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Cathelicidins and functional analogues as antisepsis molecules

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The emergence of antibiotic-resistant bacteria together with the limited success of sepsis therapeutics has lead to an urgent need for the development of alternative strategies for the treatment of systemic inflammatory response syndrome and related disorders. Immunomodulatory compounds that do not target the pathogen directly (therefore limiting the development of pathogen resistance), and target multiple inflammatory mediators, are attractive candidates as novel therapeutics. Cationic host defence peptides such as cathelicidins have been demonstrated to be selectively immunomodulatory in that they can confer anti-infective immunity and modulate the inflammatory cascade through multiple points of intervention. The human cathelicidin LL-37, for example, has modest direct antimicrobial activity under physiological conditions, but has been demonstrated to have potent antiendotoxin activity in animal models, as well as the ability to resolve certain bacterial infections. A novel synthetic immunomodulatory peptide, IDR-1, built on this same theme has no direct antimicrobial activity, but is effective in restricting many types of infection, while limiting pro-inflammatory responses. The ability of these peptides to selectively suppress harmful pro-inflammatory responses, while maintaining beneficial infection-fighting components of host innate defences makes them a good model for antisepsis therapies that merit further investigation.

Keywords: cathelicidin, host defence peptide, inflammation, pattern recognition receptor, sepsis

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1. Introduction

The comprehensive definition of sepsis and its related syndromes was debated for many decades, reflecting the intrinsically complex pathophysiology of sepsis. The concept of sepsis was recognised early in medical history, having been noted by the ancient Greeks and Romans as a life-threatening illness [1]. However, it was not until the 1990s that a consensus definition for sepsis and related syndromes was agreed upon [2]. Sepsis was defined as a systemic inflammatory response syndrome initiated by an infection. The lack of specificity of this definition and the diversity of associated clinical scenarios has led to various clarifications in defining sepsis and related disorders [3,4]. It is now generally accepted that severe sepsis and associated syndromes lead to a continuum of systemic inflammatory responses often resulting in multiple organ failure and death.

The reasonably common occurrence of severe sepsis and septic shock among hospitalised patients in North America, the increase in incidence of these maladies and the staggering associated mortality rate provide ample rationale to support an urgent need for improving sepsis management and the exploration of alternative therapies [5,6]. An estimated 750,000 annual cases of sepsis are reported in North America with a projected increase in incidence of 1.5% annually [6]. Thus, almost 3% of all hospital admissions in the US lead to severe sepsis, such that an

estimated 387,000 cases of sepsis per year are admitted to the emergency department. Sepsis is now among the 10 most common causes of death in the US and is the leading cause of hospital-associated deaths outside of coronary intensive care units [5]. The associated mortality rate can be up to 50% and exceeds 80% for individuals with septic shock and related multiple organ dysfunction [5]. It is remarkable that the annual deaths in North America due to sepsis and related systemic syndromes are higher than deaths due to myocardial infarction, colon or breast cancer, or AIDS [5-7]. The impact of this disease on healthcare costs is incredibly high, with reported costs for management of sepsis as high as $17 billion/year in the US alone [6].

Undeniably, severe sepsis and related systemic inflammatory syndromes present a major challenge to the medical community today. These syndromes are extremely complex and the various strategies developed to manipulate sepsis-associated inflammatory responses in an attempt to decrease related mortality have for the most part enjoyed little success. Even though the use of antibiotics for treating sepsis-associated infections has been largely successful in the past decades, it has not contributed in parallel to significantly improving the survival rate among individuals with severe sepsis. Indeed, antibiotic therapy, despite destroying bacteria, results in the release of associated signature molecules considered as extrinsic triggers for sepsis, including bacterial lipopolysaccharide (LPS), lipoteichoic acid (LTA) and peptidoglycan, which often exacerbates sepsis.

In light of these observations there is an urgent need for alternative therapies for sepsis that do not directly target the causative pathogen and can selectively manipulate the associated inflammatory cascade. One such candidate therapy is provided by variants or analogues of natural cationic host defence peptides, representing unique immunomodulatory molecules with selectively anti-inflammatory properties. They can suppress pro-inflammatory responses induced by various stimuli such as bacterial endotoxin (LPS), while maintaining the expression of certain innate immune genes that favour resolution of infections [8]. These selectively immunomodulatory, anti-inflammatory host defence peptides, therefore, provide templates for the development of potentially beneficial therapeutics for sepsis. This review discusses the antisepsis and immunomodulatory properties of host defence peptides, in particular the cathelicidin family, and explores their promise as therapeutics for sepsis management.

