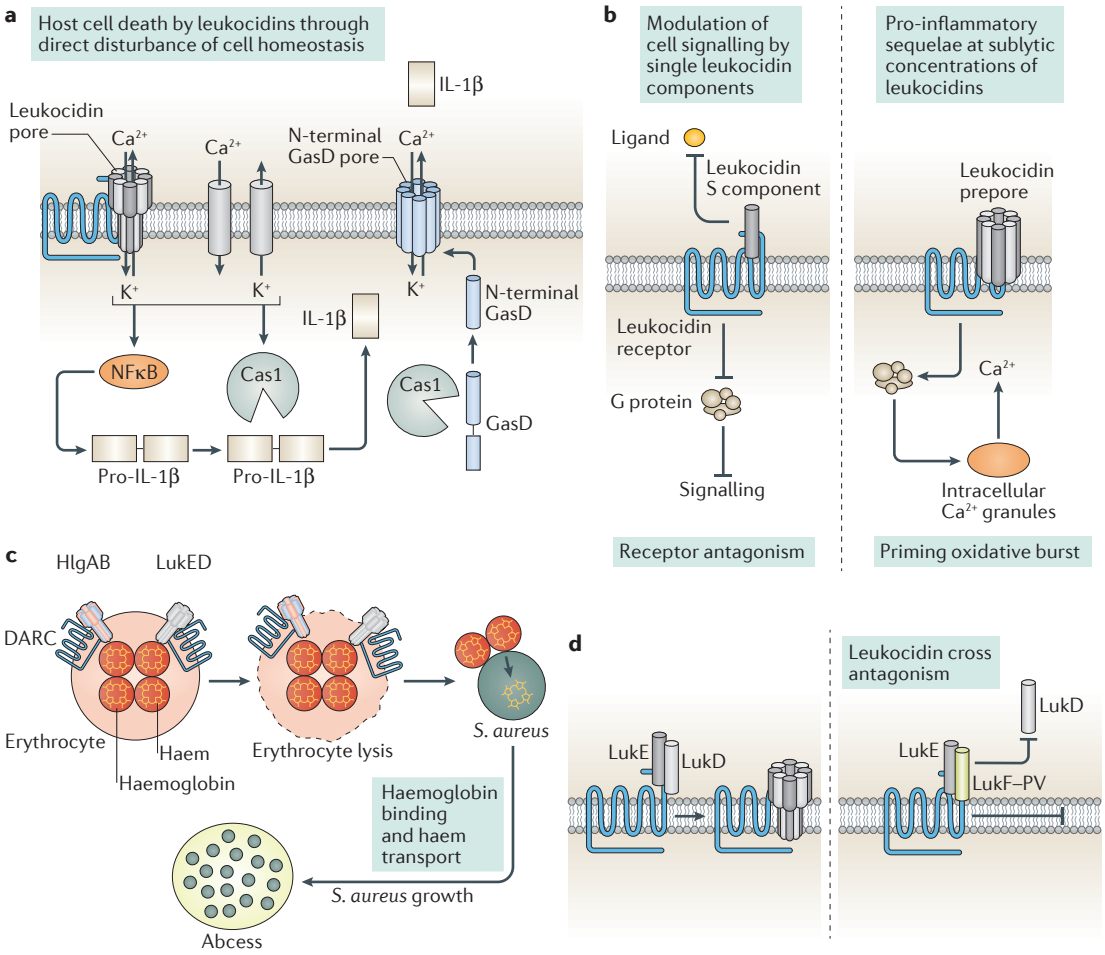


Figure 2 | **Leukocidins in pathophysiology. a** | Cell death by leukocidins. Perturbation of cell homeostasis caused by the leakage of cations through pores and by the mobilization of ions through ion channels results in osmotic imbalance and inflammatory cell death, as nuclear factor- κB (NF-κB) stimulation and inflammasome activation lead to the release of pro-inflammatory cytokines and the assembly of endogenous amino-terminal gasdermin D (GasD) pores. **b** | The modulation of host cell signalling by leukocidins. Depending on the targeted receptor, single leukocidin S components in the absence of an F component can functionally antagonize G protein-coupled receptors by preventing signalling that is induced by the endogenous receptor ligand. The significance of functional antagonism by single S components during infection is unknown. At sublytic concentrations, leukocidins prime neutrophils in a receptor-specific manner, which results in the increased production of reactive oxygen species, enhanced degranulation and phagocytosis, and enhanced bactericidal activity of phagocytes. **c** | Leukocidins promote the growth of *Staphylococcus aureus*. Haemoglobin is the most abundant source of iron in mammals, and haem iron is the preferred source of iron for metabolism in *S. aureus*. Duffy antigen receptor for chemokines (DARC) is the erythroid receptor for γ-haemolysin AB (HlgAB) and leukocidin ED (LukED). Targeting of DARC by HlgAB and LukED results in haemolysis, which promotes the growth of *S. aureus* in a haemoglobin acquisition-dependent manner. **d** | Antagonism of leukocidins through the formation of inactive hybrid complexes as a result of sequestration of the S component from its cognate F component. Antagonism of cytotoxicity by non-cognate paring has been described for LukED and PVL. Cas1, caspase 1; IL-1β, interleukin-1β; GasD, gasdermin D; Pro-IL-1β, pro-interleukin-1β.

Activation status
A state of professional phagocytes that defines the responsiveness of the cell to invading pathogens.

Neutrophil extracellular traps
Networks of extracellular fibres, primarily composed of DNA and antimicrobial peptides, that are released from neutrophils and bind to pathogens.

