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Review

Cationic host defence peptides: Innate immune regulatory peptides as a novel approach for treating infections

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Abstract. An increase in antibiotic resistance and the emergence of new pathogens has led to an urgent need for alternative approaches to infection management. Immunomodulatory molecules that do not target the pathogen directly, but rather selectively enhance and/or alter host defence mechanisms, are attractive candidates for therapeutic development. Natural cationic host defence peptides represent lead molecules that boost innate immune responses and selectively modulate pathogen-induced inflammatory responses. This review discusses recent evidence exploring the mechanisms of cationic host defence peptides as innate immune regulators, their role in the interface of innate and adaptive immunity, and their potential application as beneficial therapeutics in overcoming infectious diseases.

Keywords. Innate immunity, inflammation, host defence.

Introduction

Co-evolution of host and pathogens has lead to a variety of multifaceted survival mechanisms that are either germline-encoded (innate immunity) or acquired through adaptation to specific antigens (adaptive immunity) in the host. Among the abundant cache of defence mechanisms, cationic host defence (also referred to as "antimicrobial") peptides represent important evolutionarily conserved elements of innate immunity. They are widely distributed in nature from insects and plants, to highly evolved animal species with more complex immune systems. More than two decades ago these defence molecules were initially isolated from insect lymph, the skin of frogs, and mammalian neutrophil granules and demonstrated to have antibacterial properties [1]. Since then, interest in the distribution and application of these peptides has been escalating, leading to the discovery of more than 700 cationic peptides from numerous species. We describe in this review typical functions associated with cationic host defence peptides. However, it is worth mentioning that many of these functions have been only studied for a small subset of the known host defence peptides, and where comparative studies have been performed it has been shown that individual peptides do not necessarily have all of the described functions [2–4].

Cationic host defence peptides are diverse in their sequence and structures. They are generally amphipathic (having hydrophobic and charged, hydrophilic patches on their surfaces), small (12–50 amino acids), and have at least two positive charges (as arginine or lysine residues). They can be broadly distributed into four classes based on structure; (i) amphipathic α-helical, (ii) β-sheet structures stabilised with two or three disulphide bonds, (iii) extended structures, and

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(iv) loop structures with one disulphide bond. Cationic amphipathic peptides are able to interact with and insert into biomembranes, a property that is influenced by their hydrophobicity and net positive charge, conformational flexibility and secondary structure. Detailed studies on structure activity relationships for the directly antimicrobial peptides have revealed that there are many possible peptides that have bacterial killing activities. Indeed, many of the natural peptides are not optimised for activity and can be easily improved with appropriate amino acid substitutions [5]. On the other hand, comprehensive analyses of the impact of different structural classes of these peptides on their rapidly emerging functions in host immunity has not been performed as yet but merits further investigation. Our own preliminary unpublished data reveal considerable sequence flexibility in determining these types of functions, although individual peptides with similar properties can exhibit a range in the potency of their immunomodulatory activities.

Cationic host defence peptides are gene encoded and in mammals are expressed in a variety of cell types including monocyte/macrophages, neutrophils, epithelial cells, keratinocytes and mast cells. They are generally expressed as pro-peptides that undergo subsequent proteolytic processing to release the biologically active, mature host defence peptide. These diverse peptides are expressed differentially depending on the specific peptide and the tissue or cell type. Some peptides are constitutively expressed, while others are strongly inducible by microbial signature molecules, inflammation or tissue injury. For example, human beta-defensin-1 (hBD-1) is constitutively expressed in intestinal epithelial cells, while in contrast the expression of hBD-2 is rapidly induced in response to infection by enteric pathogens, and hBD-3 is strongly up-regulated during inflammatory disorders such as Crohn's disease [6–8]. Generally, the expression of host defence peptides is increased at the onset of infection in response to stimuli such as various exogenous and endogenous inflammatory mediators. However, the diverse expression profiles of these peptides among different species with respect to different stimuli is yet to be completely unravelled.

