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Review

The role of mammalian antimicrobial peptides and proteins in awakening of innate host defenses and adaptive immunity

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Abstract. Since we live in a dirty environment, we have developed many host defenses to contend with microorganisms. The epithelial lining of our skin, gastrointestinal tract and bronchial tree produces a number of antibacterial peptides, and our phagocytic neutrophils rapidly ingest and enzymatically degrade invading organisms, as well as produce peptides and enzymes with antimicrobial activities. Some of these antimicrobial moieties also appear to alert host cells involved in both innate host defense and adaptive immune responses. The epithelial cells are a source of constitutively produced β defensin (HBD1) and proinflammatory cytokine-inducible β defensins (HBD2 and -3) and cathelicidin (LL37). The neutrophils-derived antimicrobial peptides are released on demand from their cytoplasmic granules. They include the enzymes cathepsin G and chymase, azurocidin, α defensins and cathelicidin. In contrast, C5a and C3b are produced by activation of the serum complement cascade. The antimicrobial moieties direct the migration and activate target cells by interacting with selected G-protein-coupled seven-transmembrane receptors (GPCRs) on cell surfaces. The β defensins interact with the CCR6 chemokine GPCRs, whereas cathelicidins interact with the low-affinity FPRL-1 receptors. The neutrophil-derived cathepsin G acts on the high-affinity FMLP receptor (GPCR) known as FPR, while the receptors for chymase and azurocidin have not been identified as yet. The serum-derived C5a uses a GPCR known as C5aR to mediate its chemotactic and cell-activating effects. Consequently, all these ligand-receptor interactions in addition to mediating chemotaxis also activate receptor-expressing cells to produce other mediators of inflammation.

Key words. Defensin; chemokine; receptor; cathelicidin; complement; cathepsin G; immunity; antimicrobial.

Introduction

Our environment is contaminated by an enormous number and variety of microorganisms, and we have numerous defenses against invasion by these organisms. Skin keratinocytes and epithelial cells lining our gastrointestinal tract, genitourinary tract, and tracheobronchial tree provide an initial barrier, and phagocytic neutrophils and monocytes patrol our circulation. However, these barriers are often breached, and we would be overcome were it not for the secretion and release of numerous antimicrobial peptides by these barrier epithelial cells and phagocytes

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[1, 2] Over 400 antimicrobial peptides have been identified to date in plants, insects and animals [3–7]. Recently, some of the mammalian antimicrobial peptides have been shown to have a second major function of rapidly chemoattracting and activating host cells to engage in innate host defense and/or adaptive immune responses [8–10]. Since some of these antimicrobial peptides are stored in barrier epithelial cells and phagocytes, they can be exocytosed by degranulation in response to a number of stimuli and rapidly become available at sites of microbial invasion. The stimulants resulting in degranulation include the process of phagocytosis, microbial products such as lipopolysaccharide (LPS), cell injury and proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1 and IL-8 [8]. These latter endogenous mediators are induced in response to microbial invasion and microbial products and therefore represent products of the first line of defense that in turn can stimulate the secretion by epithelial cells of more of the inducible antimicrobial peptides [11–13].

This review will consider those antimicrobial peptides and proteins that by activating phagocytic neutrophils and monocytes/macrophages potentially enhance innate host defenses. These agents include cathepsin G, chymase, azurocidin, complement, defensins and cathelicidins. All of these agents, including the enzymes and the complement components, also have the capacity to act on antigen-presenting dendritic cells and/or T lymphocytes and can potentially promote subsequent adaptive immune responses to microbial antigens [9, 14]. We will also outline the experimental evidence showing that some of these antimicrobial peptides and proteins have potent in vivo immunoenhancing effects that may make them useful as vaccine adjuvants [15, 16].