2. Sepsis: an evolving complex portrait of pathobiology

In the nineteenth century the initiation of sepsis was linked to infection by Gram-negative bacteria and the release of endotoxin, triggering an activation of the inflammatory cascade leading to tissue damage and death [1]. Indeed, it is now recognised that more than 60% of all sepsis cases are caused by Gram-negative bacteria [9], with Gram-positive bacteria, fungi, viruses and parasites also being causative agents of sepsis [1,9,10]. The complex triggers of sepsis have only recently begun to be appreciated and are now attributed to various microbe-associated surface (endotoxic LPS, LTA, flagellin, lipoproteins, etc.) and intracellular (e.g., heat-shock proteins) molecules, which are often grouped using the rather inaccurate term pathogen-associated molecular patterns, but referred to here as microbial signatures, which are in turn recognised by pattern-recognition receptors. The interaction of microbial signatures with pattern-recognition receptors such as membrane-bound Toll-like receptors (TLR) or cytoplasmic intracellular nuclear oligomerisation domain (NOD) receptor or NOD-like receptors, results in a complex inflammatory cascade [11]. Paradoxically, sepsis is a syndrome triggered by signal transduction via pattern-recognition receptors such as TLR4 in response to microbial signature molecules such as Gram-negative LPS, whereas conversely such signal transduction is an integral part of host defence responses against pathogenic microbe assault [12,13]. Therefore, innate immunity mediated by pattern-recognition receptors has a Yin and Yang aspect, as it is crucial for marshalling host innate antimicrobial defences, but can result in sepsis as an untoward consequence of downstream events when there is excessive amplification and/or a breakdown in the concise regulation of inflammatory responses.

A chain of intracellular events is triggered as a consequence of host innate immune responses to foreign microbes. These responses are quite complicated [14] and only an outline is presented here. The interaction between microbial molecular signatures and specific pattern-recognition receptors results in the recruitment of certain adapter proteins such as the myeloid differentiation primary response protein-88 (MyD88) being an important adapter molecule, as well as other key adapter molecules such as MyD88 adapter-like protein, TIR-domain-containing adapter-inducing IFN-β (TRIF) and TRIF-related adapter molecule. This leads to the triggering of signal transduction pathways including MyD88-dependent and -independent pathways, leading to the activation of numerous other pathways and related kinase proteins such as IL-1-receptor-associated kinases (IRAKs), mitogen-activated protein kinases (MAPK); ERK1/2, p38 and JNK, TANK-binding kinase 1, Bruton's tyrosine kinase and the phosphoinositide-3-phosphate kinase. The activation of these signalling pathways leads to the activation of a slew of transcription factors including, prominently, the five NF-κB subunits, which can be associated as hetero- or homodimers into 12 transcription factors, and others such as the cAMP response element binding protein, C/EBP1, AP-1 and -2, Elk1, IFN response factors 1 and 3, early growth response 1 protein and serum response factor, which together are key to mounting innate immune responses in the host [12,15,16,17]. The consequences are the altered gene and/or protein expression of many genes. The actual complement of expressed genes varies between different TLR agonists (**Table 1**). These expressed

Table 1. Examples of differential TLR-mediated utilisation of adapter molecules and consequent differential downstream responses (summarised from [93-95]).

TLR	Use of adapters				Representative cytokine responses
	MyD88	MAL/TIRAP	TRIF	TRAM	
TLR2	Yes	Yes	No	No	TNF-α, IL-1, IL-12, IL-18
TLR3	No	No	Yes	No	TNF-α, IFN-β, IL-6, IL-12, RANTES, IP-10, MCP-1
TLR4	Yes	Yes	Yes	Yes	TNF-α, IL-1, IL-6, IL-12, IFN-β, RANTES
TLR9	Yes	(?)	No	No	TNF-α, IFN-α, IL-1, IL-18

MAL: MyD88 adapter-like protein; TIRAP: Toll/IL-1R domain containing adaptor protein; TLR: Toll-like receptor; TRAM: TRIF-related adapter molecule; TRIF: TIR-domain-containing adapter-inducing IFN-β.

genes include certain pro-inflammatory mediators such as TNF-α, IL-1, IL-6 and IFN-γ, which are presumed to play an integral role in host defence responses combating the pathogen, as well as positive regulators of innate immunity such as TNF-associated interacting protein (TNF-AIP)-2, which serve to amplify responses. Negative regulators of the pro-inflammatory signalling cascade, including IRAK-M, suppressor of cytokine signalling-1 (SOCS1), NF-κB-inhibitory ligand (NFKBIL) and Tollip, are also upregulated, often with a slight delay, to limit the duration and permit control of amplified responses, resulting in balanced, moderate and beneficial pro-inflammatory responses [17-20].

