The role of cationic antimicrobial peptides in innate host defences

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Cationic antimicrobial peptides are found in all living species. A single animal can contain >24 different antimicrobial peptides, which fall into four structural classes. These peptides are produced in large quantities at sites of infection and/or inflammation and can have broad-spectrum antibacterial, antifungal, antiviral, antiprotozoan and antisepsis properties. In addition, they interact directly with host cells to modulate the inflammatory process and innate defences.

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We are exposed daily to tens of thousands of potential pathogens through ingestion, contact with infected surfaces (animate and inanimate) and inhalation. The adaptive humoral and cellular immune responses have minimal impact on whether these pathogens will go on to cause infections. Bacteria are able to grow so rapidly that a single organism can produce 5×10^8 bacteria, a full-blown infection, in 24 hours, given a 50 min doubling time. By contrast, a primary immune response involving clonal expansion of slow-growing B and T cells takes up to seven days (three days for a secondary response) before it is even noticeable. Thus, the body depends on the innate immune response[1] to prevent onset of infection. The hallmarks of the adaptive immune response are specificity, inducibility and discrimination of self vs non-self. Although these characteristics exist to some extent in the innate immune response, pre-existing constitutive effectors (e.g. lysozyme) also play a role. The inducible effectors of the innate immune response differ from those of the adaptive immune response in that they are relatively non-specific, have conserved molecular patterns of recognition of stimulatory molecules, which include bacterial lipopolysaccharide (LPS) and lipoteichoic acid (LTA), and are rapidly induced (within minutes to hours).

The effectors of the innate immune response have traditionally been thought to include phagocytic cells, such as neutrophils and macrophages, other leukocytic cells, including mast cells, and serum proteins such as complement. It is our proposal that this scenario is incomplete, and that a group of cationic antimicrobial peptides are major players in the innate immune response[2–4]. Such peptides have relatively recently been discovered to be components of the innate immune response with broad antimicrobial activities. However, they are clearly very ancient elements of the immune responses of all species of life, and the induction pathways for these peptides in vertebrates, insects and plants[1,5] are highly conserved. Furthermore, it is becoming increasingly apparent that cationic antimicrobial peptides have many potential roles in inflammatory responses, which represent an orchestration of the mechanisms of innate immunity.

The intention of this review is to describe the role of cationic antimicrobial peptides in host innate immunity to microbial pathogens. We will discuss their diverse nature and unusually broad spectra of activity against infectious agents of all types. The induction of certain antimicrobial peptides by bacterial products during acute inflammation will be described, and the conserved induction pathways for the inducible peptides discussed. Additionally, evidence pointing to a major or essential role of such peptides in innate immunity will be briefly described, and the myriad of potential roles of antimicrobial peptides defined. Our discussion will be largely restricted to mammalian systems, although the role of such peptides in insect immunity is possibly better defined[5]. Readers are directed to other reviews for detailed information about the 500 known natural peptides, their mechanisms of action and their potential exploitation as therapeutics[4,6–8] (`http://bbcm1.univ.trieste.it/~tossi/pag1.htm`).

Properties of cationic antimicrobial peptides

Cationic antimicrobial peptides are defined here as peptides of 12–50 amino acids with a net positive charge of +2 to +7 owing to an excess of basic amino acids (arginine, lysine and histidine) over acidic amino acids[8]. Generally speaking, 50% or more of the amino acids are hydrophobic, a fact reflected by the interaction of such peptides with bacterial membranes as part of their mechanism of action[8]. Despite their small size and common physico-chemical features, cationic antimicrobial peptides have a range of 3-D structures that fit into four known classes[6]. The most common classes are β-sheet peptides stabilized by 2–4 disulfide bridges (and occasionally containing a short α-helical stretch), and unstructured peptides that fold into amphipathic α-helices upon contact with membranes. Less common are extended peptides

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Table 1. Examples of the primary amino acid sequences of mammalian cationic antimicrobial peptides

Peptide	Structural class	Sequence[a]
Rabbit α-defensin (NP-1)	β-sheet	VVC$_1$AC$_2$**RR**ALC$_3$LP**R**E**RR**AGFC$_3$**R**I**R**G**R**IHLC$_2$C$_1$**RR**
Human β-defensin 1	β-sheet	DHYNC$_1$VSSGQC$_2$LYSAC$_3$PIFT**K**IQGTC$_2$Y**R**G**K**A**K**C$_1$C$_3$**K**
Pig protegrin 1	β-sheet	**R**GG**R**LC$_1$YC$_2$**RRR**FC$_2$VC$_1$VG**R***
Human LL-37	α-helical	LLGDFF**RK**S**K**E**K**IG**K**EF**KR**IVQ**R**I**K**DFL**R**NLVP**R**TES*
Human histatin 5	α-helical	DSHA**KR**HHGY**KRK**FHE**K**HHSH**R**GY*
Cattle indolicidin	Extended	ILPW**K**WPWWPW**RR***
Pig PR39	Extended	**RRR**P**R**PPYLP**R**P**R**PPPFFPP**R**LPP**R**IPPGFPP**R**FPP**R**FP*
Cattle bactenecin	Loop	**R**LC$_1$**R**IVVI**R**VC$_1$**R**

