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Review

Cathelicidins – a family of multifunctional antimicrobial peptides

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Abstract. One component of host defence at mucosal surfaces are epithelial-derived antimicrobial peptides. Cathelicidins are one family of antimicrobial peptides characterized by conserved pro-peptide sequences that have been identified in several mammalian species. LL-37/hCAP-18 is the only cathelicidin found in humans and is expressed in inflammatory and epithelial cells. Besides their direct antimicrobial function, cathelicidins have multiple roles as mediators of inflammation influencing diverse processes such as cell proliferation and migration, immune modulation, wound healing, angiogenesis and the release of cytokines and histamine. Finally, cathelicidin antimicrobial peptides qualify as prototypes of innovative drugs that may be used to treat infection and/or modulate the immune response. This review provides an overview of antimicrobial peptides of the cathelicidin family, the structures of their genes and peptides and their biological functions.

Key words. Cathelicidin; antimicrobial peptide; host defence; innate immunity; resistance.

Introduction: antimicrobial Peptides as Effector Substances of Innate Immunity

Survival of a multicellular organism in a non-sterile environment requires a network of host defence mechanisms. The initial contact of pathogenic microorganisms with the host usually takes place at internal or external body surfaces. Animals of various phyla have developed first-line defence mechanisms to inhibit the growth and invasion of microorganisms. The first line of defence against pathogenic insult is called the innate immune system, which in mammals is followed by acquired immune responses associated with activation of T and B cells aimed against specific antigens [1, 2]. One principle of innate immunity is the production of endogenous antibiotic peptides.

Antimicrobial peptides are effector molecules of innate immunity with direct antimicrobial activity and multiple other functions [3, 4]. They have an important role in providing an initial mechanism of host defence. Antimicrobial peptides are categorized by homologous structural motifs. The most prominent families are defensins and cathelicidins. Mammalian defensins are cationic, relatively arginine rich non-glycosylated peptides that contain six cysteines forming three intramolecular disulphide bridges [5]. Peptide antibiotics of the cathelicidin family are characterized by a highly conserved signal sequence and pro-regions (‘cathelin’ = cathepsin L inhibitor) but show substantial heterogeneity in the C-terminal domain that encodes the mature peptide [6, 7].

The aim of this review is to describe the antimicrobial peptides of the cathelicidin family as an endogenous part of the innate immune system, to summarize the structures of their genes and peptide molecules and to comment on their role in health and disease.

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Structure and classification of cathelicidins

Cathelicidins are synthesized as pre-pro-peptides (fig. 1) and characterized by the conserved amino-terminal sequence of the peptide pro-piece, whereas the variable carboxy-terminal domain contains the antimicrobial activities. The pro-sequence is termed 'cathelin' after the function of this domain to inhibit the activity of cathepsin L (cathepsin L inhibitor) and is between 99 and 114 amino acids long [6] (fig. 2). The 'cathelin' protein was initially identified from pig leukocytes [8]. Molecules with a cathelin-like pro-peptide sequence have been isolated from multiple species including cow, pig, rabbit, sheep, human, mouse, monkey and horse. The function of the cathelin pro-sequence is speculative; however, one model is that it assists in the biogenesis of the mature peptide.

The carboxy-terminal domain represents the antimicrobially active peptide that varies considerably between individual molecules in sequence, length (12–100 residues) (fig. 2), and function. Based on amino acid sequences, mature cathelicidin peptides can be organized into three groups: group I – linear, α-helical peptides without cysteines, e.g. LL-37/hCAP-18 from human; group II – peptides with an even number of cysteines linked by disulphide bridges, e.g. protegrins (porcine cathelicidin peptides), and group III – peptides with an unusually high proportion of one or two amino acids, e.g. PR-39 from porcine leukocytes.

