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Cationic host defense (antimicrobial) peptides

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Members of the cationic host defense (antimicrobial) peptide family are widely distributed in nature, existing in organisms from insects to plants to mammals and non-mammalian vertebrates. Although many demonstrate direct antimicrobial activity against bacteria, fungi, eukaryotic parasites and/or viruses, it has been established that cationic peptides have a key modulatory role in the innate immune response. More recent evidence suggests that host defense peptides are effective adjuvants, are synergistic with other immune effectors, polarize the adaptive response, and support wound healing. In addition, the mechanisms of action are being unraveled, which support more effective implementation of derivatives of these endogenous peptides as therapeutic agents.

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Introduction: cationic peptides in host defense

Cationic amphipathic peptides are found in every complex species [1]. They are generally defined as having 12 to about 50 amino acids with 2–9 positively charged lysine or arginine residues and up to 50% hydrophobic amino acids. They fold into a variety of secondary structures (often after they insert into membrane bilayers) in which the charged and polar, and hydrophobic residues form patches on the surface of the molecule [2]. Early work with insects, amphibians and mammalian phagocytes demonstrated that they had direct antimicrobial activity against diverse microbes. More recently, it has become evident that they have a diverse range of functions in modulating immunity (Figure 1) which have an impact on infections and inflammation [1,3–5]. Therefore, although they are often termed antimicrobial peptides, we prefer the term used in this review to describe the breadth of their activities, namely cationic host defense peptides.

Peptides are expressed with elements of immunity

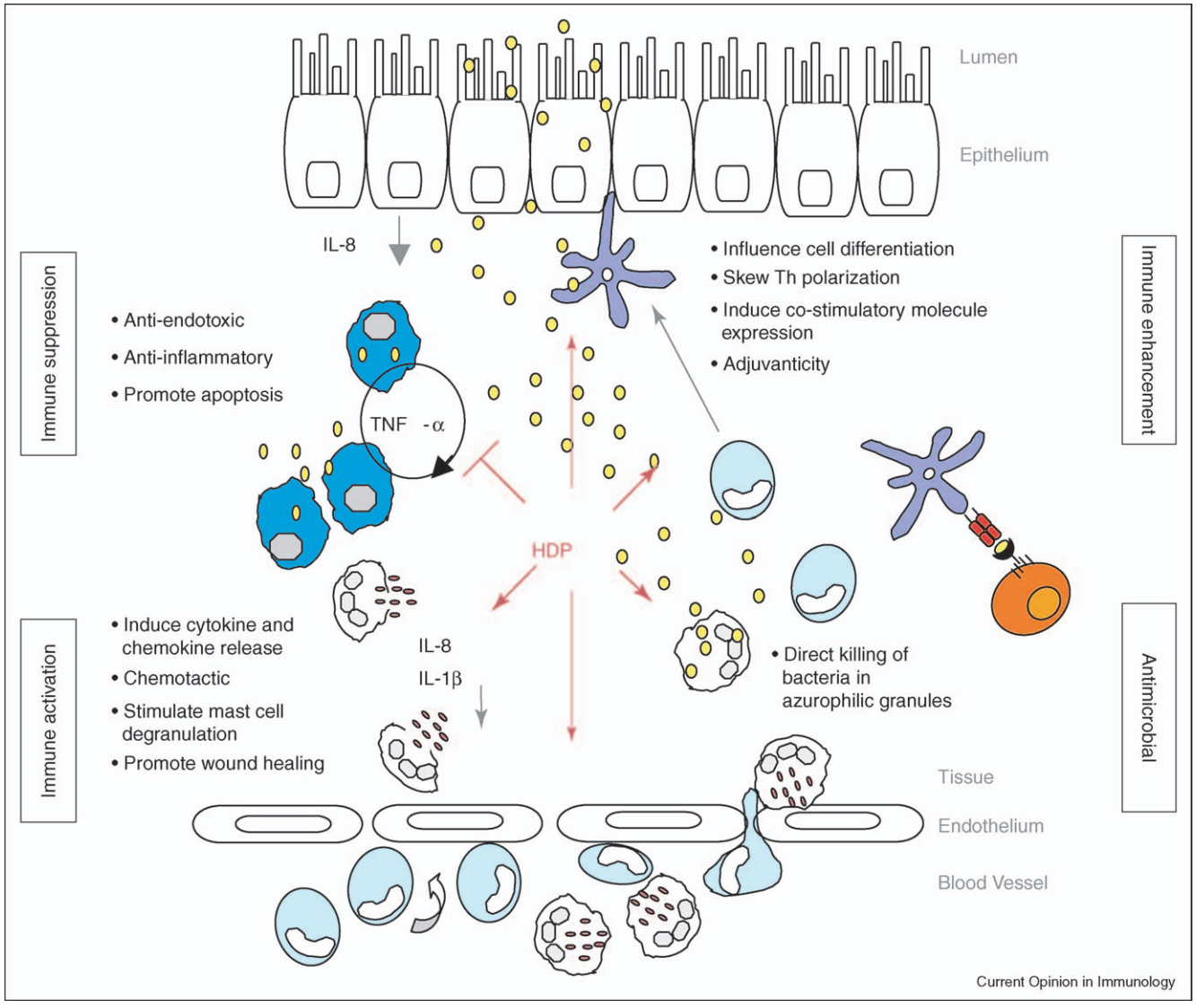
Innate immunity is the most ancient form of immune defense, conserved throughout the animal kingdom and vital to invertebrate host defenses. More recently discovered aspects of innate immunity — most notably, the family of Toll-like receptors (TLRs) — have illuminated the previously unrecognized complexity and heterogeneity within the innate immune system. Many factors support important and diverse roles for antimicrobial peptides in immunity: the robust membership, broad diversity in sequence and structure, thematic similarity of vertebrate and invertebrate antimicrobial peptides, wide distribution in cells of the immune system (leukocytes, Paneth cells) and in tissues that encounter bacterial infections (gut, trachea) and the observation that they might be either constitutive or demonstrate TLR- or inflammation-induced expression or secretion.