Cathepsin G

The acute stage of inflammation as typified by neutrophil infiltration and edema is followed by subsequent predominance of mononuclear cell infiltration at the chronic stage. In contrast, in cases of clinical cyclic or experimental neutropenia, mononuclear cell influx into inflammatory sites is significantly decreased and delayed. Restoration of circulating neutrophils reestablished the normal sequence of events in the development of the inflammatory response [17, 18]. This suggests that neutrophils might produce chemoattractant(s) for mononuclear cells and led us to identify α-defensins as chemoattractants of T cells, as will be discussed. Since the chemotactic response of monocytes to this neutrophil-derived signal could be inhibited by protease inhibitors, it was proposed that this signal might be a serine protease [19]. Infiltration of neutrophils can be induced at injection sites by such chemoattractants as formyl peptides, anaphylatoxin C5a and chemokines such as IL-8. We therefore tested the possibility that an IL-8-induced neutrophil infiltrate generates a subsequent mononuclear cell response. The injection of IL-8 subcutaneously into SCID mice that had been given human peripheral blood lymphocytes (PBLs) resulted in an initial neutrophil infiltration, followed by the subsequent appearance of a considerable infiltrate of human T cells and murine monocytes by 72 h [20]. This happened despite the fact that a human chemokine was used to attract human cells in a murine milieu, necessitating the interaction of human cells with murine adhesion proteins. This in vivo chemotactic effect on human PBL appeared to depend on the prior infiltration by murine neutrophils. Since IL-8 is capable of inducing degranulation of neutrophil azurophilic and specific granule components [20], we hypothesized that some of the granule-derived proteins may be responsible for the subsequent monocyte and T cell migration. Biochemical purification of a monocyte chemotactic factor from neutrophil granules led to the identification of cathepsin G [21]. Cathepsin G is a neutral serine proteinase that is present primarily in azurophilic granules of neutrophils, and to a lesser extent in a cytoplasmic membrane-bound form. It is referred to as a chymotrypsin-like enzyme because it hydrolyzes peptide bonds after leucine, methionine and phenylalanine residues. Cathepsin G is considered to be a rather inefficient proteinase, degrading collagen and proteoglycan more slowly than neutrophil elastase [22]. Various physiological effects are ascribed to this cathepsin G, such as antimicrobial activity, degradation of extracellular matrix, vasoregulation [22], activation of neutrophil elastase [23] and IL-8 processing [24]. The activities of cathepsin G relevant to antimicrobial immunity are outlined in figure 1. The monocyte chemotactic activity of cathepsin G appeared to be dose dependent with an optimal concentration range of 0.5–5 µg/ml. Cathepsin G appeared to be a much more potent chemoattractant for monocytes than either azurocidin or thrombin [21]. To determine the relationship of the chemotactic activity of cathepsin G to its enzymatic activity, cathepsin G

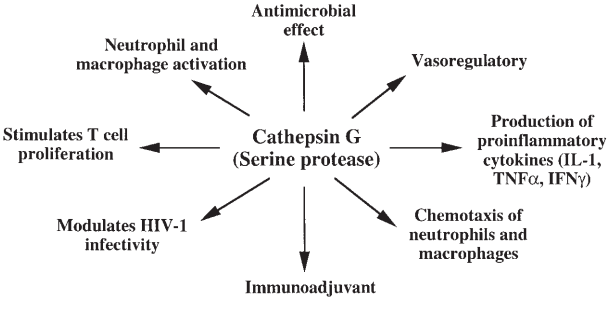

Figure 1. Activities of cathepsin G relevant to host antimicrobial immunity. Abbreviations used: HIV, human immunodeficiency virus; IL-1, interleukin-1; TNFα, tumor necrosis factor α; IFNγ, interferon γ.

was modified by diisopropylfluorophosphate (DFP) or phenylmethanesulfonyl fluoride (PMSF). The inhibition of proteolytic activity of cathepsin G by these agents also led to inactivation of its monocyte chemotactic activity, clearly indicating that proteolytic activity of cathepsin G is essential for its chemotactic activity. The polyclonal T-cell-activating (mitogenic) activity of cathepsin G [25] was also inhibited by pretreatment of cathepsin G by PMSF [O. Chertov et al., unpublished observation].

The activities of serine proteinases in the blood are regulated by specific inhibitors (serpins) [26]. Chymotryptic activity is inhibited by α_1-antichymotrypsin. At a fourfold molar ratio α_1 -antichymotrypsin almost completely inhibited monocyte chemotaxis to cathepsin G in parallel with inhibition of its enzymatic activity. α_1-Antichymotrypsin may have a regulatory role in inflammation since it behaves as an acute phase protein [27]. The plasma concentration of this acute phase protein rapidly increases severalfold above normal to about 5–7 μM during tissue injury, autoimmune diseases, malignancies and infections in response to systemic proinflammatory cytokines such as IL-1, IL-6, TNF-α and LT [27]. Despite the fact that human serum containing α_1 -antichymotrypsin efficiently inhibited the in vitro chemotactic effect of cathepsin G, subcutaneous injection of cathepsin G in mice induced local inflammatory reactions [21]. This suggests that serum inhibitor(s) are not present in sufficient concentration in the tissues during initial stages of inflammation. The fact that cathepsin G can stimulate an influx of inflammatory cells into the site of injection in spite of high concentration of proteinase inhibitors in plasma may indicate that the outcome of proteinase-inhibitor interaction, depends on the kinetics of the reaction, the rate of diffusion of inhibitors from the circulation, inactivation of proteinase inhibitors by oxidation and protection of cathepsin G enzymatic activity by DNA fragments [28].