The secondary and tertiary structures of the mature antimicrobial peptide have been examined for several molecules. Studies using circular dichroism and nuclear magnetic resonance (NMR) spectroscopy revealed the transition of CRAMP [9, 10], PAMP-36 [11], PAMP-37 [12], BMAP-27, BMAP-28 [13] and LL-37 [14] from a random coil to an ordered, mainly α-helical form. A poly(L)-proline type II structure has been proposed for PR-39 [15], and the bactenecins Bac-5 and Bac-7 [16]. Several structural features have been identified as relevant for the microbicidal function of antimicrobial peptides: size, sequence, charge, degree of structuring (helicity), overall hydrophobicity, amphipathicity and the angles subtended by hydrophobic and hydrophilic surfaces of the helical molecule [17]. High mean hydrophobicity has been correlated with increased cytotoxic activity against eukaryotic membranes [18]. There is no simple correlation between activity and charge. When the net charge becomes more positive, binding to negatively charged surfaces of microorganisms is increased; however, the formation of trans-membrane pores is inhibited [19–21]. Since all these structural features are strongly interrelated, predicting the antimicrobial or cytotoxic activity from a given amino acid sequence is difficult.

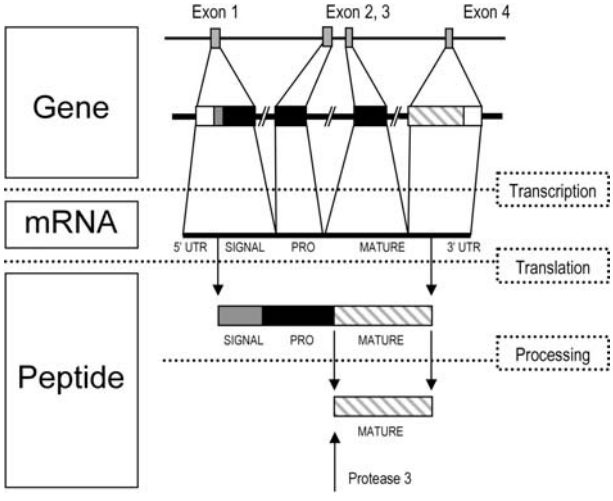

Figure 1. Structure of the gene and peptide of LL-37/hCAP-18 as a prototypical example for the cathelicidin family. The C terminus represents a part of the molecule with antimicrobial activity. The gene is represented schematically with the following individual components: White box, 5′ untranslated region (5′ UTR); grey box, signal sequence; black box, pro-sequence; shaded box, mature peptide; white box, 3′ UTR.

Biosynthesis, gene structure and expression

Cathelicidins are synthesized as pre-pro-peptides. Generally, the cathelin pro-peptide must be removed from the C-terminal peptide to unleash the microbicidal activity. Processing of the storage form to active peptide is assumed to take place in activated neutrophils during degranulation into the phagocytic vacuole or extracellular milieu [22]. Studies on bovine cathelicidins, proBac 5 and proBac 7, highlighted steps involved in the biogenesis of the nascent peptide [23]. The two pro-peptides lack antimicrobial function, likely because the cathelin-like pro-piece inactivates the C-terminal mature peptide [24]. As described below in detail, LL-37 is stored and secreted as pro-peptide and then cleaved extracellularly by protease 3 [25].

Most cathelicidins are stored in granules of neutrophils or macrophages, where they contribute to oxygen-independent mechanisms of antimicrobial activity [26, 27]. Initially, cathelicidins were isolated from myeloid cells of various species. In recent years, several cathelicidins have been identified in epithelial cells, indicating that these molecules have a role in host defense at body surfaces [28, 29]. The expression patterns of specific cathelicidins are described below.