The expression of mature (biologically active) peptides requires proteolytic cleavage [6], might be constitutive or inducible and depends on species, tissue type, cellular lineages and/or differentiation state of the cell [7,8]. Gene expression and/or protein secretion are induced by factors such as bacterial products, injury and/or inflammatory stimuli. For example, hBD expression is upregulated in monocytes (hBD-1 and -2) exposed to bacteria, lipopolysaccharide (LPS) or IFN-γ [9,10], in keratinocytes (hBD-2–4) stimulated with TNF-α, IL-1β, bacteria or IL-22 (for hBD-2 and -3) [11–13], and in intestinal, uterine or airway epithelial cells (hBD-1–3) stimulated with TLR agonists [14–17]. The 5′ flanking sequences upstream of the cathelicidin coding sequence have several potential consensus sequences for transcription factors, including nuclear factor (NF) κB, NF-IL6, acute phase response factor and IFN-γ response elements [18–20]. Synthesis of hBD-2 is induced in monocytes by IL-1β and in intestinal epithelial cells by LPS or peptidoglycan and is dependent on NFκB [14,21–23], whereas IL-22-induced hBD-2 and -3 expression in keratinocytes is dependent on the transcription factor STAT3 [13]. IL-1, TNF-α and TLR agonists also activate NF-κB, the transcription factor that is responsible for the transcription of multiple inflammatory and immunity genes in mammals. These data demonstrate that transcriptional regulation of antimicrobial peptides is dependent on the stimulus and cell type and is regulated and/or coordinated with the expression of other entities of innate immunity and acute inflammation [19,20,24].