Recently, Moriuchi et al. confirmed that cathepsin G is an efficient chemoattractant for macrophages [29]. Macrophages stimulated by the bacterial product LPS migrated more efficiently in response to cathepsin G than unstimulated cells. Cathepsin G induces the expression of proinflammatory cytokines TNF-α and IL-1β by macrophages and interferon (IFN)-γ by T cells [29]. Administration of cathepsin G together with an antigen stimulates enhanced production of immunoglobulin (Ig) G1 and IgG2a antibodies. This was associated with increased production of IFN-γ and IL-4 by lymph node lymphocytes from immunized mice. Thus, cathepsin G appears to have adjuvant effects on both T_{h1} and T_{h2} limbs of the immune response. Cathepsin G augments in vivo T cell responses to antigen and enhances both cellular and humoral adaptive immune reactions [O. Chertov, et al., unpublished observation].

Chemotaxis of macrophages to cathepsin G is mediated by G_i protein-mediated signal transduction, as suggested by inhibition of chemotaxis by pertussis toxin [21, 29]. The sensitivity of cathepsin G-induced chemotaxis of macrophages to pertussis toxin implies the involvement of a G_i-protein-coupled seven-transmembrane receptor (GPCR). In an effort to identify the GPCR used by cathepsin G, we have evaluated the capacity of prior exposure to various ligands to inhibit subsequent chemotactic responses of monocytes and neutrophils to cathepsin G. Although a number of chemoattractants had no effect, FMLP desensitized chemotactic response of monocytes to cathepsin G, implicating the high-affinity receptor for FMLP (FPR) as a receptor for cathepsin G. This was supported by experiments showing that cell lines transfected with FPR developed chemotactic responses to cathepsin G as well as to FMLP. This observation suggests that FPR mediates the chemotactic effect of cathepsin G but does not rule out the possibility that cathepsin G interacts with other GPCRs as well [O. Chertov et al., unpublished].

It was reported recently that pretreatment of macrophages with cathepsin G rendered them much more susceptible to human immunodeficiency virus-type (HIV-1) infection. In contrast, the infectivity of CD4+ T lymphocytes by HIV-1 was not affected by cathepsin G [29]. The infectivity of macrophages exposed to pertussis toxin was not enhanced by subsequent treatment with cathepsin G, suggesting the involvement of GPCRs and G_i-protein-mediated signal transduction. On the other hand, more prolonged exposure of macrophages to cathepsin G reduced HIV infection of macrophages; this effect was inhibited by a specific inhibitor of cathepsin G – α_1-antichymotrypsin. Although FPR is not known to be a coreceptor for HIV-1 cell entry, more prolonged exposure to FPR to cathepsin G may result in heterologous desensitization and downregulation of CCR5 coreceptor [30].

The physiologic importance of cathepsin G was suggested by the finding that neutrophils from vitamin A-deficient rats contain lower levels of cathepsin G [31]. The levels of other neutrophil proteases such as elastase, plasminogen activators and gelatinase, unlike cathepsin G, were not altered by vitamin A deficiency. It is known that vitamin A deficiency is associated with increased severity and rate of infections and mortality in humans. Neutrophils from vitamin A-deficient rats also have reduced chemotactic response to FMLP [32]. The relationship between cathepsin G deficiency and impaired neutrophil chemotaxis to the integrity of host defense remains to be established.

Mice with homologous deletion of cathepsin G gene have been generated and appear to be phenotypically normal [33]. Neutrophils from cathepsin $G^{-/-}$ mice have normal morphology and display normal phagocytosis and superoxide production. The cathepsin $G^{-/-}$ mice do show a decrease in their wound-healing capacity with a more prolonged influx of increased numbers of neutrophils into the site [34]. However, the chemotactic responses of their neutrophils to C5a, IL-8 and FMLP was reported to

be normal. Cathepsin G-deficient mice have also reported to be more susceptible to the lethal effects of fungal pathogens and to be more resistant to endotoxin shock [35]. These data also suggest that cathepsin G contributes to antimicrobial host defenses.