[a]One letter amino acid code with the following additions: positively charged residues at neutral pH are in bold; the subscript numbers represent amino acids that are joined by cysteine disulfides; a star at the end of a peptide implies that the peptide is known to be amidated at its carboxy terminus.

with a predominance of one or two amino acids (e.g. proline, tryptophan or histidine) and loop peptides formed by a single disulfide bond. Other cationic antimicrobial peptides are formed by proteolytic digestion of larger cationic proteins such as lactoferrin. Indeed, several cationic proteins implicated in innate immunity, including lactoferrin, bactericidal/permeability increasing protein and cathepsin G, have characteristics and activities that are reminiscent of cationic peptides. Generally speaking, the most potent cationic peptides fold into molecules that have the charged and hydrophilic portions segregated from the hydrophobic portions, either as amphipathic structures or as cationic double wing structures with a hydrophobic core separating two charged segments.

Such peptides are found in all species of life including bacteria, fungi, plants, insects, birds, crustaceans, amphibians and mammals. A single animal can contain different classes of peptides and a number of variants in a given class. For example, cattle are known to contain 38 antimicrobial peptides, including β-stranded α- and β-defensins, α-helical BMAP peptides, the extended peptide indolicidin, and the loop peptide bactenecin, as well as a variety of fragments of larger proteins. There are at least four possible reasons for this variety. First, the antimicrobial spectrum of any given peptide tends to be incomplete, suggesting that certain peptides cover deficiencies inherent in others. Second, different peptides work in synergy with each other against microorganisms. Third, the non-antimicrobial (e.g. anti-endotoxic, chemotactic and pro-inflammatory) activities of peptides appear to vary and might also complement each other. Fourth, these peptides tend to be produced by different cell types, such that a given tissue might express only a subset. Table 1 describes some examples of mammalian peptides.

Antimicrobial activity

Cationic antimicrobial peptides have a diverse range of targets (Table 2). The only defining characteristic of these targets is their possession of a membrane. Thus, viruses are targets for antimicrobial peptides, but only enveloped viruses such as HIV, herpesvirus and vesicular stomatitis virus (VSV). Other targets include Gram-negative and/or Gram-positive bacteria, fungi, parasites such as trypanosomes and plasmodia, and even cancer cells. Normal human cells are relatively resistant, but it should be mentioned that certain cationic antimicrobial peptides, such as melittin from bees, mastoparan from wasps and charybdotoxin from scorpions, are potent toxins. The basis of discrimination for the relatively non-toxic peptides appears to be the lipid composition of the target membrane (selective peptides tend to prefer membranes that have a negatively charged surface and are free of cholesterol) and the possession, by the peptide-susceptible organism, of a large transmembrane electrical potential (oriented internal negative)[8]. The activities of antimicrobial peptides can be reduced by a variety of relevant factors that exist *in vivo*, including high mono- and divalent cation concentrations, polyanions, serum, apolipoprotein A-1, serpins and proteases[6,9]. However, some peptides appear to be relatively immune to several of these agents.

Table 2. Activities of cationic antimicrobial peptides and some examples of peptides with those activities

Activity	Examples
Broad-spectrum antibacterial	Defensins, indolicidin, protegrin and LL-37
Synergy with other peptides	Defensins NP-1 and NP-5
Antifungal	Protegrin, indolicidin and histatins
Anti-endotoxin	LL-37
Anti-enveloped virus (HIV,HSV,VSV)	Indolicidin, protegrin and defensins
Anticancer	Indolicidin and defensins
Wound healing	PR39 and defensins
Antiparasite	Indolicidin and defensins