The host defence peptides originally gained prominence through initial descriptions of their direct antimicrobial functions [1]. Although some of these peptides are sufficiently potent or are found at relatively high concentrations to be considered natural antibiotics with direct microbicidal capacity, increasing evidence suggests that many mammalian cationic host defence peptides have limited microbicidal activity under physiologically relevant conditions due to modest concentrations, *e.g.* at mucosal surfaces, and strong antagonism of their antibiotic activity by physiological salt concentrations (100 mM monovalent and 2 mM divalent cations) [9]. In contrast, under such conditions, a wide range of functions have been demonstrated for these peptides in the context of host immunity (Table 1). In light of increasing recent evidence, and the fact that the peptides can indeed protect against a wide variety of infections *in vivo* (Table 1), we suggest that in many cases the anti-infective properties of cationic host defence peptides can be primarily credited to their activity as immune regulators. The immunomodulatory activities of these peptides indicate the potential application of natural and synthetic cationic host defence peptides in infection management.

Involvement of host defence peptides in immunity

Extensive research in the last decade has established that cationic host defence peptides have an eclectic range of functions, including the ability to confer protection against a variety of pathogens, limit sepsis and even the potential as novel cytotoxic agents against certain types of cancers. They are innate molecules involved in defence against infections since their absence leads to modestly increased susceptibility to infections, while overexpression or exogenous introduction protects animals.

Consistent with this, the expression of many peptides increases during infection or inflammation; for example, the expression of human β-defensin-2, is up-regulated in various cell types such as monocytes, epithelial cells and keratinocytes during bacterial infections, and upon stimulation with different bacterial components that activate the Toll-like receptor (TLR) to nuclear factor (NF)-κB pathway, and in contrast human cathelicidin LL-37 seems to be up-regulated only by endogenous inflammatory molecules [10–13]. The enhanced expression of these peptides in transgenic murine models results in increased resistance to bacterial infections [14, 15]. Similarly, in various inflammatory human clinical conditions such as psoriasis, bronchiolitis and cystic fibrosis, the concentrations of defence peptides, defensins and cathelicidins, become remarkably increased [16–19]. In contrast, *in vivo* studies with murine models have illustrated that the absence of these defence peptides can lead to modestly increased susceptibility to infections. Mice lacking the endogenous cathelicidin CRAMP are somewhat more susceptible to streptococcal infections [20], mice with a deficiency in matrilysin (required for excretion and processing of biological active mature α-defensins) have increased susceptibility to oral *Salmonella typhi-*

Table 1. Functions of mammalian cationic host defence peptides.

Functions	Examples of peptides				References
	Human	**Murine**	**Bovine/ ovine**	**Porcine**	
Anti-infective and protects against diverse pathogens *in vivo*	LL-37 HNP-1 hBD-3	CRAMP	BMAP-27 BMAP-28 SMAP-29	PMAP23	[9, 20, 54, 55, 96–99]
Anti-endotoxin activity	LL-37	CRAMP	Indolicidin SMAP-29		[2, 50, 51, 54, 56, 99, 100]
Selectively modulates pro-inflammatory responses	LL-37		BMAP-27		[2, 50, 51, 54]
Chemotactic	LL-37 HNP1/2	CRAMP	Bac2A	PR-39	[2, 54, 64, 71, 101–103]
Influences cell proliferation and differentiation	LL-37	CRAMP	Indolicidin		[2, 63, 104]
Promotes wound healing and angiogenesis	LL-37			PR-39	[73, 105–108]
Works in cooperation with other immune mediators	LL-37				[2, 9, 50]
Induces gene expression and enhances protein secretion in mammalian host cells	LL-37 hBD-1/2		BMAP-27 Indolicidin	PR-39	[50, 51, 54, 59, 63, 72, 107–109]
Influences initiation and polarisation of adaptive immunity	LL-37 HNP-1/2	CRAMP			[63, 71, 110]