Chymase

Recently, it was shown that injection of human mast cell chymase into the skin of guinea pigs or into the peritoneum of BALB/c mice induced marked neutrophilia and eosinophilia. Chymase is a major chymotrypsin-like serine proteinase expressed in the secretory granules of mast cells. Chymase-induced leukocyte infiltration in both these species was dependent on enzymatic activity. Coinjection of proteinase inhibitors or heat inactivation of the enzyme markedly reduced cell accumulation [36]. Tani et al. demonstrated that mast cell chymase acts directly on monocytes and neutrophils, resulting in their chemotactic migration [37]. Pretreatment of chymase with enzyme inhibitors reduced both its enzymatic activity and monocyte chemotactic activity to the same extent. Chymase also stimulated cell migration of T lymphocytes, although the effect appeared to be chemokinetic rather than chemotactic. These results suggest that mast cell chymase may also play a role in the accumulation of inflammatory cells in the development of chronic inflammatory responses. The general substrate specificity of cathepsin G and chymase is the same [38], which may account for the fact that they both have considerable chemotactic activity. Other serine proteases such as elastase, thrombin, trypsin and chymotrypsin exhibit only modest or no chemotactic activity. The basis for the marked chemotactic effects of chymase and cathepsin G remains to be determined.

CAP37/azurocidin

A cationic antimicrobial protein (CAP37) was first isolated in 1984 from human neutrophil granules as one of the components of oxygen-independent phagocytic defenses [39]. Several years later, another group purified and identified nine polypeptides in neutrophil granules that display antimicrobial activities, with one of them referred to as azurocidin being identical to CAP37 [40]. CAP37/azurocidin is synthesized as a precursor of 351 amino acids and processed to a glycosylated mature form of 222 amino acids [41, 42]. CAP37/azurocidin bears substantial similarities to serine proteases, especially neutrophil elastase (45% homology), but has no enzymatic activity.
Apart from its antimicrobial activity, CAP37/azurocidin has been shown to have moderate chemotactic effects on monocytes/macrophages [21, 43, 44] and T cells [8], to bind LPS [45] and to be capable of heparin binding [44]. Very recently, it has been shown that CAP37/azurocidin can serve as an opsonin [46] and as a modulator for LPS-induced monocyte activation [47], such as enhancing TNF production by monocytes in response to LPS [48]. Thus, CAP37/azurocidin, presumably like other antimicrobial peptides and proteins, may potentially play a role in promoting host antimicrobial immunity.

Complement system

The complement (C) system was discovered more than a century ago as a heat-labile 'factor' in fresh serum capable of causing lysis of bacteria and erythrocytes [49]. Despite the existence of three pathways of complement activation, namely classical [50], alternative [51] and lectin [52] pathways, all pathways converge and activate the central component, C3, leading to the covalent binding of C3b to the surface of microorganisms or aged erythrocytes and culminating in the formation of an identical terminal membrane attack complex (MAC). MAC, being composed of C5b, C6, C7, C8, and as many as 18 C9, induces pores in cell membranes of microorganisms or aged erythrocytes through which ions, small molecules and water enters bringing about osmotic lysis of the targets. Thus, the complement cascade may, in a broader sense, be considered to consist of antimicrobial proteins. In addition to its direct microbicidal effect, MAC has also been demonstrated to induce activation of numerous host cell types, resulting in degranulation, proliferation, release of inflammatory mediators, and secretion of cytokines and chemokines (e.g. IL-8 and MCP-1), as reviewed recently [53].
Besides MAC, several other products of complement activation, in particular C3a, C3b, C3d and C5a, participate in the awakening of innate host defense and adaptive immunity against microbial invasion [50, 51]. Binding of C3b to microorganisms enhances phagocytosis through interaction with complement receptor (CR) 1 and CR3 present on phagocytic cells (opsonization), thus promoting innate host defense against microbial invasion [50, 51]. C3a and C5a are potent leukocyte chemoattractants (C5a > C3a) with powerful anaphylactic actions (C3a > C5a) on phagocytic cells (neutrophils and monocytes/macrophages), eosinophils, mast cells and basophils [50, 51]. By interacting with their corresponding receptors, C3a and C5a on one hand contribute to the recruitment of neutrophils, monocytes/macrophages, eosinophils, basophils and mast cells to sites of infection, and on the other activate those leukocytes to release numerous inflammatory mediators, including lipid metabolites, cytokines and chemokines, providing another way for the complement system to augment innate antimicrobial defenses [50, 51, 54, 55].