More than just leukocytes. In addition to inducing cell death in phagocytes, HlgAB and LukED can lyse erythrocytes[32,38,87] (TABLE 1). HlgAB and LukED promote the growth of *S. aureus* following erythrocyte lysis[38,87] (FIG. 2c), which suggests that these toxins are involved in nutrient acquisition. Indeed, the lysis of erythrocytes results in the release of haemoglobin, which is the preferred iron source for the growth of *S. aureus*[6]. Iron is an essential metal for metabolism in many bacteria and is required for the pathogenesis of *S. aureus*. The ferric-uptake regulator (Fur), a major regulator of iron acquisition, regulates the expression of LukED, HlgAB and HlgCB[88], which further indicates that leukocidins may have a role in nutrient acquisition during infection with *S. aureus*[38,87].

DARC is the erythroid receptor for leukocidins[38], and the *S. aureus*-mediated lysis of human erythrocytes is DARC-dependent (FIG. 2c; TABLE 1). The effect of the haemolytic activity of LukED and HlgAB *in vivo* has been challenging to study, as mutants that diminish the haemolytic activity of leukocidins without altering the leukocidal activity of the toxins remain to be

identified. However, mice that were infected with isogenic *S. aureus* mutants that lacked LukED and HlgAB had the same phenotype as mutants that lacked components of the iron-regulated surface determinant (Isd) haem–iron acquisition system[89], which provided a link between haemolysis and the pathophysiology of *S. aureus* infection[38].

Additive versus antagonistic activities of S.* aureus *leukocidins. Many clinical isolates of *S. aureus* encode all five bi-component leukocidins. The assembly of cognate and non-cognate leukocidins, LukE with LukD or LukE with HlgB, accordingly, could result in the assembly of 13 different toxin complexes that have a range of cytotoxic activities[32,33] (TABLE 1). The differences in activities between cognate and non-cognate leukocidin subunit pairs could be due to differences in cellular receptor binding and in the formation of pores in cell membranes. Studies that investigated the killing of human monocytes by *S. aureus in vitro* revealed that isogenic strains that lack all of the leukocidins are less efficient at killing human monocytes than any single mutant strain[76]. Moreover, LukAB and PVL have an additive effect in the cytotoxic potential of *S. aureus*. As such, the leukocidin milieu at the site of infection could influence the contribution of any particular leukocidin *in vivo*[63,66].

