Review

Spotlight on Human LL-37, an Immunomodulatory Peptide with Promising Cell-Penetrating Properties

Michèle Seil 1, Carole Nagant 1, Jean-Paul Dehaye 1, Michel Vandenbranden 2 and Marc Ferdinand Lensink 3,*

1 Laboratoire de Chimie Biologique et Médicale et de Microbiologie Pharmaceutique, Institut de Pharmacie, Université Libre de Bruxelles, Boulevard du Triomphe, CP 205/3, B-1050 Brussels, Belgium
2 Laboratoire de Structure et Fonction des Membranes Biologiques, Faculté des Sciences, Université Libre de Bruxelles, Boulevard du Triomphe, CP 206/2, B-1050 Brussels, Belgium
3 Biological Nanosystems, Interdisciplinary Research Institute, University of Sciences and Technology Lille, USR3078 CNRS, 50 Avenue Halley, F-59658 Villeneuve d’Ascq, France

* Author to whom correspondence should be addressed; E-Mail: Marc.Lensink@iri.univ-lille1.fr; Tel.: +33-3-6253-1733; Fax: +33-3-6253-1701.

Received: 4 October 2010; in revised form: 23 October 2010 / Accepted: 29 October 2010 / Published: 1 November 2010

Abstract: Cationic antimicrobial peptides are major components of innate immunity and help control the initial steps of the infectious process. They are expressed not only by immunocytes, but also by epithelial cells. They share an amphipathic secondary structure with a polar cationic site, which explains their tropism for prokaryote membranes and their hydrophobic site contributing to the destructuration of these membranes. LL-37 is the only cationic antimicrobial peptide derived from human cathelicidin. LL-37 can also cross the plasma membrane of eukaryotic cells, probably through special domains of this membrane called lipid rafts. This transfer could be beneficial in the context of vaccination: the activation of intracellular toll-like receptors by a complex formed between CpG oligonucleotides and LL-37 could conceivably play a major role in the building of a cellular immunity involving NK cells.

Keywords: antimicrobial peptides; biofilm; P2X7 receptors; formyl peptide receptors; cell-penetrating peptides; LL-37; cathelicidin
1. Introduction

Epithelia are constantly exposed to potential pathogens. The digestive tract, the upper respiratory airways, the genital tract and the skin are, among others, body surfaces interacting with the surroundings and very often a door to aggression by a pathogen. The response of adaptative immunity to this aggression is highly efficient and very often successful in fighting an infection, but adaptation is slow and linked to delay. In a first step, cells producing membrane-anchored antibodies with reasonable affinity to the antigen have to be selected. In a second step, these antibodies are secreted in the extracellular medium. The tremendous dilution of these proteins decreases their concentration to extremely low values, which jeopardizes their interaction with the antigen. The dilution must be compensated by an increase of the affinity of the protein for the antigen. This affinity maturation is the consequence of a very high mutational rate of the gene, a high proliferation rate of the cells and a very strong selective pressure. This process is considered a paradigm for evolution, but at a timescale of a few days rather than a few million years [1]. A similar selection and maturation process for T lymphocytes contributes to the delay of a strong and specific attack of the pathogens. During the building and shaping of this adaptative immunity, the pathogens would have plenty of time to multiply and would reach, after a few days, such a number that they would provoke the death of the invaded body. This would render adaptative immunity a very elegant but futile response to the lethal aggression. The innate immunity prevents this fatal issue. The mechanisms of innate immunity are designed to block or at least to delay the progression of the infection. This very rapid response is triggered by the recognition by specialized cells like antigen-presenting cells of unusual molecular patterns which are specifically associated with pathogens. Among the various responses elicited by local infections, antimicrobial peptides have generated great interest since their discovery by the group of Boman in 1980 [2]. During these last three decades the number of antimicrobial peptides has been constantly increasing and by October 2010 the antimicrobial Peptide Database created by Wang and Wang [3] contained 1,628 references. These peptides are designed to kill bacteria, viruses and fungi. They interact with their targets in a rather non-specific way which explains why the pathogens have some difficulty to elaborate mechanisms of resistance against these peptides. More recently new modes of action of these peptides have been discovered and at concentrations which are bactericidal they also activate eukaryotic cells. Among other responses they contribute to the mobilization of immunocytes at the site of aggression. These peptides originally described as antimicrobials are now considered as “alarmins” [4]. In mammals two main families of antimicrobial peptides have been described: the defensins [5] and the cathelicidins [6]. Other peptides with antimicrobial properties have also been described in humans. For instance histatins are salivary peptides rich in histidine residues and which have a strong fungicidal activity [7]. Neuropeptides like VIP have also microbicidal
properties [8]. Lactoferricin is a cationic peptide which is derived from lactoferrin. It is present in mucosal secretions and in milk [9]. Dermicidin is secreted as a precursor by sweat glands and is activated by cathepsin D [10]. Some antimicrobial peptides are derived from proteins which have apparently no obvious role in innate immunity as the amyloid beta protein associated with Alzheimer’s Disease [11], proenkephalin-A [12], apolipoproteins A, B or E [13-15], growth factors [16] or members of the complement system or superoxide dismutase [17]. After a brief description of defensins and cathelicidins, this review will focus mostly on LL-37, the only human antimicrobial peptide derived from cathelicidin.

2. Defensins

Defensins are small (approximately 4 kDa), cysteine-rich, cationic peptides which have been identified in plants, insects, and a large variety of higher-level mammals [5]. They have been classified in three distinct families: α-, β- and θ-defensins. The θ-defensins are circular peptides derived from α-defensins. DEFT, the gene for θ-defensins, probably arose from duplication of the gene for α-defensin in Old World apes. It mutated after divergence of the orangutan and hominid lineages. These mutations introduced stop codons in the open reading frame and suppressed the synthesis of the peptide [18]. θ-Defensin is thus not expressed in man and it has been suggested that the absence of this defensin is responsible for our sensitivity to HIV infection [18]. The two other families of defensins are expressed in man. These peptides contain six cysteine residues forming three disulfide bridges. These bridges not only play a role in the structure of the peptides but also contribute to their resistance to intracellular and extracellular proteases [19,20]. The spacing between the cysteine residues and the topology of the disulfide bonds is the rationale for the distinction of these peptides in two families, the α-defensins and the β-defensins [21].

The genes for α- and β-defensins are located on chromosome 8. They form a cluster within a < 1 Mb region at the locus p22-23 [22]. Defensins are produced as immature peptides, the pre-prodefensins, that contain a signal sequence at the N-terminus, followed by a propeptide sequence, and the mature defensin peptide at the C-terminus [23]. After the cleavage of the signal peptide the pro-defensin is released in the endoplasmic reticulum. The α-prodefensin has an N-terminal sequence of about 40 amino acids. This prosequence is anionic which probably accounts for the binding of the mature defensin to the propeptide. α-Prodefensin acts as a chaperone and contributes to the structure of mature defensin. It also blocks the activity of the mature defensin preventing damages to the producing cells [24]. In man, the α-prodefensin has no microbicidal activity and its activation requires its proteolysis by serine proteases like trypsin [25], kallikrein [26] or metalloproteases [27]. The α-defensins have 29-35 amino acids. Four isoforms are found in granules of neutrophils and two isoforms are expressed in Paneth cells of the small intestine [28]. The β-defensins are also synthetized as prepropeptides. The N-terminal extension of the proform has a very short amino acid sequence; the proform has bactericidal activity [27] and even some cellular toxicity [29]. The mature peptides have ~ 40 residues. At least six isoforms have been isolated [30]. They are expressed in epithelia, the testis and the epididymis [31]. Both α- and β-defensins share a common motif termed the γ-core. It is composed of two antiparallel β-sheets with basic residues polarized along its axis [32].
3. Cathelicidins

The first antimicrobial peptide of this group was isolated from pig [33]. The term cathelicidins was introduced in 1995 by Zanetti et al. [34] to describe molecules containing both a cathelin domain and a C-terminal antimicrobial domain. Cathelin is an acronym for cathepsin L inhibitor. The human cathelicidin has 18 kDa (hCAP-18) and is a major protein in specific granules of neutrophils [35]. It is also present in subpopulations of lymphocytes and monocytes, in squamous epithelia, in epididymis [36] and in the lung [37,38]. Several resident cells of the skin like keratinocytes, mast cells or sebocytes also express hCAP-18 [39-41]. Plasma contains a high concentration of hCAP-18 bound to lipoproteins [42]. The pre-proregion of cathelicidins has 128-145 residues: a signal peptide with 29-30 residues and a proregion with 99-114 residues (Figure 1). This proregion shows a high intra-species identity ranging from 75 to 100% homologies between species. Four invariant cysteinyl residues in the C-terminal region of the cathelin-like domain form two intramolecular disulfide bridges.

Figure 1: Structure of LL-37. From top to bottom: Location of the cathelicidin gene on the human genome and its structure. Global structure of the pre-propeptide and primary structure of hCAP-18. Sequence of various fragments of LL-37 and model representing the secondary alpha-helicoidal structure of LL-37 [94].

Considering the conservation of this proregion during evolution, it might play an important biological function with respect to the maturation of the antimicrobial peptide which is the C-terminal domain of the protein. Zaiou et al. [43] reported that the cathelin domain had also potent antibacterial activity. The C-terminal domain of cathelicidin is very variable among species. In man, the only peptide derived from hCAP has 37 amino acids and two leucines at its N-terminal, hence the acronym LL-37 [44]. The human gene coding for hCAP-18 is named CAMP. This gene is located on
chromosome 3 at the p21 locus [45], a locus frequently deleted in gastric carcinoma [46]. This might explain why the concentration of the peptide is reduced in human gastric adenocarcinomas [47,48]. The full peptide was first isolated from bone marrow [49,50], then from the secretions of neutrophils [49]. The release of LL-37 from its precursor is mediated by proteinase 3 [51] or elastase [49]. In vagina, gastricsin, a prostatic protease, releases a peptide with 38 amino acids, ALL-38 [52]. In human sweat the mature LL-37 has been shown to be degraded to shorter peptic fragments, including RK-31, KS-30 and KR-20 [53]. These fragments are generated by two distinct kallikreins [54]. These natural and more recently synthetic fragments of LL-37 have contributed to a better understanding of the full peptide. LL-37 has broad bactericidal activity toward both Gram-negative and Gram-positive bacteria [55]. It neutralizes LPS, it has synergistic antibacterial effects with the defensins [56] and it is a chemotactic agent for neutrophils, monocytes and T cells using the formyl peptide receptor-like 1 (FPRL-1) [57]. It also interacts with a wide panel of other plasma membrane receptors and has access to some intracellular compartments of eukaryotic cells. However, LL-37 is also cytotoxic towards mammalian cells [58].

4. Effects of LL-37 on Prokaryotes

Patients with atopic dermatitis suffer not only from chronic cutaneous inflammation but are also affected by recurrent infections provoked by bacteria, viruses or fungi [59]. The skin of these patients is characterized by a decreased expression of defensins and LL-37, suggesting a role for these peptides in skin protection [60]. Drugs increasing the local concentration of antimicrobial peptides have thus been logically proposed as a treatment for atopic dermatitis [61]. The role of antimicrobial peptides is fully supported by experiments with transgenic animals. *Salmonella typhimurium* survive better in macrophages from mice which do not express the cathelicidin related antimicrobial peptide (CRAMP), the murine analog of LL-37, than from wild-type (WT) mice [62]. These mice are also more prone to infections of the skin by *Staphylococcus aureus* [40] or to meningococcal infections of the central nervous system [63] and to infections of the urinary tract [64]. Conversely, Bals *et al.* [65] demonstrated that mice overexpressing LL-37 had a lower bacterial load and reduced inflammatory response in the lung after a challenge with *Pseudomonas aeruginosa*. They also showed that the transfer of the LL-37/hCAP18 gene restored bacterial (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) killing in a human cystic fibrosis bronchial xenograft model [66]. Cathelicidin is highly expressed at barrier sites including respiratory and colonic epithelium, saliva, and skin. At these locations it constitutes an important first line defense mechanism for the innate immune system to respond to infectious aggressions [67]. It is active in the micromolar concentration range, with a higher tropism for Gram-negative than for Gram-positive bacteria [68].