The C5a receptor is also expressed by dendritic cells [56–58]. We recently demonstrated that both immature and mature dendritic cells express functional C5a receptors [59], suggesting that C5a may participate both in the recruitment of immature dendritic cells to inflammatory sites and in the trafficking of mature dendritic cells into lymphoid tissues. It is reported that B [60, 61] and T lymphocytes [62] also express C5a receptors. We therefore proposed that C5a may participate in bringing antigen-presenting dendritic cells and lymphocytes together in T cell areas and/or B cell follicles of secondary lymphoid tissues [59]. Furthermore, attachment of C3d to microbial antigens facilitates antigen uptake by follicular dendritic cells and B cells through interaction with CR2 and, to a lesser extent, CR1, thereby promoting antigen-specific humoral immune response against invading microorganisms (reviewed in [63]).Thus, the complement system also contributes to adaptive antimicrobial immune response by several distinct pathways.

The contribution of the complement system to innate host defense and adaptive immunity has been confirmed by numerous experiments utilizing complement-deficient or CR knockout mice. For example, when investigated in the cecal ligation and puncture model of acute septic peritonitis, mice deficient in C3 or C4 have 100% mortality at 24 h compared with less than 25% mortality in wild-type control mice [64]. In addition, knockout of CR1 and CR2 impairs the immune response of mice to T-dependent antigens [65]. Furthermore, knockout of C5a receptor renders mice susceptible to intrapulmonary-instilled *Pseudomonas aeruginosa* [66]. Complement products can also be used as immunoadjuvants as demonstrated initially by Dempsey et al. that coupling of multiple copies of C3d to soluble antigen significantly enhances its immunogenicity [67].

Defensins

Defensins were initially isolated from rabbit and human neutrophils in an effort to characterize phagocyte-derived oxygen-independent antimicrobial activities [68, 69]. Subsequently, defensin molecules were characterized from plants [4], insects [5], and many other vertebrate species [1, 6, 7, 70]. All defensins are cationic, microbicidal and contain six to eight highly conserved cysteine residues which form three to four pairs of intramolecular disulfide bonds. Defensins are classified into three families according to their origins (table 1). Based on their numbers and patterns of disulfide bridges, vertebrate defensins are divided into α-, β-, and θ-defensin subfamilies (table 1). The θ-defensin subfamily currently has only one member that is cyclic, with its six cysteine residues linking C1 to C6, C2 to C5, and C3 to C4 [71]. The three disulfide bonds of α-defensins are paired C1 to C6, C2 to C4, and C3 to C5 [72], whereas those of β-defensins are C1 to C5, C2 to C4, and C3 to C6 [73]. Both α- and β-defensins have similar tertiary structures, showing triple-stranded antiparallel β sheets [74, 75].

In mammalian species, more than 50 defensins have been identified which are either stored in the granules of neutrophils, monocytes/macrophages and Paneth cells, or are generated by keratinocytes and mucosal epithelial cells of the respiratory, digestive, urinary and reproductive systems. However, the number and cell source of mammalian defensins varies in different species. As many as 14 bovine β-defensins are produced by neutrophils and tongue epithelial cells [76, 77]. In the mouse, at least 17 α-defensins (also called cryptdins) are generated by Paneth cells and skin [78, 79], whereas four β-defensins are expressed by keratinocytes or various epithelial cells [80–83]. In humans, six α- and three β-defensins have thus far been characterized. Human α-defensins 1–4 [also termed human neutrophil peptides (HNPs) 1–4] are predominantly stored in the granules of phagocytes [69, 84] and have also recently been shown to be produced by B and $\gamma\delta$T lymphocytes [85]. Human Paneth cell granules express two α-defensins called human defensin (HD) 5 and 6 [86, 87]. Human β-defensins (HBDs) 1, 2 and 3 are the products of keratinocytes and various epithelial cells [11, 12, 88–90]. In both mice and humans, the genes encoding both α- and β-defensins are clustered on a syntenic location, mapping to the proximal region of chromosome 8 in each species [81–83, 90–94].

Table 1. Classification of defensins.

Classification		Origin	Intramolecular disulfide bridge	
Family	Subfamily		Number	Pattern
Plant defensins	–	Plant cells	4	C1–C8, C2–C5, C3–C6, C4–C7
Insect defensins	–	Insect cells	3	C1–C4, C2–C5, C3–C6
Vertebrate defensins	α	Mammalian leukocytes and Paneth cells	3	C1–C6, C2–C4, C3–C5
	β	Avian and mammalian epithelial cells and keratinocytes	3	C1–C5, C2–C4, C3–C6
	θ	Primate leukocytes	3	C1–C6, C2–C5, C3–C4

Defensins are synthesized as prepropeptides and processed posttranslationally into mature forms as reviewed in reference [70].