Antimicrobial peptides including cathelicidins are products of individual genes that code for the corresponding pre-pro-peptides which are cleaved to liberate the mature peptide. The structure of cathelicidin genes is conserved, with most consisting of four exons (fig. 1). The first three exons code for the signal peptide and the cathelin-like pro-sequence. The fourth exon codes for the carboxy-terminal active peptide. In species with more than one mem-

Pre - / Signal-Peptide

```
Bac-5    METQRASLSLGRCSLWLLLLGLVLPSASA
BMAP-27  METQRASLSLGRWSLWLLLLGLALPSASA
CAP-18   METHKHGPSLAWWSLLLLLLGLLMPPAIA
CRAMP    MQFQRDVPSLWLWRSLSLLLLLGLGFSQT
LL-37    METQRASLSLGRWSLWLLLLGLVVPSASA
eCATH-1  METQRNTRCLGRWSPLLLLLGLVIPPATT
PR-39    METQRASLCLGRWSLWLLLLGLVVPSASA
PG-1     METQRASLCLGRWSLWLLLLALVVPSASA
SMAP-29  METQRASLSLGRRSLWLLLLGLVLASARA
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Pro-Peptide (conserved Cathelin-like sequence)

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Bac-5    QALSYREAVLRAVDQFNERSSEANLYRLLELDPTPNDDLDPGTRKPVSFRVKETDCPRTSQQPLEQCDFKENGLVKQCVGTVTLDPSNDQFDINCNELQSV
BMAP-27  QALSYREAVLRAVDQFNERSSEANLYRLLELDPPPKEDDENPNIPKPVSFRVKETVCPRTSQQPAEQCDFKENGLVKQCVGTVTLDAVKGKINVTCEELQSV
CAP-18   QDLTYREAVLRAVDAFNQQSSEANLYRLLSMDPQQLEDAKPYTPQPVSFTVKETECPRTTWKLPEQCDFKEDGLVKRCVGTVTRYQAWDSFDIRCNRAQESPEPT
CRAMP    PSYRDAVLRAVDDFNQQSLDTNLYRLLDLDPEPQGDEDPDTPKSVRFRVKETVCGKAERQLPEQCAFKEQGVVKQCMGAVTLNPAADSFDISCNEPGAQPFRFKKFA
LL-37    QVLSYKEAVLRAIDGINQRSSDANLYRLLDLDPRPTMDGDPDTPKPVSFTVKETVCPRTTQQSPEDCDFKKDGLVKRCMGTVTLNQARGSFDISCDKDNKRFA
eCATH-1  QALSYKEAVLRAVDGLNQRSSDENLYRLLELDPLPKGDKDSDTPKPVSFMVKETVCPRIMKQTPEQCDFKENGLVKQCVGTVILGPVKDHFDVSCGEPQRV
PR-39    QALSYREAVLRAVDRLNEQSSEANLYRLLELDQPPKADEDPGTPKPVSFTVKETVCPRPTRQPPELCDFKENGRVKQCVGTVTLNPSIHSLDISCNEIQSV
PG-1     QALSYREAVLRAVDRLNEQSSEANLYRLLELDQPPKADEDPGTPKPVSFTVKETVCPRPTRQPPELCDFKENGRVKQCVGTVTLDQIKDPLDITCNEVQGV
SMAP-29  QALSYREAVLRAVDQLNEKSSEANLYRLLELDPPPKQDDENSNIPKPVSFRVKETVCPRTSQQPAEQCDFKENGLLKECVGTVTLDQVGNNFDITCAEPQSV
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Mature C-terminal peptide

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Bac-5    RFRPPIRRPPIRPPFYPPFRPPIRPPIFPPIRPPFRPPLGPFPGRR
BMAP-27  GRFKRFRKKFKKLFKKLSPVIPLLHLG
CAP-18   GLRKRLRKFRNKIKEKLKKIGQKIQGFVPKLAPRTDY
CRAMP    ISRLAGLLRKGGEKIGEKLKKIGQKIKNFFQKLVPQPEQ
LL-37    LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES
ECATH-1  KRFGRLAKSFLRMRILLPRRKILLAS
PR-39    RRRPRPPYLPRPRPPPFFPPRLPPRIPPGFPPRFPPRFPGKR
PG-1     RGGRLCYCRRRFCVCVGRG
SMAP-29  RGLRRLGRKIAHGVKKYGPTVLRIIRIAG
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Figure 2. Comparison of cathelicidin antimicrobial peptides and their pre- and prosequences. The displayed peptides are: Bac-5 [16], BMAP-27 [13], CAP-18 [93], CRAMP [9] LL-37 [27, 62, 63], eCATH-1 [94], PR-39 [30], PG-1 [79] and SMAP-29 [95].

ber of the cathelicidin family, the corresponding genes are usually arranged in clusters [30]. The biology of the promoters of only a few cathelicidins has been studied. The presence of specific binding sites for transcription factors in the promoter regions of PR-39 and LL-37/hCAP-18 is associated with upregulation of these genes during inflammation [25, 29].

Biological activities of cathelicidins

Antimicrobial activity