However, under certain circumstances this delicate balancing of inflammatory responses in the host becomes disrupted, leading to sepsis (**Figure 1**). Such unbalancing responses can result from an overwhelming pathogenic assault in a short time period, a breach in homeostasis, or predisposing factors such as genetic variability in humans, immunodeficiency or immunosuppression [10,12,21]. Even though substantial production of circulating anti-inflammatory cytokines and other pro-inflammatory antagonists has been reported during sepsis [22,23], it remains elusive as to whether their levels are sufficient to counteract the elevated pro-inflammatory effects. Indeed, the final stages of morbidity due to sepsis are often accompanied by the strong suppression of pro-inflammatory responses to below basal levels for healthy individuals, again emphasising that dysregulation of normal innate immune responses is a critical element of sepsis.

When systemic inflammatory syndrome results, it has a complex physiological scenario that is typified by altered body temperature (fever and chills) and pulse rate, upregulation of various plasma components such as pro-inflammatory cytokines, proteases, kinins, reactive oxygen species and platelet activating factors, and activation of the complement and blood coagulation cascades [3,9,10]. Worsening of sepsis to severe sepsis and septic shock is further compounded by breaches in the regulation of microcirculatory function, by tissue hypoxia, and organ dysfunction including cardiovascular failure, breathing difficulties and renal or liver failure that may be related to the upregulation of inflammatory cascades and neuroendocrine systems [3,10,24]. With high frequency this can lead to multiple organ failure and death.

An intrinsic difficulty associated with the management of severe sepsis is the prompt action required to identify the basis for the inflammatory syndrome, linking it to the causal presence of infection. Even though > 80 biomarkers have been investigated for sepsis so far [25], relatively few are being used for diagnosis. This lack of biomarkers is critical given the rapid progression of sepsis and consequent short time span for administration of interventions to effectively control the infection and execute effective treatments. Several mediators in the inflammatory cascade that contribute to sepsis have been identified and targeted for the development of beneficial therapeutics, but most of the therapeutic candidates that have shown success in animal models have not translated into success in human clinical trials. With the exception of activated protein C (Xigris) which is an anticoagulant, specific therapies for sepsis have largely failed [1,26]. Therefore, it seems worthwhile to further investigate natural immunomodulatory mechanisms used to control or balance innate immune responses as a means of revealing alternative strategies for the effective management of sepsis and related inflammation. Cationic host defence peptides, including cathelicidins and other host defence peptides, which can selectively suppress pro-inflammatory responses without abrogating the beneficial immune responses that are required for anti-infective immunity, are discussed below as a prototype for antisepsis therapeutics.

3. Host defence peptides: evolutionarily conserved components of innate immunity

Cationic host defence (also termed antimicrobial) peptides are key components of innate immunity and have been isolated from a wide variety of species. More than 800 natural peptides that have diverse sequences and secondary structures are now known. In general, they are 12 – 50 amino acids in length, have a net positive charge due to the presence of 2 – 9 lysine and/or arginine residues and fold into amphipathic/amphiphillic