4.1. Structure of LL-37 and Biophysical Studies

At the structural level, efforts were made to understand how LL-37 and related peptides interact with the membrane and what type of perturbation they may cause. To have access to the molecular level, simplified systems were used, comprising phospholipid bilayers made of a limited series of commonly found phospholipids. Since cathelicidins have a positive balance of cationic amino acid side-chains, the focus was put on the possible difference between zwitterionic and acidic phospholipid-containing membranes.
The interpretation of initial circular dichroic studies on the peptide diluted in pure water led to conclude that LL-37 adopted a random conformation in aqueous solution. It became rapidly obvious that the peptide could structure itself either in the presence of the salts found in physiological fluids [58] or at higher concentrations of the peptide which seemed to favour structuring oligomerization as demonstrated by chemical cross-linking experiments [69]. In the presence of a phospholipid bilayer or short-tail phospholipid micelles, LL-37 preferentially adopts an alpha-helical conformation as shown by FTIR [69,70], circular dichroism [70-73] and NMR [73-76]. The mean orientation of the helix is closely parallel to the bilayer surface. This was shown independently by solid-state NMR on macroscopically aligned DMPC bilayer samples where the helical part of the structure forms an angle of about 72° with the bilayer normal [73] and by polarized ATR-FTIR spectroscopy on oriented bilayers [69,70]. As the alpha-helix is amphipatic, it is expected to bury its hydrophobic residues in the hydrophobic part of the bilayer while the polar/charged residues should stay at the level of the lipid polar headgroups. This topology is consistent with NMR data [69,74]. How this topology could explain the toxic effect of cathelicidins is less clear (see next paragraph on the mode of action of LL-37 on prokaryotes). The weak point of the spectroscopic techniques used to determine the peptide topology results from their inability to detect minor but perhaps significant subpopulations of other topological arrangements that would be responsible for the membrane-destabilizing or lytic activity. Furthermore, the main question of course is to evaluate the role of a direct membrane-destabilizing effect over a more indirect effect that relies on the activation of some cell signalling cascades, possibly via membrane receptors (see the section on the effects of LL-37 on eukaryotic cells). Experimental results on liposomes do support the existence of a direct permeabilizing effect on phospholipid bilayers [69,74,77], but only a few lipid compositions were used and therefore the role of some specific membrane lipids might have been overlooked. The question of a selective destabilizing effect of cathelicidins towards bacterial versus eukaryotic membranes was partly addressed at the lipid level, using lipids present in both types of organisms, but which are not fully exposed to the outer membrane leaflet, such as the acidic phospholipid phosphatidylserine (PS), phosphatidylglycerol (PG) and the non-bilayer forming unsaturated phosphatidylethanolamine (PE), the latter being abundant in prokaryotes. Whereas it was initially demonstrated that similar leakage occurred in zwitterionic palmitoyl-oleoyl-phosphatidylcholine (POPC) vesicles as well as in charged palmitoyl-oleoyl-phosphatidyl serine/palmitoyl-oleoyl-phosphatidylcholine (POPS/POPC) vesicles when considering K⁺ permeabilization [69], further studies demonstrated a preference for negatively charged vesicles composed of palmitoyl-oleoyl-phosphatidylglycerol (POPG) as compared to neutral zwitterionic POPC vesicles when larger molecules such as calcine were considered [71]. In another study, a mixture of neutral lipids (PC/sphingomyelin/cholesterol) (PC:SM:CHOL) seemed equally susceptible as acidic lipids phosphatidylglycerol/diposphatidylglycerol towards a leakage assay of a mixture of 8-amino-napththalene-1,3,6-trisulfonic acid/p-xylene-bis-pyridinium bromide (ANTS/DPX) with a slight preference for the PC:SM:CHOL mixture at low peptide concentration, especially with the orang-utan orthologue of the human LL-37 [70].

In accordance with typical antimicrobial peptide behavior, cholesterol diminished LL-37 induced leakage in the study of Zhang et al. [71], whereas it has no such effect in the study of Morgera et al. [70]. From theses results and others, it seems difficult at present to confirm any clear lipid preference for LL-37 that would explain a selective effect on bacteria over mammalian cells [78]. Clearly, other
features of the membrane lipid composition should be taken into account, such as the presence of LPS or peptidoglycans in the bacterial wall or complex glucosaminoglycans in the case of mammalian cells and perhaps the transmembrane electrical potential. Other parameters than membrane leakage could play a significant role such as lipid clustering [79] or membrane thickening effects [80]. Many studies failed to show a clear discrimination in the toxic effect on prokaryotes and eukaryotes, although some LL-37 orthologues seem generally at least an order of magnitude more effective towards bacteria [72]. Demonstration of such an effect is complicated by the way toxicity is measured in both cases. Indeed, while the focus is put on the peptide concentration, its ratio to the total lipid mass may vary considerably, bacteria being usually tested at a lower cell concentration [81].

4.2. Mode of Action of LL-37 on Prokaryotes

LL-37 is a peptide with 35% hydrophobic residues. It has a high content of basic residues (five arginines; six lysines) and at neutral pH it has a positive charge (+6). In spite of their very diverse primary structures, antimicrobial peptides seem to share some common structural characteristics (see previous paragraph on the structure of LL-37 and biophysical studies). They all form amphipathic secondary structures with a cationic and a hydrophobic face [82,83]. This characteristic seems mandatory for their interaction with the bacterial membranes [84,85,70]. Three mechanisms have been suggested for the permeation of the target cell membrane by antibacterial peptides. A barrel stave mechanism involves the formation of transmembrane channels in a voltage-dependent manner with nonpolar domains of the molecules facing the membrane lipids and forming a hydrophilic pore spanning the membrane [86]. In the aggregate channel model, intracellular components leak out of the cells through transient pores formed by peptides in unstructured clusters in the membrane. In the carpet-like mechanism the antimicrobial peptides cover the cell membrane. The bending of the lipid bilayer on itself forms holes and destroys the integrity of the membrane [87]. LL-37 binds to bacterial membrane [88]. At high pH or in the presence of detergents or lipids LL-37 forms an alpha-helix covering residues 2 to 31 [58,69,89,90] with a cationic and a hydrophobic side. The formation of this helix increases the rigidity of the entire molecule and correlates with its lytic potency [70]. Only residues 33 to 37 are mobile and do not contribute to the interaction with the membrane [91]. These authors also showed that the interaction with a membrane stabilizes the alpha-helix formed by LL-37. Oren et al. [69] have suggested that LL-37 permeabilizes the bacterial membrane by a carpet-like mechanism. LL-37 is able to aggregate and to form a tetramer [92]. KR-20, the fragment of LL-37 after removal of the 17 residues from the N-terminal is still able to aggregate. In contrast, deletion of the 14 C-terminal residues greatly reduces the aggregation of LL-23 [92]. KE-18 also forms an alpha-helix [50,93] and inhibits the interaction between LPS with CD14 [94]. When LL-37 is cut into two fragments, only the C-terminal fragment (IG-25) retains the toxic activity on bacteria and eukaryotic cells [91]. The same group also showed that removal of residues not essential for the interaction of this peptide with micelles generates FK-16, the fragment of LL-37 running from F17 to V32. This fragment has an even better activity against prokaryotes and nucleated cells than the full peptide or than IG-25. Removal of the C-terminal tripeptide from FK-16 yields FK-13, a peptide nearly devoid of any activity on bacteria and eukaryotic cells. KR-12 has three arginines and two lysines. It covers the cationic rich region of LL-37. This peptide is the smallest fragment of LL-37 that is still active against Gram-negative bacteria [90]. A longer fragment, GF-17 has antibacterial activity against both Gram-
positive and Gram-negative bacteria [95]. This difference is best explained by different interactions with membrane lipids. KR-12 functions by separating the anionic and zwitterionic lipids in the membrane of Gram-negative bacteria. This lateral-phase separation is impossible in Gram-positive bacteria which have mostly anionic lipids. RI-10, the fragment obtained after removal of the amino acid at both ends of KR-12 has no activity [90]. The longer fragment (GF-17) can not only promote the lateral-phase separation but also the permeabilization of anionic membranes explaining why it is active on both types of bacteria. LL-37 has lost the ability to induce lipid segregation probably because of the rigidity of its alpha-helix. The formation of intramolecular ionic interactions might also prevent cationic residues to interact with anionic lipids.

4.3. Interaction of LL-37 Derivatives with Lipid Bilayers

As discussed in the previous section, the mode of action of LL-37 requires the peptide to adopt an amphipathic helix conformation. However, shortened versions like KR-12, FK-16, and especially KR-20, are not amphipathic per se, and may resemble classical cell-penetrating peptides in both structure and mode of action. These peptides, with the 16-residue penetratin as their prototypical representative, have been the subject of many studies over the past decade [96] and are suggested to have a common mechanism of action [97]. Despite continued effort, the precise mechanism of internalization remains at present unknown. A receptor and endocytosis-independent pathway is hypothesized [98] with peptide-lipid interactions governing the translocation mechanism [99]. For pep-1, a cell-penetrating peptide with particularly high uptake efficiency rates, initial peptide adsorption was shown to be a crucial factor in the translocation mechanism [100]. Molecular dynamics simulations of penetratin have shown this association to be a fast process, driven by electrostatic interactions, with the presence of negatively charged lipids significantly enhancing binding of the peptide to the membrane [101]; binding to phospholipids was also shown to be tight for CADIY [102], a secondary amphipathic peptide used for delivery of siRNA [103]. Surprisingly perhaps, NMR studies of several homeodomain-derived cell-penetrating peptides in membrane-mimicking SDS micelles show uptake efficiency to be highest for the least deeply inserted peptide [104], although this does confirm the importance of electrostatic surface interactions. It is now commonly accepted that cell-penetrating peptides destabilize the lipid bilayer and that this destabilization is due to specific peptide-lipid interactions. This may lead to the formation of aqueous toroidal pores [105]. Adsorption of penetratin on zwitterionic lipid bilayers, as modelled by molecular dynamics simulation, shows the peptide to introduce surface curvature, leading to the formation of lipid vesicles that encapsulate the protein [106]. However, these simulations were performed in the absence of negatively charged phospholipids and may lead to an alternative translocation mechanism involving a non-lamellar lipid morphology [107]. Investigation of the role of lipid composition on membrane perturbation confirms the capacity to increase membrane curvature and form lipid vesicles, but also demonstrates a remodelling of the membrane [97] and a general ability to induce phospholipid domain separation [98]. The role of electrostatics is evidenced by the importance of negatively charged lipids [100,101,108] and their interaction with the cationic peptides [105,109] and in fact hydrophobic forces seem to play no major role in binding [110]. The free energy cost of insertion of a single penetratin helix into a DPPC bilayer was estimated to be about 75 kJ/mol [106], making spontaneous diffusion across the bilayer a prohibitively slow process, but local charge matching and pairing of the ion charges between peptide
and lipids show an important improvement of the theoretical transfer energies [111]. The formation of a peptide-lipid structure was also proposed for the energy-independent translocation of pep-1 [99]. The key of the mechanism of action of cell-penetrating peptides is then to lie in their abundance of cationic residues, where arginine seems to favour internalization over lysine [110-112]. Although membrane pore formation remains a controversial issue [99], solid-state NMR measurements provide direct evidence for toroidal pores in the action of PG-1, an arginine-rich antimicrobial peptide [108], with the driving force for this formation being the complexation between the arginine guanidinium and lipid phosphate functional groups [113]. Although arginine-phosphate interactions play a stronger role in the lipid-assisted translocation of cell-penetrating peptides versus lysine [109], they are not required for efficient internalization [112]. Nevertheless, both uptake efficiency and bilayer destabilization are directly related to arginine content [111,114]. The stronger interaction with lipid phosphate groups of arginine over lysine results in a more efficient destabilization of the lipid bilayer, a condition that seems to be required for any of the proposed translocation mechanisms [114]. Complexation of arginine and phosphate combined with bilayer undulations allow the insertion of charged side chains in the bilayer core that nucleate the formation of a transient, short-lived pore [105,115], allowing for the diffusion of arginine-rich cell-penetrating peptides and their cargo.

