CellPress

Host defense peptides: front-line immunomodulators

Sarah C. Mansour*, Olga M. Pena*, and Robert E.W. Hancock

Centre for Microbial Diseases and Immunity Research, 2259 Lower Mall Research Station, University of British Columbia, Vancouver, BC, V6T 1Z4, Canada

Although first studied for their antimicrobial activity, host defense peptides (HDPs) are now widely recognized for their multifunctional roles in both the innate and adaptive immune responses. Their diverse immunomodulatory capabilities include the modulation of pro- and anti-inflammatory responses, chemoattraction, enhancement of extracellular and intracellular bacterial killing, cellular differentiation and activation of the innate and adaptive compartments, wound-healing, and modulation of autophagy as well as apoptosis and pyroptosis. We review the various immunomodulatory roles of HDPs and their synthetic analogs, the innate defense regulators (IDRs). We discuss their potential as host-directed therapies, the hurdles they face in clinical development, and propose ways forward.

The quest for new antimicrobial agents

The discovery of penicillin paved the way for a new era of antimicrobial therapies, giving rise to a multitude of antibiotics for the treatment of infectious diseases. However, exacerbated by excessive and sometimes improper use, penicillin-resistant isolates emerged, driving the development of other antibiotics such as methicillin, a narrow-spectrum drug used to treat Gram-positive infections. In 1960, only one year after its initial clinical use [1], methicillin-resistant *Staphylococcus aureus* (MRSA) emerged. Over the past 4 decades there has been an explosion of antimicrobial-resistant bacteria including the frequent occurrence of so-called 'superbugs' such as vancomycin-resistant enterococci (VRE), multidrug-resistant *Pseudomonas*, *Klebsiella*, and *Acinetobacter*, and fluoroquinolone-resistant *Pneumococcus* [2]. In light of these developments, the need to discover novel antimicrobial therapies and approaches has become imperative. The exploitation of the broad immunomodulatory properties of naturally occurring HDPs has thus attracted considerable attention.

In the present review we focus on the most recently described roles of host defense peptides as well as the therapeutic progress of natural and synthetic cationic peptides in experimental animal models. We explore detailed mechanisms by which HDPs interact with cells of the innate and adaptive immune system, discuss the major hurdles impeding the use of these peptides in the clinic, and propose potential solutions.

Nature strikes back

Host defense peptides, also known as antimicrobial peptides (AMPs), are evolutionarily conserved molecules of the innate immune system. Found in all complex living organisms, HDPs have been commonly studied for their modest antimicrobial properties, targeting a broad spectrum of pathogens including bacteria, viruses, fungi, and protozoa [3,4]. Naturally occurring HDPs can vary in size, ranging from 12 to 50 (or more) amino acids, are generally cationic owing to the presence of excess lysine and arginine, and contain about 50% hydrophobic amino acids [3,5]. These properties allow them to interact with membranes, and many peptides are capable of penetrating the cell membrane. HDPs associate preferentially with membranes of bacteria-like composition (i.e., negatively charged) in model systems, but many peptides are able to translocate into host cells. For this reason the precise basis for their selectivity is still poorly understood [6]. It has been suggested that in bacteria they tend to translocate intracellularly owing to the presence of a large electrical potential gradient (part of the proton motive force) of about −120 mV [7]. Their direct antimicrobial mechanism of action against bacteria often involves multiple different targets including bacterial membrane integrity, inhibition of essential processes that utilize membrane-associated intermediates such as cell wall peptidoglycan biosynthesis and possibly cell division, inhibition of cytosolic RNA, protein, or DNA synthesis, and/or inhibition of cytosolic enzymes/chaperones [6,8]. Given these diverse range of targets, HDPs are less likely to select for resistance mechanisms in bacteria [8]. Furthermore, specific peptides have recently been described to possess multi-species anti-biofilm activity which is independent of their activity against free-swimming (planktonic bacteria) owing to their ability to target the stress response signal guanosine tetraphosphate [9].