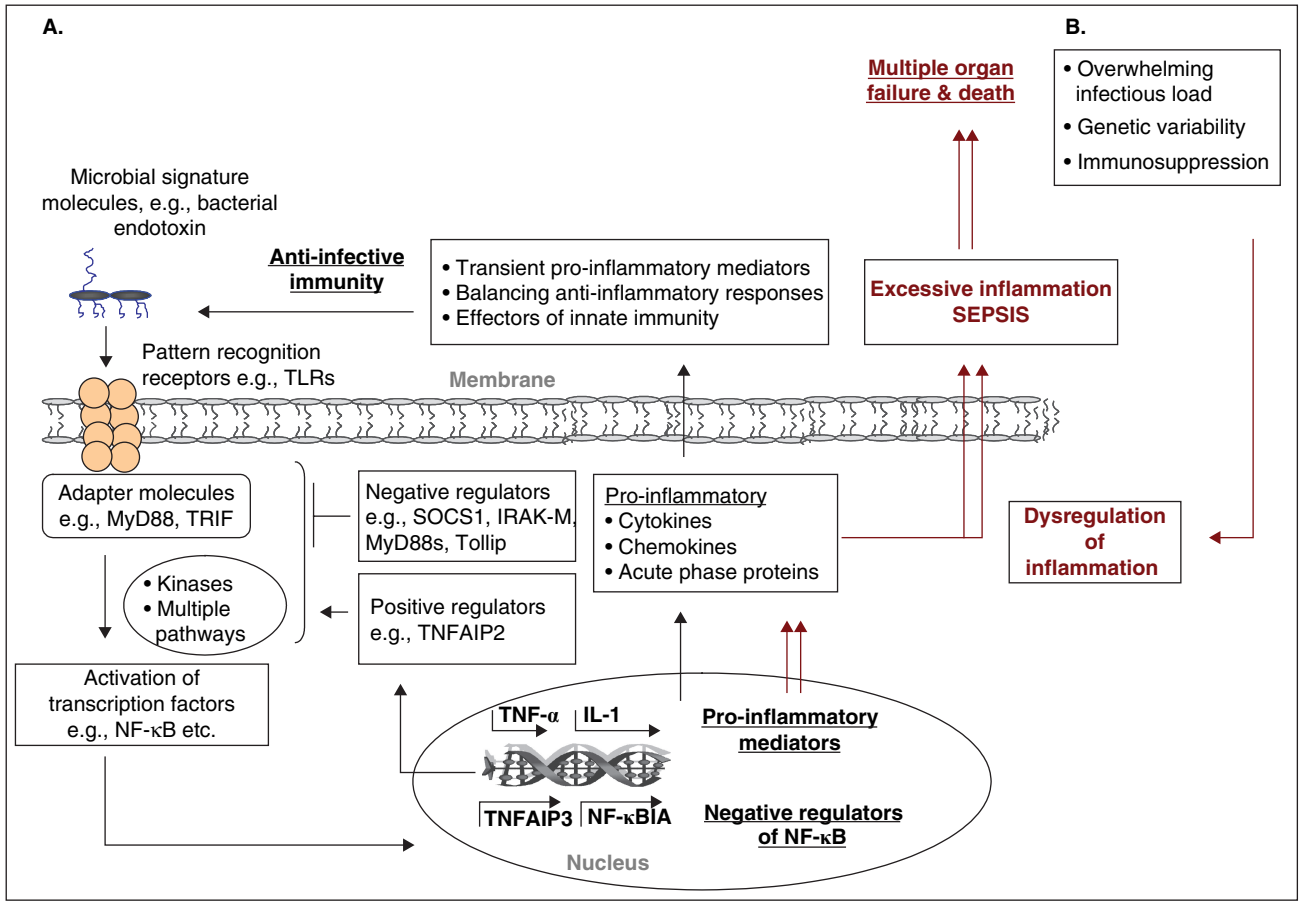

Figure 1. Dysregulation of inflammation and consequent pathogenesis of sepsis. Sepsis is the overall result of the dysregulation of the delicately balanced inflammatory cascade resulting from the recognition of microbial signature molecules in the event of an infection by pattern recognition receptors on host cells. **A.** The recognition of microbe-associated molecular signatures, for example, bacterial endotoxin, by pattern recognition receptors (such as TLR4) results in the induction of downstream signalling cascade employing different adapter molecules, kinases and so on, resulting in activation of transcription factors such as NF-κB; consequently induction of pro-inflammatory mediators such as various cytokines results in efficient resolution of infection. Simultaneously there is also upregulation of negative regulators, the efficient functioning of which results in controlled pro-inflammatory anti-infective immunity. **B.** Breakdown of the delicate regulation of the inflammatory cascade due to several factors including overwhelming infectious load, host genetic variabilities and host immunosuppression leads to the dysregulation of the inflammatory cascade leading to amplified and excessively prolonged pro-inflammatory responses. The resultant excessive pro-inflammation in the absence of meticulous regulation results in sepsis, which can further lead to dysfunction of multiple organs and death.
TLR: Toll-like receptor.

structures as a result of a high proportion of hydrophobic residues (as much as ≥ 50%) [27,28]. Many of these peptides are unstructured in free solution and fold into their final structures on contact with membranes. The two most common secondary structures are amphipathic α-helices and β-sheets held together by two to four disulfide bridges; others include looped structures containing one disulfide bridge and extended structures with a high proportion of certain amino acids such as histidine, proline or tryptophan [27,28].

Cathelicidins are α-helical cationic host defence peptides found in various locations throughout the body, are produced by many types of epithelial cells, as well as leukocytes such as monocytes, T cells, B cells and NK cells and are found in high concentrations in the specific granules of neutrophils [29], as well as many other body locations (e.g., at mucosal surfaces) and fluids (e.g., gastric juices, saliva, semen, sweat, plasma, airway surface liquid and breast milk). The cathelicidins are characterised by an amino-terminal signal sequence, a highly conserved 'cathelin' pro-domain and a carboxy-terminal functional domain [30]. Proteolytic cleavage of the signal and cathelin domains releases the mature, functional form of the peptide, which can have any of the above described secondary structures [30]. Cathelicidin homologues have been identified in mammals (human, rhesus monkey, mouse, rat, guinea-pig, cow, sheep, goat, rabbit, pig, dog and horse) and more recently in chickens and hagfish [31-33]. Some species have multiple

cathelicidins with different structures; for example, cattle cathelicidins include α-helical BMAPs, extended indolicidin and Bac5, and looped bactenecin. In contrast, the only human cathelicidin is hCAP18 (human cationic antibacterial protein of 18 kDa), which is processed into a 37-amino acid, mature peptide (LL-37) by protease 3 [34]. Varying lengths of hCAP18-derived peptides have been detected; however, LL-37 is the most extensively characterised immunomodulatory cathelicidin peptide.