4.4. Mechanisms of Resistance of Prokaryotes to LL-37

LL-37 interacts with the membrane of bacteria mostly by electrostatic interactions. The outer membrane of many Gram-negative bacteria contains LPS with lipid A, an anionic lipid forming the outer leaflet of the membrane. The transfer of aminoarabinose [116] to lipid A or its acylation [117] constitutes an alteration of the LPS which decreases the net negative charges of the membrane. Gram-positive bacteria are enveloped by a thick wall rich in teichoic acid and peptidoglycan which explains its electronegativity. Coupling of alanine via an ester bond to the chain of teichoic acid introduces the positive charge of the amino group of the amino acid [118]. The synthesis of a derivative of phosphatidylglycerol containing a residue of lysine is another mechanism developed by Gram-positive bacteria to resist to LL-37 [119]. Some bacteria secrete proteases which inactivate LL-37 [120]. In some cases the resistance to the peptide is mediated by an efflux pump driven by a proton-motive-force and which usually confers resistance to dyes or disinfectants [121]. Other bacteria like *Streptococcus pyogenes* secrete a protein which binds to and neutralizes LL-37 [122]. Inhibition is sometimes exerted at the transcriptional level [123].

4.5. Effect of LL-37 on Biofilms

Bacteria spend most of their life not in planktonic conditions but within a biofilm. In a first step the bacteria adhere to a support. Planktonic and adherent bacteria have a different transcriptome [124]. The cells engaged in the process of biofilm formation express enzymes involved in the synthesis and secretion of exopolysaccharides and proteins contributing to the expansion of the biofilm [125]. The expansion phase of the biofilm impairs the transport of nutrients and oxygen. This phase is paralleled by an increased number of bacteria which exacerbates the metabolic stress exerted on bacteria. This is followed by the rupture of the biofilm and the release of bacteria which recover their initial transcriptome and start to colonize the support at various other locations [126]. The development of a biofilm is very often associated with a decreased sensitivity to treatment [127,128]. The resistance to antibiotics can be
Pharmaceuticals 2010, 3

secondary to interaction of the drug with the extracellular matrix which decreases its concentration in the vicinity of the bacteria [129]. Bacteria also start to express some proteins contributing to their resistance to antibiotics [130]. The polysaccharides which are major components of the biofilms are rich in negative charges and neutralize cationic antimicrobial peptides by electrostatic interactions [131,132]. However, recent results suggest that biofilms might not always be an absolute protection for the bacteria against antimicrobial peptides. BMPA-28, which is a 28-amino acid cathelicidin, also inhibited the formation of a biofilm by Staphylococcus aureus on intravenous catheters [133]. Overhage et al. [134] reported that very low concentrations of LL-37 inhibited the formation of biofilms by Pseudomonas aeruginosa in vitro. It also affected preexisting biofilms. Instillation of LL-37 also inhibited the formation of a biofilm by Pseudomonas aeruginosa in a model of sinusitis developed in rabbits [135]. More recently, Hell et al. [136] reported that infra-bactericidal concentrations of LL-37 inhibited the attachment of Staphylococcus epidermidis in wells of microtitre plates and the formation of a biofilm by these bacteria. Amer et al. [137] used a similar protocol to demonstrate that very low concentrations of LL-37 also inhibited the formation of a biofilm by Francisella.

5. Effects of LL-37 on Eukaryotic Cells

The original description of cationic peptides as antimicrobial agents put the emphasis on their deleterious effects on bacteria. The specificity of cationic antimicrobial peptides for bacterial membranes was explained by the anionic properties of bacterial surfaces [138]. The high cholesterol content of the plasma membrane of eukaryotic cells did not prevent the binding of LL-37 but blocked its penetration in the bilayer [139]. Any effect on eukaryotic cells was considered as a side-effect inasmuch as high concentrations of the peptides proved to be cytolytic on these cells [140]. It was thus mandatory to design peptides with the highest bactericidal activity at concentrations without any toxic activity on eukaryotic cells. The discovery that LL-37 was chemotactic for neutrophils, monocytes and T cells created a new bridge between innate and adaptive immunity. It was suggested that the peptide could bind to the formyl peptide receptor-like 1 (FPRL-1), a G-protein coupled receptor (GPCR) [57]. In line with this hypothesis CRAMP was chemotactic for HEK-293 cells transfected with the human or murine form of FPRL-1 confirming that these receptors mediated the response to LL-37 [57]. The activation of FPRL-1 by LL-37 was also responsible for the anti-apoptotic effect of the peptide on neutrophils [141]. The peptide promoted angiogenesis by interacting with the FPRL-1 receptor expressed by endothelial cells [142]. Two other GPCR have more recently been linked to LL-37. Zhang et al. [143] reported that LL-37 could selectively down-regulate the CXCR2 receptor on human neutrophils. LL-37 also elicited all the responses coupled to this receptor suggesting that it was a full agonist. Brandenburg et al. [144] observed that a metabotropic purinergic receptor, the P2Y11 receptor of glial cells, was activated by LL-37. This interaction provoked the expression of various cytokines and the activation of phosphorylation pathways. The activation of mast cells by LL-37 might also be mediated by a GPCR. Indeed, the peptide was chemotactic for these cells and stimulated their degranulation [145] and their production of prostaglandins [146]. These responses were blocked by inhibitors of GPCR signalling pathways [147]. Elssner et al. [148] reported that LL-37 could stimulate the release of IL-1β by LPS-primed monocytes. This response was blocked by inhibitors of P2X7 receptors. It was not secondary to the release of ATP since apyrase, an enzyme which catabolizes extracellular ATP, had no effect on the response to LL-37. These results suggested that the P2X7
receptor was a target for LL-37. This receptor is an ionotropic purinergic receptor with two transmembrane domains [149]. After activation with ATP, they form a homotrimer or even a heterotrimer with P2X<sup>4</sup> receptors [150]. More recently, Tomasinsig <i>et al.</i> [151] correlated the ability of cathelicidins to activate P2X<sub>7</sub> receptors with their ability to form an alpha-helix and to aggregate. LL-37 also provokes cell proliferation and wound healing [152]. It can also be the trigger for tumour proliferation [153-156]. The mechanism involved in the response is complex. It might be secondary to the activation of a still-uncharacterized GPCR responsible for the activation of a transmembrane metalloprotease which belongs to the family of ADAM [157]. This activated protease could contribute to the ectodomain shedding of ligands which might transactivate receptors for EGF (EGFR) and promote cell proliferation.

These multiple effects of LL-37 and the diversity of plasma membrane receptors which might be affected by the peptide raised some doubts on a direct interaction between these receptors and the peptide [40,158]. For instance LL-37 might regulate P2X<sub>7</sub> receptors by modifying the physico-chemical state of the membrane rather than by directly interacting with the receptor. Such a model has been proposed by Zughaier <i>et al.</i> [159] and by Di Nardo <i>et al.</i> [160] to explain the regulation of TLR4 by LL-37. This hypothesis is supported by the decrease of the anisotropy of plasma membranes of eukaryotic cells exposed to the peptide [161]. This decrease reflects a modification (increase) of the fluidity of the plasma membrane. LL-37 also modifies the cellular metabolism of phospholipids by activating phospholipase A<sub>2</sub> and by inhibiting phospholipase D [161]. According to Rao [162], these effects are generally observed with membrane active peptides and are best explained by a modification of the packaging of the lipid. The LL-37 might thus regulate the cellular function by modifying the physico-chemical state of the membrane rather than by interacting with a plasma membrane receptor, a model proposed by Perregaux <i>et al.</i> [163], who suggested that the cationic peptides activate macrophages by perturbing their plasma membrane. LL-37 might also regulate the activity of P2X<sub>7</sub> receptors by binding to the intracellular C-terminal domain of the receptor [164]. Indeed, contrary to the six other P2X receptors, P2X<sub>7</sub> has a very long C-terminal extension through which this receptor interacts with intracellular proteins [165]. In 2004 Sandgren <i>et al.</i> reported that the peptide could cross the plasma membrane [166]. The peptide used special domains of the plasma membrane called lipid rafts. Rafts are rich in cholesterol and sphingolipids explaining why these zones are rather rigid [167]. Such a transport might explain how LL-37 interacts with an intracellular receptor. It has indeed been recently proposed that in monocytes LL-37 binds to glyceradehyde 3-phosphate dehydrogenase, an enzyme of the glycolytic pathway [168]. According to these authors, this interaction regulates the p38 MAPK and cytokine production.

6. LL-37, a Cell-Penetrating Peptide

LL-37 belongs to the cell-penetrating peptide family [166,169]. These peptides have been classified into arginine-rich peptides and amphipathic peptides [170]. Penetratin, the peptide derived from the third helix of the homeodomain of the drosophile transcription factor Antennapedia without the N-terminal glutamate (RQIKIWFQNRRMKWKK) (peptide 43-58) [171], and the HIV1-Tat derived nonapeptide (RKKRRQRRR) [172], the first cell-penetrating peptides described, belong to the arginine-class of peptides. Similar sequences are found in the A chain of the Shiga toxin from <i>Shigella dysenteriae</i> (RFRQIQRGFR) or in the A chain of ricin from <i>Ricinus communis</i> (RTRIRYNRR) [173].
In solution, these cell-penetrating peptides adopt a random coil structure [174,175]. In a lipidic environment like membranes they adopt alpha-helical or beta-sheet secondary conformations [176-178]. Penetratin has a chameleon-like behaviour and modifies its conformation according to the lipid/peptide ratio or the charges on the surface. At high ratio or in the presence of low surface charge, the peptide has mostly an alpha-helical structure which transforms to beta-sheet at lower ratio or higher surface charge [179]. These peptides are internalized through two major mechanisms [180]. They might cross the plasma membrane through translocation, an energy-independent mechanism [181,182], or be internalized by endocytosis which originates at lipid rafts [183]. The internalization mechanism of the two classes of peptides differs: arginine-rich peptides seem to follow the endocytosis and vesicular pathway [184]. The intracellular location of the peptides has been a matter of debate. It was originally suggested that they had access to the nucleus [185], but this is apparently a post-fixation artefact [186] and the peptides seem to localize either in a compartment of endosomal vesicles or in the whole cytoplasm [187]. This is a very important issue when considering pharmaceutical applications of these peptides. They have indeed been considered as Trojan horses for the transmucosal or transmembrane transfer of molecules like peptides or proteins, polysaccharides, ions, nucleic acids [188]. These peptides share with LL-37 its antimicrobial or antifungal properties [189,190]. Conversely Lande et al. [191] showed that LL-37 was able to form a complex with human DNA and to transfer the nucleic acid to the endosomes. Zhang et al. [192] confirmed that LL-37 could mediate the cell delivery of oligonucleotides. Recently, Hurtado and Au Peh [193] reported that LL-37 promoted the rapid delivery of bacterial CpG motifs to human B lymphocytes and pDC. This response was independent of the amphipathic and bactericidal properties of LL-37 since GL-37, a scrambled analog, could reproduce the response to LL-37. These results suggested that the combination of CpG with LL-37 was a very efficient combination to stimulate TLR9. This intracellular receptor is expressed by macrophages, conventional dendritic cells and pDC and B lymphocytes. Its activation triggers a cascade of events which greatly improves adaptive immunity [194]. Indeed, stimulated pDC secrete type I IFN [195] which activates cytotoxic and helper T cells [196] and NK cells [197]. Activated cDC stimulate pDC and NK cells [198]. They also promote the differentiation of Th0 lymphocytes to the Th1 phenotype [199]. The activated B lymphocytes proliferate and secrete immunoglobulins of the M or G type rather than allergic immunoglobins [200]. The injection of a mixture of CpGs and LL-37 to mice which had been treated with ovarian cancer cells enhanced the survival of the animals when compared to treatment with either CpG or LL-37 alone [201]. Tumours grow faster in cathelicidin knockout mice. NK cells from these mice had impaired cytotoxic activity toward tumour cells [202]. These promising results suggest that LL-37 or some analogs might be very potent adjuvants [203,204]. They should however be confirmed inasmuch as Nijnik et al. [205] reported that LL-37 inhibited the immune responses in response to IFN-γ.

