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Review

The human cathelicidin hCAP18/LL-37: A multifunctional peptide involved in mycobacterial infections

Patricia Méndez-Samperio*

Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, IPN., Prol. Carpio y Plan de Ayala, 11340 Mexico, D.F., Mexico

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ABSTRACT

Antimicrobial peptides are predominantly small cationic polypeptides that are classified together on the basis of these molecules to directly kill or inhibit the growth of microorganisms including mycobacteria, and to activate mechanisms of cellular and adaptive immunity. Various families of antimicrobial peptides have been identified, including the cathelicidins. The cathelicidin family is characterised by a conserved N-terminal cathelin domain and a variable C-terminal antimicrobial domain that can be released from the precursor protein after cleavage by proteinases. LL-37 is the C-terminal part of the only human cathelicidin identified to date called human cationic antimicrobial protein (hCAP18), which is mainly expressed by neutrophils and epithelial cells. The cathelicidin hCAP18/LL-37 is a multifunctional molecule that may mediate various host responses, including bactericidal action, chemotaxis, epithelial cell activation, angiogenesis, epithelial wound repair and activation of chemokine secretion. The antimicrobial peptide LL-37 is produced from human cells during infection of mycobacteria and exerts a microbicidal effect. The discussion will (1) describe recent work on the antimicrobial and immunomodulatory functions of the cathelicidin hCAP18/LL-37, (2) highlight the effectiveness of the cathelicidin hCAP18/LL-37 as a potent component in antimycobacterial immune responses and (3) summarise current progress in the understanding of the therapeutic application of hCAP18/LL-37 and its derivates antimicrobial peptides in mycobacterial infection.

Contents

1. Introduction

Over the last 21 years, around 1500 cationic antimicrobial peptides of different origins have been identified or predicted [106]. Cathelicidins, which are one of the major antimicrobial peptide families, are characterised by a highly conserved N-terminal signal peptide known as the cathelin domain and a structurally variable cationic antimicrobial peptide at the C-terminus. Humans contain only one cathelicidin family member. This peptide was independently identified in 1995 by three groups; it was deduced from myeloid bone marrow cDNA [1,20,48] and isolated from neutrophils [20]. The name hCAP18 refers to an 18 kDa peptide derived by extracellular proteolysis from the C-terminal end of the human CAP (Cationic Antimicrobial Protein) [21]. This peptide contains two disulfide bonds between cysteine residues C85–C96 and C107–C124. The alternative designation "FALL-39" was used originally on the assumption that Phe–Ala–Leu–Leu (FALL) was the N-terminal residues of the putative antimicrobial domain. This

* Tel.: +52 5 729 60 00x62499; fax: +52 5 729 60 00x46211.
E-mail addresses: pmendezs@bios.encb.ipn.mx, pmendezs@encb.ipn.mx.

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name was then changed to LL-37 when the antimicrobial peptide was isolated from neutrophils and found to consist of 37 amino acids beginning with two leucines [36]. Currently, the term hCAP18 is accepted to indicate the propeptide, whereas LL-37 denotes a mature peptide which has potent and broad-spectrum antimicrobial activity when released from the C-terminus of hCAP18.

The cathelicidin hCAP18/LL-37 is encoded by only one cathelicidin gene, which is located on chromosome 3 (3p21.3). LL-37 is present in the human organism at a very early stage of development, since has been detected at approximately 5 μg/ml in tracheal aspirates of newborns [82], and during the perinatal period [26,56]. The hCAP18 gene is expressed in the squamous epithelia of the airways, mouth, tongue, esophagus and intestine [6,7,33,50,87]. The cathelicidin hCAP18/LL-37 peptide is constitutively synthesised in spleen, liver, stomach, intestine and bone marrow. In addition, this peptide is secreted in sweat [67], saliva [68], wound fluid [31] and in seminal plasma [4].

Cationic antimicrobial peptides have a positive charge provided by Arg and Lys residues, between 12 and 50 amino acids in length, and form an amphipathic structure [41,115]. LL-37 is a positively charged molecule (+6 at physiological pH of ~7.4) with a high content of Arg and Lys amino acids and adopts an α-helical structure in solutions with ionic composition similar to human plasma [27,42]. The stability of the helix has been attributed to peptide concentration-dependent aggregation induced by ionic and hydrophobic interactions that are favored by the presence of negatively charged residues in the LL-37 sequence [75]. It has been demonstrated that at a physiological concentration of 2 μg/ml LL-37 has its antimicrobial activities at sites of its epithelial expression. The physiological significance of the biological activities of LL-37 is mainly dependent on the peptide concentration and the composition of the media at the specific site *in vivo*. It has also been documented that in adults, concentration of LL-37 in the airway fluids is estimated to be 1.2 μg/ml, and that in humans, LL-37 prevents infection in pulmonary system [82]. Importantly, the presence of the cathelicidin hCAP18/LL-37 in human airways as well as its biological activities of this peptide against various microorganisms has been documented [7]. It is well known that physiological levels of divalent cations and serum are strongly antagonist for the antimicrobial and immunomodulatory properties of LL-37 [15]. Moreover, it has been reported that LL-37 has no direct antimicrobial activity *in vitro* under physiologically relevant salt and peptide concentrations but under the same conditions, has immunomodulatory effects in model systems of mucosal surfaces [38,84,86]. A major criticism of host defense peptide research is that many of the antimicrobial and immunomodulatory effects observed in culture occur at concentrations that are much higher than would be expected *in vivo*. In general, the structure of LL-37 peptide in aqueous solutions is relatively disordered. However, this peptide can switch to an α-helix upon contact with the bacterial wall [70]. In addition, some serum cytokines show synergistic effects on the immunomodulatory properties of the cathelicidin LL-37, indicating that the immunomodulatory activity of this peptide can be augmented by other immune mediators [113]. Moreover, it is well known that in humans, LL-37, in cooperation with other cationic antimicrobial peptides, prevents infection in digestive, genitourinary and pulmonary systems [19]. Furthermore, the *in vitro* antimicrobial activity is enhanced in the presence of α or β defensins [74] further suggesting that these peptides synergize under *in vivo* conditions to form an efficient barrier against microbial invasion. In this regard, the synergistic antimicrobial activity of LL-37 and hBD-2 has been demonstrated to efficiently kill or inhibit the growth of Group B *Streptococcus*, an important neonatal pathogen [26], suggest that these peptides provide innate immunity during development of cellular immune response mechanisms in the newborn period.

Regarding the fate of hCAP18/LL-37, relatively high levels of uncleaved hCAP18 are present in plasma (approximately 1.2 μg/ml) [92], where the propeptide circulates bound to lipoproteins through the antimicrobial domain [85,94,107]. Interestingly, Schmidtchen et al. [85] showed that both the mature LL-37 and hCAP18 could be degraded in human wound fluid by elastase-producing *Pseudomona aeruginosa*. Investigations of the physiological processing of the myeloid-derived hCAP18 indicate that the propeptide is cleaved to generate the antimicrobial peptide LL-37 in exocytosed material from neutrophils, and is not detected in phagocytic vacuoles, despite the fact that all known azurophilic serine proteases, including proteinase 3 and cathepsin G, can cleave hCAP18 *in vitro* [95]. This result correlates with the presence of a proteinase 3-compatible (Ala–Leu) cleavage site, as deduced from cDNA, between the cathelin portion and the antimicrobial domain of hCAP18. Interestingly, data from Sorensen et al. [96] suggested that cathelicidins are processed differently in different physiological contexts within the same organism, since have shown timely cleavage of epididymal-derived hCAP18 in seminal plasma by the prostate-derived protease pepsin C in the presence of vaginal fluid at low pH.