Despite the well-described antimicrobial properties of HDPs, this activity can be considerably affected by the physiological conditions present in the body including the strong antagonism of divalent cations and polyanions (e.g., heparin) found in the blood, organs, mucosa and body fluids. For example, under physiological salt conditions (~100–200 nM), the antimicrobial activity of human HDP cathelicidin is severely dampened [10]. Moreover, under pathological conditions that generate excessive

Corresponding author: Hancock, R.E.W. (bob@hancocklab.com).
*These authors contributed equally to this work.

1471-4906/
 http://dx.doi.org/10.1016/j.it.2014.07.004

Table 1. Cellular expression and target distribution of HDPs.

HDP	Produced by	Acts on	Refs
α-Defensins	Neutrophils, Paneth cells	Epithelial cells, monocytes	[80–82]
β-Defensins	Neutrophils, epithelial cells (keratinocytes)	Monocytes, dendritic cells, T cells	[83–86]
LL-37	Neutrophils, monocytes, mast cells, epithelial cells	Neutrophils, monocytes, macrophages, dendritic cells, T cells, mast cells, epithelial cells, vascular endothelium, mesenchymal stromal cells	[33,71,87–92]

mucus, the antimicrobial activity of HDPs is greatly hindered by polysaccharides [11]. However, HDP-mediated protection has been observed in several *in vivo* studies, thus suggesting that the antagonistic effect exerted by physiological conditions may affect direct antimicrobial activity, but possibly not the broad range of immunomodulatory activities exhibited by these peptides – which help to refine host defenses to respond more favorably to infection [12–15]. These findings suggest that the immunomodulatory activities of HDPs might be the most relevant biological functions in the context of microbial infections.

Host defense peptides and their synthetic cousins

HDPs are produced by a variety of immune cells of hematopoietic and epithelial origin [15] (Table 1). In humans, HDPs are generally grouped into two major families; β-structured defensins and variably-structured cathelicidins. Most mammalian defensins are further grouped into two classes, α and β, that differ based on their patterns of disulphide bonding. Genomic studies have revealed the presence of dozens of defensin genes, pseudogenes, copy-number polymorphisms, and new defensin-like families in mouse and human [16]; however, here we deal with the best-studied examples in human.

There are six prominent α-defensins described in human: four produced and stored in the secretory granules of neutrophils (human neutrophil peptides, HNPs) and two expressed as propeptides in granules of Paneth cells [17]. When stimulated by proinflammatory molecules, both neutrophils and Paneth cells degranulate, releasing α-defensins into the local environment. Human β-defensins (HBDs) are another group of defensins that play a major role in preventing the colonization of microbes such as bacteria, viruses, parasites, and fungi on different epithelial surfaces [17]. Some β-defensins are produced constitutively, such as human β-defensin 1 (HBD1), which is released predominantly by keratinocytes, possibly as a constitutive defense. Other β-defensins such as HBD2-4, produced by the mucosal, gastrointestinal, and urogenital epithelia, can be induced by proinflammatory stimuli such as lipopolysaccharide (LPS) and tumor necrosis factor α (TNF-α) [17].

In human, there is only one cathelicidin precursor, hCAP-18, which is processed by serine protease 3 to generate α-helical LL-37, a 37 amino acid linear peptide [18]. LL-37 has been identified in many cells, tissues, and organs, as well as on the mucosa and in secretions, and is one the best-studied HDPs. Moreover, during infection and inflammation, degranulating neutrophils release relatively high levels of LL-37 into the local environment. LL-37 has demonstrated a broad range of immunomodulatory functions *in vivo*, including the direct and indirect recruitment of neutrophils, monocytes, and T cells, as well as very strong anti-endotoxin activity [19,20]. Individuals with morbus Kostmann, a disease characterized by a deficiency of LL-37 and reduced production of human neutrophil peptide (HNP) 1-3, exhibit increased susceptibility to severe periodontal disease, demonstrating the importance of these peptides in host defenses [21].