7. Conclusions and Perspectives

LL-37 was originally described as an antimicrobial peptide. The development of analogs of the peptide with increased resistance to proteases and less cytotoxicity on eukaryotic cells, or the synthesis of molecules mimicking LL-37 is a promising strategy to eradicate multiresistant bacteria [206]. Low, non-cytotoxic concentrations of LL-37 have now proven to be also effective on eukaryotic cells. The topic application of the peptide on skin wounds or bedsores should promote wound healing. But the
immunomodulatory properties of the peptide secondary to its regulation of intracellular receptors probably offer the most fascinating perspectives.

Acknowledgements

This work was supported by grants 3.4.528.07.F and 3.4577.10 from the Fonds National de la Recherche Scientifique (FNRS) to JPD and MV. CN is a research fellow and MV a research associate of the FNRS.

References

1. Neuberger M.S. Novartis Medal Lecture. Antibodies: a paradigm for the evolution of molecular recognition. Biochem. Soc. Trans. 2002, 30, 341-350.
2. Hultmark, D.; Steiner, H.; Rasmussen, T.; Boman, H.G. Insect immunity. Purification and properties of three inducible bactericidal proteins from hemolymph of immunized pupae of Hyalophora cecropia. Eur. J. Biochem. 1980, 106, 7-16.
3. Wang, G.; Li, X.; Wang, Z. APD2: the updated antimicrobial peptide database and its application in peptide design. Nucleic. Acids Res. 2009, 37, D933-D937.
4. Oppenheim, J.J.; Tewary, P.; de la Rosa, G.; Yang, D. Alarmins initiate host defense. Adv. Exp. Med. Biol. 2007, 601, 185-194.
5. Ganz, T. Defensins: antimicrobial peptides of innate immunity. Nat. Rev. Immunol. 2003, 3, 710-720.
6. Bals, R.; Wilson, J.M. Cathelicidins-a family of multifunctional antimicrobial peptides. Cell. Mol. Life Sci. 2003, 60, 711-720.
7. Kavanagh, K.; Dowd, S. Histatins: antimicrobial peptides with therapeutic potential. J. Pharm. Pharmacol. 2004, 56, 285-289.
8. Brogden, K.A.; Guthmiller, J.M.; Salzet, M.; Zasloff, M. The nervous system and innate immunity: the neuropeptide connection. Nat. Immunol. 2005, 6, 558-564.
9. Gifford, J.L.; Hunter, H.N.; Vogel, H.J. Lactoferricin: a lactoferrin-derived peptide with antimicrobial, antiviral, antitumor and immunological properties. Cell. Mol. Life Sci. 2005, 62, 2588-2598.
10. Baechle, D.; Flad, T.; Cansier, A.; Steffen, H.; Schittek, B.; Tolson, J.; Herrmann, T.; Dihazi, H.; Beck, A.; Mueller, G.A.; Mueller, M.; Stevanovic, S.; Garbe, C.; Mueller, C.A.; Kalbacher, H. Cathepsin D is present in human eccrine sweat and involved in the postsecretory processing of the antimicrobial peptide DCD-1L. J. Biol. Chem. 2006, 281, 5406-5415.
11. Soscia, S.J.; Kirby, J.E.; Washicosky, K.J.; Tucker, S.M.; Ingelsson, M.; Hyman, B.; Burton, M.A.; Goldstein, L.E.; Duong, S.; Tanzi, R.E.; Moir, R.D. The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. PLoS One 2010, 5, e9505.
12. Goumon, Y.; Lugardon, K.; Kieffer, B.; Lefèvre, J.F.; Van Dorsselraer, A.; Aunis, D.; MetzBoutigue, M.H. Characterization of antibacterial COOH-terminal proenkephalin-A-derived peptides (PEAP) in infectious fluids. Importance of enkelytin, the antibacterial PEAP209-237 secreted by stimulated chromaffin cells. J. Biol. Chem. 1998, 273, 29847-29856.
13. Dobson, C.B.; Sales, S.D.; Hoggard, P.; Wozniak, M.A.; Crutcher, K.A. The receptor-binding region of human apolipoprotein E has direct anti-infective activity. J. Infect. Dis. 2006, 193, 442-450.
14. Kelly, B.A.; Harrison, I.; McKnight, A.; Dobson, C.B. Anti-infective activity of apolipoprotein domain derived peptides in vitro: identification of novel antimicrobial peptides related to apolipoprotein B with anti-HIV activity. BMC Immunol. 2010, 11, 13.

15. Srinivas, R.V.; Venkatachalapathi, Y.V.; Rui, Z.; Owens, R.J.; Gupta, K.B.; Srinivas, S.K.; Anantharamaiah, G.M.; Segrest, J.P.; Compans, R.W. Inhibition of virus-induced cell fusion by apolipoprotein A-I and its amphipathic peptide analogs. J. Cell. Biochem. 1991, 45, 224-237.

16. Malmsten, M.; Davoudi, M.; Schmidtchen, A. Bacterial killing by heparin-binding peptides from PRELP and thrombospondin. Matrix Biol. 2006, 25, 294-300.

17. Pasupuleti, M.; Walse, B.; Nordahl, E.A.; Mörgelin, M.; Malmsten, M.; Schmidtchen, A. Preservation of antimicrobial properties of complement peptide C3a, from invertebrates to humans. J. Biol. Chem. 2007, 282, 2520-2528.

18. Nguyen, T.X.; Cole, A.M.; Lehrer, R.I. Evolution of primate theta-defensins: a serpentine path to a sweet tooth. Peptides 2003, 24, 1647-1654.

19. Maemoto, A.; Qu, X.; Rosengren, K.J.; Tanabe, H.; Henschen-Edman, A.; Craik, D.J.; Ouellette, A.J. Functional analysis of the alpha-defensin disulfide array in mouse cryptdin-4. J. Biol. Chem. 2004, 279, 44188-44196.

20. Rajabi, M.; de Leeuw, E.; Pazgier, M.; Li, J.; Lubkowski, J.; Lu, W. The conserved salt bridge in human alpha-defensin 5 is required for its precursor processing and proteolytic stability. J. Biol. Chem. 2008, 283, 21509-21518.

21. Ganz, T.; Lehrer, R.I. Defensins. Curr. Opin. Immunol. 1994, 4, 584-589.

22. Jia, H.P.; Schutte, B.C.; Schudy, A.; Linzmeier, R.; Guthmiller, J.M.; Johnson, G.K.; Tack, B.F.; Mitros, J.P.; Rosenthal, A.; Ganz, T.; McCray, P.B., Jr. Discovery of new human beta-defensins using a genomics-based approach. Gene 2001, 263, 211-218.

23. Yang, D.; Chertov, O.; Bykovskaia, S.N.; Chen, Q.; Buffo, M.J.; Shogan, J.; Anderson, M.; Schröder, J.M.; Wang, J.M.; Howard, O.M.; Oppenheim, J.J. Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science 1999, 286, 525-528.

24. Michaelson, D.; Rayner, J.; Couto, M.; Ganz, T. Cationic defensins arise from charge neutralized propeptides: a mechanism for avoiding leukocyte autotoxicity? J. Leukoc. Biol. 1992, 51, 634-639.

25. Ghosh, D.; Porter, E.; Shen, B.; Lee, S.K.; Wilk, D.; Drazba, J.; Yadav, S.P.; Crabb, J.W.; Ganz, T.; Bevins, C.L. Paneth cell trypsin is the processing enzyme for human defensin-5. Nat. Immunol. 2002, 3, 583-590.

26. Shaw, J.L.; Petráki, C.; Watson, C.; Bocking, A.; Diamandis, E.P. Role of tissue kallikrein-related peptidases in cervical mucus remodeling and host defense. Biol. Chem. 2008, 389, 1513-1522.

27. Wilson, C.L.; Schmidt, A.P.; Pirilä, E.; Valore, E.V.; Ferri, N.; Sorsa, T.; Ganz, T.; Parks, W.C. Differential processing of α- and β-defensin precursors by matrix metalloproteinase-7 (MMP-7). J. Biol. Chem. 2009, 284, 8301-8311.

28. Bevins, C.L. Paneth cell defensins: key effector molecules of innate immunity. Biochem. Soc. Trans. 2006, 34, 263-266.
29. Yamaguchi, Y.; Nagase, T.; Tomita, T.; Nakamura, K.; Fukuara, S.; Amano, T.; Yamamoto, H.; Ide, Y.; Suzuki, M.; Teramoto, S.; Asano, T.; Kangawa, K.; Nakagata, N.; Ouchi, Y.; Kurihara, H. Beta-defensin overexpression induces progressive muscle degeneration in mice. *Am. J. Physiol. Cell. Physiol.* **2007**, *292*, C2141-C2149.

30. Huang, L.; Ching, C.B.; Jiang, R.; Leong, S.S. Production of bioactive human beta-defensin 5 and 6 in *Escherichia coli* by soluble fusion expression. *Protein Expr. Purif.* **2008**, *61*, 168-174.

31. Yanugu, S.; Hamil, K.G.; French, F.S.; Hall, S.H. Antimicrobial actions of the human epididymis 2 (HE2) protein isoforms, HE2alpha, HE2beta1 and HE2beta2. *Reprod. Biol. Endocrinol.* **2004**, *2*, 61.

32. Yount, N.Y.; Yeaman, M.R. Multidimensional signatures in antimicrobial peptides. *Proc. Natl Acad. Sci. USA* **2004**, *101*, 7363-7368.

33. Agerberth, B.; Lee, J.Y.; Bergman, T.; Carlquist, M.; Boman, H.G.; Mutt, V.; Jörnvall, H. Amino acid sequence of PR-39. Isolation from pig intestine of a new member of the family of proline-arginine-rich antibacterial peptides. *Eur. J. Biochem.* **1991**, *202*, 849-854.

34. 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.

35. Sørensen, O.; Arnljots, K.; Cowland, J.B.; Bainton, D.F.; Borregaard, N. The human antibacterial cathelicidin, hCAP-18, is synthesized in myelocytes and metamyelocytes and localized to specific granules in neutrophils. *Blood* **1997**, *90*, 2796-2803.

36. Malm, J.; Sørensen, O.; Persson, T.; Frohm-Nilsson, M.; Johansson, B.; Bjartell, A.; Lilja, H.; Stählé-Bäckdahl, 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*, 4297-4302.

37. Bals, R.; Wang, X.; Zasloff, M.; Wilson, J.M. 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*, 9541-9546.

38. Agerberth, B.; Grunewald, J.; Castaños-Velez, E.; Olsson, B.; Jörnvall, H.; Wigzell, H.; Eklund, A.; Gudmundsson, G.H. Antibacterial components in bronchoalveolar lavage fluid from healthy individuals and sarcoidosis patients. *Am. J. Respir. Crit. Care Med.* **1999**, *160*, 283-290.