murium [21], and β-defensin knockout mice show increased susceptibility to staphylococcal infections [22]. Similarly, the lack and/or low expression of certain defence peptides in humans leads to an increased susceptibility to infections (although it is worth noting that the syndromes involved are complex). For example, a deficiency of the sole human cathelicidin LL-37, as a result of attempts to restore a neutrophil deficiency in so-called morbus Kostmann syndrome, is associated with severe periodontal inflammation and infections [23], while atopic dermatitis is associated with a deficiency of defensins and cathelicidin LL-37 [24], and recurrent bacterial infections are associated with a deficiency in defensins [25]. Overall, experimental systems simulating physiologically relevant conditions [9], as well as *in vivo* animal model infection studies [26], have convincingly demonstrated that cationic host defence peptides are able to limit or clear infections. In addition to exhibiting protective capabilities against a diverse range of pathogens from bacteria, fungi, parasites and viruses [26–33], and protecting against systemic inflammatory syndrome (discussed below), these peptides are also thought to be beneficial in other scenarios such as wound healing [34] and counteracting tumours [35, 36]. The potential for beneficial applications of cationic host defence peptides has continued to grow with increased interest in elucidating the mechanisms associated with the protective functions of these defence peptides.

Assessment of direct antimicrobial properties of cationic host defence peptides

One of the earliest known properties of cationic host defence peptides, namely their capacity for antimicrobial activity, has been widely discussed. At their antimicrobial concentrations, cationic peptides interact with multiple bacterial targets, including microbial membrane components, leading to disruption of cytoplasmic membrane integrity, or intracellular bacterial targets, resulting in microbial killing [1, 37–39]. Even though all cationic peptides are able to interact with bacterial cytoplasmic membranes and some strongly perturb bilayers, there is often no absolute correlation between their ability to disrupt membrane integrity and antimicrobial activity [40]. Indeed, some peptides exert their effects by targeting intracellular components of bacteria, protozoa and fungi without disrupting their membranes [41–43]. Within the host, some cationic peptides may protect against infection by targeting pathogens directly, especially in situations where the peptides are found in very large concentrations, *e.g.* as observed for α-defensins that are at mg/ml concentrations in the granules of phagocytes or the crypts of the intestine [44], or when they demonstrate very high salt-resistant antimicrobial activity, *e.g.* pig protegrin [45]; however, not all protective cationic peptides are necessarily working through direct microbicidal action. For example, human cathelicidin LL-37, which is known to be protective against bacterial infections *in vivo*, can exhibit antimicrobial activity in phosphate buffer but does not reduce bacterial load in physiologically relevant tissue culture medium [9]. In addition,

synthetic cationic peptides with no direct antimicrobial activity have been demonstrated to be effective in protecting against tissue damage and bacterial infections *in vivo* [9]. The designation "antimicrobial" must therefore be carefully considered in the light of two general observations. First, given the modest to low concentrations of peptides present at many body surfaces and fluids, and under physiologically relevant conditions, *e.g.* high concentrations of mono- and divalent cations, and the presence of various host factors, especially negatively charged polysaccharides, such as glycosaminoglycans like heparin, there would be strong antagonism of the direct antimicrobial activities of many peptides [9, 46–48]. Secondly, a wide range of alternative functions have been demonstrated under these conditions for host defence peptides that lead to balanced and selective modulation of host innate immune functions, and subsequent transition to adaptive immunity (discussed below). While these immunomodulatory functions do not lead to direct antimicrobial activity, but rather recruit and enhance bodily defences against infection, they can influence the outcome of infection. We have proposed that the selective immunomodulatory activities of host defence peptides are as important, or more important, than direct antimicrobial activity for their protective mechanisms.

Anti-endotoxin properties of cationic host defence peptides

Inflammatory responses in the host, triggered concurrently with the onset of infection, are beneficial for combating pathogenesis. However, when there is either an excessive pathogenic stimulus or a breakdown in the regulation of meticulously coordinated inflammatory responses, uncontrolled inflammation can lead to systemic inflammatory syndrome or sepsis. Cationic host defence peptides derived from various sources, including the insect-derived cecropin-melittin hybrid peptide CEMA [49], human cathelicidin LL-37 [50], bovine cathelicidin BMAP-27 [51], bovine indolicidin [52], and small synthetic cationic peptides [52], all result in significant reduction of endotoxin-induced inflammatory responses, and can protect against endotoxaemia *in vivo* [9, 53–55]. This leads to the speculation that the anti-endotoxin activity exhibited by these peptides may be conserved across species.