It is important to note that the cathelicidin hCAP18/LL-37 often show overlapping tissue distribution and may have its immunomodulatory activities in response to distinct stimuli within the same tissue [37]. It has been reported that the hCAP18 peptide is found in the phagocytic vacuoles of activated neutrophils [93], and in psoriatic skin [74]. Importantly, it has been reported that the cathelicidin hCAP18/LL-37 is stored in neutrophil granules at a molar concentration as high as 40 μM [92]. The expression of LL-37 is induced during the course of bacterial infection or inflammation in a variety of tissues [109], and is differentially regulated in several inflammatory conditions [25,32]. LL-37 is produced by mast cells and recruits mast cells [23], thereby participating in innate immunity both by direct antimicrobial activity and by recruitment of cellular defenses [112]. In fact, it is well known that the cathelicidin hCAP18/LL-37 participate actively in linking innate and adaptive immunity [63]. The LL-37 peptide has been shown by RT-PCR, *in situ* hybridization and immunohistochemical analysis to be produced by monocytes, T-lymphocytes, B-lymphocytes, NK cells, epithelial cells [2,69] and mast cells [23], in addition to immature neutrophils [93].

2. Functions of hCAP18/LL-37 in immunity

2.1. Antimicrobial and cytotoxic activities

Antimicrobial peptides exhibit potent activity against microorganisms by the direct lysis of bacteria through the permeabilization of cellular membranes [52]. The antimicrobial peptides are amphipathic structures that have a positively charged hydrophilic face and a hydrophobic face. The LL-37 peptide kills target organisms by disrupting membrane integrity [104], since LL-37 has a high content of basic and hydrophobic amino acids [78]. It is important to consider that the surface of microorganisms tends to display a preponderance of negative charge and that the cationic nature of the cathelicidin hCAP-18/LL-37 is attracted by electrostatic forces to the negative phospholipid headgroups on the membrane surface in bacterias. When a critical concentration of the cathelicidin hCAP-18/LL-37 accumulate on the bacterial surface, alteration of membrane structure leads to formation of ion channels or aqueous pores, leading to microbial death through hypoosmotic lysis [75]. A proof of this was provided by biophysical experiments using model systems [39,42,75] showing that LL-37 performs its bactericidal action by electrostatic binding of its cationic molecules to the bacterial wall. Insertion of the peptide into the cell membrane

results in leakage of the cell cytoplasm into the extracellular space causing death of the bacterial cell [28,70].

Several studies have demonstrated that LL-37 has potent, direct antimicrobial activity against different bacterial strains, including *Klebsiella pneumoniae* [90], *Staphylococcus aureus* [103] and *Neisseria gonorrhoeae* [13]. Moreover, Putsep et al. [77] showed that LL-37 deficiency in neutrophils from patients with morbus Kostmann correlates with the occurrence of chronic periodontal disease in these patients, indicating an important role of LL-37 in prevention of oral bacterial infections. The involvement of the cathelicidin LL-37 in prevention of oral bacterial infections is further supported by the ability of this peptide to efficiently kill common periodontopathogens [97,99]. These authors have suggested that the cathelicidin LL-37 may be considered as a therapeutic agent against mixed bacterial infections such as periodontitis [97]. Importantly, LL-37 is the only active form of cathelicidin in the skin, and the concept that endogenous expression of LL-37 protects from skin infections comes from the demonstration that mice null for the CRAMP (murine cathelin-related antimicrobial peptide) cathelicidin gene are much more susceptible to skin infection by group A *Streptococcus* than wild-type mice are [73]. It was also recently demonstrated that LL-37 inhibits the formation of *P. aeruginosa* bacterial biofilms, a pattern of bacterial growth associated with antibiotic resistance, indicating a new mechanism for antimicrobial activity of the cathelicidin LL-37 [76].

Regarding an important role of LL-37 in prevention of bacterial infections, this role has been investigated by using a genetic approach based on adenoviral-mediated hCAP-18 gene transfer into respiratory epithelia [8,9]. In support of this, it has been reported that transfer of hCAP-18/LL-37 gene into mouse airways results in a decreased bacterial load in *P. aeruginosa*-infected mice [9]. Moreover, these authors reported that systemic over expression of this gene protects D-GalN-sensitised mice injected with LPS or *E. coli* from septic death [9]. However, it is important to note that this activity may depend largely on the LPS-neutralizing properties of LL-37 [86].

Recent observations have indicated that the minimal inhibitory concentration of antimicrobial peptides against microorganisms is rarely found *in vivo* conditions. It has been reported that LL-37 has a minimal inhibitory concentration of between 1 and 30 μg/ml against a variety of common bacteria in media of low ionic strength [55]. However, in addition to the concentration of LL-37, this peptide acts synergistically with other classes of antimicrobial peptides to effectiveness of antimicrobial activity. Furthermore, LL-37 can function as potent immune regulator acting as chemokines and/or inducing cytokine production. The following section detail studies with functions of LL-37 as a human host defense peptide that can work in synergy with the endogenous inflammatory mediators.

2.2. Functions as immunoregulatory agent

In addition to its bactericidal activity or inhibiting microbial growth, the cathelicidin hCAP18/LL-37 exhibit broad-spectrum antimicrobial activity against microorganisms, including chemotactic activity for human peripheral blood neutrophils, monocytes and CD4 T cells [3,22] in a dose-dependent manner, responding at optimal concentrations ranging from 10^{-5} to 10^{-7} M [17,72]. Currently, there have been a number of receptors associated with LL-37-induced immunomodulation [117]. However, the receptor to which LL-37 has been proposed to bind directly is the N-formylpeptide receptor-like-1, a G protein-coupled receptor (FPRL-1) [48]. It has been reported that LL-37-mediated chemotaxis can be inhibited by an agonist of this receptor or by pertussis toxin. However, other LL-37-mediated effects such as MAPK activation, mast cell chemotaxis and IL-8 production are not pertussis toxin-sensitive, indicating that LL-37 may mediate these events through other receptors or other mechanisms of action.

In contrast with the antimicrobial and cytotoxic activities which are mediated by membrane perturbation and are serum-sensitive [42], the chemotactic activity of LL-37 is not significantly affected by serum, but depends on binding to FPRL1 [22], at serum concentrations that are known to inhibit the *in vitro* antimicrobial functions of LL-37 [42], indicating that the two activities are mediated by different mechanisms. In addition, it has been suggested that LL-37 has an important role in host defense against microorganisms by participating in the recruitment of immune cells that express functional FPRL1. It is important to consider that the chemotactic concentrations of LL-37 are higher than those of some chemokines. Thus, cellular recruitment by LL-37 may be active only when a threshold LL-37 concentration is reached following upregulation of the gene in epithelial cells infected with microorganisms. A study by Niyonsaba and Ogawa [71] showed that LL-37 has chemotactic effects to attract rat peritoneal mast cells with an optimal concentration of 5 μg/ml, and to induce histamine release and intracellular Ca^{2+} mobilization in these cells, indicating the potential involvement of LL-37 in mast cell recruitment at inflammatory sites. It is well known that mast cells are among the first inflammatory cells to encounter invading pathogens, and can initiate site specific inflammation by phagocytosing and killing opsonised bacteria [23]. In this regard, LL-37-induced mast cell degranulation would lead to release of inflammatory mediators, including neutrophil chemoattractants and histamine [71], which would increase vascular permeabilization thus favoring neutrophil infiltration of inflamed tissues [91]. Importantly, these authors demonstrated the role of neutrophil-secreted LL-37 in the induction of chemotactic migration of inflammatory monocytes *in vivo* [91]. In the context of the immunoregulatory activities of LL-37 in infected epithelia, this peptide may act as a direct antimicrobial effector and as a mediator of positive amplification loops of innate and immune responses, by participating in the recruitment of different types of leukocytes. Importantly, Koczulla and Bals [45] reported that LL-37 shows rapid, potent and broad-spectrum immunomodulatory activity, since this peptide acts synergistically with other antimicrobial peptides, stimulates endothelial cell proliferation by binding to FPRL-1, and functions as a chemoattractant for neutrophils, monocytes and CD4 T cells.