39. Lee, D.Y.; Yamazaki, K.; Rudsil, J.; Zouboulis, C.C.; Park, G.T.; Yang, J.M.; Gallo, R.L. Sebocytes express functional cathelicidin antimicrobial peptides and can act to kill *propionibacterium acnes*. *J. Invest. Dermatol.* **2008**, *128*, 1863-1866.

40. Braff, M.H.; Zaiou, M.; Fierer, J.; Nizet, V.; Gallo, R.L. Keratinocyte production of cathelicidin provides direct activity against bacterial skin pathogens. *Infect. Immun.* **2005**, *73*, 6771-6781.

41. Di Nardo, A.; Braff, M.H.; Taylor, K.R.; Na, C.; Granstein, R.D.; McInturff, J.E.; Krutzik, S.; Modlin, R.L.; Gallo, R.L. Cathelicidin antimicrobial peptides block dendritic cell TLR4 activation and allergic contact sensitization. *J. Immunol.* **2007**, *178*, 1829-1834.

42. Sørensen, O.; Bratt, T.; Johnsen, A.H.; Madsen M.T.; Borregaard, N. The human antibacterial cathelicidin, hCAP-18, is bound to lipoproteins in plasma. *J. Biol. Chem.* **1999**, *274*, 22445-22451.

43. Zaiou, M.; Nizet, V.; Gallo, R.L. Antimicrobial and protease inhibitory functions of the human cathelicidin (hCAP18/LL-37) prosequence. *J. Invest. Dermatol.* **2003**, *120*, 810-816.
44. Agerberth, B.; Gunne, H.; Odeberg, J.; Kogner, P.; Boman, H.G.; Gudmundsson, G.H. FALL-39, a putative human peptide antibiotic, is cysteine-free and expressed in bone marrow and testis. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 195-199.

45. Larrick, J.W.; Lee, J.; Ma, S.; Li, X.; Francke, U.; Wright, S.C.; Balint, R.F. Structural, functional analysis and localization of the human CAP18 gene. *FEBS Lett.* **1996**, *398*, 74-80.

46. Yustein, A.S.; Harper, J.C.; Petroni, G.R.; Cummings, O.W.; Moskaluk, C.A.; Powell, S.M. Allelotype of gastric adenocarcinoma. *Cancer Res.* **1999**, *59*, 1437-1441.

47. Hase, K.; Murakami, M.; Cole, S.P.; Horibe, Y.; Ohtake, T.; Obonyo, M.; Gallo, R.L.; Eckmann, L.; Kagnoff, M.F. Expression of LL-37 by human gastric epithelial cells as a potential host defense mechanism against *Helicobacter pylori*. *Gastroenterology* **2003**, *125*, 1613-1625.

48. Wu, W.K.; Sung, J.J.; To, K.F.; Yu, L.; Li, H.T.; Li, Z.J.; Chu, K.M.; Yu, J.; Cho, C.H. The host defense peptide LL-37 activates the tumor-suppressing bone morphogenetic protein signaling via inhibition of proteasome in gastric cancer cells. *J. Cell. Physiol.* **2010**, *223*, 178-186.

49. Gudmundsson, G.H.; Agerberth, B.; Odeberg, J.; Bergman, T.; Olsson, B.; Salcedo, R. The human gene FALL39 and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes. *Eur. J. Biochem.* **1996**, *238*, 325-332.

50. Larrick, J.W.; Hirata, M.; Balint, R.F.; Lee, J.; Zhong, J.; Wright, S.C. Human CAP18: a novel antimicrobial lipopolysaccharide-binding protein. *Infect. Immun.* **1995**, *63*, 1291-1297.

51. Sørensen, O.E.; Follin, P.; Johnsen, A.H.; Calafat, J.; Tjabringa, G.S.; Hiemstra, P.S.; Borregaard, N. Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. *Blood* **2001**, *97*, 3951-3959.

52. Sørensen, O.; Gram, L.; Johnsen, A.H.; Andersson, E.; Bangsbøll, S.; Tjabringa, G.S.; Hiemstra, P.S.; Malm, J.; Egesten, A.; Borregaard, N.E. 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*, 28540-28546.

53. Murakami, M.; Lopez-Garcia, B.; Braff, M.; Dorschner, R.A.; Gallo, R.L. Postsecretory processing generates multiple cathelicidins for enhanced topical antimicrobial defense. *J. Immunol.* **2004**, *172*, 3070-3077.

54. Yamasaki, K.; Schauber, J.; Coda, A.; Lin, H.; Dorschner, R.A.; Schechter, N.M.; Bonnart, C.; Descargues, P.; Hovnanian, A.; Gallo, R.L. Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. *FASEB J.* **2006**, *20*, 2068-2080.

55. Turner, J.; Cho, Y.; Dihn, N.-N.; Waring, A.J.; Lehrer, R.I. Activities of LL-37, a cathelin-associated antimicrobial peptide of human neutrophils. *Antimicrob. Agents Chemother.* **1998**, *42*, 2206-2214.

56. Nagaoka, I.; Hirota, S.; Yomogida, S.; Ohwada, A.; Hirata, M. Synergistic actions of antibacterial neutrophil defensins and cathelicidins. *Inflamm. Res.* **2000**, *49*, 73-79.

57. Yang, D.; Chen, Q.; Schmidt, A.P.; Anderson, G.M.; Wang, J.M.; Wooters, J.; Oppenheim, J.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*, 1069-1074.
58. Johansson, J.; Gudmundsson, G.H.; Rottenberg, M.E.; Berndt, K.D.; Agerberth, B. Conformation-dependent antibacterial activity of the naturally occurring human peptide LL-37. *J. Biol. Chem.* 1998, 273, 3718-3724.

59. Leung, D.Y.; Boguniewicz, M.; Howell, M.D.; Nomura, I.; Hamid, Q.A. New insights into atopic dermatitis. *J. Clin. Invest.* 2004, 113, 651-657.

60. Ong, P.Y.; Ohtake, T.; Brandt, C.; Strickland, I.; Boguniewicz, M.; Ganz, T.; Gallo, R.L.; Leung, D.Y. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *New Engl. J. Med.* 2002, 347, 1151-1160.

61. Büchau, A.S.; Schauber, J.; Hultsch, T.; Stuetz, A.; Gallo, R.L. Pimecrolimus enhances TLR2/6-induced expression of antimicrobial peptides in keratinocytes. *J. Invest. Dermatol.* 2008, 128, 2646-2654.

62. Rosenberger, C.M.; Gallo, R.L.; Finlay, B.B. Interplay between antibacterial effectors: a macrophage antimicrobial peptide impairs intracellular *Salmonella* replication. *Proc. Natl. Acad. Sci. USA* 2004, 101, 2422-2427.

63. Bergman, P.; Johansson, L.; Wan, H.; Jones, A.; Gallo, R.L.; Gudmundsson, G.H.; Hökfelt, T.; Jonsson, A.B.; Agerberth, B. Induction of the antimicrobial peptide CRAMP in the blood-brain barrier and meninges after meningococcal infection. *Infect. Immun.* 2006, 74, 6982-6991.

64. Chromek, M.; Slamová, Z.; Bergman, P.; Kovács, L.; Podracká, L.; Ehrén, I.; Hökfelt, T.; Gudmundsson, G.H.; Gallo, R.L.; Agerberth, B.; Brauner, A. The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection. *Nat. Med.* 2006, 12, 636-641.

65. Bals, R.; Weiner, D.J.; Moscioni, A.D.; Meegalla, R.L.; Wilson, J.M. Augmentation of innate host defense by expression of a cathelicidin antimicrobial peptide. *Infect. Immun.* 1999, 67, 6084-6089.

66. Bals, R.; Weiner, D.J.; Meegalla, R.L.; Wilson, J.M. Transfer of a cathelicidin peptide antibiotic gene restores bacterial killing in a cystic fibrosis xenograft model. *J. Clin. Invest.* 1999, 103, 1113-1117.

67. Zanetti, M. The role of cathelicidins in the innate host defenses of mammals. *Curr. Issues Mol. Biol.* 2005, 7, 179-196.

68. Gutsmann, T.; Larrick, J.W.; Seydel, U.; Wiese, A. Molecular mechanisms of interaction of rabbit CAP18 with outer membranes of gram-negative bacteria. *Biochemistry.* 1999, 38, 13643-13653.

69. Oren, Z.; Lerman, J.C.; Gudmundsson, G.H.; 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, 501-513.

70. Morgera, F.; Vaccari, L.; Antcheva, N.; Scaini, D.; Pacor, S.; Tossi, A. Primate cathelicidin orthologues display different structures and membrane interactions. *Biochem. J.* 2009, 417, 727-735.

71. Zhang, X.; Oglecka, K.; Sandgren, S.; Belting, M.; Esbjörner, E.K.; Nordén, B.; Gräslund, A. Dual functions of the human antimicrobial peptide LL-37-target membrane perturbation and host cell cargo delivery. *Biochim. Biophys. Acta* 2010, doi: dx.doi.org/10.1016/j.bbamem.2009.12.011.

72. Tomasinsig, L.; Morgera, F.; Antcheva, N.; Pacor, S.; Skerlavaj, B.; Zanetti, M.; Tossi, A. Structure dependence of biological activities for primate cathelicidins. *J. Pept. Sci.* 2009, 15, 576-582.

73. Henzler Wildman, K.A.; Lee, D.K.; Ramamoorthy, A. Mechanism of lipid bilayer disruption by the human antimicrobial peptide, LL-37. *Biochemistry* 2003, 42, 6545-6558.
74. Porcelli, F.; Verardi, R.; Shi, L.; Henzler Wildman, K.A.; Ramamoorthy, A.; Veglia, G. NMR structure of the cathelicidin-derived human antimicrobial peptide LL-37 in dodecylphosphocholine micelles. Biochemistry 2008, 47, 5565-5572.
75. Porcelli, F.; Buck, B.; Lee, D.K.; Hallock, K.J.; Ramamoorthy, A.; Veglia, G. Structure and orientation of pardaxin determined by NMR experiments in model membranes. J. Biol. Chem. 2004, 279, 45815-45823.
76. Wang, G. Structure, dynamics and mapping of membrane-binding residues of micelle-bound antimicrobial peptides by natural abundance 13C NMR spectroscopy. Biochim. Biophys. Acta 2010, 1798, 114-121.
77. Neville, F.; Cahuzac, M.; Konovalov, O.; Ishitsuka, Y.; Lee, K.Y.; Kuzmenko, I.; Kale, G.M.; Gidalevitz, D. Lipid headgroup discrimination by antimicrobial peptide LL-37: insight into mechanism of action. Biophys. J. 2006, 90, 1275-1287.
78. Wu, M.; Maier, E.; Benz, R.; Hancock, R.E. Mechanism of interaction of different classes of cationic antimicrobial peptides with planar bilayers and with the cytoplasmic membrane of Escherichia coli. Biochemistry 1999, 38, 7235-7242.
79. Epand, R.M.; Epand, R.F.; Arnusch, C.J.; Papahadjopoulos-Sternberg, B.; Wang, G.; Shai, Y. Lipid clustering by three homologous arginine-rich antimicrobial peptides is insensitive to amino acid arrangement and induced secondary structure. Biochim. Biophys. Acta 2010, 1798, 1272-1280.
80. Pabst, G.; Grage, S.L.; Danner-Pongratz, S.; Jing, W.; Ulrich, A.S.; Watts, A.; Lohner, K.; Hickel, A. Membrane thickening by the antimicrobial peptide PGLa. Biophys. J. 2008, 95, 5779-5788.
81. Matsuzaki, K. Control of cell selectivity of antimicrobial peptides. Biochim. Biophys. Acta 2009, 1788, 1687-1692.
82. Gennaro, R.; Zanetti, M. Structural features and biological activities of the cathelicidin-derived antimicrobial peptides. Biopolymers 2000, 55, 31-49.
83. Hancock, R.E.; Diamond, G. The role of cationic antimicrobial peptides in innate host defences. Trends Microbiol. 2000, 8, 402-410.
84. Lehrer, R.I.; Lichtenstein, A.K.; Ganz, T. Defensins: antimicrobial and cytotoxic peptides of mammalian cells. Annu. Rev. Immunol. 1993, 11, 105-128.
85. Risso, A. Leukocyte antimicrobial peptides: multifunctional effector molecules of innate immunity. J. Leukoc. Biol. 2000, 68, 785-792.
86. Boheim, G.; Hanke, W.; Eibl, H. Lipid phase transition in planar bilayer membrane and its effect on carrier- and pore-mediated ion transport. Proc. Natl. Acad. Sci. USA 1980, 77, 3403-3407.
87. Heller, W.T.; Waring, A.J.; Lehrer, R.I.; Harroun, T.A.; Weiss, T.M.; Yang, L.; Huang, H.W. Membrane thinning effect of the beta-sheet antimicrobial protegrin. Biochemistry 2000, 39, 139-145.
88. Henzler Wildman, K.A.; Martinez, G.V.; Brown, M.F.; Ramamoorthy, A. Perturbation of the hydrophobic core of lipid bilayers by the human antimicrobial peptide LL-37. Biochemistry 2004, 43, 8459-8469.
89. Turner, J.; Cho, Y.; Dihn, N.-N.; Waring, A.J.; Lehrer, R.I. Activities of LL-37, a cathelin-associated antimicrobial peptide of human neutrophils. Antimicrob. Agents Chemother. 1998, 42, 2206-2214.
90. Wang, G. Structures of human host defense cathelicidin LL-37 and its smallest antimicrobial peptide KR-12 in lipid micelles. J. Biol. Chem. 2008, 283, 32637-32643.
91. Li, X.; Li, Y.; Han, H.; Miller, D.W.; Wang, G. Solution structures of human LL-37 fragments and NMR-based identification of a minimal membrane-targeting antimicrobial and anticancer region. *J. Am. Chem. Soc.* 2006, 128, 5776-5785.