Host defence peptides have been proposed to play a role in the delicate balancing and regulation of inflammatory responses. They suppress endotoxin-induced pro-inflammatory gene expression, protein secretion of inflammatory mediators, *e.g.* tumour necrosis factor-α (TNF-α), and endotoxin-induced nuclear translocation of NF-κB subunits, while maintaining other pro-inflammatory responses such as the production and release of several chemokines, resulting in the overall selective suppression of pro-inflammatory responses [50, 56]. Since these defence molecules do not appear to target a single inflammatory mediator, but rather are multifaceted in their action and selective in their responses, it makes them attractive anti-endotoxin agents. It is now well documented that cationic host defence peptides such as the human cathelicidin LL-37, can selectively suppress pro-inflammatory responses induced by the signature Gram-negative bacterial component lipopolysaccharide (LPS) as well as other TLR-agonists such as the Gram-positive signature lipoteichoic acid (LTA), in various mammalian species [50, 51, 54]. Some biological properties of host defence peptides including direct antimicrobial activity are strongly antagonised by the presence of autologous serum [57]. In contrast, we have shown that the anti-endotoxin property of human cathelicidin LL-37 was maintained in the presence of autologous serum when the peptide was added either simultaneously (Fig. 1a) or post stimulation with bacterial endotoxin (Fig. 1b). Similarly, the immunomodulatory activities of LL-37 are preserved in whole human blood [54], in which it can induce the production of macrophage chemoattractant protein-1 (MCP-1) without inducing pro-inflammatory cytokine TNF-α, and can suppress endotoxin-induced TNF-α production. Despite the previously described association of the G protein-coupled receptors FPRL-1 in the direct chemotactic activity of human cathelicidin LL-37 [58], we have shown that the anti-endotoxin property of this peptide appears to be independent of any G protein-coupled receptor (Fig. 2). This is consistent with previous observations describing the bioactivity of LL-37 in human monocytic cells [59]. Indeed, it is yet to be elucidated whether the anti-endotoxin property exhibited by host defence peptides is receptor-mediated.

There are two central emerging themes regarding the mechanism of the anti-endotoxic activity of the cationic host defence peptides. The first concerns their ability to directly bind to LPS, which appears to be only partly responsible for this anti-endotoxic activity, and the second relates to their ability to modulate signalling through the endotoxin-induced TLR to the NF-κB pathway. The complex mechanisms of the anti-endotoxic activity for these peptides are slowly being unravelled, with emerging evidence that these peptides act through both routes via multiple points of intervention [49, 50, 56, 60]. The overall anti-endotoxic effect of cationic host defence peptides

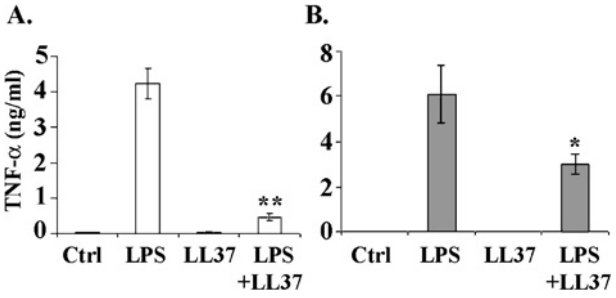

Figure 1. Human cathelicidin LL-37 suppresses LPS-induced secretion of TNF-α in presence of autologous serum. The concentration of the proinflammatory cytokine TNF-α (Y-axis) was monitored in the tissue culture supernatant of THP-1 monocytic cell line in RPMI media with 5% human serum by ELISA (e-biosciences). The cells were stimulated with LPS (100 ng/ml) purified from *Pseudomonas aeruginosa* H103 in the presence or absence of LL-37 (20 μg/ml) for 4 h. The peptide was added (*a*) simultaneously with LPS, (*b*) after 30 min of LPS treatment. The results are an average of three independent experiments ± standard deviation and represents unpublished data from the authors. Simultaneous addition of the peptide with LPS resulted in LPS-induced TNF-α inhibition of 89± 2.3% with $p<0.01$ (**), and delayed addition of peptide 30 min after LPS stimulation resulted in 48.5±17.9% inhibition with $p<0.05$ (*).