In addition to its direct chemotactic capacity, other important function of LL-37 to antimicrobial defense depends on its capacity to induce the secretion of chemokines including CXCL8 (IL-8) and CCL2 (MCP-1), which act indirectly in the recruitment of dendritic cells, monocytes and neutrophils to the site of injury. In recent years, LL-37 was shown to act in synergy with GM-CSF in the production of CXCL-8 by monocytes [14]. A more recent study reported that LL-37-enhanced production of CXCL-8 is under the control of ERK and p38 MAPKs in myeloid and epithelial cells [44]. In support of this, Zuyderduyn et al. [118] reported that human airway smooth muscle cells also respond to LL-37 by secreting CXCL8. In addition, data from experiments with lung epithelial cell lines demonstrates that LL-37 acts as a human host defense peptide that can induce CXCL-8 release [101,102]. Also, it should be considered that in addition to their capacity to induce chemokine secretion, the *in vivo* contribution of LL-37 to antimicrobial defense depends on its capacity to induce the secretion of proinflammatory cytokines, suggesting that direct migration of immune cells, and may be induced by this LL-37 through modulation of gene expression in target cells. In support of this, it has been demonstrated that LL-37 promotes the expression and release of inflammatory mediators such as IL-1β from monocytes via the activation of P2X7, which is a nucleotide receptor [3]. More recently, data from Yu et al. [113] showed a synergistic interaction between LL-37 and IL-1β to increase immune responses by activation of AKT, CREB and NF-

Table 1
Summary of biological activities of the LL-37 peptide against mycobacteria.

Activity	Mechanisms and signaling TLR activation	References
Antimicrobial activity	Membrane disruption and TLR activation	[34,51,54,57,58,80]
Chemotaxis of cells	FPRL1 receptor, Ca^{2+}-flux	[89]
Neutrophil antimicrobial functions	ERK1/2 and p38 MAPKs activation	[80,89]
Induction of immune mediators	PI3K, MAPKs and NF-kB activation	[108,110,111]
Regulation of inflammatory response	TLR and NF-kB signaling pathways	[110,111]

ERK1/2, extracellular signal-regulated kinases; FPRL1, N-formylpeptide receptor-like-1; MAPKs, mitogen-activated protein kinases; NF-kB, nuclear factor-kB; PI3K, phosphatidylinositol 3-kinase; TLR, Toll-like receptor.

κB pathways in human cells. Importantly, these authors reported that LL-37 synergistically enhances the IL-1β-induced production of chemokines such as CCL2 in human monocytes. Similarly, LL-37 synergistically increases the IL-1β-induced secretion of IL-6, IL-10 and CCL2 in human peripheral blood mononuclear cells. Furthermore, data from experiments with peptidoglycan and lipopeptides highlight that LL-37 stimulates IL-6 production by human dendritic cells [24]. In one recent report, it has been acknowledged that LL-37 strongly augments plasmacytoid dendritic cell IFN-α to self-DNA [47]. Importantly, it has been demonstrated the ability of the cathelicidin LL-37 to alter transcriptional responses, as assessed by gene array-based studies [86]. According to these studies, LL-37 at 50–100 μg/ml regulates a number of genes in the murine macrophage cell line RAW 264.7 and in the human epithelial cell line A549, some of which are proinflammatory cytokines and/or chemokines [86].

In addition to its capability to augment host defenses via indirectly amplify cell recruitment through modulation of gene expression in cells LL-37 can play a role in repair of damaged tissue by promoting reepithelialization of healing skin [38]. Furthermore, it has been demonstrated that LL-37 stimulates angiogenesis [116], induces proliferation of lung epithelial cells, and accelerates wound closure of the airway epithelium [100]. Importantly, it has been demonstrated that the immunomodulatory properties of cationic peptides, including their ability to induce chemotaxis [18,49,100], to induce histamine release by mast cells [100], to promote angiogenesis [2], and to modulate dendritic cell differentiation [2,16], may occur under physiological conditions. Furthermore, the fact that high concentrations of hCAP-18 have been detected in seminal plasma (range of 41.8–142.8 μg/ml from 10 healthy donors [55]) compared with the plasma lipoprotein-associated polypeptide (approximately 1.2 μg/ml plasma) [92], suggesting that this peptide could be present in sufficiently large amounts in some *in vivo* settings.

3. Role of hCAP18/LL-37 in the immune response against mycobacterial infection

At present, the importance of several antimicrobial peptides, including the cathelicidin hCAP18/LL-37, in protective immunity against mycobacterial infection has been demonstrated from their direct antimicrobial activity against mycobacteria (Table 1). In this context, a study by Martineau et al. showed that LL-37-induced a dose-dependent reduction in *Mycobacterium tuberculosis* CFU in iron-depleted broth that was maximal (15.7-fold) at 100 μg/ml ($P = 0.004$) [57]. In addition, these authors showed that a total of 200 μg/ml of the antimicrobial peptide LL-37 reduced the growth of *M. tuberculosis* in culture by 75.7% and 20 μg/ml reduced growth by 52.4%, indicating that this antimicrobial peptide may participate in the control of mycobacterial growth [58]. In support of this, it has been shown that the human monocytic cell line THP-1 express cathelicidin when incubated with $1,25D_3$, and that cathelicidin is required for $1,25D_3$-mediated antimicrobial activity against intracellular *M. tuberculosis* in human monocytes [54]. Importantly, these authors reported that $1,25D_3$-induced antimicrobial activity was completely inhibited in the presence of siRNA against cathelicidin, instead leading to enhanced intracellular growth of its bactericidal activity mycobacteria. A more recent study reported that blockade of LL-37 significantly increased the intracellular *Mycobacterium ulcerans* growth in keratinocytes, suggesting an important role of this antimicrobial peptide for cutaneous innate immune responses [51].

Besides its capacity to inhibit mycobacterial growth, the cathelicidin hCAP18/LL-37 exhibits its bactericidal activity against *M. tuberculosis*. Interestingly, Liu et al. [53] showed that Toll-like receptor (TLR) activation of monocytes induced killing of *M. tuberculosis* through LL-37 participation, and a vitamin-D mediated human antimicrobial responses against *M. tuberculosis* through the cathelicidin LL-37. These data are supported by a publication that demonstrated that the cathelicidin hCAP18/LL-37 has its direct antimicrobial activity and as an initiator of host response via TLRs [34]. Furtermore, Rivas-Santiago et al. [80] showed that human cells produce significant levels of LL-37 when infected with *M. tuberculosis* and that this peptide has important bactericidal activities against *M. tuberculosis* infection. Interestingly, these authors reported double immunolabeling of LAM and LL-37 inside alveolar macrophages phagosomal vesicles, indicating that many bacteria were killed and LL-37 could participate in this process [80] (Table 1).