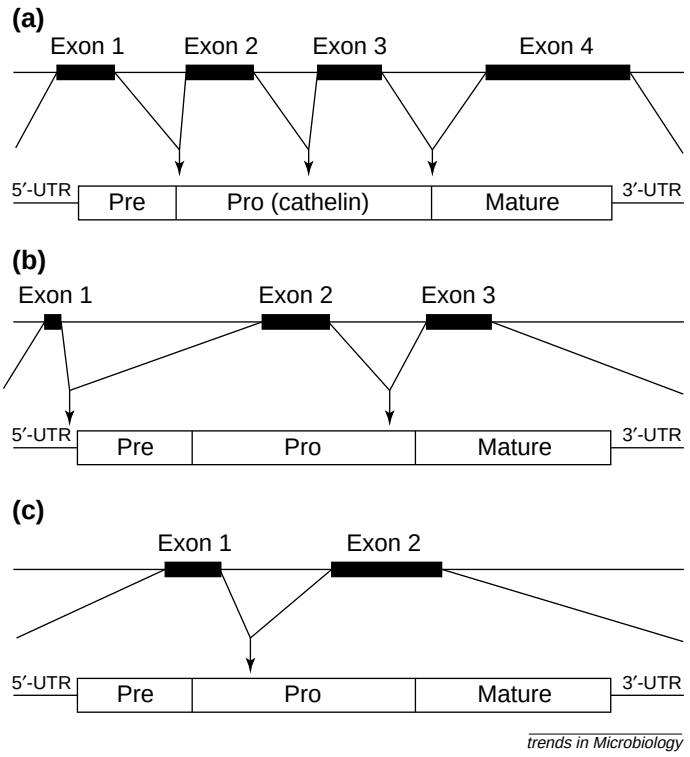

Fig. 1. Gene structures of mammalian antimicrobial peptides. **(a)** cathelicidins, **(b)** myeloid α-defensins and **(c)** β-defensins and epithelial α-defensins. The pre-regions represent signal sequences involved in secretion. The anionic pro-regions protect the cationic peptide portion prior to release of the mature peptide. Abbreviation: UTR, untranslated region.

Cationic antimicrobial peptides have independent activities, but these activities can be improved in synergy with other factors. Synergy between different peptides has already been mentioned; however, the peptides also work synergistically with lactoferrin, lysozyme and other proteins present in body fluids or tissues[10]. Cationic antimicrobial peptides also show synergy with a diverse range of conventional antimicrobials[7], leading one to suspect that there are other molecules, especially at sites of inflammation, which will act in synergy with cationic peptides.

Genetics of antimicrobial peptides

Antimicrobial peptides are encoded by single genes that comprise highly homologous gene families (Fig. 1). These gene families localize to chromosomal clusters that appear to reflect the evolution of different subclasses[11], characterized by tissue of expression and inducibility. In humans, the defensin locus is found on chromosome 8p21–23, where both α- and β-defensins co-localize[12]. The genes for α- and β-defensins maintain high sequence identity within each group, but are widely diverse from each other. Characterization of defensin mRNA has indicated that these peptides are synthesized as precursor molecules, which include a signal sequence followed by a propeptide region. The propeptide region is further processed to the mature peptide. Within each defensin family there is remarkably high conservation of both amino acid and mRNA sequences in the region encoding the signal sequence. In the mRNA, this sequence identity extends into the 5′-untranslated segment where there is a region of >90% identity between defensins of unrelated species (Fig. 2).

Cathelicidins are a family of structurally divergent antimicrobial peptides from diverse species that are classified together because their propeptide regions contain a conserved domain with homology to that of cathelin, a porcine cysteine-protease inhibitor[13]. Unlike defensins, cathelicidins are stored as inactive propeptide precursors and processed upon stimulation. In porcine and bovine neutrophils, processing occurs via neutrophil elastase, which then releases the active peptide into the extracellular fluid[14]. Cathelicidin peptides include protegrins from pigs, bactenecin and indolicidin from cows, and CAP18 from rabbits. In humans only one cathelicidin – LL-37/hCAP18 (Ref. 10) – has been found to date. Cathelicidins are encoded by genes of four exons, with the active mature peptide, which ranges in size from 12–79 amino acids, located in the fourth exon[15] (Fig. 1). As observed with the genes encoding defensins, cathelicidin-encoding genes contain a highly homologous 5′ region that encodes the conserved cathelin domain[16]. This has enabled researchers to identify new cathelicidin sequences in numerous species, including cow, pig, sheep, horse, mice, guinea pig and rabbit. Cathelicidin genes are found as clusters on sheep, cow and pig chromosomes; in humans, the single cathelicidin gene is located on chromosome 3.

Gene expression

It has been observed that the tissue of expression and inducibility of antimicrobial peptides reflects their gene structure. By direct sequence comparison, it was found that human enteric defensins (HD5 and -6), which are expressed in the Paneth cells of the small intestine, are encoded by two-exon genes and appear to be the evolutionary precursor of the myeloid defensins (HNP-1/3, 2 and 4)[11]. The myeloid defensins are each encoded by three exons, and appear to be under the control of haematopoietic regulatory elements, resulting in their constitutive expression in the promyelocyte[17]. By contrast, epithelial defensins are found in both constitutively expressed and inducible forms. Specifically, enteric (α-) defensin genes are expressed at high levels in normal intestine in the mouse, human and rat[18]. The expression of at least one of these genes is induced in the rat intestine after haemorrhagic shock[19]. One other enteric α-defensin gene, *HD5*, was found to be variably expressed in the female reproductive tract[20], where expression in the endometrium correlated with the phase of the menstrual cycle.