92. Li, Y.; Li, X.; Li, H.; Lockridge, O.; Wang, G. A novel method for purifying recombinant human host defense cathelicidin LL-37 by utilizing its inherent property of aggregation. *Protein Expr. Purif.* 2007, 54, 157-165.

93. Kirikae, T.; Hirata, M.; Yamasu, H.; Kirikae, F.; Tamura, H.; Kayama, F.; Nakatsuka, K.; Yokochi, T.; Nakano, M. Protective effects of a human 18-kilodalton cationic antimicrobial protein (CAP18)-derived peptide against murine endotoxemia. *Infect. Immun.* 1998, 66, 1861-1868.

94. Nagaoka, I.; Hirota, S.; Niyonsaba, F.; Hirata, M.; Adachi, Y.; Tamura, H.; Tanaka, S.; Heumann, D. Augmentation of the lipopolysaccharide-neutralizing activities of human cathelicidin CAP18/LL-37-derived antimicrobial peptides by replacement with hydrophobic and cationic amino acid residues. *Clin. Diagn. Lab. Immunol.* 2002, 9, 972-982.

95. Epand, R.F.; Wang, G.; Berno, B.; Epand, R.M. Lipid segregation explains selective toxicity of a series of fragments derived from the human cathelicidin LL-37. *Antimicrob. Agents Chemother.* 2009, 53, 3705-3714.

96. Howl, J.; Nicholl, I.D.; Jones, S. The many futures for cell-penetrating peptides: how soon is now? *Biochem. Soc. Trans.* 2007, 35, 767-769.

97. Shaw, J.E.; Epand, R.F.; Hsu, J.C.; Mo, G.C.; Epand, R.M.; Yip, C.M. Cationic peptide-induced remodelling of model membranes: direct visualization by *in situ* atomic force microscopy. *J. Struct. Biol.* 2008, 162, 121-138.

98. Lamazière, A.; Maniti, O.; Wolf, C.; Lambert, O.; Chassaing, G.; Trugnan, G.; Ayala-Sanmartin, J. Lipid domain separation, bilayer thickening and pearling induced by the cell penetrating peptide penetratin. *Biochim Biophys Acta* 2010, 1798, 2223-2230.

99. Henriques, S.T.; Quintas, A.; Bagatolli, L.A.; Homblé, F.; Castanho, M.A. Energy-independent translocation of cell-penetrating peptides occurs without formation of pores. A biophysical study with pep-1. *Mol. Membr. Biol.* 2007, 24, 282-293.

100. Henriques, S.T.; Castanho, M.A.; Pattenden, L.K.; Aguilar, M.I. Fast membrane association is a crucial factor in the peptide pep-1 translocation mechanism: a kinetic study followed by surface plasmon resonance. *Biopolymers* 2010, 94, 314-322.

101. Lensink, M.F.; Christiaens, B.; Vandekerckhove, J.; Prochiantz, A.; Rosseneu, M. Penetratin-membrane association: W48/R52/W56 shield the peptide from the aqueous phase. *Biophys. J.* 2005, 88, 939-952.

102. Konate, K.; Crombez, L.; Deshayes, S.; Decaffmeyer, M.; Thomas, A.; Brasseur, R.; Aldrian, G.; Heitz, F.; Divita, G. Insight into the cellular uptake mechanism of a secondary amphipathic cell-penetrating peptide for siRNA delivery. *Biochemistry* 2010, 49, 3393-3402.

103. Crombez, L.; Aldrian-Herrada, G.; Konate, K.; Nguyen, Q.N.; McMaster, G.K.; Brasseur, R.; Heitz, F.; Divita, G. A new potent secondary amphipathic cell-penetrating peptide for siRNA delivery into mammalian cells. *Mol. Ther.* 2009, 17, 95-103.

104. Balayssac, S.; Burlina, F.; Convert, O.; Bolbach, G.; Chassaing, G.; Lequin, O. Comparison of penetratin and other homeodomain-derived cell-penetrating peptides: interaction in a membrane-mimicking environment and cellular uptake efficiency. *Biochemistry* 2006, 45, 1408-1420.
105. Herce, H.D.; Garcia, A.E. Cell penetrating peptides: how do they do it? J. Biol. Phys. 2007, 33, 345-356.

106. Yselyevsky, S.; Marrink, S.J.; Mark, A.E. Alternative mechanisms for the interaction of the cell-penetrating peptides penetratin and the TAT peptide with lipid bilayers. Biophys. J. 2009, 97, 40-49.

107. Afonin, S.; Frey, A.; Bayerl, S.; Fischer, D.; Wadhwani, P.; Weinkauf, S.; Ulrich, A.S. The cell-penetrating peptide TAT(48-60) induces a non-lamellar phase in DMPC membranes. Chemphyschem 2006, 7, 2134-2142.

108. Tang, M.; Waring, A.J.; Hong, M. Phosphate-mediated arginine insertion into lipid membranes and pore formation by a cationic membrane peptide from solid-state NMR. J. Am. Chem. Soc. 2007, 129, 11438-11446.

109. Su, Y.; Doherty, T.; Waring, A.J.; Ruchala, P.; Hong, M. Roles of arginine and lysine residues in the translocation of a cell-penetrating peptide from (13)C, (31)P, and (19)F solid-state NMR. Biochemistry 2009, 48, 4587-4595.

110. Gonçalves, E.; Kitas, E.; Seelig, J. Binding of oligoarginine to membrane lipids and heparan sulfate: structural and thermodynamic characterization of a cell-penetrating peptide. Biochemistry 2005, 44, 2692-2702.

111. Esbjörner, E.K.; Lincoln, P.; Nordén, B. Counterion-mediated membrane penetration: cationic cell-penetrating peptides overcome Born energy barrier by ion-pairing with phospholipids. Biochim. Biophys. Acta. 2007, 1768, 1550-1558.

112. Amand, H.L.; Fant, K.; Nordén, B.; Esbjörner, E.K. Stimulated endocytosis in penetratin uptake: effect of arginine and lysine. Biochem. Biophys. Res. Commun. 2008, 371, 621-625.

113. Pantos, A.; Tsogas, I.; Paleos, C.M. Guanidinium group: a versatile moiety inducing transport and multicompartmentalization in complementary membranes. Biochim. Biophys. Acta. 2008, 1778, 811-823.

114. Schmidt, N.; Mishra, A.; Lai, G.H.; Wong, G.C. Arginine-rich cell-penetrating peptides. FEBS Lett. 2010, 584, 1806-1813.

115. Herce, H.D.; Garcia, A.E.; Litt, J.; Kane, R.S.; Martin, P.; Enrique N.; Rebolledo, A.; Milesi, V. Arginine-rich peptides destabilize the plasma membrane, consistent with a pore formation translocation mechanism of cell penetrating peptides. Biophys. J. 2009, 97, 1917-1925.

116. Gunn, J.S.; Lim, K.B.; Krueger, J.; Kim, K.; Guo, L.; Hackett, M.; Miller, S.I. PmrA-PmrB-regulated genes necessary for 4-aminoarabinose lipid A modification and polymyxin resistance. Mol. Microbiol. 1998, 27, 1171-1182.

117. Guo, L.; Lim, K.B.; Poduje, C.M.; Daniel, M.; Gunn, J.S.; Hackett, M.; Miller, S.I. Lipid A acylation and bacterial resistance against vertebrate antimicrobial peptides. Cell 1998, 95, 189-198.

118. Peschel, A.; Otto, M.; Jack, R.W.; Kalbacher, H.; Jung, G.; Götz, F. Inactivation of the dlt operon in Staphylococcus aureus confers sensitivity to defensins, protegrins, and other antimicrobial peptides. J. Biol. Chem. 1999, 274, 8405-8410.

119. Peschel, A.; Jack, R.W.; Otto, M.; Collins, L.V.; Staubitz, P.; Nicholson, G.; Kalbacher, H.; Nieuwenhuizen, W.F.; Jung, G.; Tarkowski, A.; van Kessel, K.P.; van Strijp, J.A. Staphylococcus aureus resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MrpF is based on modification of membrane lipids with l-lysine. J. Exp. Med. 2001, 193, 1067-1076.
120. Guina, T.; Yi, E.C.; Wang, H.; Hackett, M.; Miller, S.I. A PhoP-regulated outer membrane protease of *Salmonella enterica* serovar typhimurium promotes resistance to alpha-helical antimicrobial peptides. *J. Bacteriol.* **2000**, *182*, 4077-4086.

121. Shafer, W.M.; Qu, X.; Waring, A.J.; Lehrer, R.I. Modulation of *Neisseria gonorrhoeae* susceptibility to vertebrate antibacterial peptides due to a member of the resistance/ nodulation/division efflux pump family. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 1829-1833.

122. Frick, I.M.; Akesson, P.; Rasmussen, M.; Schmidtchen, A.; Björck, L. SIC, a secreted protein of *Streptococcus pyogenes* that inactivates antibacterial peptides. *J. Biol. Chem.* **2003**, *278*, 16561-16566.

123. Islam, D.; Bandholtz, L.; Nilsson, J.; Wigzell, H.; Christensson, B.; Agerberth, B.; Gudmundsson, G. Downregulation of bactericidal peptides in enteric infections: a novel immune escape mechanism with bacterial DNA as a potential regulator. *Nat. Med.* **2001**, *7*, 180-185.

124. Waite, R.D.; Paccanaro, A.; Papakonstantinopoulou, A.; Hurst, J.M.; Saqi, M.; Littler, E.; Curtis, M.A. Clustering of *Pseudomonas aeruginosa* transcriptomes from planktonic cultures, developing and mature biofilms reveals distinct expression profiles. *BMC Genom.* **2006**, *7*, 162.