Additionally, the important role of the cathelicidin hCAP18/LL-37 in the innate immune response against mycobacteria has been demonstrated, as concluded from the observation that mycobacterial infection induced the gene expression and protein secretion of LL-37 in several human cell types. It is well known that in mycobacterial infection alveolar macrophages are the main effector cell type in *M. tuberculosis* destruction in the lung, and that when bacilli enter the airways, the first cells that encounter the bacteria are alveolar macrophages and epithelial cells [3]. In this regard, Rivas-Santiago et al. [80] reported that alveolar macrophages infected with *M. tuberculosis* showed a significant percentage of LL-37-immunostained cells in a dose-dependent manner. In addition, these authors demonstrated that when lung epithelial cells were infected with *M. tuberculosis*, high levels of LL-37 were produced, primarily after 18 h, in a dose-dependent manner [80]. Interestingly, we showed that treatment of A549 epithelial cells with *M. bovis* bacillus Calmette-Guérin (BCG) upregulates LL-37 mRNA expression in a dose- and time-dependent manner [61]. This observation was supported by the fact that the quantitative analysis of LL-37 gene expression correlated with our Western blotting results [61]. A more recent study reported that exposing epidermal keratinocytes, which function in primary host defense against mycobacterial infection, to *M. ulcerans* resulted in significant production of the mRNA and protein expression of LL-37 [51].

It has also been demonstrated that neutrophils are one of the first cells to arrive at the infection site during mycobacterial infection and contribute substantially to innate resistance to tuberculosis infection [12,58]. Moreover, it has been reported that LL-37 is found at extremely high concentrations in the specific granules of neutrophils [92]. Furthermore, it has been demonstrated that neutrophil granules contain a variety of antimicrobial peptides at high levels, which have antimicrobial and chemotactic activities

against *M. tuberculosis* [89,98] (Table 1). Thus, during mycobacterial infection, high concentrations of LL-37 will be released at sites of neutrophil accumulation. In regard to mycobacteria-induced LL-37 expression, Rivas-Santiago et al. [80] have found that neutrophils efficiently produced LL-37 when infected with *M. tuberculosis*.

It is important to consider that others and we have previously shown that there is induction of human beta-defensin (HBD)-2 mRNA in epithelial A549 cells after infection with *M. tuberculosis* [79] and *M. bovis* BCG [59,60,62], indicating that different antimicrobial peptides may act synergistically against mycobacterial infection. In this context, it has been demonstrated that cathelicidin expression was required for the *M. tuberculosis*-induced release of ROS and the production of proinflammatory cytokines/chemokines, indicating a positive circuit of inflammation in response to *M. tuberculosis* [110]. These authors also demonstrated a regulatory mechanism for TLR2-dependent innate responses to *M. tuberculosis* involving crosstalk between nitric oxide synthesis and TLR2 and the expression of cathelicidin in human monocytes after mycobacterial infection [51]. In addition, Lee et al. showed that ROS generation and LL-37 expression are required for the antimicrobial activity against intracellular *M. ulcerans* in human keratinocytes [51]. In this regard, we showed previously that the production of cathelicidin LL-37 is coincident with the presence of superoxide generation [64]. Therefore, suggesting that the cathelicidin LL-37 and ROS may act synergistically to enhance direct killing of mycobacteria.

An understanding of the different biological functions of cathelicidin LL-37 in the immune resistance to mycobacteria requires an in depth knowledge of the signaling mechanisms regulating its production. Therefore, the discussion will describe the most recent findings regarding the transcriptional regulation of hCAPp18/LL-37. At present, it has been reported that a ligand for TLR-2, TLR-4 and TLR-9 induced strong production of LL-37 in monocytes stimulated with purified DNA from *M. tuberculosis* [80]. A more recent study reported that TLRs-2 and -4 and Dectin-1 are essential for the *M. ulcerans*-mediated expression of LL-37 in keratinocytes [51], suggesting that these receptors may provide an important mechanism for the mycobacteria-mediated expression of LL-37 in human cells.

On the other hand, some studies demonstrate that *M. tuberculosis* mediated cathelicidin expression is dependent on NADPH oxidase activity and superoxide production in human monocytic THP-1 cells [114]. In addition, we showed that *M. bovis* BCG-mediated upregulation of cathelicidn LL-37 is influenced by NADPH/ROS signaling pathways in human epithelial cells [64]. Taken together, these data suggest that the NADPH/ROS pathway play an essential role in mediating the mycobacteria-induced cathelicidin LL-37 production.

Recent studies have demonstrated that cathelicidin LL-37 expression is regulated via the MAPK signaling pathway. Schauber et al. [83] showed that inhibition of the ERK1/2 pathway blocked sodium butyrate-induced cathelicidin gene expression in colonic, gastric, and hepatic cells. A latter study demonstrated that the inhibition of p38 kinase pathway significantly decreased cathelicidin LL-37 gene expression in human keratinocytes [81]. Recently, our data showed that *M. bovis* BCG-stimulated LL-37 production can be regulated by both the ERK1/2 and p38 MAPK signaling pathways [61], indicating that the MEK1/2 and p38 MAPK signaling pathways play a critical role in the regulation of inducible LL-37 gene expression. In addition, our previous results demonstrated that infection of A549 cells by *M. bovis* BCG, the only tuberculosis vaccine currently available, results in the expression of cathelicidin LL-37 at both the mRNA and protein levels, and that this expression is at least in part mediated through activation of signaling protein of p38MAPK and MEK1/2 [61].

The current literature indicates that the cathelicidin LL-37 gene has potential binding sites for several transcription factors, including NF-kB, NF–interleukin-6 and AP-1 [108,111]. Therefore, it is possible the participation of some of these transcription factors in mycobacteria-induced LL-37 expression in human cells. Currently, several studies are exploring this possibility. The comprehension of the molecular signaling mechanisms in response to mycobacterial infection will assist in the rational design of more effective adjuvants.

4. Therapeutic use of the cathelicidin LL-37 in mycobacterial infection

At present, the antimycobacterial activities of the antimicrobial peptides have highlighted their potential as alternative therapeutic agents against mycobacteria. In one animal study, it has been reported the therapeutic potential of human neutrophil peptide 1 against tuberculosis [88]. In this context, Ashitani et al. [5] found that α-defensins may act as therapeutic agents against human tuberculosis. Recently, we have reported that the biological effects of the cathelicidin LL-37 to inhibit mycobacterial growth in human cells will aid in the development of novel therapeutic agents for treatment of mycobacterial infections, and to develop an alternative/adjunct therapeutic strategy against tuberculosis [61]. A latter study demonstrated that the cathelicidin LL-37 may be considered as preventive and therapeutic agent against mixed bacterial infections such as periodontitis by eliminating the pathogens themselves [97]. In addition, it has been shown previously that systemic administration of peptide derivatives of the cathelicidin hCAP18/LL-37 blunts the clinical consequences of septic shock in animal models, suggesting the approach from LL-37 gene transfer as a new strategy for developing treatment of infections [35]. In support of this, it has been shown that augmentation of the cathelicidin hCAP18/LL-37 by exogenous administration or transfer of the hCAP18 gene into mouse airways has been a useful experimental strategies to assess capability of this peptide to protect from microbial infection [8,10,11].

With respect to aid the clearance of intracellular infections, it has been proposed various strategies, including liposomes to burst upon endocytosis through the incorporation of pH-sensitive elements into the membrane bilayer [105]. However, despite being able to deliver a variety of molecule types *in vitro*, their *in vivo* use requires further development. It has also been proposed the use of microspheres composed of polymers, since may have some utility as an appropriate delivery system that can aid longevity of the therapeutic peptide to be more effective *in vivo*. This approach has produced some promising results in reducing the frequency of dosing of therapeutic peptides [29,43]. Currently, the *in vivo* use of antimicrobial peptides has mainly been by the direct application of these molecules via the topical route. In this regard, different methods for improving the resistance of peptides to proteases including the modification of the C-terminus and N-terminus of the peptides through amidation and acetylation and the replacement of amino acids considering the α-helical antimicrobial peptides to retain their biological activity, have been reported [40].