The ability of cathelicidins to inhibit growth of bacteria is the most prominent function of these molecules. The standard assays for antibiotic activity involve conventional microbiological dilution assays to determine minimal inhibitory or bactericidal concentrations, inhibition zone assays on agar plates and fluorescence assays to monitor death of microorganisms. Structure/function studies are often limited by the availability of purified active materials. Screening assays have been used to identify cathelin antimicrobial peptides in biological samples, such as wound fluid and lung lavage liquid. In these studies, peptides and proteins were separated by chromatography and fractions assayed for antimicrobial activity [31, 32]. There are limitations in extrapolating in vitro antibacterial activity with antibiotic function in vivo. Antimicrobial peptides represent single components of complex biological networks involved in host defence and cell regulation. Knowledge of local microenvironments would be essential in understanding the role of cathelicidins in biological settings.

Mechanism of antimicrobial activity

The spectrum of organisms susceptible to cathelicidin peptides is broad and involves bacteria, protozoa, fungi and viruses. The activity is found at concentrations in the micromolar range. Antimicrobial activity occurs through several mechanisms (fig. 3). Interactions between the peptide and surface membranes of the target organisms play a key role. The initial binding is thought to depend on electrostatic interactions between the positively charged peptides and the negatively charged molecules at the surface of the target. The biophysical properties of the membrane are perturbed by direct interactions with the peptide. Membrane-active properties of antimicrobial peptides have been analyzed in model systems by demonstrating induction of leakage across lipid bilayers of artificial liposomes [33–36].

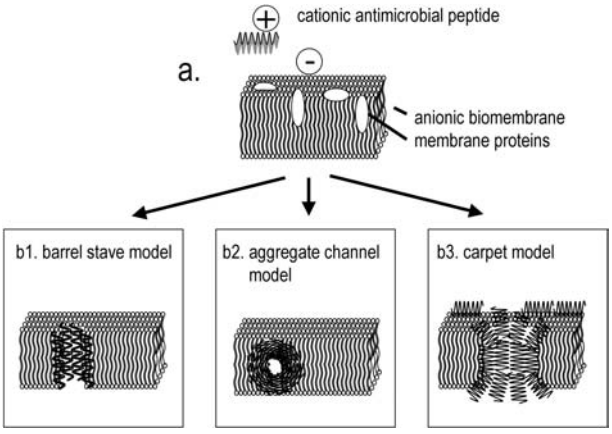

Figure 3. Mechanisms of action of antimicrobial peptides. After electrostatic interactions between the negatively charged bacterial wall and the positively charged peptides (*a*), the peptides associate with the membranes, leading to a destabilization of the membrane and subsequent cell death of the microorganism (*b*). Several models of action have been developed (as explained in the text): b1, barrel stave model; b2, aggregate channel model and b3, carpet model.

Three main mechanisms have been suggested for peptide permeation of the target cell membrane [18]. (i) 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 [37] (fig. 3). (ii) The aggregate channel model involves arrangement of peptides in unstructured clusters in the membrane allowing the dynamic formation of pores for short periods of time and the leakage of intracellular components. Antimicrobial peptides can also enter the intracellular space through this mechanism [38] (fig. 3). (iii) A carpet-like mechanism describes covering of the microbial cell membrane by a lawn of antimicrobial peptides (fig. 3). The integrity of the membrane collapses via holes that form by the bending of the lipid bilayer back on itself [39, 40]. Cathelicidins interact with both the inner and outer membrane of Gram-negative microorganisms [41, 42]. The term 'self-promoted' uptake describes mechanisms by which antimicrobial peptides interact with lipopolysaccharide (LPS) at the surface of Gram-negative bacteria to accumulate at the surface and to enter the microbial cell (43). The situation for Gram-positive bacteria is less clear; however, several Gram-positive organisms are susceptible to cathelicidins at low concentrations [44].