Two classes of β-defensins can be defined by observing expression patterns. Constitutively expressed β-defensins, such as hBD-1 in humans and the 13 bovine neutrophil β-defensins (BNBD-1–13), are characterized by two exons surrounding a large intron[12] (G. Diamond, unpublished). hBD-1 is expressed at high levels in the genitourinary tract, and

(a)

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Human       HNP-1   CCCAGCCATGAGGACCCTCGCCATCTTGCTGCCATTCTCCTGGTGGCCCTGCAGGCCCAGGCTGAGC
            HNP-3   ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
            HD-5    ::::::::::::::A:::::::::::::::::::::::::::::::::::::::::::::::::::T
            HD-6    ::T::::::::A::::::A::::::CA::::TG::::::C::::::::C::::::A:::::::::
Rabbit      MCP-1   ::::::::::::::::::::TC:G:::::::::::::::::::::::::::::::::::::::::::
            MCP-2   ::::::::::::::::::::TC:G:::::::::::::::::::::::::::::::::::::::::::
Mouse       CRY-1   :G::::::::A::AA::A:T:C:::CTT:::::C::G:::::C:T:GT:::::::T::::::::TT
Guinea pig  GCNP1   ::::::::::::::::G::C:TC::::::::::TG:::T:::C::A:::::AT::::::::::::::
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(b)

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Cow         TAP     ATGAGGCTCCATCACCTGCTCCTCGCGCTCCTCTTCCTGGTCCTGTCTGCTTGGTCAGGATTTACTCAAGGAGTA
            BNBD-4  :::::::::::::::::::::::::AG:::::::::::::::::::::::G:::::::::::::::::A:::::
Sheep       SBD-1   :::::::::::::::::::::::::T:C::T:::::G::::::::::::::G:::::::::::::::::::::::
Goat        GBD-1   :::::::::::::::::::::::::T:C::T:::::G::::::::::::::G:::::::::::::::::::::::
Human       HBD-2   ::::::G::TTGT:T::C:::T::T::T:::::::A:AT:::::ATGC::CTTC::::TG::TT:GGT::T:::
Rabbit      RBD-2   ::::::A:::::T::::T:::T::T:AT:T:::C:GG::C:G:::::GC:CCTT::::CC:::::::::A:T::C
Mouse       MBD-1   ::::::A:::::T::::T:::T:TG:AT:T:::C:GG::C:G:::::TC:ACCTG:::CT::::GCA::AA:::C
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Fig. 2. Sequence identity of the 5′ regions of the **(a)** α-defensin and **(b)** β-defensin mRNAs. The initiator codon ATG is underlined. A colon indicates an identical base.

at lower levels in other tissues, but its mRNA levels do not change with microbial challenge or inflammatory state[16]. By contrast, the expression of tracheal antimicrobial peptide (TAP), a β-defensin found in the bovine airway, is upregulated by infectious agents and inflammatory mediators in primary culture systems. Specifically, a 15-fold increase in the steady-state levels of mRNA encoding TAP was observed upon incubation with 100 ng ml^{-1} *Escherichia coli* LPS (Ref. 21). This indicates that airway epithelial cells can respond to pathogens by the production of antimicrobial agents. These cells also upregulate the expression of TAP in response to numerous infectious and inflammatory agents, including phorbol 12-myristate 13-actetate[21], tumour necrosis factor α (TNF-α)[22], interleukin (IL)-1β, muramyl dipeptide, LTA (Ref. 23) and interferon γ (IFN-γ) (G. Diamond, unpublished). In addition, the homologous β-defensin lingual antimicrobial peptide (LAP), originally described as a peptide whose expression in the tongue epithelium was upregulated at sites of inflammation, underwent an increase in expression in the airway in a coordinated fashion with TAP (Ref. 22). These genes are much smaller, mostly owing to a relatively small intron.

In humans, a TAP homologue, β-defensin 2 (hBD 2) was initially discovered to be expressed in psoriatic skin[24]. This peptide is also expressed in other inflamed tissues, including the airway, where it has been observed that primary airway epithelial cells upregulate hBD-2 mRNA in response to IL-1β (Ref. 25). A similar induction in an air–liquid-interface culture of human TEC was found in response to *Pseudomonas aeruginosa* LPS, as well as TNF (G. Diamond, unpublished). The homologous mouse β-defensin 2 (mBD2) also undergoes upregulation in response to LPS.

TAP and hBD-2 gene expression is induced by bacterial LPS through an epithelial-cell-expressed CD14-mediated signal transduction pathway[21]. The induction occurs via activation of the p65/p50 heterodimer of nuclear factor (NF)-κB, which binds to an NF-κB consensus sequence upstream from the TAP gene[23]. A similar pathway is activated in the LPS upregulation of the hBD-2 gene in human airway cells. These data suggest that inflammation and infection mediate a peptide-based host response in mucosal tissues through transcriptional regulation.