Defensins are another group of cationic host defence peptides that can be divided into α- and β-subfamilies in mammals according to the arrangement of their three characteristic disulfide bonds and three β-sheets [35,36]. In general, α-defensins are stored in the azurophilic granules of neutrophils and in intestinal Paneth cells and β-defensins are produced by epithelial cells, keratinocytes, monocytes and macrophages. Similar to cathelicidins, defensins are synthesised as inactive prepropeptides, and release of mature peptides involves proteolytic cleavage by trypsin or matrix metalloprotease 7 in humans and mice, respectively [35].

Many cationic host defence peptides are induced under conditions of inflammation, infection or injury, whereas others are constitutively expressed [37-40]. A positive correlation has been observed between enhanced expression of one of these peptides [41], or exogenous application [29], and resistance to infection, whereas decreased expression is associated with modestly increased susceptibility to infection [42-44]. A wide range of functions have been associated with these peptides ranging from an ability to directly kill (or inactivate) a variety of microorganisms to a large variety of selective immunomodulatory functions (as discussed below). The 800 natural host defence peptides isolated so far [45-47] provide an invaluable resource as templates for the design and generation of small synthetic cationic peptides that can be optimised for their anti-infective and selective immunomodulatory properties, while avoiding the cytotoxicity that plagues some natural peptides [48-50].

4. Antisepsis properties of cathelicidins

Cathelicidins such as human LL-37, bovine BMAP-28 and sheep SMAP-29 have been demonstrated to protect against sepsis in various animal models, as determined by parameters such as reduced bacterial load in the blood and other organs, lower endotoxin and pro-inflammatory cytokine TNF-α levels in plasma, and reduced endotoxic shock and death of animals [51-53]. Consistent with this, cathelicidins including human LL-37 and bovine BMAP-27, as well as small synthetic peptide analogues, significantly reduced pro-inflammatory responses induced by endotoxin (LPS) and protected against endotoxaemia [8,54-57], indicating that the antiendotoxin attribute of these peptides may be conserved across species. This can be modelled in tissue culture, as the exposure of murine macrophages to human cathelicidin LL-37 has been demonstrated to desensitise them to stimulation by LPS [58], which lends further support to their potential role as antisepsis agents.

Some studies have emphasised that the anti-infective property of these peptides may be due to the direct killing of bacteria and consequent reduction of bacterial load [59-62]. However, at the modest concentrations found at, for example, mucosal surfaces, the presence of physiological salt concentrations (100 mM monovalent and 2 mM divalent cations) and host factors such as negatively charged glycosaminoglycan polysaccharides, the microbicidal ability of several cathelicidins is negated, although their immunomodulatory properties remain intact (e.g., human cathelicidin LL-37) [54]. Similarly the ability of these peptides to protect against endotoxaemia in murine models cannot be ascribed to any direct antimicrobial property, as the peptides can protect against challenge by LPS alone (i.e., where no intact organisms are present).

Many of these cationic peptides have the capability to bind to LPS and the Gram-positive endotoxin lipoteichoic acid [59], and it was suggested originally that this LPS binding property was responsible for the antiendotoxin nature of these peptides [62]. There are several lines of evidence that argue against the concept that binding of bacterial endotoxin by cathelicidins is solely responsible for protection against endotoxaemia. First, the micromolar binding affinity of cathelicidins such as LL-37 to LPS is more than two orders of magnitude lower than the affinity of natural components involved in sepsis/LPS responses including lipopolysaccharide binding protein, bactericidal/permeability increasing protein and receptor component CD14 [56]. Second, cathelicidins added both pre and post LPS stimulation have demonstrated the capacity to suppress LPS-induced pro-inflammatory responses [8,57]. Third, fluorescence labelling studies have demonstrated co-localisation of LPS and host defence peptide at the surface of host cells rather than displacement of one by the other [63]. Fourth, functional genomic studies analysing transcriptional profiles have demonstrated that host defence peptides, while suppressing some pro-inflammatory responses (e.g., TNF-α), influence such LPS-induced responses to substantially different extents (rather than equally reducing all pro-inflammatory responses) and in contrast enhance expression of certain genes that may be termed as 'pro-inflammatory' (e.g., chemokine IL-8), as well as several other chemokines that are beneficial in counteracting infections [8,55,57,64].

Thus, there appear to be at least two separate mechanisms to explain the antiendotoxic property of cathelicidins including their ability to bind bacterial endotoxin [65] and, perhaps more importantly, their ability to selectively modulate pro-inflammatory cascades through multiple points of intervention [8,55,66]. The authors of this review have proposed that the antiendotoxin and anti-infective functions of some of these peptides are most likely due to their ability to selectively modulate immune signalling pathways, and thereby selectively suppress the ability of TLR agonists such as bacterial LPS to upregulate inflammatory responses during infection [67]. These authors hypothesise that the repertoire of emerging

immunomodulatory properties of cathelicidins reflect this subtle modulation of pro-inflammatory mechanisms by substantially reducing many, but not all, endotoxin-induced pro-inflammatory responses, thereby eliminating uncontrolled inflammation associated with sepsis.