Interactions described above in the three models lead to loss of membrane function including breakdown of membrane potential, loss of metabolites and ions and alteration of membrane permeability. The partial selectivity of cathelicidins for prokaryotic cells seems to depend on unique lipid compositions associated with membranes of microorganisms which are distinct from membranes found on eukaryotic cells. Other mechanisms of antimicrobial activity include the inhibition of protein and/or RNA synthesis by Bac5, Bac7 and PR-39 [16, 45]. The antimicrobial spectra of individual peptides depend on their structures and amino acid sequences. Peptides rich in Pro or Arg residues are usually more active against Gram-negative than Gram-positive bacteria. Other linear (helical or Trp-rich) and Cys-containing peptides are generally active against Gram-positive and Gram-negative organisms. The antimicrobial activity of cathelicidin peptides is synergistic with other host defence molecules, such as lysozyme and lactoferrin [28].

Several cathelicidin peptides bind to bacterial endotoxin and inactivate its biological activity [46, 47]. This property has been used to reduce LPS mortality in murine models of endotoxaemia by application of LL-37/hCAP-18-derived peptides [48]. We used overexpression of LL-37/hCAP-18 by gene transfer in various model systems to demonstrate that endogenously produced antimicrobial peptides function in host defence in vivo and that this approach might have therapeutic implications [49, 50]. In contrast to the antimicrobial activity of the peptide, the uncleaved pro-peptide of rabbit CAP-18 still blocks biological activities of LPS [51].

Microbial resistance

The development of microbial resistance against antimicrobial peptides is rare. Several mechanisms have been described that can result in decreased susceptibility of bacteria to cathelicidins [52]. Gonococcal susceptibility to the lethal action of protegrin 1, LL-37 and other antimicrobial peptides is modulated by an energy-dependent efflux system, part of the resistance/nodulation/division efflux pump family [53]. Increase in the phosphocholine content of cell walls of *Haemophilus influenzae* decreased the susceptibility to LL-37 [54]. When exposed to the environment found in the airways of cystic fibrosis patients, *Pseudomonas aeruginosa* is capable of modifying the structure of LPS attached to the outer membrane [55]. These modifications in endotoxin decrease the susceptibility of these bacteria to cationic antimicrobial peptides. Infection or chronic inflammation seem also to be responsible for the transcriptional downregulation of antimicrobial peptides. Gastrointestinal *Shigella* infections in humans are associated with reduced expression of LL-37 in epithelial cells [56].

Other functions of cathelicidins

Besides their antimicrobial and anti-LPS activities, cathelicidins have a variety of other functions (fig. 4), such as chemoattraction of immune cells, release of histamine from mast cells, modulation of inflammation, or induction of angiogenesis. As examples, LL-37 and PR-39

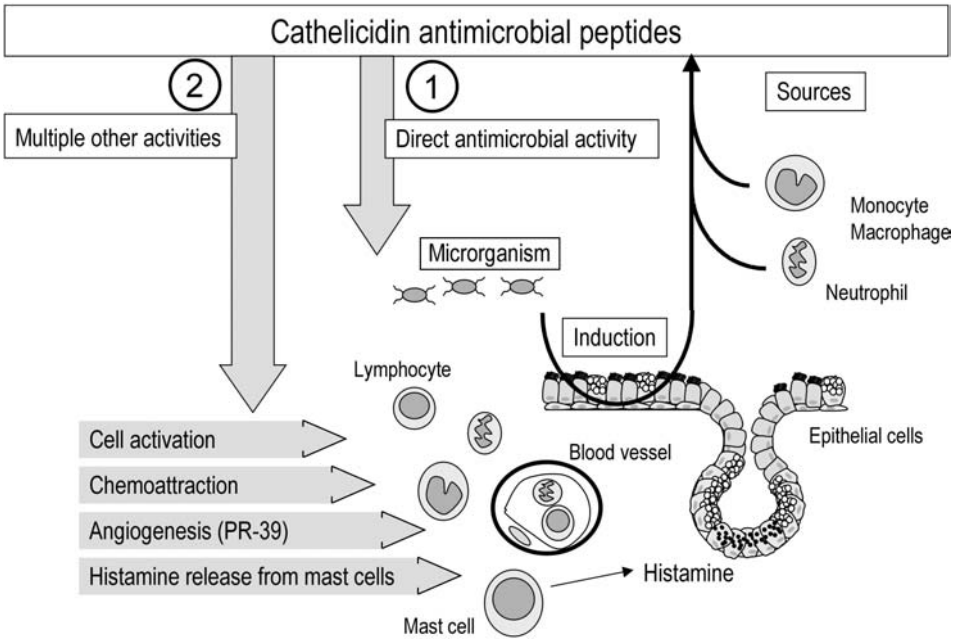

Figure 4. Biological functions of cathelicidin antimicrobial peptides. Cathelicidins are secreted by several cell types during infection and inflammation. For example, LL-37/hCAP-18 is found in airway surface fluid and originates from epithelial cells as well as professional inflammatory cells, such as macrophages, neutrophils and lymphocytes. The cathelicidin peptides have direct antimicrobial activity ①. Additionally, they regulate cellular responses including cell proliferation, cell migration of inflammatory cells, release of cytokines and angiogenesis ②. Cathelicidins are multifunctional peptides that link host defence with inflammation and angiogenesis and activate the adaptive immune system.