It is well known that the cathelicidin hCAP18/LL-37 is an important member of the host defense system, as it has a broad ability to kill microorganisms by disrupting the structure of microbial cell membranes, functions in inflammation, wound repair, and regulation of the adaptive immune system [18,30,45]. To date, there are several patents which have been related to the method of identifying peptides that have antimicrobial activity, including the use of cathelicidin LL-37 and its derivatives and/or synthetic analogs for wound healing [115].

A different approach utilises natural antimicrobial peptides as templates for the development of synthetic analogs with optimised biological functions, such as pexiganan acetate a synthetic analog of the cathelicidin LL-37 [46]. This approach represents an important

alternative for bacterial resistance to conventional antibiotics, such as the multidrug resistant mycobacterial pathogens. In addition, it has been reported that synthetic analogs from the cathelicidin LL-37 can be designed to enhance innate immunity through non-inflammatory mechanisms [65,66].

It is important to consider that further studies are needed to investigate the costs of peptide production, the possible toxicities that might accompany systemic administration of the cathelicidin LL-37, the develop of novel LL-37 analogs proposed as a rationale target for the therapy of various mycobacterial diseases, and the transition of these synthetic analogs from the laboratory into the clinic. However, the combination of the therapeutic use of the cathelicidin LL-37 or their synthetic analogs and conventional antimycobacterial drugs (e.g. isoniazid and rifampicin) might be a result of reduced the prolonged (6–9 mo) treatment courses in antituberculosis drug therapy which results hepatotoxic.

5. Conclusions

Substantial progress has been achieved in the past decade with respect to the mechanisms of the cathelicidin LL-37 functions and their complex role in our immune system. Along with the capacity of this peptide to directly kill mycobacteria and/or inhibiting mycobacterial growth, this peptide also boost specific innate immune responses and exert selective immunomodulatory functions on the host. Furthermore, their ability to interact and synergize *in vitro* to enhance the antimicrobial effects in experiments reflecting *in vivo* conditions has given insight into the full potential of the complex mixture of substances found at infection sites, strongly suggesting that the combined effort of different peptides greatly increases the global antimycobacterial action. Additionally, the capacity of LL-37 to modulate different responses connected with host defense, such as chemotaxis of inflammatory and immune cells, and activation of defense cells, indicates a high level of integration of this effector molecule with other innate immune mechanisms.

Since the regulatory signaling pathways of mycobacteria-induced LL-37 production can potentially be exploited therapeutically, in the near future, it will be important issues to clarify. For example, an important issue to be elucidated is the clear dissection of the mechanisms that control the tissue-specific expression of the hCAP18/LL-37 gene. It will also be possible to gain further insights into the establishment of the receptors as well as signaling pathways mediating the different aspects of peptide activity and to explore its interactions with other immune mediators in the wider context of immune response. In addition, further *in vivo* studies are needed to determine how these effects globally impact host processes. Finally, an important challenge over the next decade will be an intensification of clinical studies of the cathelicidin LL-37, due to the growing problem of drug resistant in mycobacteria. A better understanding of expression and regulation of the cathelicidin LL-37 in mycobacterial infection will potentially assist the practical use of this peptide as an important therapeutic agent against mycobacterial infectious diseases.

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References

[1] Agerberth B, Gunne H, Odeberg J, Kogner P, Boman HG, Gudmundsson GH. FALL-39, a putative human peptide antibiotic, is cysteine-free and expressed in bone marrow and testis. Proc Natl Acad Sci USA 1995;92:195–9.
[2] Agerberth B, Charo J, Werr J, Olsson B, Idali F, Lindbom L, Kiessling R, Jornvall H, Wigzell H, Gudmundsson GH. The human antimicrobial and chemotactic peptides LL-37 and alpha-defensins are expressed by specific lymphocyte and monocyte populations. Blood 2000;96:3086–93.
[3] Agerberth B, Gudmundsson GH. Host antimicrobial defence peptides in human disease. Curr Top Microbiol Immunol 2006;306:67–90.
[4] Andersson E, Sorensen OE, Frohm B, Borregaard N, Egesten A, Malm J. Isolation of human cationic antimicrobial protein-18 from seminal plasma and its association with prostasomes. Hum Reprod 2002;17:2529–34.
[5] Ashitani J, Mukae I, Hiratsuka T, Nakazato M, Kumamoto K, Matsakura S. Elevated levels of α-defensins in plasma and BAL fluid of patients with active pulmonary tuberculosis. Chest 2002;121:519–26.
[6] Ashitani J, Matsumoto N, Nakazato M. Elevated levels of antimicrobial peptides in bronchoalveolar lavage fluid in patients with chronic eosinophilic pneumonia. Respiration 2007;74:69–75.
[7] Bals R, Wang X, Zasloff M, Wilson JM. The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. Proc Natl Acad Sci USA 1998;95:9541–6.
[8] Bals R, Weiner DJ, Meegalla RL, Wilson JM. Transfer of a cathelicidin peptide antibiotic gene restores bacterial killing in a cystic fibrosis xenograft model. J Clin Invest 1999;103:1113–7.
[9] Bals R, Weiner DJ, Moscioni AD, Meegalla RL, Wilson JM. Augmentation of innate host defense by expression of a cathelicidin antimicrobial peptide. Infect Immun 1999;67:6084–9.
[10] Bals R. Epithelial antimicrobial peptides in host defense against infection. Respir Res 2000;1:141–50.
[11] Bals R, Wilson JM. Cathelicidins-a family of multifunctional antimicrobial peptides. Cell Mol Life Sci 2003;60:711–20.
[12] Barrios-Payan J, Aguilar-Leon D, Lascurain-Ledezma R, Hernandez-Pando R. Neutrophil participation in early control and immune activation during experimental pulmonary tuberculosis. Gac Med Mex 2006;142:273–81.
[13] Bergman P, Johansson L, Asp V, Plant L, Gudmundsson GH, Jonsson AB, Agerberth B. Neisseria gonorrhoeae downregulates expression of the human antimicrobial peptide LL-37. Cell Microbiol 2005;7:1009–17.
[14] Bowdish DM, Davidson DJ, Speert DP, Hancock RE. The human cationic peptide LL-37 induces activation of the extracellular signal-regulated kinase and p38 kinase pathways in primary human monocytes. J Immunol 2004;172:3758–65.
[15] Bowdish DME, Davidson DJ, Hancock REW. A re-evaluation of the role of host defence peptides in mammalian immunity. Curr Protein Pept Sci 2005;6:35–51.
[16] Bowdish DM, Davidson DJ, Hancock RE. Immunomodulatory properties of defensins and cathelicidins Curr Top Microbiol Immunol 2006;306:27–66.
[17] Braff MH, Zaiou M, Fierer J, Nizet V, Gallo RL. Keratinocyte production of cathelicidin provides direct activity against bacterial skin pathogens. Infect Immun 2005;73:6771–81.
[18] Carretero M, Escámez MJ, García M, Duarte B, Holguín A, Retamosa L, Jorcano JL, Río MD, Larcher F. In vitro and in vivo wound healing promoting activities of human cathelicidin LL-37. J Invest Dermatol 2008;128:223–36.
[19] Chromek M, Slamova Z, Bergman P, Kovacs L, Podracka L, Ehren I, Hokfelt T, Gudmundsson GH, Gallo RL, Agerberth B, Brauner A. The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection. Nat Med 2006;12:636–41.
[20] Cowland JB, Johnsen AH, Borregaad N. hCAP-18, a cathelin/pro-bactenecin-like protein of human neutrophil specific granules. FEBS Lett 1995;368:173–6.
[21] De Smet K, Contreras R. Human antimicrobial peptides: defensins, cathelicidins and histatins. Biotechnol Lett 2005;27:1337–47.
[22] De Yang, Chen Q, Schmidt AP, Anderson GM, Wang JM, Wooters J, Oppenheim J, Chertov O. LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. J Exp Med 2000;192:1069–74.
[23] Di Nardo A, Vitiello A, Gallo RL. Cutting edge: mast cell antimicrobial activity is mediated by expression of cathelicidin antimicrobial peptide. J Immunol 2003;170:2274–8.
[24] Di Nardo A, Braff MH, Taylor KR, Na C, Granstein RD, McInturff JE, Krutzik S, Modlin RL, Gallo RL. Cathelicidin antimicrobial peptides block dendritic cell TLR4 activation and allergic contact sensitization. J Immunol 2007;178:1829–34.
[25] Dorschner RA, Pestonjamasp VK, Tamakuwala S, Ohtake T, Rudisill J, Nizet V, Agerberth B, Gudmundsson GH, Gallo RL. Cutaneous injury induces the release of cathelicidin anti-microbial peptides active against group A Streptococcus. J Invest Dermatol 2001;117:91–7.
[26] Dorschner RA, Lin KH, Murakami M, Gallo RL. Neonatal skin in mice and humans expresses increased levels of antimicrobial peptides: innate immunity during development of the adaptive response. Pediatr Res 2003;53:566–72.
[27] Durr UH, Sudheendra US, Ramamoorthy A. LL-37, the only human member of the cathelicidin family of antimicrobial peptides. Biochim Biophys Acta 2006;1758:1408–25.
[28] Eckmann L. Defence molecules in intestinal innate immunity against bacterial infections. Curr Opin Gastroenterol 2005;21:147–51.
[29] Elvin SJ, Eyles JE, Howard KA, Ravichandran E, Somavarappu S, Alpar HO, Williamson ED. Protection against bubonic and pneumonic plague with a single dose microencapsulated sub-unit vaccine. Vaccine 2006;24:4433–9.