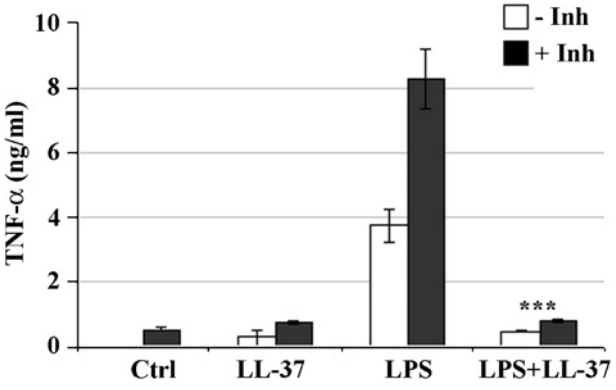

Figure 2. G protein-coupled receptors have no effect on LL-37 suppression of LPS-mediated pro-inflammatory cytokine TNF-α in human PBMC. The effect of LL-37 on the production of LPS-induced pro-inflammatory cytokine TNF-α was monitored by ELISA (e-biosciences) in the absence or presence of pertussis toxin (Inhibitor; Inh). The inhibitor (100 ng/ml) was added to human PBMC 30 min prior to exposure to the various stimuli. Human PBMC were treated with LPS (100 ng/ml) purified from *P. aeruginosa* H103 in the presence or absence of LL-37 (20 μg/ml) for 4 h. Results are an average of three independent experiments using PBMC from three independent donors ± standard deviation and represents unpublished data from the authors. LPS-induced TNF-α was inhibited by 85±2% in the absence of inhibitor and by 89±0.9% in the presence of inhibitor, $p<0.001$ (***).

leads to the speculation that they are involved, not only in suppressing inflammation in the presence of pathogenic challenge, but also might play a critical role in maintaining homeostasis by limiting inflammatory responses that could otherwise be triggered by the presence of commensals, which contain the same conserved signature molecules (*e.g.* LPS, LTA, CpG DNA, *etc.*) that are involved in initiating inflammation through TLRs.

Cationic host defence peptides function as innate defence regulators

Although cationic host defence peptides suppress certain pro-inflammatory responses, they also have the ability to enhance certain other immune responses, traditionally thought of as pro-inflammatory, which are beneficial in cell recruitment to the site of infection and influencing subsequent immune responses. They induce the production of several cytokines and chemokines, as well as serving as chemokines for the directed chemotaxis of certain cell types including monocytes, neutrophils, T cells and eosinophils, and influence cell differentiation in pre- and immature dendritic cells [9, 48, 58, 61–64]. Thus, cationic host defence peptides have apparently paradoxical functions in regulating or modulating immune responses. We propose that this diversity of functions exhibited by these peptides within an inflammatory milieu results in selective suppression of pro-inflammatory responses and the overall balancing of inflammation in the host, resulting in a net anti-infective response without excessive, potentially harmful inflammation (Fig. 3). Thus, this offers an alternative view of the mechanism of protection exerted by cationic host defence peptides, an area of intense research and speculation in the last decade. We discuss some of these infection-fighting activities below.

Chemotactic activity

Cationic host defence peptides are produced by a variety of cell types [65–69]. Following pathogenic entry, local tissue cells secrete chemokines such as IL-8 [70] and MCP-1/3, which attract other immune effector cells, including neutrophils, resulting in, among other consequences, the additional release of host defence peptides, *e.g.* α-defensins and LL-37, at the site of the infection [71]. The secreted defence peptides can in turn, directly or indirectly, promote the further recruitment of effector cells such as neutrophils, monocytes/macrophages, immature dendritic cells and T cells. Therefore, a positive loop of responses is created that assists in the orchestration of innate immune functions.

At low to modest (physiological) concentrations, the peptides themselves can induce chemotaxis of immune effector cells either by inducing the production of chemokines or at slightly higher concentrations can