Defensins (mature form) are multifunctional and their activities relevant to host antimicrobial immunity are outlined in figure 2. Numerous studies demonstrate that defensins, when used at concentrations above 2 μM, have the capacity to kill a broad spectrum of microorganisms, including bacteria, protozoa, fungi and some enveloped viruses in vitro under low salt (<0.15 M of NaCl) and serum-free conditions (for details, see [6, 7, 70]). In vivo under physiological conditions, this direct microbial killing is likely to occur only in the phagocytic vacuoles of phagocytes (including neutrophils and monocytes/macrophages) after the ingestion of microorganisms or on the surfaces of skin and mucosal epithelia, thereby contributing to innate host defenses against microbial infection.

In addition to their direct microbicidal activity, a number of other activities of defensins (fig. 2) may also enhance host innate antimicrobial defenses. Human α-defensins are capable of enhancing phagocytosis by macrophages [95]. Human, rabbit and guinea pig α-defensins are able to induce degranulation of mast cells, resulting in the release of mast cell granule products including histamine [96, 97|. Human α-defensins can stimulate bronchial epithelial cells to augment IL-8 gene transcription and IL-8 production [98, 99]. Since mast cell granule products increase neutrophil influx [100, 101] and IL-8 is a potent neutrophil chemotactic factor [102, 103], defensin-induced mast cell degranulation and IL-8 production at inflammatory sites would promote the recruitment and accumulation of neutrophils. This scenario is supported by the facts that subcutaneous injection of human α-defensins causes an infiltration of neutrophils as well as mononuclear cells at the site of injection [8] and that in vivo administration of α-defensins increases the ability of mice to resist local infection by enhancing neutrophil recruitment to infected tissues [104].

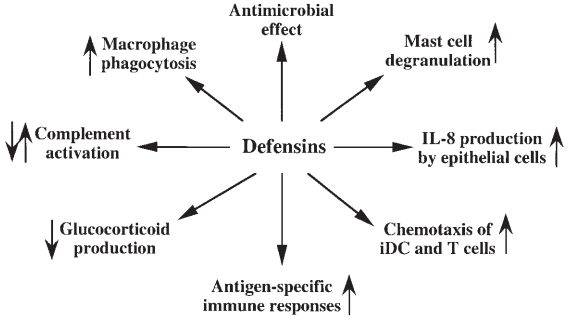

Figure 2. Schematic illustration of the activities of defensins relevant to host antimicrobial immunity. Defensins can enhance (↑) suppress (↓) or regulate (↓↑) the production of molecules as well as the activation and/or migration of immune cells. iDC, immature dendritic cells.

Degranulation by the recruited neutrophils release defensins [8, 20, 105] and consequent generation of more IL-8 [102, 103]; both result in a positive feedback loop that enhances neutrophil accumulation at sites of infection. Furthermore, human α-defensins can bind to complement C1q [106] and enhance [107] or suppress [108] the activation of the classical pathway of complement in vitro, raising the possibility that defensins in vivo may participate in the regulation of complement activation. The capacity of defensins to kill microorganisms, to enhance phagocytosis, to promote neutrophil recruitment and to regulate complement activation indicates that defensins contribute to innate host defenses against microbial invasion.

Our studies indicate that defensins also participate in awakening of host adaptive immunity (fig. 2). The earliest suggestion that defensins may play a role in adaptive immunity is perhaps the finding that HNP1 and -2 are chemotactic for human T cells both in vitro and in vivo [8]. Subsequent studies revealed that human neutrophil α-defensins are selectively chemotactic at nanomolar concentrations for resting CD4/CD45RA and CD8 T cells, whereas human β-defensins are chemotactic for CD45RO memory T cells [10, 109]. Although in vivo α-defensins are synthesized by immature myeloid cells (especially metamyelocytes and myelocytes) [110] and stored in granules of neutrophils, monocytes and macrophages, they can be released into extracellular environment by neutrophil disruption or degranulation [8, 20, 105]. Production of β-defensins 2 and 3 is induced by contact with microbes or bacterial products such as LPS or proinflammatory cytokines such as IL-1 and TNF, whereas β-defensin 1 is a constitutive product of epithelial cells and keratinocytes [11–13, 77, 80–83, 89–91]. Consequently, both α- and β-defensins are presumably present at sites of microbial invasion, forming chemotactic gradients. Thus, defensins in vivo are likely to play a role in recruiting effector T cells to inflammatory sites, thereby contributing to the effector phase of adaptive immunity.