In most species, cathelicidins are expressed in myeloid precursor cells, although expression in mature peripheral porcine neutrophils has been reported[26]. Surprisingly, porcine cathelicidins are also expressed in a number of lymphoid tissues in young pigs (four-weeks old), but expression disappears in adults[26]. A single cathelicidin, LL-37/hCAP18, is found in humans, expression of which is widespread, including myeloid precursors, testis, keratinocytes and the airway epithelium[10]. The promoter region of the gene encoding porcine PR-39 exhibits several potential transcription-factor-binding sites, including NF-IL6, suggesting regulatory controls similar to (and possibly coordinated with) defensins. Indeed, cathelicidins and defensins exhibit synergistic antimicrobial activities[27], suggesting that they both participate in a combined host defence response.

In vivo evidence for a host defence role

The case for a primary role for antimicrobial peptides in host defences is becoming increasingly convincing. Defensins have been shown to be the most predominant protein species in neutrophils, representing nearly 15% of total protein in these dedicated anti-infective cells. Other antimicrobial peptides are found at mucosal and epithelial surfaces and in the gut, lungs, kidneys and skin. Their induction during inflammation correlates with a primary role in assisting and/or directing inflammatory responses and with the previously described results of *in vitro* expression

studies. Indeed, increased levels of antimicrobial peptides have been observed in a number of clinical and laboratory-induced infectious and inflammatory states. Pigs subjected to *Salmonella* infection exhibited a threefold increase in circulating cathelicidin (PR-39) levels[28]. Similarly, plasma levels of hBD-2 were increased fourfold in patients with bacterial pneumonia[29]. Experimentally induced infection of calves with *Cryptosporidium parvum* increased enteric β-defensin levels fivefold[30]. Cows testing positive for infection with *Mycobacterium paratuberculosis* exhibited increased expression of β-defensins in the intestine[31]. Similar upregulation was also experimentally induced by intratracheal instillation of *Pasteurella haemolytica* into a single lobe of a cow lung, where an increase in β-defensin expression in the airway epithelium correlated with the infection. In mice, intratracheal instillation of *P. aeruginosa* was sufficient to increase expression of mouse β-defensin 3 (mBD-3) in the tracheal epithelium as well as the intestinal tract[32]. In humans, inflamed intestinal epithelium exhibits high levels of hBD-2 expression relative to normal colon[33], as does inflamed gingival epithelium[34].

Functional studies have supported the above evidence for a role for antimicrobial peptides in host defence, though these experiments are complicated by the numerous defensin genes often expressed in the same tissues, as well as the redundant defence mechanisms within the innate immune system. The first example was seen in *Drosophila*, where mutations of the Toll pathway leading to antifungal peptide gene expression led to increased susceptibility to lethal fungal, but not bacterial, infections[35]. Conversely, mutations affecting the induction of antibacterial peptides reduced survival in response to bacterial challenge. Most recently, an innovative technique was devised to address the issue of multiple defensin genes in mice. Rather than attempting to knock out all 20 defensins expressed in the mouse small intestine, Wilson *et al.*[36] identified the single enzyme necessary for processing the preprodefensins to the active mature form. Genetic inactivation of this single gene (matrilysin) led to no production of active defensin in the small intestine, and subsequently a tenfold increase in the susceptibility to infection by orally introduced virulent bacteria was observed.

Such studies cannot be done in humans. However, patients with specific granule deficiency syndrome, and who are therefore completely lacking in α-defensins, suffer from frequent and severe bacterial infections[4,9]. Similarly, a group of HIV patients with lower salivary levels of histatin peptides showed a higher incidence of oral candidiasis and fungal infection[9]. Other studies have connected the chronic lung infections of cystic fibrosis patients with the high salt concentrations in the airways (caused by a mutation in the cystic fibrosis transmembrane regulator/chloride channel), as the antimicrobial substances secreted by lung epithelial cells tend to be salt sensitive[8]; however, these studies remain controversial.

Platelets play a role in innate host defences, in part because they possess antimicrobial peptides called platelet microbicidal peptides. Interestingly, for *Staphylococcus aureus* and *Staphylococcus epidermidis* it has been shown that bacteria susceptible to these peptides have a reduced propensity to cause endocarditis in humans compared with peptide-resistant isolates[37].

Although they provide only indirect confirmation, animal model studies in which cationic peptide levels are artificially boosted either through injection or transgenic overexpression, clearly reveal that such peptides are antimicrobial in the context of the intact host. A wide variety of animal model studies and early clinical trials have demonstrated that both natural and synthetic exogenously added peptides will protect against local or systemic infection by bacteria and fungi[7]. Furthermore, peptides will block LPS-mediated induction of the cytokine TNF in galactosamine-sensitized mice, thus preventing endotoxaemia and death[38,39]. These studies have been extended by demonstrating that mice transgenic for the synthetic α-helical peptide Shiva 1a (Ref. 40) are resistant to infection by *Brucella abortus*. Conversely, overexpression of antimicrobial peptides can provide increased resistance to infections. Overexpression of human cathelicidin genes in the mouse airway by adenovirus-mediated gene transfer results in the increased ability to prevent infections[41]. Overexpression of defensins in cell cultures also provides these cultures with increased resistance to bacterial and viral infections[42]. In plants, upregulation of antimicrobial-peptide gene expression also increases their resistance to pathogens[43]. Overall, these results provide a compelling rationale for the importance of cationic antimicrobial peptides in host defences against infection.