125. Hall-Stoodley, L.; Stoodley, P. Developmental regulation of microbial biofilms. *Curr. Opin. Biotechnol.* **2002**, *13*, 228-233.

126. Monds, R.D.; O'Toole, G.A. The developmental model of microbial biofilms: ten years of a paradigm up for review. *Trends Microbiol.* **2009**, *17*, 73-87.

127. Tré-Hardy, M.; Vanderbist, F.; Traore, H.; Devleeschouwer, M.J. *In vitro* activity of antibiotic combinations against *Pseudomonas aeruginosa* biofilm and planktonic cultures. *Int. J. Antimicrob. Agents* **2008**, *31*, 329-336.

128. Nickel, J.C.; Ruseska, I.; Wright, J.B.; Costerton, J.W. Tobramycin resistance of *Pseudomonas aeruginosa* cells growing as a biofilm on urinary catheter material. *Antimicrob. Agents Chemother.* **1985**, *27*, 619-624.

129. Mah, T.F.; Pitts, B.; Pellock, B.; Walker, G.C.; Stewart, P.S.; O'Toole, G.A. A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance. *Nature* **2003**, *426*, 306-310.

130. Høiby, N.; Bjarnsholt, T.; Givskov, M.; Molin, S.; Ciofu, O. Antibiotic resistance of bacterial biofilms. *Int. J. Antimicrob. Agents* **2010**, *35*, 322-332.

131. Vuong, C.; Voyich, J.M.; Fischer, E.R.; Braughton, K.R.; Whitney, A.R.; DeLeo, F.R.; Otto, M. Polysaccharide intercellular adhesin (PIA) protects *Staphylococcus epidermidis* against major components of the human innate immune system. *Cell. Microbiol.* **2004**, *6*, 269-275.

132. Vuong, C.; Kocianova, S.; Voyich, J.M.; Yao, Y.; Fischer, E.R.; DeLeo, F.R.; Otto, M. A crucial role for exopolysaccharide modification in bacterial biofilm formation, immune evasion, and virulence. *J. Biol. Chem.* **2004**, *279*, 54881-54886.

133. Cirioni, O.; Giacometti, A.; Ghiselli, R.; Bergnach, C.; Orlando, F.; Mocchegiani, F.; Silvestri, C.; Licci, A.; Skerlavaj, B.; Zanetti, M.; Saba, V.; Scalise, G. Pre-treatment of central venous catheters with the cathelicidin BMAP-28 enhances the efficacy of antistaphylococcal agents in the treatment of experimental catheter-related infection. *Peptides* **2006**, *27*, 2104-2110.

134. Overhage, J.; Campisano, A.; Bains, M.; Torfs, E.C.; Rehm, B.H.; Hancock, R. E. Human host defense peptide LL-37 prevents bacterial biofilm formation. *Infect. Immun.* **2008**, *76*, 4176-4182.
135. Chennupati, S.K.; Chiu, A.G.; Tamashiro, E.; Banks, C.A.; Cohen, M.B.; Bleier, B.S.; Kofonow, J.M.; Tam, E.; Cohen, N.A. Effects of an LL-37-derived antimicrobial peptide in an animal model of biofilm Pseudomonas sinusitis. Am. J. Rhinol. Allergy 2009, 23, 46-51.
136. Hell, E.; Giske, C.G.; Nelson, A.; Römling, U.; Marchini, G. Human cathelicidin peptide LL37 inhibits both attachment capability and biofilm formation of Staphylococcus epidermidis. Lett. Appl. Microbiol. 2010, 50, 211-215.
137. Amer, L.S.; Bishop, B. M.; van Hoek, M.L. Antimicrobial and antibiofilm activity of cathelicidins and short, synthetic peptides against Francisella. Biochem. Biophys. Res. Comm. 2010, 396, 246-251.
138. McPhee, J.B.; Hancock, R.E. Function and therapeutic potential of host defence peptides. J. Pept. Sci. 2005, 11, 677-687.
139. Sood, R.; Kinnunen, P.K. Cholesterol, lanosterol, and ergosterol attenuate the membrane association of LL-37(W27F) and temporin L. Biochim. Biophys. Acta 2008, 1778, 1460-1466.
140. Ciornei, C.D.; Sigurdardóttir, T.; Schmidtchen, A.; Bodelsson, M. Antimicrobial and chemoattractant activity, lipopolysaccharide neutralization, cytotoxicity, and inhibition by serum of analogs of human cathelicidin LL-37. Antimicrob. Agents Chemother. 2005, 49, 2845-2850.
141. Nagaoka, I.; Tamura, H.; Hirata, M. An antimicrobial cathelicidin peptide, human CAP18/LL-37, suppresses neutrophil apoptosis via the activation of formyl-peptide receptor-like 1 and P2X7. J. Immunol. 2006, 176, 3044-3052.
142. Koczulla, R.; von Degenfeld, G.; Kupatt, C.; Krötz, F.; Zahler, S.; Gloe, T.; Issbrücker, K.; Unterberger, P.; Zaiou, M.; Leberher, C.; Karl, A.; Raake, P.; Pfoesser, A.; Bookstegers, P.; Welsch, U.; Hiemstra, P.S.; Vogelmeier, C.; Gallo, R.L.; Clauss, M.; Bals, R. An angiogenic role for the human peptide antibiotic LL-37/hCAP-18. J. Clin. Invest. 2003, 111, 1665-1672.
143. Zhang, Z.; Cherryholmes, G.; Chang, F.; Rose, D.M.; Schraufstatter, I.; Shively, J.E. Evidence that cathelicidin peptide LL-37 may act as a functional ligand for CXCR2 on human neutrophils. Eur. J. Immunol. 2009, 39, 3181-3194.
144. Brandenburg, L.O.; Jansen, S.; Wruck, C.J.; Lucius, R.; Pufe, T. Antimicrobial peptide rCRAMP induced glial cell activation through P2Y receptor signalling pathways. Mol. Immunol. 2010, 47, 1905-1913.
145. Yoshioka, M.; Fukuishi, N.; Kubo, Y.; Yamanobe, H.; Ohsaki, K.; Kawasaki, Y.; Murata, M.; Ishizumi, A.; Nishii, Y.; Matsui, N.; Akagi, M. Human cathelicidin CAP18/LL-37 changes mast cell function toward innate immunity. Biol. Pharm. Bull. 2008, 31, 212-216.
146. Niyonsaba, F.; Someya, A.; Hirata, M.; Ogawa, H.; Nagaoka, I. Evaluation of the effects of peptide antibiotics human β-defensins-1/-2 and LL-37 on histamine release and prostaglandin D2 production from mast cells. Eur. J. Immunol. 2001, 31, 1066-1075.
147. Chen, X.; Niyonsaba, F.; Ushio, H.; Nagaoka, I.; Ikeda, S.; Okumura, K.; Ogawa, H. Human cathelicidin LL-37 increases vascular permeability in the skin via mast cell activation, and phosphorylates MAP kinases p38 and ERK in mast cells. J. Dermatol. Sci. 2006, 43, 63-66.
148. Elssner, A.; Duncan, M.; Gavrilin, M.; Wewers, M.D. A novel P2X7 receptor activator, the human cathelicidin-derived peptide LL37, induces IL-1 beta processing and release. J. Immunol. 2004, 172, 4987-4994.
149. Skaper, S.D.; Debetto, P.; Giusti, P. The P2X7 purinergic receptor: from physiology to neurological disorders. *FASEB J.* 2010, 24, 337-345.

150. Weinhold, K.; Krause-Buchholz, U.; Rödel, G.; Kasper, M.; Barth, K. Interaction and interrelation of P2X7 and P2X4 receptor complexes in mouse lung epithelial cells. *Cell. Mol. Life Sci.* 2010, 67, 2631-2642.

151. Tomasinsig, L.; Pizzirani, C.; Skerlavaj, B.; Pellegrini, P.; Gulinelli, S.; Tossi, A.; Di Virgilio, F.; Zanetti, M. The human cathelicidin LL-37 modulates the activities of the P2X7 receptor in a structure-dependent manner. *J. Biol. Chem.* 2008, 283, 30471-30481.

152. Shaykhiev, R.; Beisswenger, C.; Kändler, K.; Senske, J.; Püchner, A.; Damm, T.; Behr, J.; Bals, R. Human endogenous antibiotic LL-37 stimulates airway epithelial cell proliferation and wound closure. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2005, 289, L842-L848.

153. Coffelt, S.B.; Tomchuck, S.L.; Zwezdaryk, K.J.; Danka, E.S.; Scandurro, A.B. Leucine leucine-37 uses formyl peptide receptor-like 1 to activate signal transduction pathways, stimulate oncogenic gene expression, and enhance the invasiveness of ovarian cancer cells. *Mol. Cancer Res.* 2009, 7, 907-915.

154. Coffelt, S.B.; Waterman, R.S.; Florez, L.; Höner zu Bentrup, K.; Zwezdaryk, K.J.; Tomchuck, S.L.; LaMarca, H.L.; Danka, E.S.; Morris, C.A.; Scandurro, A.B. Ovarian cancers overexpress the antimicrobial protein hCAP-18 and its derivative LL-37 increases ovarian cancer cell proliferation and invasion. *Int. J. Cancer.* 2008, 122, 1030-1039.

155. von Haussen, J.; Koczulla, R.; Shaykhiev, R.; Herr, C.; Pinkenburg, O.; Reimer, D.; Wiewrodt, R.; Biesterfeld, S.; Aigner, A.; Czubayko, F.; Bals, R. The host defence peptide LL-37/hCAP-18 is a growth factor for lung cancer cells. *Lung Cancer* 2008, 59, 12-23.

156. Weber, G.; Chamorro, C.I.; Granath, F.; Liljegren, A.; Zreika, S.; Saidak, Z.; Sandstedt, B.; Stähle, M. Human antimicrobial protein hCAP18/LL-37 promotes a metastatic phenotype in breast cancer. *Breast Cancer Res.* 2009, 11, R6.

157. Ohtsu, H.; Dempsey, P.J.; Eguchi, S. ADAMs as mediators of EGF receptor transactivation by G protein-coupled receptors. *Am. J. Physiol. Cell. Physiol.* 2006, 291, C1-C10.

158. Seil, M.; Kabré, E.; Nagant, C.; Vandenbranden, M.; Fontanils, U.; Marino, A.; Pochet, S.; Dehaye, J.P. Regulation by CRAMP of the responses of murine peritoneal macrophages to extracellular ATP. *Biochim. Biophys. Acta.* 2010, 1798, 569-578.

159. Zughaier, S.M.; Shafer, W.M.; Stephens, D.S. Antimicrobial peptides and endotoxin inhibit cytokine and nitric oxide release but amplify respiratory burst response in human and murine macrophages. *Cell. Microbiol.* 2005, 7, 1251-1262.

160. Di Nardo, A.; Braff, M.H.; Taylor, K.R.; Na, C.; Granstein, R.D.; McInturff, J.E.; Krutzik, S.; Modlin, R.L.; Gallo, R.L. Cathelicidin antimicrobial peptides block dendritic cell TLR4 activation and allergic contact sensitization. *J. Immunol.* 2007, 178, 1829-1834.

161. Pochet, S.; Tandel, S.; Querriére, S.; Tré-Hardy, M.; Garcia-Marcos, M.; De Lorenzi, M.; Vandenbranden, M.; Marino, A.; Devleeschouwer, M.; Dehaye, J.P. Modulation by LL-37 of the responses of salivary glands to purinergic agonists. *Mol. Pharmacol.* 2006, 69, 2037-2046.

162. Rao, N.M. Differential susceptibility of phosphatidylcholine small unilamellar vesicles to phospholipases A2, C and D in the presence of membrane active peptides. *Biochem. Biophys. Res. Commun.* 1992, 182, 682-688.
163. Perregaux, D.G.; Bhavsar, K.; Contillo, L.; Shi, J.; Gabel, C.A. Antimicrobial peptides initiate IL-1β posttranslational processing: a novel role beyond innate immunity. *J. Immunol.* **2002**, *168*, 3024-3032.