Peptides combat infection

The role for antimicrobial peptides in immunity is supported by *in vivo* evidence in humans and mice correlat-

Figure 1



Functions of cationic host defense peptides (HDPs). Within cytoplasmic granules, HDPs are antimicrobial agents that directly kill microbes, whereas secreted HDPs interact with cells of the innate immune system to indirectly eradicate pathogens (yellow circles represent bacteria/bacterial products). HDPs promote immune responses to infection yet simultaneously limit the magnitude of the inflammatory response (suppress production of pro-inflammatory cytokines such as TNF-α). The multifunctional properties of HDPs make them attractive as therapeutic agents.

ing the expression of antimicrobial peptides with susceptibility or resistance to bacterial infections. Patients with specific granule deficiency syndrome lack α-defensins and suffer from severe and frequent bacterial infections [25]. Others with a condition known as morbus Kostmann suffer from frequent oral bacterial infections and severe periodontal disease which correlates with a deficiency in the human cathelicidin peptide LL-37 and human α-defensins (HNP1-3) [26]. Low expression of LL-37, human β-defensin (hBD)-2 and hBD-3 in skin lesions caused by atopic dermatitis coincides with enhanced susceptibility to skin infections [27,28]. Conversely, hBD-2 and hBD-3 expression are enhanced in psoriasis [29] and in bronchoalveolar inflammation [30,31]. The expression of cathelicidins (LL-37 and mouse cathelicidin-related antimicrobial peptide [CRAMP]) in skin keratinocytes varies with infection and/or injury [32].

In rodent models, cathelicidins can control bacterial load and prevent mortality when administered after bacterial challenge [33–35]. Matrix metalloproteinase-7, β-defensin-1 and CRAMP gene knockout mice are more susceptible to, and fail to clear, infections [36–40]. It should be noted that, although these observations are consistent with a role for cationic peptides in host defense, they are not definitive in distinguishing between a direct

antimicrobial function for these peptides, an immunomodulatory role or both in contributing to defense. Further, these observations correlate the expression of cathelicidins and defensins with states of infection and inflammation and clearly establish a role for antimicrobial peptides in innate defense, clearance, prevention and protection against bacterial assault.

Direct antimicrobial activity

The protective effects of the peptides have been attributed, in part, to the direct antimicrobial killing properties of purified peptides against bacterial, fungal or viral pathogens observed *in vitro* [1,5,41]. Most active antimicrobial peptides are able to interact with bacterial membranes, as described by four separate models [2,5]. With substantial local perturbation of the cytoplasmic membrane bilayer, ion-permeable channels are created, leading to increased cytoplasmic membrane permeability and bacterial cell death. Conversely, a substantial number of antimicrobial peptides, including polyphemusin, a very potent horseshoe crab antimicrobial peptide [42], can traverse the membrane [2] and induce killing by acting on one or more anionic intracellular targets [42–45]. Although there is a broad range of potencies, virtually all cathelicidins and defensins have direct antimicrobial activity *in vitro* under the appropriate conditions. Although some peptides might retain bactericidal activity *in vitro* under physiological conditions, the direct killing activity is often antagonized by physiological salt conditions, monovalent and divalent ions and serum [46••]. There is no doubt that some peptides (e.g. α-defensins in neutrophil granules) are present at concentrations that virtually guarantee that they act in a directly microbicidal fashion; however, the low concentrations and antagonism by physiological salt concentrations (e.g. at mucosal surfaces) is consistent with an interpretation that at least some cationic peptides act by alternative means *in vivo*.

Immune system modifiers

More recent findings have established that, at physiological concentrations of peptides, salt and serum, antimicrobial peptides stimulate a broad range of biological effects relevant to inflammation, innate immunity and adaptive immunity [4,46••,47,48,49••] in innate immune cells (neutrophils and epithelial cells) and in cells that bridge the innate and adaptive immune systems (monocytes, macrophages and dendritic cells [DCs]). Mammalian host defense peptides have been shown to boost, inhibit or complement cellular functions such as chemotaxis, apoptosis, gene transcription and cytokine production [47,50]. Such biological effects probably promote bacterial clearance, although not via direct killing. Further, the evidence suggests that the peptides have roles in immunity beyond antimicrobial activity, in suppressing bacterial-induced cytokine production (anti-inflammatory), and stimulating wound healing, angiogenesis and adjuvanticity (all of which have been demonstrated in animal or tissue models [51]), explaining the increasing use of the term 'host defense peptides'.