[30] Fahy RJ, Wewers MD. Pulmonary defense and the human cathelicidin hCAP-18/LL-37. Immunol Res 2005;31:75–89.
[31] Frohm M, Gunne H, Bergman AC, Agerberth B, Bergman T, Boman A, Liden S, Jornvall H, Boman HG. Biochemical and antibacterial analysis of human wound and blister fluid. Eur J Biochem 1996;237:86–92.
[32] Frohm M, Agerberth B, Ahangari G, Stahle-Backdahl M, Liden S, Wigzell H, Gudmundsson GH. The expression of the gene coding for the antimicrobial Peptide LL-37 is induced in human keratinocytes during inflammatory disorders. J Biol Chem 1997;272:15258–63.
[33] Frohm Nilsson M, Sandstedt B, Sorensen O, Weber G, Borregaard N, Stahle-Backdahl M. The human cationic antimicrobial protein (hCAP-18), a peptide antibiotic, is widely expressed in human squamous epithelia and colocalizes with interleukin-6. Infect Immun 1999;67:2561–6.
[34] Gilliet M, Lande R. Antimicrobial peptides and self-DNA in autoimmune skin inflammation. Curr Opin Immunol 2008;20:401–7.
[35] Giroir B, Quint P, Barton P, Kirsch E, Kitchen L, Goldstein B, Nelson B, Wedel N, Carroll S, Scanon P. Preliminary evaluation of recombinant amino-terminal fragment of human bactericidal/permeabilityincreasing protein in children with severe meningicoccal sepsis. Lancet 1997;350:1439–42.
[36] Gudmundsson GH, Agerberth B, Odeberg J, Bergman T, Olsson B, Salcedo R. The human gene FALL-39 and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes. Eur J Biochem 1996;238: 325–32.
[37] Hase K, Eckmann L, Leopard JD, Varki N, Kagnoff MF. Cell differentiation is a key determinant of cathelicidin LL-37/human cationic antimicrobial protein 18 expression by human colon epithelium. Infect Immun 2002;70:953–63.
[38] Heilborn JD, Nilsson MF, Kratz G, Weber G, Sorensen O, Borregaard N, Stahle-Backdahl M. The cathelicidin anti-microbial peptide LL-37 is involved in re-epithelialization of human skin wounds and is lacking in chronic ulcer epithelium. J Invest Dermatol 2003;120:379–89.
[39] Henzler Wildman KA, Lee DK, Ramamoorthy A. Mechanism of lipid bilayer disruption by the human antimicrobial peptide, LL-37. Biochemistry 2003;42:6545–58.
[40] Hong SY, Oh JE, Lee KH. Effect of D-amino acid substitution on the stability, the secondary structure, and the activity of membrane-active peptide. Biochem Pharmacol 1999;58:1775–80.
[41] Jenssen H, Hamill P, Hancock REW. Peptide antimicrobial agents. Clin Microbiol Rev 2006;19:491–511.
[42] Johansson J, Gudmundsson GH, Rottenberg ME, Berndt KD, Agerberth B. Conformation-dependent antibacterial activity of naturally occurring human Peptide LL-37. J Biol Chem 1998;273:3718–24.
[43] Kemp SF, Fielder PJ, Attie KM, Blethen SL, Reiter EO, Ford KM, Marian M, Dao LN, Lee HJ, Saenger P. Pharmacokinetic and pharmacodynamic characteristics of a longacting growth hormone (GH) preparation (nutropin depot) in GHdeficient children. J Clin Endocrinol Metab 2004;89:3234–40.
[44] Khine AA, Del Sorbo L, Vaschetto R, Voglis S, Tullis E, Slutsky AS, Downey GP, Zhang H. Human neutrophil peptides induce interleukin-8 production through the P2Y6 signaling pathway. Blood 2006;107:2936–42.
[45] Koczulla AR, Bals R. Antimicrobial peptides: current status and therapeutic potential. Drugs 2003;63:389–406.
[46] Lamb HM, Wiseman LR. Pexiganan acetate. Drugs 1998;56:1047–52.
[47] Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang YH, Homey B, Cao W, Wang YH, Su B, Nestle FO, Zal T, Mellman I, Schröder JM, Liu YJ, Gilliet M. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature 2007;449:564–9.
[48] Larrick JW, Morgan JG, Palings I, Hirata M, Yen MH. Complementary DNA sequence of rabbit CAP18-a unique lipopolysaccharide binding protein. Biochem Biophys Res Commun 1991;179:170–5.
[49] Larrick JW, Hirata M, Balint RF, Lee J, Zhong J, Wright SC. Human CAP18: a novel antimicrobial lipopolysaccharide binding protein. Infect Immun 1995;63:1291–7.
[50] Laube DM, Yim S, Ryan LK, Kisich KO, Diamond G. Antimicrobial peptides in the airway. Curr Top Microbiol Immunol 2006;306:153–82.
[51] Lee JS, Yang CS, Shin DM, Yuk JM, Son JW, Jo EK. Nitric oxide synthesis is modulated by 1,25-dihydroxyvitamin D3 and interferon-gamma in human macrophages after mycobacterial infection. Immune Netw 2009;9:192–202.
[52] Lehrer R, Lichtenstein A, Ganz T. Defensins: antimicrobial and cytotoxic peptides of mammalian cells. Annu Rev Immunol 1993;11:105–28.
[53] Liu PT, Stenger S, Li H, Wenzel L, Tan B, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zügel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006;311:1770–3.
[54] Liu PT, Stenger S, Tang DH, Modlin RL. Cutting edge: vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin. J Immunol 2007;179:2060–3.
[55] Malm J, Sorensen O, Persson T, Frohm-Nilsson M, Johansson B, Bjartell A, Lilja H, Stahle-Backdahl M, Borregaard N, Egesten A. The human cationic antimicrobial protein (hCAP-18) is expressed in the epithelium of human epididymis, is present in seminal plasma at high concentrations, and is attached to spermatozoa. Infect Immun 2000;68:4297–302.
[56] Marchini G, Lindow S, Brismar H, Stabi B, Berggren V, Ulfgren AK, Lonne-Rahm S, Agerberth B, Gudmundsson GH. The newborn infant is protected by an innate antimicrobial barrier: peptide antibiotics are present in the skin and vernix caseosa. Br J Dermatol 2002;147:1127–34.