164. Wewers, M.D.; Sarkar, A. P2X7 receptor and macrophage function. *Purinergic Signal.* **2009**, *5*, 189-195.

165. Denlinger, L.C.; Fisette, P.L.; Sommer, J.A.; Watters, J.J.; Prabhu, U.; Dubyk, G.R.; Proctor, R.A.; Bertics, P.J. Cutting edge: the nucleotide receptor P2X7 contains multiple protein- and lipid-interaction motifs including a potential binding site for bacterial lipopolysaccharide. *J. Immunol.* **2001**, *167*, 1871-1876.

166. Sandgren, S.; Wittstrup, A.; Cheng, F.; Jönsson, M.; Eklund, E.; Busch, S.; Belting, M. The human antimicrobial peptide LL-37 transfers extracellular DNA plasmid to the nuclear compartment of mammalian cells via lipid rafts and proteoglycan-dependent endocytosis. *J. Biol. Chem.* **2004**, *279*, 17951-17956.

167. Garcia-Marcos, M.; Dehaye, J.P.; Marino, A. Membrane compartments and purinergic signalling: the role of plasma membrane microdomains in the modulation of P2XR-mediated signalling. *FEBS J.* **2009**, *276*, 330-340.

168. Mookherjee, N.; Lippert, D.N.; Hamill, P.; Falsafi, R.; Nijnik, A.; Kindrachuk, J.; Pistolic, J.; Gardy, J.; Miri, P.; Naseer, M.; Foster, L.J.; Hancock, R.E. Intracellular receptor for human host defense peptide LL-37 in monocytes. *J. Immunol.* **2009**, *183*, 2688-2696.

169. Sebbage, V. Cell-penetrating peptides and their therapeutic applications. *Bioscience Horizons* **2009**, *2*, 64-72.

170. Hällbrink, M.; Kilk, K.; Elmquist, A.; Lundberg, P.; Lindgren, M.; Jiang, Y.; Pooga, M.; Soomets, U.; Langel, U. Prediction of cell-penetrating peptides. *Int. J. Pep. Res. Ther.* **2005**, *11*, 249-259.

171. Derossi, D.; Joliot, A.H.; Chassaing, G.; Prochiantz, A. The third helix of the Antennapedia homeodomain translocates through biological membranes. *J. Biol. Chem.* **1994**, *269*, 10444-10450.

172. Vivès, E.; Brodin, P.; Lebleu, B. A truncated HIV-1 Tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus. *J. Biol. Chem.* **1997**, *272*, 16010-16017.

173. Fischer, R.; Köhler, K.; Fotin-Mleczek, M.; Brock, R. A stepwise dissection of the intracellular fate of cationic cell-penetrating peptides. *J. Biol. Chem.* **2004**, *279*, 12625-12635.

174. Chaloin, L.; Vidal, P.; Heitz, A.; Van Mau, N.; Mery, J.; Divita, G.; Heitz, F. Conformations of primary amphipathic carrier peptides in membrane mimicking environments. *Biochemistry* **1997**, *36*, 11179-11187.

175. Vidal, P.; Chaloin, L.; Heitz, A.; Van Mau, N.; Mery, J.; Divita, G.; Heitz, F. Interactions of primary amphipathic vector peptides with membranes. Conformational consequences and influence on cellular localization. *J. Membr. Biol.* **1998**, *162*, 259-264.

176. Magzoub, M.; Eriksson, L.E.; Gräslund, A. Comparison of the interaction, positioning, structure induction and membrane perturbation of cell-penetrating peptides and non-translocating variants with phospholipid vesicles. *Biophys. Chem.* **2003**, *103*, 271-288.

177. Barany-Wallje, E.; Andersson, A.; Gräslund, A.; Maler, L. NMR solution structure and position of transportan in neutral phospholipid bicelles. *FEBS Lett.* **2004**, *567*, 265-269.
178. Deshayes, S.; Plenat, T.; Aldrian-Herrada, G.; Divita, G.; Le Grimellec, C.; Heitz, F. Primary amphipathic cell-penetrating peptides: structural requirements and interactions with model membranes. *Biochemistry* **2004**, *43*, 7698-7706.

179. Magzoub, M.; Eriksson, L.E.; Gräslund, A. Conformational states of the cell-penetrating peptide penetratin when interacting with phospholipid vesicles: effects of surface charge and peptide concentration. *Biochim. Biophys. Acta* **2002**, *1563*, 53-63.

180. Duchardt, F.; Fotin-Mleczek, M.; Schwarz, H.; Fischer, R.; Brock, R. A comprehensive model for the cellular uptake of cationic cell-penetrating peptides. *Traffic* **2007**, *8*, 848-866.

181. Pooga, M.; Hällbrink, M.; Zorko, M.; Langel, U. Cell penetration by transportan. *FASEB J.* **1998**, *12*, 67-77.

182. Lindgren, M.; Hällbrink, M.; Prochiantz, A.; Langel, U. Cell-penetrating peptides. *Trends Pharmacol. Sci.* **2000**, *21*, 99-103.

183. Fittipaldi, A.; Ferrari, A.; Zoppe, M.; Arcangeli, C.; Pellegrini, V.; Beltram, F.; Giacca, M. Cell membrane lipid rafts mediate caveolar endocytosis of HIV-1 Tat fusion proteins, *J. Biol. Chem.* **2003**, *278*, 34141-34149.

184. Simeoni, F.; Morris, M.C.; Heitz, F.; Divita, G. Insight into the mechanism of the peptide-based gene delivery system MPG: implications for delivery of siRNA into mammalian cells. *Nucleic Acids Res.* **2003**, *31*, 2717-2724.

185. Suzuki, T.; Futaki, S.; Niwa, M.; Tanaka, S.; Ueda, K.; Sugirua, Y. Possible existence of common internalization mechanisms among arginine rich peptides. *J. Biol. Chem.* **2002**, *277*, 2437-2443.

186. Drin, G.; Cottin, S.; Blanc, E.; Rees, A.R.; Temsamani, J. Studies on the internalization mechanism of cationic cell-penetrating peptides. *J. Biol. Chem.* **2003**, *278*, 31192-31201.

187. Richard, J.P.; Melikov, K.; Vives, E.; Ramos, C.; Verbeure, B.; Gait, M.J.; Chernomordik, L. V.; Lebleu, B. Cell penetrating peptides: a reevaluation of the mechanism of cellular uptake. *J. Biol. Chem.* **2003**, *278*, 585-590.

188. Dietz, G.P.; Bähr, M. Delivery of bioactive molecules into the cell: the Trojan horse approach. *Mol. Cell. Neurosci.* **2004**, *27*, 85-131.

189. Almeida, P.F.; Pokorny, A. Mechanisms of antimicrobial, cytolytic, and cell-penetrating peptides: from kinetics to thermodynamics. *Biochemistry* **2009**, *48*, 8083-8093.

190. Rivas, L.; Luque-Ortega, J.R.; Fernandez-Reyes, M.; Andreu, D. Membrane-active peptides as anti-infectious agents. *J. Appl. Biomed.* **2010**, *8*, 159-167.

191. Lande, R; Gregorio, J.; Facchinetti, V.; Chatterjee, B.; Wang, Y. H.; Homey, B.; Cao, W.; Wang, Y-H.; Su, B.; Nestle, F.O.; Zal, T.; Mellman, I.; Schröder, J.M.; Liu, Y.J.; Gilliet, M. Plasmacytoid dendritic cells sense self-DNA coupled with anti-microbial peptide. *Nature* **2007**, *449*, 564-569.

192. Zhang, X., Ogłąćka, K., Sandgren, S., Belting, M., Esbjörner, E.K., Nordén, B., Gräslund, A. Dual functions of the human antimicrobial peptide LL-37-Target membrane perturbation and host cell cargo delivery. *Biochim. Biophys. Acta* **2009**, *1798*, 2201-2208.

193. Hurtado, P.; Peh, C. A. LL-37 promotes rapid sensing of CpG oligodeoxynucleotides by B lymphocytes and plasmacytoid dendritic cells. *J. Immunol.* **2010**, *184*, 1425-1435.

194. Kumagai, Y.; Takeuchi, O.; Akira, S. TLR9 as a key receptor for the recognition of DNA. *Adv. Drug Deliv. Rev.* **2008**, *60*, 795-804.
195. Ito, T.; Amakawa, R.; Inaba, M.; Hori, T.; Ota, M.; Nakamura, K.; Takebayashi, M.; Miyaji, M.; Yoshimura, T.; Inaba, K.; Fukuhara, S. Plasmacytoid dendritic cells regulate Th cell responses through OX40 ligand and type I IFNs. *J. Immunol.* **2004**, *172*, 4253-4259.

196. Kolumam, G.A.; Thomas, S.; Thompson, L.J.; Sprant, J.; Murali-Krishna, K. Type I interferons act directly on CD8 T cells to allow clonal expansion and memory formation in response to viral infection. *J. Exp. Med.* **2005**, *202*, 637-650.

197. Lee, S.H.; Biron, C.A. Here today--not gone tomorrow: roles for activating receptors in sustaining NK cells during viral infections. *Eur. J. Immunol.* **2010**, *40*, 923-932.

198. Fitzgerald-Bocarsly, P.; Feng, D. The role of type I interferon production by dendritic cells in host defense. *Biochimie* **2007**, *89*, 843-855.

199. Ueno, H.; Schmitt, N.; Palucka, A.K.; Banchereau, J. Dendritic cells and humoral immunity in humans. *Immunol. Cell. Biol.* **2010**, *88*, 376-380.

200. Lin, L.; Gerth, A.J.; Peng, S.L. CpG DNA redirects class-switching towards "Th1-like" Ig isotype production via TLR9 and MyD88. *Eur. J. Immunol.* **2004**, *34*, 1483-1487.

201. Chuang, C.M.; Monie, A.; Wu, A.; Mao, C.P.; Hung, C.F. Treatment with LL-37 peptide enhances antitumor effects induced by CpG oligodeoxynucleotides against ovarian cancer. *Hum. Gene Ther.* **2009**, *20*, 303-313.

202. Büchau, A.S.; Morizane, S.; Trowbridge, J.; Schauer, J.; Kotol, P.; Bui, J.D.; Gallo, R.L. The host defense peptide cathelicidin is required for NK cell-mediated suppression of tumor growth. *J. Immunol.* **2010**, *184*, 369-378.

203. Kindrachuk, J.; Jenssen, H.; Elliott, M.; Townsend, R.; Nijnik, A.; Lee, S.F.; Gerds, V.; Babiuk, L.A.; Halperin, S.A.; Hancock, R.E. A novel vaccine adjuvant comprised of a synthetic innate defence regulator peptide and CpG oligonucleotide links innate and adaptive immunity. *Vaccine* **2009**, *27*, 4662-4671.

204. Easton, D.M.; Nijnik, A.; Mayer, M.L.; Hancock, R.E. Potential of immunomodulatory host defense peptides as novel anti-infectives. *Trends Biotechnol.* **2009**, *27*, 582-590.

205. Nijnik, A.; Pistolic, J.; Wyatt, A.; Tam, S.; Hancock, R.E. Human cathelicidin peptide LL-37 modulates the effects of IFN-gamma on APCs. *J. Immunol.* **2009**, *183*, 5788-5798.

206. Nagant, C.; Tré-Hardy, M.; El-Ouaaliti, M.; Savage, P.; Devleeschouwer, M.; Dehaye, J.P. Interaction between tobramycin and CSA-13 on clinical isolates of Pseudomonas aeruginosa in a model of young and mature biofilms. *Appl. Microbiol. Biotechnol.* **2010**, *88*, 251-263.

© 2010 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).