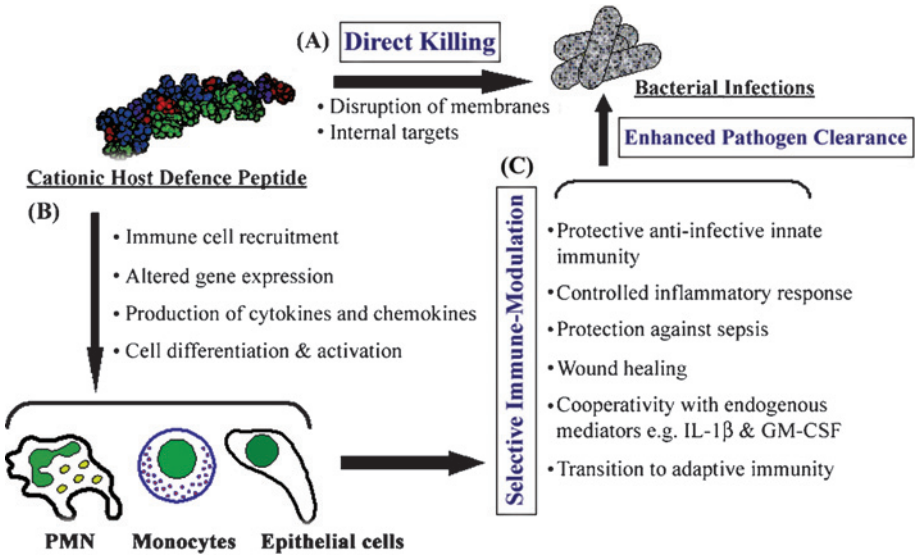

Figure 3. Anti-infective properties of cationic host defence peptides. Cationic host defence peptides protect against pathogens by being either directly antimicrobial or by selectively boosting host immune responses. (*a*) Certain host defence peptides that are present at high concentrations or are highly salt resistant exhibit direct antimicrobial properties either by disrupting the bacterial membrane or by targeting intracellular components of the pathogen. (*b*) Most cationic host defence peptides induce a variety of responses in host innate immune cells such as monocytes/macrophages, polymorphonuclear leukocytes, and epithelial cells. They alter gene expression of the host cells, and induce production of chemokines and cytokines, promote immune cell recruitment to the site of infection, and influence cell differentiation and activation. (*c*) The outcome of the selective immunomodulation by these peptides in the host results in induction of innate immune responses, leading to protection against infection, selective suppression of pro-inflammatory responses ensuring protection against sepsis, induction of wound healing, and the subsequent initiation and polarisation of adaptive immunity. The overall effect of host defence peptides is to promote optimal anti-infective efficacy and a balanced immunomodulatory response without exacerbated pro-inflammation.

exhibit direct chemokine activity, thereby recruiting leukocytes to the sites of infection required for innate and subsequent adaptive immune responses [9, 48, 58, 61–64]. An important characteristic of cationic host defence peptides is, therefore, their ability to selectively induce the gene expression and production of chemokines/cytokines that are essential for immune effector functions, including CXCL8/IL-8, CCL2/MCP-1, and Interferon (IFN)-α. The uptake of peptide, *e.g.* human cathelicidin LL-37, into the cytosol has been described to be required for chemokine production [72]; however, whether a specific receptor is involved remains elusive. Even though the direct chemokine activity of human cathelicidin LL-37 was shown to be mediated by the G protein-coupled formylpeptide receptor-like-1 [58], other responses induced by this peptide in monocytic cells have been shown to be independent of any G protein-coupled receptors. Other trans-activated or direct binding receptors have also been described in a variety of cell types for LL-37 [72], indicating that the bioactivities may be mediated by a diversity of receptors. Interestingly, some of host defence peptides, *e.g.* β-defensins, interact with chemokine receptors such as the macrophage inflammatory protein-3α (MIP-3α) receptor on host immune cells, further reinforcing the notion of chemotactic properties for host defence peptides.

Orchestration of innate immune functions

Other immunomodulatory functions influenced by cationic host defence peptides, in addition to the above-mentioned roles in attraction of other host cells, include cellular differentiation and proliferation, extension of the life span of neutrophils through suppression of apoptosis, activation and degranulation of mast cells, wound repair, stimulation of angiogenesis, and enhancement of the ability of dendritic cells to take up and present antigens [63, 73–76]. Many of these functions have now been demonstrated in either complex organ systems or in mice, indicating that they are operative *in vivo*. Overall, the immunomodulatory functions mediated by cationic host defence peptides cannot be considered independently of other immune responses. These peptides appear to function cooperatively with other immune effector molecules such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or IL-1β [50, 59] within the context of the inflammatory environment. This then results in a complex network of immune mediators and downstream signalling pathways that are required for the overall effective functioning of defence mechanisms.