Leukocidins have also been shown to synergize with other virulence factors to contribute to immune subversion and pathogenesis. For example, the killing of neutrophils by PVL is enhanced by the action of PSMs[8,90]. Similarly, LukAB has been found to synergize with PVL and PSMs to promote the escape of *S. aureus* from macrophages after phagocytosis[91]. Very few studies have investigated the synergy between leukocidins and other virulence factors *in vivo*. Early studies demonstrated that α-toxin, an important PFT for the pathogenesis of *S. aureus*[13], and HlgAB and HlgCB together caused septic arthritis more readily than strains that just produced either α-toxin or HlgAB and HlgCB alone[92]. Similar results were observed in a rabbit model of endophthalmitis[93]. More recently, a study that aimed to identify virulence factors that are involved in subverting macrophage functions demonstrated that *S. aureus* growing as a biofilm can evade phagocytosis owing to the combined activities of LukAB and α-toxin[94]. Importantly, these *in vitro* observations were confirmed *in vivo,* as the production of LukAB and α-toxin resulted in enhanced pathogenesis in a mouse model of orthopaedic implant infection[94]. Thus, it is clear that leukocidins are able to synergize with other *S. aureus* virulence factors to enhance their cytotoxic potential and their contribution to virulence.

Bi-component leukocidins have also been found to antagonize each other[87]. This antagonism is most evident for PVL and LukED. PVL has been shown to block the LukED-mediated lysis of erythrocytes by forming complexes with LukED at the plasma membrane of erythrocytes that impair pore formation[87] (FIG. 2d). In a series of *in vivo* bloodstream infections in mice, it was found that *S. aureus* strains that produced PVL and LukED were less virulent than strains that produced LukED alone[87]. Interestingly, these findings could explain previous studies in which deletion of the *pvl* locus resulted in increased virulence in several murine[87,95–97] and rabbit models of *S. aureus* infection[98,99].

The virulence of *S. aureus* infection is remarkable, given that the organism colonizes many individuals as a commensal[1]. In a murine model of intranasal infection, it was shown that uncoupling the LukED antagonism by PVL promoted colonization of the lungs by *S. aureus*[87] (FIG. 2d). Thus, in addition to contributing to acute infection, interactions between leukocidins could contribute to colonization. However, the role of bi-component toxins in colonization remains to be fully understood.

Together, these findings highlight the possibility that bi-component leukocidins exert different activities that result in different phenotypes when they are expressed at the same time, and that to fully understand the contribution of these toxins to the pathophysiology of *S. aureus* we need to study them as a group.

Therapeutics

Owing to the increase in antibiotic resistance and the lack of new classes of antibiotic that are available to treat bacterial infections, there is a clinical need to develop alternative antimicrobial strategies and an effective *S. aureus* vaccine. An important consideration in the development of new antibiotics and vaccines that target *S. aureus* is that bi-component leukocidins kill phagocytes that are required for the clearance of the pathogen[9].

The cellular receptors that are used by leukocidins are candidate drug targets for severe *S. aureus* infections (FIG. 3). Blocking interactions between receptors and leukocidins by means of receptor antagonists has protected neutrophils against the action of some leukocidins *in vitro*[10,36,38]; however, the development of a combination therapy in which several receptor–leukocidin interactions are targeted could lead to adverse reactions resulting from suppressed host immune responses.

Several monoclonal antibodies (mAbs) that neutralize one or more toxins are currently being evaluated in clinical and preclinical trials[22,100,101] and could be used to treat severe infections during the active phase or for the prevention of infections in high-risk groups (FIG. 3). An engineered bi-specific tetravalent mAb was shown to neutralize PVL and HlgCB *in vitro* and in an inflammation model in rabbits[102]. More recently, a mAb that neutralizes α-toxin, PVL, HlgAB, HlgCB and LukED was reported to inhibit leukocidin-induced cytotoxicity *in vitro* and to confer protection against *S. aureus* infection in mice[103] and rabbits[104]. This broad-neutralizing mAb recognizes a shared conformational epitope in α-toxin and the leukocidin F components. Owing to leukocidin structural dissimilarities, this mAb does not neutralize LukAB[103]. However, the identification of a series of mAbs that specifically neutralize LukAB was recently described[22]; this included naturally occurring mAbs that were isolated from children who were infected with *S. aureus*[105]. The role of LukAB in the intracellular

Endophthalmitis
Inflammation of the internal eye that is most commonly of infectious origin.

Biofilm
A bacterial community that is typically enclosed in an extracellular polymeric substance matrix.