The induction of adaptive antimicrobial immunity is initiated in infected tissues by immature dendritic cells that phagocytize microbial antigens [111–113]. Both α- and β-defensins have the capacity to chemoattract immature, but not mature, dendritic cells [10, 14, 109]. The chemotactic activity of human β-defensins for immature dendritic cells and memory T cells is mediated by human CC chemokine receptor 6 [10, 14], which is selectively expressed by immature dendritic cells [114, 115]. The receptor that mediates the chemotactic activity of human α-defensins has not been identified yet. Nevertheless, the formation of localized α- and β-defensin gradients at sites of microbial entry presumably facilitates the recruitment of immature dendritic cells to sites of antigen deposition, thereby enhancing antigen uptake, processing, presentation and ultimately the induction of antigen-specific im-

munity. This scenario is supported by studies showing that human α-defensins markedly enhance antigen-specific immune responses when administered simultaneously with antigens in vivo [15, 16]. Defensins also promote dendritic cell maturation. As dendritic cells mature, they process antigens and display antigenic epitopes on their surfaces in the form of antigen-major histocompatibility class (MHC) class II complexes [59, 111, 112, 114]. Mature dendritic cells then migrate via afferent lymphatics to the T cell areas of lymphoid tissues (lymph nodes, spleen and Peyer's patches), where they present antigens to activate antigen-specific naive T cells [111–113, 116].

Several features of defensins make them useful candidates for immunoadjuvants (i) Both α- and β-defensins are able to induce the migration of immature dendritic cells [10, 14, 109]. (ii) Human α-defensins have been shown to enhance antigen-specific immune responses when administered together with antigens in vivo [15, 16], and human β-defensins may have a similar effect. (iii) Defensins are endogenous products and small in size, so they are likely to be nonimmunogenic. (iv) In addition to being produced by recombinant technology, since defensins are small in size, they can also be chemically synthesized in highly pure forms in reasonable amounts [117, 118].

Nanomolar concentrations of α-defensins have also been reported to inhibit the production of immunosuppressive adrenal steroid hormones [119–121] by blocking the adrenocorticotropin receptor [121, 122]. During systemic infections, α-defensin levels in plasma can reach up to 100 μg/ml, a concentration sufficient to interfere with the production of adrenal glucocorticoids [123, 124]. Since glucocorticoids are potent immunosuppressive mediators, α-defensins may thus also enhance systemic innate host defense and adaptive immunity in vivo by inhibiting the production of glucocorticoids.

The importance of defensins in host innate defense and adaptive immunity against microbial infection has been supported by several clinical and experimental studies. Cystic fibrosis patients manifest exacerbation of chronic microbial infection of the lung due to the inhibition of the activities of both α- and β-defensins by abnormally high salinity of their airway surface fluid [125–127]. Patients with Chediak-Higashi syndrome and specific granule deficiency, two disorders characterized by a deficiency in neutrophil defensins, also have increased susceptibility to recurrent bacterial infection [128, 129]. A very recent study showing that genetic knockout of the gene of matrilysin, which is involved in the processing of murine α-defensins, prevents the production of mature murine α-defensins and reduces the resistance of mice to bacterial challenge [130]. The fact that matrilysin-deficient mice become more susceptible to orally administered *Salmonella typhimurium* provides direct support for the importance of defensins in host defense [130].

Cathelicidins

Cathelicidins comprise another family of antimicrobial proteins in mammals [6, 7, 131]. Cathelicidins consist of a putative N-terminal signal peptide, a highly conserved cathelin (cathepsin L inhibitor)-like domain in the middle of the molecule and a less conserved C-terminal antimicrobial domain (fig. 3). About 30 cathelicidin members have been identified from various mammalian species; however, humans produce only one cathelicidin, called hCAP18 [6, 7, 131–135]. The C-terminal antimicrobial domain is cleaved off by appropriate proteases. For example, human cathelicidin/hCAP18 is cleaved by neutrophil elastase to liberate its C-terminal antimicrobial domain, a peptide called LL-37 because it begins with two leucine residues and is 37 amino acid residues long [136]. The C-terminal antimicrobial peptides of cathelicidins are markedly variable in structure. Some are α helical (e.g. hCAP18/LL37 and rabbit CAP18) [136–138]. Others (e. g. porcine PR-39 and bactenecins) are proline/arginine-rich, showing a polyproline-type structure [139, 140]. Porcine protegrins, on the other hand, have β-sheet structures [141, 142]. Cathelicidins are primarily stored in the granules of neutrophils of various species and can be released extracellularly upon neutrophil activation [131, 133]. However, hCAP18/LL-37 is also expressed by epithelial cells [132, 143], monocytes, NK cells, B cells and γδT cells [85], and is induced in keratinocytes in response to inflammatory stimuli [137, 144].