Key signalling pathways that regulate downstream innate immune gene functions are either activated

and/or modulated to varying extents in response to cationic host defence peptides. Human cathelicidin LL-37, as well as β-defensins, can activate the mitogen-activated protein kinases (MAPK) p38 and extracellular-signal-regulated kinase-1/2 (ERK1/2) in mast cells, keratinocytes and monocytes [59, 75, 77]. In addition, the signal transducer and activator of transcription 3 (STAT3) pathway has been recently implicated in the transactivation of the epidermal growth factor receptor in keratinocytes by LL-37 [76]. These results together indicate that host defence peptides can directly influence the activation of transcription factors that are involved in the regulation and expression of innate immune genes, consistent with functional genomic studies [50, 51, 54]. Microarray experiments have revealed several hundred genes induced by, for example, LL-37 ([54] and Mookherjee and Hancock, unpublished data), demonstrating the complexity of these responses, although the specific roles of many of these genes in the broad range of immune functions induced by peptides (Table 1) is yet to be determined. On the other hand, multiple pathways are also involved in the induction of specific human β-defensins in keratinocytes and epithelial cells, including the NF-κB, AP-1, Janus kinase (JAK) and phosphatidylionisitol-3 kinase (PI3K) pathways [78–82], indicating that these defensins are a component of the innate immune network.

Role in adaptive immunity

Mediators of innate immunity are known to play an instructional role in the specific development of lymphocytes that trigger adaptive immune responses [83]. The cellular components of innate immunity that are rapidly recruited to the site of pathogenic challenge result in the induction of a cascade of immune mediators, which include cytokines, chemokines and host defence peptides. Specialised innate immune cells, including immature dendritic cells that function as professional antigen-presenting cells, are activated as a direct result of the functioning of innate immune mediators. This subsequently leads to the activation of specific immune-enhancing cytokines and subsets of T and B lymphocytes, resulting in the initiation and development of antigen-specific adaptive immune responses. Various cationic host defence peptides, such as human α-defensins HNP-1 and HNP-2, murine β-defensins, porcine cathelicidin PR-39, and the human cathelicidin LL-37, are chemoattractant to immature dendritic cells and T cells [63, 71, 84, 85]. They can act as adjuvants by interacting with various receptors on these cell types and influencing the magnitude and polarisation of antigen-specific adaptive immunity [68, 86].

Recent studies have provided functional evidence to directly demonstrate the role of cationic host defence peptides in adaptive immunity. It has been shown that lymphocytes working in synergy with cationic defence peptides result in an increased clearance of invading microbes [87]. *In vitro* studies with human defensins have demonstrated that they can enhance cellular proliferation and cytokine responses of $CD4^+$ T cells, *e.g.* through IFN-γ, IL-10 and IL-6 induction as well as modulation of the expression of co-stimulatory molecules [88]. Similarly, human cathelicidin LL-37 can influence the differentiation of immature dendritic cells and cell polarisation (to favour Th1 responses) [63], while other peptides influence responses in other fashions [89], thus indicating the ability of these peptides to link innate and adaptive responses. Consistent with this, *in vivo* studies employing co-administration of human defensins with antigens in mice resulted in the enhanced production of antigen-specific serum antibodies. This provides evidence for peptide involvement in humoral (Th2-dependent) responses, and suggests that host defence peptides may possess adjuvant-like properties [89]. Similarly, human α-defensins and murine β-defensins can promote the induction of antigen-specific cytotoxic T lymphocytes, thereby enhancing Th1-dependent cellular responses, and potentially anti-tumour immunity [88, 90]. Overall, recent evidence demonstrates that cationic host defence peptides are elements that play a role in the interface between innate and adaptive immune responses [4, 48]. These peptides serve as signals influencing initiation, polarisation, and amplification of adaptive immune responses.

The focus on understanding the extent and mechanisms of immunomodulatory functions of cationic host defence peptides has intensified in the last decade, leading to the description of a plethora of novel biological properties (Table 1). As the biology of these peptides as innate defence regulators is increasingly understood, it is likely that there will be increased activity in developing host defence peptides as potential therapeutics and adjuvants.