[57] Martineau AR, Newton SM, Wilkinson KA, Kampmann B, Hall BM, Nawroly N, Packe GE, Davidson RN, Griffiths CJ, Wilkinson RJ. Neutrophil-mediated innate immune resistance to mycobacteria. J Clin Invest 2007;117:1988–94.
[58] Martineau AR, Wilkinson KA, Newton SM, Floto RA, Norman AW, Skolimowska K, Davidson RN, Sørensen OE, Kampmann B, Griffiths CJ, Wilkinson RJ. IFN-gamma- and TNF independent vitamin D-inducible human suppression of mycobacteria: the role of cathelicidin LL-37. J Immunol 2007;178:7190–8.
[59] Méndez-Samperio P, Miranda E, Trejo A. Mycobacterium bovis Bacillus Calmette-Guérin (BCG) stimulates human beta-defensin-2 gene transcription in human epithelial cells. Cell Immunol 2006;239:61–6.
[60] Méndez-Samperio P, Alba L, Trejo A. Mycobacterium bovis-mediated induction of human beta-defensin-2 in epithelial cells is controlled by intracellular calcium and p38MAPK. J Infect 2007;54:469–74.
[61] Méndez-Samperio P, Miranda E, Trejo A. Expression and secretion of cathelicidin LL-37 in human epithelial cells after infection by Mycobacterium bovis Bacillus Calmette-Guérin. Clin Vaccine Immunol 2008;15:1450–5.
[62] Méndez-Samperio P, Miranda E, Trejo A. Regulation of human beta-defensin-2 by Mycobacterium bovis bacillus Calmette-Guérin (BCG): involvement of PKC, JNK, and PI3K in human lung epithelial cell line (A549). Peptides 2008;29:1657–63.
[63] Méndez-Samperio P. Role of antimicrobial peptides in host defense against mycobacterial infections. Peptides 2008;29:1836–41.
[64] Méndez-Samperio P, Pérez A, Torres L. Role of reactive oxygen species (ROS) in Mycobacterium bovis bacillus Calmette Guérin-mediated up-regulation of the human cathelicidin LL-37 in A549 cells. Microb Pathog 2009;47:252–7.
[65] Mookherjee N, Rehaume LM, Hancock RE. Cathelicidins and functional analogues as antisepsis molecules. Expert Opin Ther Targets 2007;11:993–1004.
[66] Mookherjee N, Lippert DN, Hamill P, Falsafi R, Nijnik A, Kindrachuk J, Pistolic J, Gardy J, Miri P, Naseer M, Foster LJ, Hancock RE. Intracellular receptor for human host defense peptide LL-37 in monocytes. J Immunol 2009;183:2688–96.
[67] Murakami M, Ohtake T, Dorschner RA, Schittek B, Garbe C, Gallo RL. Cathelicidin anti-microbial peptide expression in sweat, an innate defense system for the skin. J Invest Dermatol 2002;119:1090–5.
[68] Murakami M, Ohtake T, Dorschner RA, Gallo RL. Cathelicidin antimicrobial peptides are expressed in salivary glands and saliva. J Dent Res 2002;81:845–50.
[69] Nell MJ, Tjabringa GS, Vonk MJ, Hiemstra PS, Grote JJ. Bacterial products increase expression of the human cathelicidin hCAP-18/LL-37 in cultured human sinus epithelial cells. FEMS Immunol Med Microbiol 2004;42:225–31.
[70] Nijnik A, Pistolic J, Wyatt A, Tam S, Hancock RE. Human cathelicidin peptide LL-37 modulates the effects of IFN-gamma on APCs. J Immunol 2009;183:5788–98.
[71] Niyonsaba F, Ogawa H. Protective roles of the skin against infection: implication of naturally occurring human antimicrobial agents beta-defensins, cathelicidin LL-37 and lysozyme. J Dermatol Sci 2005;40:157–68.
[72] Niyonsaba F, Nagaoka I, Ogawa H. Human defensins and cathelicidins in the skin: beyond direct antimicrobial properties. Crit Rev Immunol 2006;26:545–76.
[73] Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, Dorschner RA, Pestonjamasp V, Piraino J, Huttner K, Gallo RL. Innate antimicrobial peptide protects the skin from invasive bacterial infection. Nature 2001;414:454–7.
[74] Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, Gallo RL, Leung DY. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. N Engl J Med 2002;347:1151–60.
[75] Oren Z, Lerman JC, Gudmundsson GH, Agerberth B, Shai Y. Structure and organization of the human antimicrobial peptide LL-37 in phospholipid membranes: relevance to the molecular basis for its non-cell-selective activity. Biochem J 1999;341:501–13.
[76] Overhage J, Campisano A, Bains M, Torfs EC, Rehm BH, Hancock RE. Human host defense peptide LL-37 prevents bacterial biofilm formation. Infect Immun 2008;76:4176–82.
[77] Putsep K, Carlsson G, Boman HG, Andersson M. Deficiency of antibacterial peptides in patients with morbus Kostmann: an observation study. Lancet 2002;360:1144–9.
[78] Ramanathan B, Davis EG, Ross CR, Blecha F. Cathelicidins: microbicidal activity, mechanisms of action, and roles in innate immunity. Microbes Infect 2002;4:361–72.
[79] Rivas-Santiago B, Schwander SK, Sarabia C, Diamond G, Klein-Patel ME, Hernandez-Pando R, Ellner JJ, Sada E. Human beta-defensin 2 is expressed and associated with Mycobacterium tuberculosis during infection of human alveolar epithelial cells. Infect Immun 2005;73:4505–11.
[80] Rivas-Santiago B, Hernandez-Pando R, Carranza C, Juarez E, Contreras JL, Aguilar-Leon D, Torres M, Sada E. Expression of cathelicidin LL-37 during Mycobacterium tuberculosis infection in human alveolar macrophages, monocytes, neutrophils, and epithelial cells. Infect Immun 2008;76:935–41.
[81] Sayama K, Komatsuzawa H, Yamasaki K, Shirakata Y, Hanakawa Y, Ouohara K, Tokumaru S, Dai X, Tohyama M, Ten Dijke P, Sugai M, Ichijo H, Hashimoto K. New mechanisms of skin innate immunity: ASK1-mediated keratinocyte differentiation regulates the expression of beta-defensins, LL37, and TLR2. Eur J Immunol 2005;35:1886–95.
[82] Schaller-Bals S, Schulze A, Bals R. Increased levels of antimicrobial peptides in tracheal aspirates of newborn infants during infection. Am J Respir Crit Care Med 2002;165:992–5.