have been found to modulate the activity of immune and inflammatory cells. As discussed below in more detail, the peptide LL-37 binds to formyl peptide receptor-like 1 (FPRL1), a G protein-coupled, seven-transmembrane cell receptor found on various cell types including macrophages, neutrophils and subsets of lymphocytes [57]. The receptor is activated by high concentrations of N-formylmethionyl-leucyl-phenylalanine (fMLP) and several endogenous and exogenous ligands [58] and likely mediates cellular effects of LL-37, such as chemoattraction of neutrophils, monocytes and T cells as well as the activation of mast cells [59]. PR-39 is involved in several cellular processes, such as chemoattraction, angiogenesis and inflammation [60, 61]. The intracellular activities of PR-39 are described below.

Detailed description of selected cathelicidins

The only human cathelicidin: LL-37/hCAP-18

The amino acid sequence of the only cathelicidin isolated from humans, LL-37 or hCAP-18, was originally predicted from a cDNA clone. The peptide was finally isolated from neutrophil-specific granules [27, 62, 63]. The mature carboxy-terminal sequence of hCAP-18 is called LL-37 (37 amino acid residues long, the two N-terminal amino acids are leucines). Initially LL-37/hCAP-18 was isolated from myelocytes and metamyelocytes and localized to specific granules of neutrophils [64]. In addition, LL-37/hCAP-18 was found in epithelial cells of the testis, skin [29, 65], gastrointestinal tract [28] and respiratory tract [28]. Recently, LL-37/hCAP-18 was described in natural killer (NK) cells, $\gamma\delta$ T cells, B cells and macrophages including alveolar macrophages [59]. In human airways, which have been a major focus of research on antimicrobial peptides, LL-37 originates from surface and glandular epithelial cells, lymphocytes, neutrophils and macrophages resident in the airways [28]. The level of LL-37 has been determined in different body fluids, where it can reach high concentrations in serum [66], airway surface fluid [32, 49, 67] or wound and blister fluid [65] during infection and inflammation, ranging from 1 to 5 µg/ml. Expression of LL-37/hCAP-18 is upregulated in several cell types by inflammatory mediators, such as interleukin (IL)-6 [29]. Expression in intestinal epithelial cells is also dependent on the cell differentiation status [68]. LL-37 can be induced in keratinocytes [69]. Sequence analysis of the promoter of the gene shows that this region contains putative binding sites for many transcription factors including nuclear factor NF-κB, IL-6, acute-phase response factor, activator protein 4 and CCAAT/enhancer-binding protein (C/EBP). Besides this information based on sequence analysis, little is known about the constitutive or regulated expression of LL-37. The gene encoding LL-37/hCAP-18 contains four exons and is localized to chro-

mosome 3 [26]. After cleavage of the signal peptide during transit through the endoplasmic reticulum, the mature peptide can be released from the precursor by protease action. Intracellular sorting of the pro-peptides in myeloid cells depends on the differentiation status of the cells [70]. After secretion by neutrophils, the mature C-terminal LL-37 is processed by the activity of protease 3 [25].