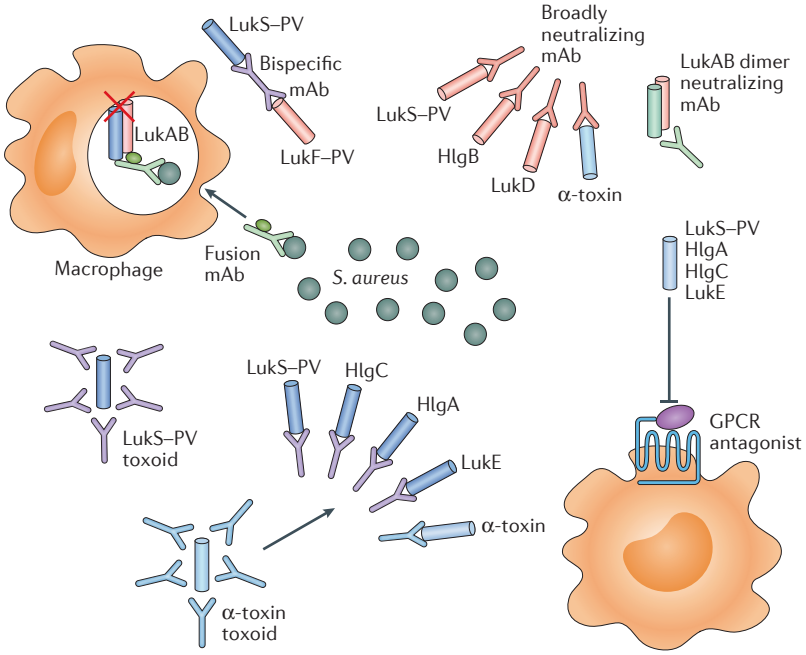


Figure 3 | **Leukocidins and anti-staphylococcal therapies and vaccines.** Antagonists of the receptors that are targeted by leukocidins can protect target cells against cytotoxicity. Bispecific monoclonal antibodies (mAbs) can neutralize both S components and F components. Broadly neutralizing mAbs target several pore-forming toxins (PFTs). A leukocidin AB (LukAB)-neutralizing mAb targets the LukAB dimer in solution. Fusion mAbs combine opsonophagocytic activity with the neutralization of leukocidins and can potentially act intracellularly. Toxoids that are derived from mutant proteins induce a broadly neutralizing antibody response. GPCR, G protein-coupled receptor; HlgA, γ-haemolysin A; PV, Panton–Valentine; *S. aureus*, *Staphylococcus aureus*.

compartment, in which it promotes bacterial escape following phagocytosis[37,63], is likely to pose challenges for the neutralization of this toxin. Nevertheless, this obstacle could be overcome by combining opsonization and leukocidin neutralization using a fusion antibody platform[101] (FIG. 3).

Many individuals have large quantities of antibodies that target bi-component leukocidins[70,106]. Studies that have investigated protection by pre-existing leukocidin-specific antibodies have reported conflicting results[97,106–109], which has made it difficult to evaluate the potential of a vaccine that solely targets leukocidins. The complex pathophysiology of *S. aureus*, together with the absence of a single dominant virulence factor, necessitates a multivalent approach, and targeting bi-component leukocidins could improve the capacity of phagocytes to clear infection (FIG. 3). It remains to be determined whether a vaccine that induces broad immunity against all staphylococcal β-barrel PFTs is technically feasible. Recently, a vaccine candidate that was based on a mutant LukS–PV toxoid resulted in a cross-neutralizing immune response against all human leukocidins, except LukAB, in mice[71,110]. A successful multivalent vaccine will probably need to be complemented with additional targets, such as other immune modulators or cell surface proteins[4,100,111]. We hypothesize that previous active and passive immunization strategies against *S. aureus* have been unsuccessful in clinical trials owing to the exclusive focus on opsonophagocytosis and the reliance on suboptimal animal models of infection[101,111,112]. Currently, there are no reliable animal models to study the pathophysiology of *S. aureus*[48,111] (BOX 3).

Outlook

The identification of host receptors that determine the cellular tropism and species specificity of bi-component leukocidins has advanced our understanding of how these toxins target and kill host cells. Additional advances include uncovering the consequences of receptor binding on host cell function. The notion that leukocidins have multiple cellular effects in a concentration-dependent manner (lytic versus sublytic effects), suggests that the level of toxins produced during infection with *S. aureus* could have broad effects on the host immune response. Moreover, the discovery that leukocidins can target signalling receptors suggests that these toxins can act as ligands, or even as antagonists, during infection[10,34–36]. It is possible that an uncharacterized role of the leukocidins is to block the function of their targeted receptors, thus preventing the migration of leukocytes.