5. Selective immunomodulatory properties of cathelicidins

A broad range of immune functions are influenced by mammalian cathelicidins (**Table 2**). In the presence of bacterial signature molecules such as LPS or LTA, mammalian cathelicidins can suppress various induced pro-inflammatory responses [8,55,57]. They themselves do not induce pro-inflammatory cytokine such as TNF-α, but instead, under physiological conditions, enhance other beneficial immune responses, some of which have been traditionally classified as pro-inflammatory. They can induce chemotactic activity either by directly chemoattracting neutrophils, monocytes, macrophages, T cells and dendritic cells [40,68,69], or by selectively enhancing the gene expression and production of several chemokines, for example, IL-8, macrophage chemoattractant protein 1 and 3, Gro-α and RANTES, which mediate cell recruitment to the site of infection [54,70-72]. Other functions associated with these peptides include the selective induction of many genes associated with innate immunity, modulation of phosphorylation cascades, suppression of neutrophil apoptosis and promotion of wound healing and angiogenesis. In addition, some of these peptides promote neutrophil and mast cell degranulation and stimulation of epithelial cell apoptosis. Recent studies have demonstrated that cathelicidins can influence the cellular activation and differentiation of, for example, immature dendritic cells, thereby playing a key role in the interface of innate and adaptive immunity, acting as adjuvants and further influencing the polarisation of subsequent adaptive immune responses [73]. Genomic microarray studies have demonstrated that the human cathelicidin LL-37 can alter the expression of several hundred genes that are involved in immunity ([55] and Mookherjee and Hancock, unpublished data). Cathelicidins act through modulation of multiple innate immune signal transduction pathways including the activation of p38 and ERK1/2 MAPK pathways [74,75], transient activation of NF-κB (Yu and Hancock, unpublished data) and activation of the signal transducer and activator of transcription factor 3 (STAT3) [76]. The beneficial immunomodulatory functions of cathelicidins appear to be cooperatively enhanced in the presence of endogenous immune mediators such as GM-CSF and IL-1β [8,74]. It appears clear that cathelicidins are important as defence molecules and that their primary function may be the maintenance and recovery of homeostasis in the face of stimulation of innate immunity either by the normal flora or by invading pathogens. However, comprehensive studies to unravel the impact of different cathelicidins on host immunity, and an in-depth exploration of their selective immunomodulatory functions, are imperative to realise their potential as antisepsis agents and promote their further development as therapeutics.

6. Emerging immunomodulatory and antisepsis properties of defensins

Defensins are directly chemotactic towards monocytes, immature dendritic cells, naive T cells, TNF-activated neutrophils and mast cells [40,77,78]. Defensins have not been as extensively studied with respect to their antisepsis activity as have the cathelicidins, but their disulfide-bridged structure makes them worthy of consideration because of their resistance to degradation.

Defensin induction occurs in response to a number of stimuli, including antimicrobial agents, pro-inflammatory cytokines and thermal and septic injury; however, their role in infection and sepsis has not been well characterised. Two 9-mer derivates, denoted peptide A and B, of the *Allomyrina dichotoma* beetle defensin both inhibited LPS- or LTA-induced TNF-α production in a murine macrophage cell line, inhibited LPS-induced nitric oxide production in murine peritoneal macrophages and conveyed protection against methicillin-resistant *Staphylococcus aureus* infection in mice [79,80]. However, only peptide A conferred significant protection against LPS-induced sepsis and subsequent death in D-galactosamine-sensitised mice [81]. These mice had lower serum levels of TNF-α, asparate aminotransferase and alanine aminotransferase, and less liver damage, as measured by necrosis and haemorrhage [81]. Even though peptide A had a higher affinity for LPS than peptide B [81], the results could not be explained simply by scavenging of LPS by the peptide. Incubation of peptide A with LPS prior to administration to the mice decreased the protective effects [81]. This is similar to the aforementioned results observed by pretreatment with LL-37, implicating a mechanism(s) of host defence peptides that is much more complicated than simply binding and sequestering LPS.

Computational analysis has identified a novel human β-defensin gene, *Defb123*, that inhibits LPS-induced TNF-α production as well as LPS-induced p38 and p42/44 MAPK activation in a murine macrophage cell line [82]. Further *in vivo* studies showed that synthetic Defb123 protects against LPS-induced death in D-galactosamine-sensitised mice [82]. As Defb123 has not been isolated in its natural form, whether this reflects a biological phenomenon remains to be seen; however, it does shed more light onto the biological function of defensins.