LL-37 is active against Gram-positive and Gram-negative bacteria in micromolar concentrations. The antimicrobial activity is associated with the α-helical conformation of the molecule [14, 71]. The activity of LL-37 in solutions is dependent on pH and electrolyte composition. An empirically identified medium E provides conditions for α-helical folding associated with increased activity. Mechanisms of activity likely follow the carpet-like processes involving detergent-like effects as described above [14]. The cytotoxic concentrations of LL-37 on mammalian cells are only three times higher than the concentrations needed to kill bacteria. Serum inactivates the cytotoxic and antimicrobial activities of LL-37, however, without effecting functions such as chemoattraction of neutrophils and macrophages. LL-37 is bound by apolipoprotein-A1, which inhibits the antimicrobial and cytotoxic activities of the peptide. We overexpressed LL-37 by means of recombinant adenovirus in a cystic fibrosis xenograft model to correct the disease-specific defect of antimicrobial activity in lung secretions [49]. In subsequent studies of murine models of pneumonia and endotoxic shock, overexpression of LL-37 reduced morbidity and mortality [50].

Other functions of LL-37 as mentioned previously were identified recently, e.g. LL-37 binding to FPRL1 on myeloid and several other cell types [57], chemotactic activity for polymorphonuclear leukocytes, neutrophils, monocytes and CD4 T lymphocytes [57, 59] and stimulation of mast cells to release histamine [72]. Human LL-37 has closely related homologues in rhesus monkey [73, 74].

Mouse cathelicidin: CRAMP

Cathelin-related antimicrobial peptide (CRAMP) is the only cathelicidin isolated from mice. An RT-PCR approach was used to identify putative mouse cathelicidins and a cDNA of 562 bp was found [9, 75]. The corresponding gene, named *cnlp* (cathelin-like protein), was mapped to chromosome 9 at a region of conserved synteny to which genes for cathelicidins have been mapped in pig and human. The transcript of CRAMP was found in bone marrow of fetal and adult animals as well as in testis, spleen, stomach and the gastrointestinal tract. Circular dichroism and NMR spectral analysis showed that CRAMP forms an amphipathic α-helical structure [10]. The synthesized peptide is active against Gram-positive and Gram-negative bacteria. Derivates of CRAMP have been synthesized and shown to have antimicrobial activity [76]. Mice with a disrupted *cnlp* gene coding for CRAMP showed increased susceptibility to skin infections with group A *Streptococcus* [77]. The results of this study clearly demonstrated that cathelicidin antimicrobial peptides have a host defence function in vivo. Based on its gene and peptide structure, cellular processing [78], antimicrobial spectrum and tissue expression, CRAMP seems to be closely related to LL-37.

Porcine cathelicidins: protegrins and PR-39

Analysis of the porcine genome revealed genes encoding several cathelicidin peptides, including the proline-arginine rich (PR) PR-39 (mature peptide is 39 amino acids in length) and the protegrins.

Protegrins are rich in cysteines with two intramolecular disulphide bonds and β sheet structures, which makes the mature peptide similar in structure to peptides of the defensin group. Protegrin peptides were identified from porcine leukocytes and the corresponding cDNA isolated by RT-PCR cloning [79–81]. Based on sequence analysis, protegrins closely resemble the tachyplesins, antimicrobial peptides found in the haemocytes (amoebocytes) of horseshoe crabs [79]. Protegrins are active against Gram-positive and Gram-negative bacteria. Unlike other cathelicidins or defensins, the antimicrobial activity of protegrins is not significantly inhibited by increased concentrations of salt [82]. This unique property has initiated attempts to use protegrin as a broad-spectrum antibiotic.