Another important consideration is that the activities and effects of leukocidins could vary in different target cells. A careful examination of how bi-component leukocidins target and kill different human phagocytes is required, particularly the pathways that are involved in the death of different phagocytes. The expression profiles and the tissue distribution of the known leukocidin receptors support the view that the targets of these toxins are leukocytes. However, the receptors are also expressed in non-myeloid cells, which suggests that leukocidins have the potential to contribute to the pathogenesis of *S. aureus* in a manner that does not involve targeting leukocytes.

S. aureus is a major pathogen of clinical significance, and leukocidins are a prime example that *S. aureus* has evolved as a human-specific pathogen. New *in vivo* models are required that better mimic the human host (BOX 3). The use of humanized mice has been informative[113,114]; however, more work is needed to fully recapitulate the function of human neutrophils in mice. The identification of human specific receptors and recent advances in genome engineering with CRISPR–Cas[115] suggest that developing a mouse model that is compatible with all leukocidins could be possible. Such a model would overcome the current lengthy and variable process of murine humanization, and could enable the study of the role of leukocidins as a group to be assessed in *S. aureus* infection. These advances are likely to have major implications for the development of effective vaccines and novel therapeutics to prevent and treat infection with *S. aureus*.

A question that has been challenging to address is why does *S. aureus* produce so many different toxins that target leukocytes? These toxins are differentially regulated *in vitro* and *in vivo*[63,116], which supports the hypothesis that *S. aureus* senses host cues to coordinate the production of leukocidins in a tissue-dependent

Opsonization
A process in which a pathogen is coated with immunoglobulins or complement for ingestion and elimination by phagocytes.

Toxoid
A toxin the toxicity of which has been inactivated chemically or through mutation, whereas its immunogenic properties are maintained.

Opsonophagocytosis
A process of opsonization of a microorganism that results in enhanced phagocytosis of the opsonized microorganism by phagocytes.

manner. Moreover, the understanding that leukocidins are able to synergize and antagonize the activities of other leukocidins, stresses the need for further studies that consider that the contribution of leukocidins to infection could change depending on the infection site, the clinical isolate of *S. aureus*, and the host.

From the perspective of the host, the genes that encode two leukocidin receptors, CCR5 and DARC, are under selective pressure. Cells of individuals that lack these receptors are resistant to leukocidins that target CCR5 and DARC[10,38]. Moreover, numerous polymorphisms have been found in genes that encode other leukocidin receptors[117]. Future work that investigates the effect of these polymorphisms on host susceptibility to leukocidins could provide insight into observed differences in the susceptibility of humans to infection with *S. aureus*.

With recent advances in our understanding of how *S. aureus* bi-component leukocidins target host cells, we are now able to address how these toxins contribute to the pathophysiology of *S. aureus*, which could lead to the development of novel anti-staphylococcal agents.

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Acknowledgements
The authors apologize to those colleagues whose relevant work was not included in this Review owing to space constraints. The authors thank all the trainees that contributed to the work on leukocidins in their laboratories and A. Lubkin for editing the manuscript. The work on leukocidins in the Torres laboratory is supported, in part, by the US National Institutes of Allergy and Infectious Diseases (award numbers AI099394, AI105129 and HHSN272201400019C). V.J.T. is a Burroughs Wellcome Fund Investigator in the pathogenesis of infectious diseases.

Competing interests statement
The authors declare competing interests: see Web version fordetails.

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CORRIGENDUM

Anti-trypanosomatid drug discovery: an ongoing challenge and a continuing need

Mark C. Field, David Horn, Alan H. Fairlamb, Michael A. J. Ferguson, David W. Gray, Kevin D. Read, Manu De Rycker, Leah S. Torrie, Paul G. Wyatt, Susan Wyllie & Ian H. Gilbert

Nature Reviews Microbiology **15**, 217–231 (2017)

In figure 2b of this article, the structures of Pafuramidine and DB75 were incorrect. The mistakes have been corrected in the PDF and online. The authors apologize to readers for any confusion caused.