Transgenic expression of the human intestinal defensin, HD5, in mice protected against oral *Salmonella typhimurium* infection [83]. Consistent with this, overexpression of rat β-defensin 2 in the rat lung protected against infection by *Psedomonas aeruginosa* and against sepsis induced by cecal ligation and double puncture of the lung, thus increasing the survival rate [84]. Inflammation was reduced in

Table 2. Known functions of mammalian cathelicidins (summarised from [56,67,96,97]).

Examples of cathelicidins	Function	Demonstrated *in vivo*
Human LL-37; bovine BMAP-18, BMAP-27, BMAP-28, BMAP-29 and indolicidin; murine CRAMP; ovine SMAP-29; porcine PMAP-23	Protection against bacterial infections	Yes
Human LL-37; Bovine BMAP-28 and Indolicidin; Murine CRAMP; Ovine SMAP-29	Protection against sepsis	Yes
Human LL-37; bovine BMAP-27; murine CRAMP; ovine SMAP-29	Reduction of endotoxin-induced pro-inflammatory responses	Yes
Human LL-37; bovine Bac2A; murine CRAMP; porcine PR-39	Chemotatic	No
Human LL-37; bovine BMAP-27 and indolicidin; porcine PR-39	Induction of cytokines and chemokines	Yes
Human LL-37; bovine BMAP-27	Induction of innate immune signalling pathways	No
Human LL-37; murine CRAMP; bovine BMAP-27	Alteration of transcriptional responses in host cells such as monocytes and epithelial cells	No
Human LL-37	Induction of anti-inflammatory responses	No
Human LL-37; murine CRAMP; bovine indolicidin	Induction of cell proliferation and differentiation	No
Human LL-37	Promotion of non-opsonic phagocytosis	No
Human LL-37; porcine PR-39	Promotion of angiogenesis and wound healing	Yes
Human LL-37; murine CRAMP	Influence in the initiation and polarisation of adaptive immunity	Yes
Human LL-37	Induction of mast-cell degranulation	No

both cases, leading to less lung damage in rats overexpressing β-defensin 2 [84]. Again, the underlying mechanism of defensins needs to be studied further, but their classification as immunomodulatory and potential antisepsis agents is warranted.

7. Conclusion

Even though sepsis was conceptualised as a systemic response to infection as early as 1892 [85], and has been studied extensively since that time, there is an urgent need to improve management of the disease today, with > 200,000 deaths in North America alone. This has become particularly pressing as: i) apart from activated protein C, the various clinical trials targeting single inflammatory mediators or the initiating bacterial signature molecule have failed [1]; ii) there has been a steady increase in incidence of antibiotic-resistant pathogens resulting in increased instances of therapeutic failure that correspondingly boost the potential for sepsis; and iii) the sepsis-associated mortality rate remains exceptionally high. Thus, there is great interest in examining alternative approaches for treatment of sepsis. The authors of this paper propose that one approach could involve host defence peptides and their synthetic functional analogues, which work through complex modulatory mechanisms whereby they suppress endotoxin-induced pro-inflammatory gene expression and protein production by selectively regulating LPS-induced signalling pathways. At the same time they maintain expression of certain innate immune genes that are beneficial in chemotaxis, and activation and differentiation of leukocytes and are required for resolution of infection. Clearly, the mechanism of antiendotoxin activity of such peptides is selective and multifaceted, neither targeting the pathogen directly nor a single inflammatory mediator, thus making them attractive candidates as antisepsis agents.

8. Expert opinion

Sepsis and related syndromes have been defined in an oversimplified fashion over the years, implying that such maladies might be able to be addressed by a single strategy. The pathobiology of sepsis results from the host's response to recognition of pathogen molecular signatures, followed by the dysregulation of the host's inflammatory cascade and, as a consequence, overwhelming exacerbated inflammatory responses. However, such dysregulation is poorly understood and is extremely complex; for example, a recent evaluation of

innate immune signalling pathways demonstrated that even a focused network of NF-κB–MAPK interactions employs > 750 biomolecules with > 1250 related interactions [86]. The improved understanding of such dysregulation is imperative to permit the intelligent design and development of alternate therapeutics. Sepsis management remains a huge challenge and is compounded by various factors including the lack of consistently predictive biomarkers that distinguish sepsis, individual and genetic variability associated with increased susceptibility to infection, the complexity of individual cases with a variety of pathological abnormalities and underlying disorders, the uncertain determinants of progression from infection to full-blown systemic inflammatory response syndrome, and the rapidity of progression and consequent short time period in which interventions can be employed. The long list of therapies that have failed to decrease sepsis-related mortality emphasises the need for alternate approaches to the management and treatment of sepsis and related inflammatory syndromes.