PR-39 peptide was initially isolated from pig small intestine on the basis of antimicrobial activity, and its cDNA was subsequently cloned from bone marrow [83, 84]. The gene encoding PR-39 was mapped to chromosome 13, which is homologous to human chromosome 3, where the LL-37/hCAP-18 gene is localized [83, 85]. The peptide is found mainly in neutrophils and is present in wound fluid. PR-39 is highly active against Gram-negative bacteria. Its mechanisms of action differ from other cathelicidin peptides [15, 83]. PR-39 crosses membranes rapidly and exerts its activity inside microbial cells by blocking bacterial DNA and protein synthesis. The mature peptide is also capable of rapidly crossing eukaryotic cell membranes [86] to bind to SH3 domains of the cytosolic component of the NADPH complex protein $p47^{phox}$ and the signalling adapter protein $p130^{cas}$ [87]. Recent evidence indicates that PR-39 stimulates angiogenesis by inhibiting the ubiquitin-proteasome-dependent degradation of hypoxia-inducible factor 1a protein [60]. This selective inhibitory effect of the peptide in the proteasome pathway (reversible binding to the a7 subunit of the 26s proteasome) also leads to an anti-inflammatory activity by blocking the degradation of the NF-kB inhibitor IkBa [88]. PR-39 has not been found to exhibit direct cytotoxic effects. Furthermore, PR-39 is chemoattractive for neutrophils in a

calcium-dependent and pertussis toxin-inhibitable reaction and contributes to wound healing by stimulating the expression of syndecans, cell surface heparan sulphate proteoglycans [88]. These multiple biological activities of PR-39 have been used to prevent post-ischemic microvascular dysfunction, partly through the inhibition of neutrophil adhesion and oxidant production [89, 90].

Outlook: cathelicidins, innate immunity and novel drugs

Cathelin-related antimicrobial peptides have emerged as effector substances of the innate immune system involving not only activities as endogenous antibiotics but also as mediators of inflammation, wound healing and tissue repair. Several important topics will have to be addressed in the future. (i) Identification of novel antimicrobial peptides. Families of cathelicidin peptides are likely to exist in mammalian species. Progress in the human genome project will also reveal ways to shortcut conventional bioscreening procedures for the identification of these family members. (ii) Analysis of the biologically relevant functions of antimicrobial peptides. Besides in vitro experiments that give the first insight into the function of peptide antibiotics, a broader approach involving genetic animal models is necessary to interpret in vitro data in the context of a whole organism. (iii) Evaluation of the function of antimicrobial peptides in diseases might provide information about the corresponding pathogenesis. (iv) Development of antimicrobial peptides as drugs. Studying the biology of antimicrobial peptides may allow the development of novel therapeutics including anti-infectious, anti-inflammatory, or pro-angiogenic drugs [91, 92]. Several naturally occurring peptides have been used to develop prototypical antibiotic drugs that are currently being evaluated in phase I–III clinical trials [18].

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