The activities of cathelicidins are summarized in figure 3. The function of the cathelin domain is unclear. The C-ter-

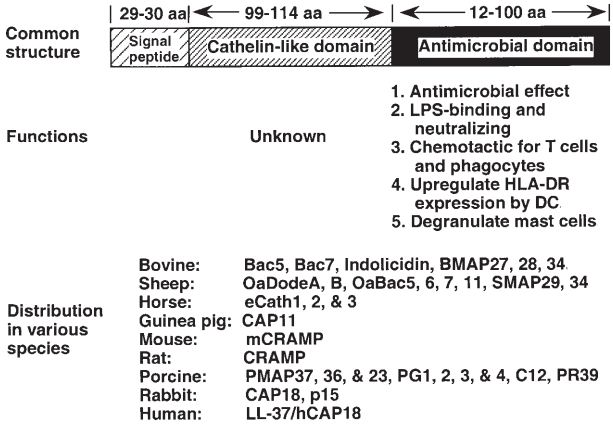

Figure 3. The structure, function and species distribution of cathelicidins. Many cathelicidins have been identified in various mammalian species (lower panel). All cathelicidins have a common primary structure (upper panel) that contains an N-terminal signal peptide, a highly conserved cathelin-like domain in the middle and a highly variable C-terminal antimicrobial domain. The function of the cathelin-like domain is not known, whereas several activities have been identified for the C-terminal antimicrobial domain of cathelicidins (middle panel). DC, dendritic cells.

minal antimicrobial peptides of all cathelicidins are microbicidal against a broad spectrum of microorganisms, including bacteria, fungi and leptospirae, with a wide overlap in specificity, but they exhibit significant differences in potency from one another [131]. Therefore, cathelicidins are generally considered to contribute to host innate antimicrobial defense. In addition, some cathelicidins (e. g. hCAP18/LL-37 and rabbit CAP18) bind LPS with high affinity and neutralize its biological activity [134, 135, 138, 145]. This has pathophysiological relevance, since both rabbit CAP18 [106–142], and truncated LL-37, a 27-residue LPS-binding fragment corresponding to hCAP18 [109–135], can protect galactosamine-sensitized mice from LPS-mediated lethality [134, 146]. Thus, cathelicidins may also ameliorate the symptoms of endotoxic shock and thereby contribute to host innate defense.

The effects of cathelicidins on host leukocytes have recently been reported. PR-39, one member of the porcine cathelicidins, is capable of inducing chemotaxis of and mobilizing Ca^{2+} in porcine neutrophils [147]. We and others have found very recently that hCAP18/LL-37 is chemotactic for human neutrophils, monocytes and T cells [9, 10, 85]. The T cell chemotactic activity of LL-37 shows selectivity since it induces the migration of CD4, but not CD8, T cells [85]. LL-37 is also able to induce Ca^{2+} mobilization in leukocytes [9, 10]. The capacity of LL-37 to induce Ca^{2+} mobilization in monocytes can be cross-desensitized by ligands specific for human formyl peptide receptor-like 1 (FPRL1), which led us to the identification of FPRL1 as a receptor for LL-37 [9, 10]. Since interaction of a chemotactic ligand with its corresponding receptor results in the activation of target cells [54], LL-37 is thus an endogenous activator of FPRL1-expressing cells.

Cathelicidins are presumably present at the sites of microbial entry due to extracellular release or secretion (see above) to form a chemotactic gradient which results in the recruitment and activation of various subsets of leukocytes. This contributes to the elimination of invading pathogens, thereby contributing to innate host defense. The recruitment to inflammatory sites and activation of in situ effector T cells would enhance the effector phase of host adaptive immunity against infection. Although LL-37 does not seem to activate dendritic cells [9], it is also reported to be capable of degranulating mast cells [148] and to enhance HLA-DR expression by human dendritic cells [149], suggesting that it may have an enhancing effect on the induction phase of adaptive immunity. The participation of cathelicidins in host innate and adaptive immunity against microbial invasion has been demonstrated by an in vivo study showing that adenoviral vector targeted systemic overexpression of cathelicidin/LL-37 in vivo results in decreased bacterial load and mortality of experimental mice following challenge with *Pseudomonas aeruginosa* or *Escherichia coli* [150].

Concluding remark

It appears as though a number of granule proteins with antimicrobial activities also have the capacity to act on proinflammatory cells or immune cells. Since exocytosis of granules results in the rapid release of their contents, these molecules are potentially the first to alert the innate and immune host defense systems. Although not all antimicrobial peptides nor all granule proteins have these capabilities, we predict more of these interesting moieties will be identified in the future.

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