There are three major aspects to consider in the development of alternate approaches to sepsis management. First, molecules for combating sepsis-related pathogenesis should not lead to the emergence of antibiotic-resistant pathogens; therefore, ideally they should not target the infectious agent directly, with the side benefit that they will not disrupt the infecting bacteria and release of signature molecules that can trigger sepsis. Second, as targeting of single inflammatory mediators in the cascade leading to sepsis has been unsuccessful, it is desirable to develop compounds that can suppress pro-inflammatory responses through multiple points of intervention. Third, the ideal antisepsis agent would be the one that can maintain the beneficial aspects of inflammatory processes that are required assist the resolution of infections while selectively suppressing harmful pro-inflammatory responses. In these respects, host defence peptides offer attractive models as antisepsis agents as they marry these desirable qualities. In several animal models of sepsis, mammalian cathelicidins and host defence peptide analogues significantly decreased serum pro-inflammatory cytokines and endotoxin levels, which did not correlate *per se* with the presence of viable bacteria [56], indicating that unlike antibiotics, these peptides provide antisepsis protection by modulating host immune responses rather than killing the infectious agent. However, despite a reasonable amount of animal model data demonstrating efficacy in models of sepsis, such peptides have not as yet entered the clinic for testing in this context, and they remain interesting conceptual leads against sepsis rather than having proven potential.

It is worth noting that although human peptide LL-37 is a very potent antiendotoxin, it has significant potential limitations as a novel antisepsis therapeutic agent due to its known adverse effects such as cytotoxicity (induction of apoptosis in epithelial cells) and the induction of mast cell degranulation and release of histamine from mast cells; another significant factor is its cost of synthesis. Therefore, natural cathelicidins are more likely to be used as templates to design shorter synthetic peptide variants, to optimise their protective efficacy and their desired immunomodulatory functions, without associated harmful effects. Eventually, the ability to synthesise large numbers of peptides [49,50] will permit the development of algorithms for *in silico* prediction of the optimised properties of these synthetic peptides, thus effectively streamlining the screening process. The limitation in this approach at present is the optimisation of the screen in order to make it high throughput.

The potential use of synthetic variants of cathelicidin host defence peptides will require some development, but is perhaps not that far fetched. It has been demonstrated that a synthetic 13-amino acid cationic peptide, IDR-1, with no direct antimicrobial activity has the capacity to confer protection against bacterial infections *in vivo*, while reducing pro-inflammatory cytokines in response to infection and/or endotoxin [87]. Similarly, a synthetic derivative of human cathelicidin LL-37 was shown to decrease tissue damage and associated pro-inflammatory responses in murine lung infection model [88]. Recent studies using human cathelicidin LL-37 as a template to design synthetic peptides with lower cytotoxicity and decreased apototic properties demonstrated that these synthetic variants were able to chemoattract neutrophils, reduce LPS-induced pro-inflammatory responses and protect mice in models of endotoxic shock [89,90]. In addition, pretreatment of catheters with either natural cathelicidin or cationic synthetic peptides demonstrated protection against bacterial infections, associated biofilm formation and bacteraemia [91,92], demonstrating their promise when used prophylactically. The use of prosthetics increases the risks of sepsis and the pretreatment of devices such as catheters (or patients with such devices inserted), with immunomodulatory, anti-infective peptides, might be a promising approach for the prevention of sepsis through hospital-acquired infections. Investigation of synthetic anti-infective, immunomodulatory cationic host defence peptides with no direct antimicrobial activity is desirable for potential clinical use, as the development of microbial resistance is limited.

The potential of host defence peptides as novel therapeutics has greatly increased the interest surrounding their research. It is critical that their mechanism of action in protecting against infections, and their ability to influence host immune responses, be completely evaluated, permitting an improved understanding of structure–function relationships, as that may impinge on their use as therapeutics. In addition, as some the natural cathelicidins are induced under inflammatory conditions and interact in a complex fashion with endogenous inflammatory mediators [8,74], it will be essential to better define the conditions under which these peptides are normally induced and the influence of the inflammatory milieu on their optimal functioning.

In conclusion, even though it is well established that cathelicidins and their peptide analogues can indeed be

effective in protecting against severe sepsis and related inflammatory syndromes, translation of this concept to the clinic will ultimately depend on an improved understanding of their underlying mechanisms. This, along with the parallel evaluation of complex sepsis-related pathogenesis, can in part be achieved by utilising global systems biology approaches to address the overall effect of the peptides in the presence of microbial signature molecules [14]. Another barrier is the limited correspondence between animal models of septic shock and the course of sepsis in humans, thus limiting the use of such models in predicting the efficacy of these peptides for treatment in the clinic. To permit the development of optimised synthetic variants of cathelicidin host defence peptides for beneficial prophylaxis or therapy of sepsis, several additional restrictions must be taken into consideration, including unknown pharmacodynamics, the development of appropriate markers for screening and possible toxicities including adverse immunotoxicities. Given the attributes of these peptides as selectively immunomodulatory compounds, their relative ease of synthesis and most importantly their multifaceted mechanism of intervention in conferring anti-infective and anti-inflammatory protection, the development of these host defence peptides as antisepsis agents remains of great interest.

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