[83] Schauber J, Iffland K, Frisch S, Kudlich T, Schmausser B, Eck M, Menzel T, Gostner A, Luhrs H, Scheppach W. Histone-deacetylase inhibitors induce the cathelicidin LL-37 in gastrointestinal cells. Mol Immunol 2004;41:847–54.
[84] Schauber J, Gallo RL. Antimicrobial peptides and the skin immune defense system. J Allergy Clin Immunol 2008;122:261–6.
[85] Schmidtchen A, Frick IM, Andersson E, Tapper H, Bjorck L. Proteinases of common pathogenic bacteria degrade and inactivate the antibacterial peptide LL-37. Mol Microbiol 2002;46:157–68.
[86] Scott MG, Davidson DJ, Gold MR, Bowdish D, Hancock RE. The human antimicrobial peptide LL-37 is a multifunctional modulator of innate immune responses. J Immunol 2002;169:3883–91.
[87] Selsted ME, Ouellette AJ. Mammalian defensins in the antimicrobial immune response. Nat Immunol 2005;6:551–7.
[88] Sharma S, Verma I, Khuller GK. Therapeutic potential of human neutrophil peptide 1 against experimental tuberculosis. Antimicrob Agents Chemother 2001;45:639–40.
[89] Silva MT, Silva MN, Appelberg R. Neutrophil-macrophage cooperation in the host defence against mycobacterial infections. Microb Pathog 1989;6:369–80.
[90] Smeianov V, Scott K, Reid G. Activity of cecropin P1 and FALL-37 against urogenital microflora. Microbes Infect 2000;2:773–7.
[91] Soehnlein O, Zernecke A, Eriksson EE, Rothfuchs AG, Pham CT, Herwald H, Bidzhekov K, Rottenberg ME, Weber C, Lindbom L. Neutrophil secretion products pave the way for inflammatory monocytes. Blood 2008;112:1461–71.
[92] Sorensen O, Cowland JB, Askaa J, Borregaard N. An ELISA for hCAP-18, the cathelicidin present in human neutrophils and plasma. J Immunol Methods 1997;206:53–9.
[93] Sorensen O, Arnljots K, Cowland JB, Bainton DF, Borregaard N. The human antibacterial cathelicidin, hCAP-18, is synthesized in myelocytes and metamyelocytes and localized to specific granules in neutrophils. Blood 1997;90:2796–803.
[94] Sorensen O, Bratt T, Johnsen AH, Madsen MT, Borregaard N. The human antibacterial cathelicidin, hCAP-18, is bound to lipoproteins in plasma. J Biol Chem 1999;274:22445–51.
[95] Sorensen OE, Follin P, Johnsen AH, Calafat J, Tjabringa GS, Hiemstra PS, Borregaard N. Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. Blood 2001;97:3951–9.
[96] Sorensen OE, Gram L, Johnsen AH, Andersson E, Bangsboll S, Tjabringa GS, Hiemstra PS, Malm J, Egesten A, Borregaard N. Processing of seminal plasma hCAP-18 to ALL-38 by gastricsin: a novel mechanism of generating antimicrobial peptides in vagina. J Biol Chem 2003;278:28540–6.
[97] Lee Sung-Hoon, Jun Hye-Kyoung, Lee Hae-Ri, Chung Chong-Pyoung, Choi Bong-Kyu. Antibacterial and lipopolysaccharide (LPS)-neutralising activity of human cationic antimicrobial peptides against periodontopathogens. Int J Antimicrob Agents 2010;35:138–45.
[98] Tan BH, Meinken C, Bastian M, Bruns H, Legaspi A, Ochoa MT, Krutzik SR, Bloom BR, Ganz T, Modlin RL, et al. Macrophages acquire neutrophil granules for antimicrobial activity against intracellular pathogens. J Immunol 2006;177:1864–71.
[99] Tanaka D, Miyasaki KT, Lehrer RI. Sensitivity of Actinobacillus actinomycetemcomitans and Capnocytophaga spp. to the bactericidal action of LL-37: a cathelicidin found in human and epithelium. Oral Microbiol Immunol 2000;15:226–31.
[100] Termen S, Tollin M, Olsson B, Svenberg T, Agerberth B, Gudmundsson GH. Phylogeny, processing and expression of the rat cathelicidin rCRAMP: a model for innate antimicrobial peptides. Cell Mol Life Sci 2003;60: 536–49.
[101] Tjabringa GS, Aarbiou J, Ninaber DK, Drijfhout JW, Sorensen OE, Borregaard N, Rabe KF, Hiemstra PS. The antimicrobial peptide LL-37 activates innate immunity at the airway epithelial surface by transactivation of the epidermal growth factor receptor. J Immunol 2003;171:6690–6.
[102] Tjabringa GS, Rabe KF, Hiemstra PS. The human cathelicidin LL-37: a multifunctional peptide involved in infection and inflammation in the lung. Pulm Pharmacol Ther 2005;18:321–7.
[103] Travis SM, Anderson NN, Forsyth WR, Espiritu C, Conway BD, Greenberg EP, McCray Jr PB, Lehrer RI, Welsh MJ, Tack BF. Bactericidal activity of mammalian cathelicidin-derived peptides. Infect Immun 2000;68:2748–55.
[104] Turner J, Cho Y, Dinh NN, Waring AJ, Lehrer RI. Activities of LL-37, a cathelin-associated antimicrobial peptide of human neutrophils. Antimicrob Agents Chemother 1998;42:2206–14.
[105] Vives E, Schmidt J, Pelegrin A. Cell-penetrating and cell targeting peptides in drug delivery. Biochim Biophys Acta 2008;1786:126–38.
[106] Wang G, Li X, Wang Z. APD2: the updated antimicrobial peptide database and its application in peptide design. Nucleic Acids Res 2009;37:D933–7.
[107] Wang Y, Agerberth B, Lothgren A, Almstedt A, Johansson J. Apolipoprotein A-I binds and inhibits the human antibacterial/cytotoxic peptide LL-37. J Biol Chem 1998;273:33115–8.
[108] Whitmarsh AJ, Davis RJ. Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. J Mol Med 1996;74:589–607.
[109] Woo JS, Jeong JY, Hwang YJ, Chae SW, Hwang SJ, Lee HM. Expression of cathelicidin in human salivary glands. Arch Otolaryngol Head Neck Surg 2003;129:211–4.
[110] Yang CS, Shin DM, Kim KH, Lee ZW, Lee CH, Park SG, Bae YS, Jo EK. NADPH oxidase 2 interaction with TLR2 is required for efficient innate immune responses to mycobacteria via cathelicidin expression. J Immunol 2009;182:3696–705.
[111] Yang D, Chertov O, Oppenheim JJ. The role of mammalian antimicrobial peptides and proteins in awakening of innate host defenses and adaptive immunity. Cell Mol Life Sci 2001;58:978–89.
[112] Yoshioka M, Fukuishi N, Ku Y, Yamanobe H, Ohsaki K, Kawasoe Y, Murata M, Ishzumi A, Nishi Y, et al. Human cathelicidin CAP18/LL-37 changes mast cell function toward innate immunity. Biol Pharm Bull 2008;31:212–6.
[113] Yu J, Mookherjee N, Wee K, Bowdish DM, Pistolic J, Li Y, Rehaume L, Hancock RE. Host defense peptide LL-37, in synergy with inflammatory mediator IL-1beta, augments immune responses by multiple pathways. J Immunol 2007;179:7684–91.
[114] Yuk JM, Shin DM, Lee HM, Yang CS, Jin HS, Kim KK, Lee ZW, Lee SH, Kim JM, Jo EK. Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. Cell Host Microbe 2009;6:231–43.
[115] Zaiou M. Multifunctional antimicrobial peptides: therapeutic targets in several human diseases. J Mol Med 2007;85:317–29.
[116] Zanetti M, Gennaro R, Romeo D. Cathelicidins: a novel protein family with a common proregion and a variable C-terminal antimicrobial domain. FEBS Lett 1995;374:1–5.
[117] Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2001;415:389–95.
[118] Zuyderduyn S, Ninaber DK, Hiemstra PS, Rabe KF. The antimicrobial peptide LL-37 enhances IL-8 release by human airway smooth muscle cells. J Allergy Clin Immunol 2006;117:1328–35.