Considerable efforts have been made to exploit these natural and powerful immune functions of HDPs by generating synthetic peptide analogs, such as the innate defense regulators (IDRs) that are conceptually based on natural peptides. Three prominent examples of IDRs are IDR-1, IDR-1002, and IDR-1018, each of which has an exceptional ability to stimulate cellular recruitment and suppress potentially harmful inflammation, thus accounting for their protective effects during murine bacterial infections and inflammation [22–25]. These protective effects have been shown in mouse models upon infection with *Staphylococcus aureus* including MRSA, vancomycin-resistant enterococci (VRE), *Plasmodium berghei*, multi-drug-resistant *Mycobacterium tuberculosis*, and herpes simplex virus [22–28].

Immunological properties of host defense peptides

The immunomodulatory activities of HDPs and IDRs include modulating pro- and anti-inflammatory responses through altering signaling pathways [29], directly [30] and indirectly [31] recruiting effector cells including phagocytes, enhancing extracellular [32] and intracellular [33] bacterial killing, promoting polarized dendritic cell maturation [34] and macrophage differentiation [12], and modulating wound repair [35], apoptosis [36], and pyroptosis [37]. These immunomodulatory activities, which are outlined in Figure 1, are discussed below in detail.

Modulation of pro- and anti-inflammatory responses

HDPs can dampen proinflammatory responses such as those produced by particular Toll-like receptor (TLR) ligands. For example, Mookherjee *et al.* [29] demonstrated that LL-37 is able to modulate TLR-mediated responses, reducing proinflammatory mediators such as TNF-α, in response to LPS and lipoteichoic acid (LTA). These effects were seen either when LL-37 was added before, in combination with, or after 30 minutes of TLR stimulation, demonstrating that the observed effect was not due only to peptide binding to and neutralization of LPS, as previously suggested [38,39]. To characterize further the transcriptional changes orchestrated by LL-37 during LPS treatment, a comprehensive microarray analysis was performed in human monocytes, demonstrating a direct but selective impact of LL-37 on the TLR to nuclear factor κB (NF-κB) pathway, regulating genes such as *TNFAIP2* (TNF-α induced protein 2) and the activation of NF-κB1 p105/p50. Quantitative PCR (qPCR) and western Blot experiments