Immune activation

Cathelicidins and defensins secreted at sites of infection and/or injury are chemotactic for effector cells, induce the transcription and secretion of chemokines and induce histamine release from mast cells [52–54]. Together, this promotes recruitment of innate and adaptive immune cells required for the cellular and humoral responses to pathogens. α-Defensins and hBD-2, -3 and -4 are chemotactic for monocytes, (memory and naïve) T cells and immature DCs [55–58]. Bovine, human, mouse and pig cathelicidins are chemotactic for virtually all subsets of peripheral blood cells *in vitro* [51,59] and *in vivo* [59,60]. For example, LL-37 induces IL-8 release which in turn promotes the chemotaxis of neutrophils and release of high concentrations of LL-37. A similar scenario is observed in invertebrates, where the circulating hemocytes migrate through chemotaxis to the site of injury, where they release peptides. Host defense peptides also stimulate the release of particular cytokines; for example, a peptide derived from the *Limulus polyphemus* anti-LPS factor induced the release of antiviral and immunomodulating cytokines, IFN-α, IFN-γ, IL-2 and IL-13, but not TNF-α or IL-6 [61], from human peripheral blood mononuclear cells. Further, this peptide increased survival in mice following a lethal dose of *Pseudomonas aeruginosa* which was correlated with diminished systemic TNF-α and elevated mRNA synthesis of IL-2, IL-12 and IL-13, but not IL-4 and IL-10, in the spleen and liver [61,62]. LL-37 induces the release of IL-6, IL-8, TNF-α, granulocyte-macrophage colony-stimulating factor and IL-1β in human keratinocytes [63••] and enhances TNF-α and IL-6 secretion in LL-37-derived immature DCs [64]. Mouse β-defensin-2-matured DCs also secrete proinflammatory (Th1-polarizing) cytokines IL-12, IL-1α, IL-1β and IL-6 [65].

Inflammatory and immune suppression

Although mammalian host defense peptides directly stimulate certain innate immune functions considered to be proinflammatory (such as chemotaxis of leukocytes, and induction of cytokine, chemokine and histamine release), the peptides can also protect the host against detrimental, potentially lethal effects, particularly those resulting from an excessive TLR-induced inflammatory response [33,66••]. Cathelicidins suppress the transcription of the genes for proinflammatory cytokines (e.g. TNF-α and IL-6) and the release of proinflammatory mediators induced by LPS and other bacterial products, and prevent sepsis in rodents after bacterial challenge. The endotoxin-neutralizing activities of host defense peptides might also implicate cathelicidins in maintaining homeostasis, particularly in commensal-rich regions of the gut, and/or in dampening excessive inflammation. In addition, cathelicidins prevent the release of toxic components that

cause excess tissue damage and inflammation (e.g. the proline-arginine [PR]-rich porcine cathelicidin, PR-39, inhibits the production of reactive oxygen species, whereas bovine myeloid antimicrobial peptide-28 induces apoptosis of activated [infected] lymphocytes), and actively promote tissue regeneration. Cathelicidins and defensins promote cell proliferation, vasculogenesis and wound repair [67–70]. These neutralizing and resolving effects of peptides defend the host against the destructive components of inflammation.

Immune enhancement

There is also some indication that cathelicidins and defensins can act at the interface of innate and adaptive immunity [4], modulating DC function [64] and antigen-specific immune responses. LL-37 induces differentiation of primary monocyte-derived DCs, increases endocytic capacity, modifies phagocytic receptor expression and function, upregulates co-stimulatory molecule expression (CD86) and enhances Th1 cytokine secretion (IL-12) by LPS-stimulated DCs. Likewise, mouse BD2 stimulates DC maturation and upregulates the expression of co-stimulatory molecules (CD40, CD80 and CD86), major histocompatibility complex class II and chemokine receptor CCR7 (CCR7 can regulate trafficking towards T cell-rich areas) on DCs [65]. In this manner, the peptides might enhance aspects of adaptive immunity, supporting the differentiation of certain cell lineages and possibly altering the cytokine milieu and, in turn, the polarization (Th1 or Th2) of the response. IL-22 upregulates hBD-2 and -3 expression in keratinocytes [13], IL-4 release from Th2 cells and upregulation of major histocompatibility complex I antigen expression and acute phase proteins. The low expression of hBD-2 and -3 mRNA in skin lesions from patients with atopic dermatitis has been correlated with the elevated expression of Th2 cytokines [28]. These data provide evidence that host defense peptide expression, and potentially function, correlates with aspects of the adaptive immune response. Further, LL-37 has synergy with cytokines, such as granulocyte-macrophage colony-stimulating factor and IL-1β [46,66••], thereby enabling low concentrations of the peptide to influence the immune response. Used as adjuvants, LL37, CRAMP and mouse BD2 enhanced antigen-specific humoral and cellular responses [59,71••,72]. These peptides might be effective adjuvants as a result of their ability to elicit several responses, including the recruitment, differentiation and activation of effector cells at the site of infection. However, the effects of peptides on lymphocyte function (B cell activation and antibody production, cytotoxic T cell and natural killer cell killing and Th cell function) are, as yet, poorly described in the literature.