Other effects of peptides on hosts

The action of cationic antimicrobial peptides is not limited to direct killing of microorganisms. Instead, they have an impressive variety of additional activities that impact particularly on the quality and effectiveness of innate immune responses and inflammation (Table 3; Fig. 3). Unfortunately, this information remains somewhat sketchy at present, as only a subset of cationic antimicrobial peptides have been examined for any given activity. Thus, in Table 3, as both natural and synthetic peptides have common physico-chemical natures, we describe all of the agents whether natural or synthetic that have been defined to influence inflammatory processes. However, in Fig. 3, we have only summarized those activities observed with natural peptides. These include many elements of inflammatory responses that are frequently ascribed to other agents, and in many cases it remains to be discovered whether these peptides are central players, supporters or even simple bystanders. One might suspect a key role, however, given the very high concentrations that have been recorded at sites of inflammation (e.g. 300 $\mu g\ ml^{-1}$ or more in the sputum of cystic fibrosis patients[44]; 20–100 $\mu g\ ml^{-1}$ in

Table 3. Effect of cationic antimicrobial peptides on host cells and inflammatory/immune processes[a]

Peptide[b]	Effect	Possible role in immunity	Refs
LL-37; PR-39; TAP; LAP; α- and β-defensins	Induction during inflammation	Synthesis triggered by situations that might involve infection	22,28–34
LL-37; **CP-28**	Directly stimulate transcription of >30 genes in macrophage cell line	Modulation of the activity of phagocytic cells	45
CAP11; Magainin 2	Promote histamine release from mast cells	Stimulate increase in blood vessel permeability	9,10
α-defensins	Induce IL-8 in airway epithelial cells	Recruitment of neutrophils	53
PR-39	Chemotactic activity for neutrophils	Increase phagocytic activity	10,47
LL-37; HNP-1, -2 and –3; histone H2B fragments	Induction in wounds and blisters	Wound healing (?)	9,10
Guinea-pig defensins	Increased expression of CD11b,c and ICAM-1	Increase adherence of neutrophils	9,10
Rabbit defensins NP-1,2	Promote non-opsonic phagocytosis by macrophages	Bacterial clearance	45
CA(1–8)M(1–18)	Influence signalling pathways in macrophages; induce iNOS synthesis	Bacterial clearance; activation of macrophages	51
Defensin I and II	Inhibit fibrinolysis by tissue plasminogen activator	Limiting spread of infection	9,10
Defensin; SB-37; **Shiva**, **Vishnu**	Stimulate mitogenic effect for fibroblasts and epithelial cells; stimulate fibroblast growth	Wound healing	46
PR-39	Induce cell surface matrix proteogylcans, syndecans 1 and 4	Wound healing	47
Histatin 3; pro Bac 7	Inhibition of furin proprotein convertase/cathepsin L protease	Inhibit tissue injury during inflammation	50
BMAP-27; BMAP-28; **CA(1–8)M(1–18)**; Lactoferricin	Apoptosis in the U937 and RAW264.7 macrophage lines and in *in vitro*-activated human lymphocytes	Elimination of cells with intracellular bacteria, virus-infected cells and cancer cells	10,49,52
Defensins	Attenuates steroidogenesis	Blunt the release of immunosuppressive cortisol during stress from infection; upregulated tissue inflammation	4,9
CAP11	Increased neutrophil adhesion; inhibits phagocytosis of opsonized zymosan particles	Modulation of neutrophil functions	10
ProBac 7; defensins	Chemotactic activity for monocytes	Recruitment of monocytes, which differentiate into inflammatory macrophages	9,10,49
CAP18(106–137); LL-37; **CP-28** and many other peptides	Neutralize LPS (endotoxin) responses in macrophages and animal models	Antisepsis; feedback inhibition of endotoxin responses	10,38,39
Many peptides	Neutralize LTA responses in macrophages	Antisepsis	45
LL-37; **CP-28**	Neutralize CpG responses in macrophages	Antisepsis	c
LL-37; **CP-28**	Selectively suppress expression of >40 LPS-induced genes in a macrophage cell line	Antisepsis; reduce cytokine expression	45
HNP-1, -2; LL-37	Chemotactic activity for T cells	Recruit T helpers and initiate cellular immune responses	48
Nisin	Induce increase in CD4 and CD8 lymphocytes in mice on short-term administration to diets	Increase cellular immunity and T-cell help	9
Defensins; HNP-1, -2 and -3	Enhancement of IFN-γ, IL5, K6, K10 and proliferative responses by T-helper cells cytokine secretion, increased systemic IgG but not IgA	Promotion of acquired systemic immune defenses (not mucosal immune defences)	52

[a]Abbreviations: iNOS, intracellular nitric oxide synthase; IFN, interferon; Ig, immunoglobulin; IL, interleukin; LAP, lingual antimicrobial peptide; LTA, lipoteichoic acid; LPS, lipopolysaccharide; TAP, transporters associated with antigen processing.
[b]Natural peptides are in normal typeface whereas synthetic peptides are indicated in bold.
[c]R.E.W. Hancock and M.G. Scott, unpublished.