Cationic host defence peptides as beneficial therapeutics

The wide repertoire of evolutionarily conserved cationic host defence peptides found in nature provides a valuable resource that can be used as a template for designing small synthetic peptides to be exploited for beneficial therapeutic applications. To date there have been three major approaches utilised

to design synthetic peptides: (1) by making changes to enhance or maintain the amphiphilic secondary structures and physical properties compatible with endogenous cationic (antimicrobial) peptides, (2) by random mutations of synthetic genes encoding cationic peptides, including truncations, and (3) by robotic synthesis of library of peptides (so-called peptide arrays) on cellulose membranes based on both systematic and random substitutions as well as peptide scrambling [45, 91, 92]. Such approaches have only been described for optimisation of antimicrobial activities to date.

The high and sometimes excessive use of broad-spectrum antibiotics has seen the emergence of multi-drug-resistant bacteria. Cationic host defence peptides that do not target the pathogen directly but instead selectively modulate the host immune system are extremely promising, as the likelihood for development of resistance is very low [93], providing an alternative approach to treating infections. Overall, we propose that there are at least three avenues via which cationic host defence peptides could be potentially exploited in the light of their emerging selective immunomodulatory bioactivity. (1) Cationic host defence peptides that are not directly microbicidal, but rather protect against infections by virtue of their selective immunomodulatory properties, could be developed as beneficial therapeutics against infections, including multidrug-resistant and emerging pathogens. (2) Since cationic host defence peptides have potent anti-inflammatory properties but can in addition selectively modulate and/or maintain certain host immune responses, they may be developed to combat acute, induced or chronic inflammatory disorders. (3) Since these immunomodulatory peptides are known to influence the initiation and polarisation of adaptive responses [4, 48, 63], they maybe developed as potential adjuvants. In all of the above-mentioned approaches, cationic host defence peptides could be used as stand-alone therapeutics or, more likely, in conjunction with existing drugs.

The focus in the development of cationic host defence peptides for clinical applications has been on small peptides containing the biologically active core of the endogenous molecules, therefore limiting related toxicity components and improving efficacy along with lowered cost of goods. There are a few biotech companies that have recently started to focus on the therapeutic potential of host defence peptides. Promising results to date from clinical trials (including Phase IIIa trials) have been demonstrated by utilising these peptides in topical treatment as direct antimicrobials for the prevention of catheter colonisation and catheter-associated infections [94, 95]. Other than this, only a modest number of antimicrobial peptides have been tested in clinical-efficacy trials, and relatively few are still viable [95]. The limitations associated with their development are essentially unknown pharmacodynamics and toxicology (including potential immunotoxicities), as well as costs of goods, all of which must be taken into consideration. The development of small synthetic cationic peptides as potential therapeutics appears viable as they are relatively easy to synthesise and can stimulate immune defences by multiple mechanisms providing an alternative approach to treating infections.

Conclusions

The emergence of bacterial resistance to antibiotics has led to an increased urgency in the health sciences to explore alternative means of combating pathogenic assault. Cationic host defence peptides are fast emerging as attractive candidates for treatment of a variety of pathogenic conditions, since they do not appear to target the pathogen directly, nor do they modify a single inflammatory mediator. Rational development of synthetic peptides with systematic or random substitution of sequences using known endogenous host defence peptides as templates is promising, as these defence peptides appear to possess parallel functions across species. The central theme is that host defence peptides boost specific innate immune responses and exert selective immunomodulatory effects on immune cells upon exposure to pathogenic challenge. Our hypothesis is that these peptides function primarily by subtly regulating innate immune responses in the host, creating an overall balance by subduing exacerbated inflammatory responses, while maintaining certain beneficial aspects of inflammation required for combating pathogenesis. They are thus involved in the orchestration of host innate immunity, in such a way as to also influence the development of specific adaptive responses. This holistic functional profile in the context of host defence makes them attractive agents for follow-up investigations as potential beneficial therapeutics for the future. Further exploration of possible applications and functional mechanisms of natural host defence peptides, and the subsequent development of synthetic defence peptides as a therapy for infections, as well as for selectively modulating acute and/or chronic inflammation, may lead to a paradigm shift in the management of infections.

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