Mechanism of immune modulation

The biological effects of host defense peptides, similarly to their expression and secretion, are often induced by inflammatory stimuli (including conserved microbial components of endogenous or pathogenic origin) and are influenced by the physiological setting, including the concentration of the peptide, the cellular environment and soluble components of the extracellular milieu. The mechanism by which host defense peptides exert immune-modulating effects probably involves multiple mechanisms, including direct binding to endotoxic LPS and known or putative surface receptors and/or intracellular signaling molecules and receptors. It has been reported that cathelicidins bind to a variety of receptors [73–75,76•,77], activate components of the mitogen-activated protein kinase signal transduction pathways [59,75,77,78], induce Ca^{2+} mobilization [52,59], bind to SH3-domain-containing proteins [79,80] and inhibit LPS-induced NFκB translocation [66••,81]. Mouse BD2 activates NFκB and might function as an endogenous ligand of TLR4 signaling [65]. The ligand–receptor interactions of cathelicidins are not well understood, and, although some functions are dependent on known receptors (e.g. LL-37 directly mediates chemotaxis of human peripheral blood neutrophils, monocytes and T cells through formyl peptide receptor-like 1 [73]), other biological functions are not associated with known receptors.

Therapeutics

Functional similarities among the antimicrobial host defense peptides of distant evolutionary species indicate that the study of both vertebrate and invertebrate peptides could permit the development of new design templates for anti-infectious agents in humans [5,82]. Although not widely investigated at present, there are an increasing number of reports of the immunomodulatory effects of natural and synthetic host defense peptides on mammalian hosts, including neutralization of LPS, induction of signal transduction, gene transcription and release of reactive oxygen species [82,83], and interest is high for developing these as a novel therapy for human infectious diseases, through the selective boosting of innate immunity [51]. Several companies are actively pursuing the host defense peptides as novel antimicrobial therapeutics, with the indolicidin-like peptides of Migenix (formerly Micrologix) having advanced to Phase IIIb clinical trials for prevention of catheter-associated infections [84•]. The functional redundancy between species, the antiseptic and antimicrobial activities, adjuvant properties and low toxicity make them attractive therapeutic agents [1,48,82,84•]. Further, functionally active domains are apparently localized to different regions of the peptide (demonstrated for the antimicrobial and immunomodulatory functions), enabling the development of peptides with potent and specific functions [51,63••].

Conclusions

In summary, there is emerging evidence that host defense peptides actively participate in all stages of host immune defenses: exerting antimicrobial activity through direct killing and/or stimulation of biological functions in

immune effector cells during the inflammatory and immune response, by communication with cells at the interface of the innate and adaptive immune system, and in the control and resolution of the inflammatory response.

A more detailed analysis of host defense peptides will aid our understanding of how these peptides participate in the recognition and neutralization of pathogens, which will assist in the development of a new anti-infective therapeutic strategy. These studies will expand on current structure–function analyses and will build on reports of immune modulating functions by elucidating the mechanisms of action of the peptides. Of highest importance, future studies will exploit and build on the diverse nature of peptides and adhere to physiologically relevant conditions, ultimately validating, *in vivo*, host defense peptide functions that protect against bacterial challenge and suppress potentially harmful inflammation.

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