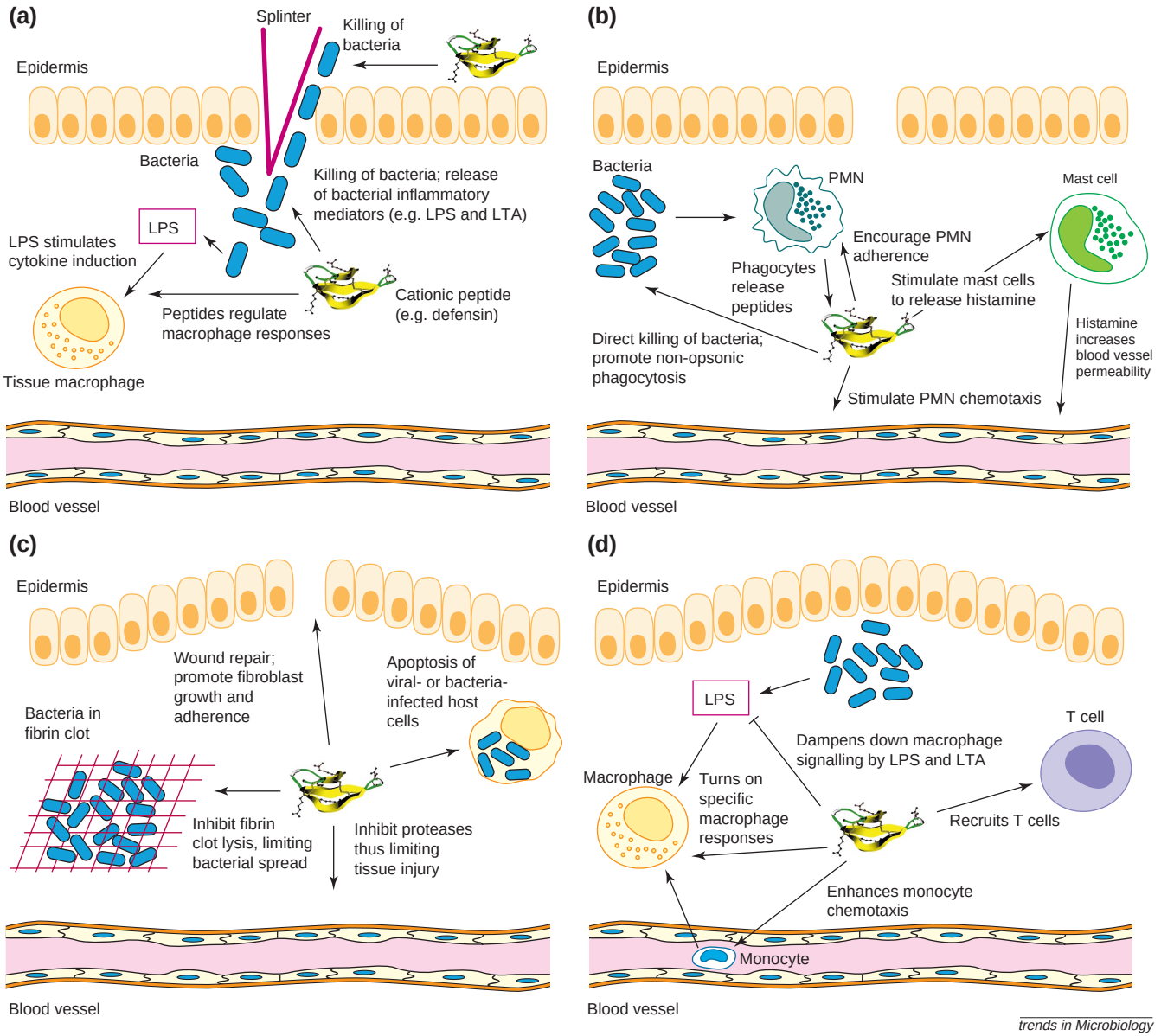

Fig. 3. Proposed role of antimicrobial peptides in acute **(a–c)** and chronic **(d)** inflammation. The specific roles of individual peptides (shown as the molecular structure of human defensin 1) in the various events depicted have been demonstrated for individual separate natural peptides (Table 3) but have not been demonstrated with any single peptide. Thus the overall scheme presented is a chimera of these separate effects. Abbreviations: LPS, lipopolysaccharide; LTA, lipoteichoic acid; PMN, polymorphonuclear leukocyte.

the dorsal tongue; up to 170 μg ml^{-1} in the plasma of septic individuals; 13 μg ml^{-1} in the saliva of patients with oral squamous cell carcinoma; and 13 μg ml^{-1} in the pleural effusion fluid of patients with empyma).

Cationic antimicrobial peptides have been reported to be involved in many aspects of innate host defences associated with acute inflammation (Fig. 3a–c), including: initial lysis of bacterial cells to release inflammatory stimuli; mast cell degranulation leading to histamine release and consequent vasodilation; increasing chemotaxis of neutrophils and T-helper cells resulting in leukocyte recruitment to the infection site; promotion of non-opsonic phagocytosis; inhibition of fibrinolysis by tissue plasminogen activator, thus reducing the spreading of bacteria; tissue/wound repair through promotion of fibroblast chemotaxis and growth; and inhibition of tissue injury by inhibiting certain proteases such as furin and cathepsin (Table 3). If acute inflammatory responses are insufficient to result in bacterial clearance, then chronic inflammation and the adaptive immune responses are initiated (Fig. 3d). Some roles that cationic antimicrobial peptides might play in this process are: acting as chemotaxins for monocytes; recruitment of T cells through chemotaxis; enhancement of chemokine production and the proliferative response of T-helper cells, leading to increased immunoglobulin (Ig)G but not IgA production; suppression of cytokine production and other responses of macrophages to LPS, LTA and bacterial CpG DNA; turning on of specific

macrophage genes; and stimulation of apoptosis of macrophages and activated lymphocytes, resulting in potential elimination of infected cells.

Thus, there is an array of potential roles of antimicrobial peptides in inflammation. One very interesting activity of these peptides is their ability to neutralize the responses of macrophages to endotoxic LPS. LPS interaction with macrophage receptors is known to stimulate cytokine production[39]. With a low LPS dose, the level of cytokines induced is moderate and this promotes immune defences. At high doses, cytokines (especially TNF) are highly induced and, in turn, can cause sepsis (also known as systemic inflammatory response syndrome). As LPS also turns on the production of cationic antimicrobial peptides[21,22], which in turn can neutralize LPS (Refs 38,39), it could be that they form part of the body's mechanisms for limiting septic responses to LPS (Ref. 45).

Conclusions

It is becoming clear that cationic antimicrobial peptides are an important and significant component of host defences against infection. Many such peptides are encoded by mammals and are inducible under specific conditions that reflect infection, utilizing signal transduction pathways that are conserved in many eukaryotes, and are also used in induction of other innate defences. The importance of such peptides in defence against infections is attested to by experiments utilizing transgenic animals engineered to produce reduced amounts of peptides and by rare peptide-deficiency syndromes in humans that tend to increase the risk of infection. Conversely, the *in vivo* potency of such peptides is clearly indicated by the ability of exogenously added or transgenically overexpressed peptides to protect against infections and endotoxaemia (sepsis). Although direct antimicrobial action is an obvious effect of such peptides on infectious agents, the peptides appear to be involved in the orchestration of many aspects of innate immunity and the inflammatory response. Thus, cationic antimicrobial peptides are an integral and important component of early host defences against infection.

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Viral mechanisms of immune evasion

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During the millions of years they have coexisted with their hosts, viruses have learned how to manipulate host immune control mechanisms. Viral gene functions provide an overview of many relevant principles in cell biology and immunology. Our knowledge of viral gene functions must be integrated into virus–host interaction networks to understand viral pathogenesis, and could lead to new antiviral strategies and the ability to exploit viral functions as tools in medicine.

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Viruses must be extremely successful predators as they depend on living cells for replication. Almost all living species represent prey for a viral invader. Viruses have coevolved with their hosts and therefore have limited pathogenicity in an immunocompetent natural host. In turn, probably as a result of the constant evolutionary pressure from viral invaders, higher vertebrates have developed a complex immune system. Only in the last decade have we caught a glimpse of what viruses do beyond invading cells for replication. For millions of years viruses have studied cell biology and immunology the hard way, to acquire and defend an ecological niche. It is remarkable that, in the process, individual virus families have targeted many common immunological principles.

Viruses that belong to different families are subject to different constraints. Owing to the low fidelity of RNA polymerase, the genome size of RNA viruses is limited. Although this confers the advantage of being able to use mutation to escape immune control, there is little room in the genome to allow immune defenses to be encoded by individual genes. The proteins encoded by RNA viruses are therefore multifunctional. This particular constraint is less rigid for DNA viruses as their genome size allows a larger number of genes to be devoted to host control. In the case of herpesviruses and poxviruses, these genes probably account for >50% of the total genome.

Viruses can exist in two forms: extracellular virion particles and intracellular genomes. Virions are more

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