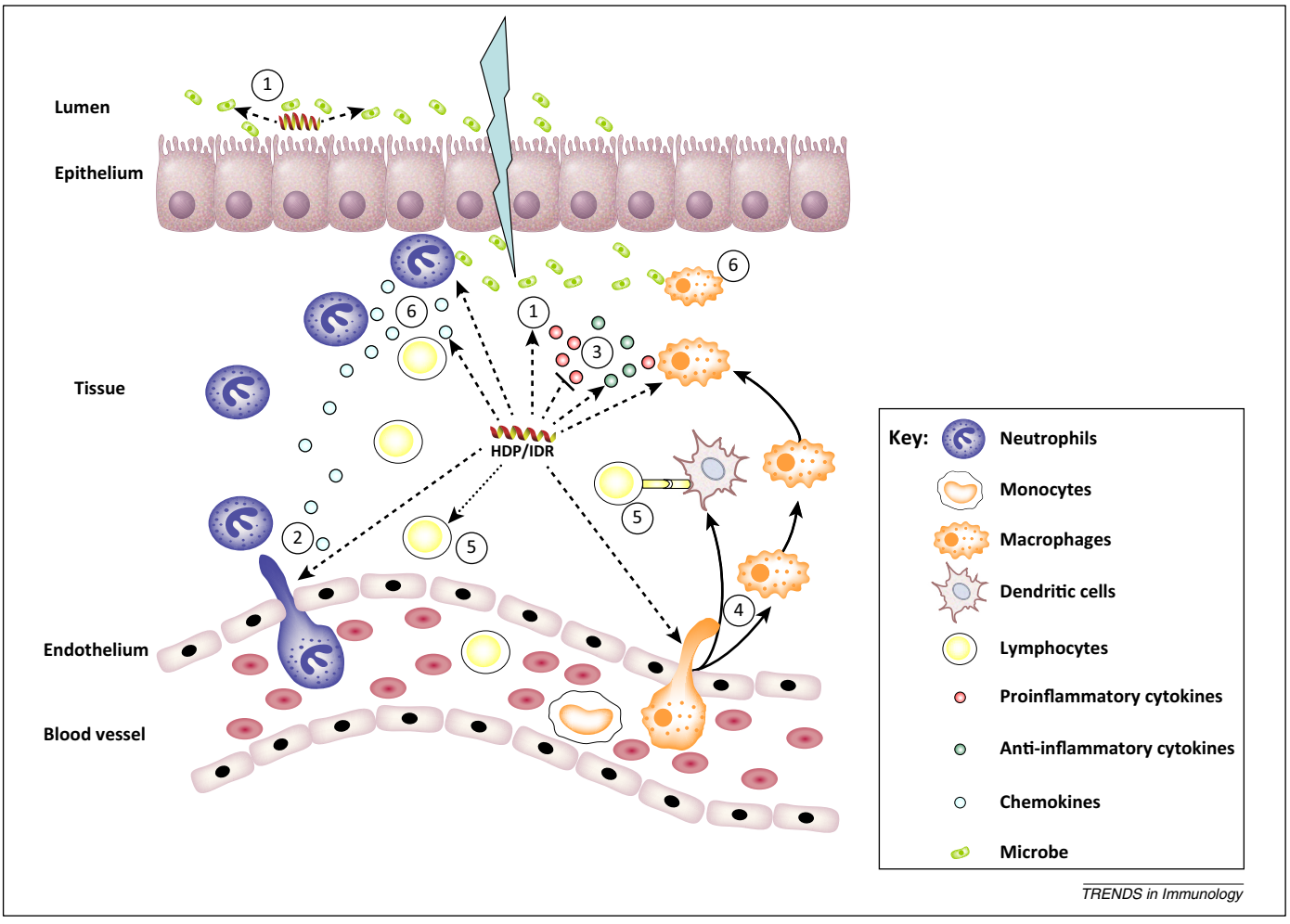

Figure 1. Immune functions of host defense peptides (HDPs)/innate defense regulators (IDRs). The different immune actions of HDPs. HDPs released by epithelial cells and immune cells such as neutrophils in high concentrations might provide direct antimicrobial or anti-biofilm activity against invading bacteria (1). At lower concentrations HDPs indirectly or directly promote the recruitment of immune cells such as neutrophils and monocytes (2), suppress the induced production of proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-8, and enhance the production of anti-inflammatory mediators including IL-10 and chemokines such as monocyte chemotactic protein 1 (MCP-1) (3), induce macrophage and dendritic cell differentiation and activation (4), leading to the modulation of adaptive immunity and inducing the recruitment of T cells, and (5) regulate specific cell activities including autophagy and the formation of neutrophil extracellular traps (NETs) (6).

further confirmed that LL-37 suppresses LPS-induced nuclear translocation of NF-κB subunits p50 and p65 [29]. Using proteomics, pull-down, co-localization, short interfering RNA (siRNA)-mediated knockdown and mobility shift analyses in monocytes, Mookherjee and colleagues additionally demonstrated that LL-37 binds to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and uses it as an intracellular receptor to promote cellular responses that are modulated through the p38 mitogen-activated protein kinase (MAPK) pathway [40].

Nijnik *et al.* also demonstrated that LL-37 is capable of inhibiting T helper 1 (Th1)-type responses produced in response to interferon (IFN)-γ by suppressing the production of TNF-α and interleukin (IL)-12 in monocytes, macrophages, and dendritic cells, as well as inhibiting the activation of class-switching in splenic B cells [41]. These inhibitory effects were mediated through suppression of signal transducer and activator of transcription 1 (STAT1)-independent signaling events, which involved both the p65 NF-κB and p38 MAPK subunits [41].

Interestingly, through the interaction of multiple pathways, namely phosphoinositide 3-kinase (PI3K), MAPK, and NF-κB, LL-37 can exert a very different effect on the production of IL-1β, another proinflammatory mediator. When combined, LL-37 and IL-1β synergize and enhance the recruitment of new monocytes and macrophages to the site of the infection. This was attributed to the selective upregulation of key chemokines including monocyte chemotactic protein 1 (MCP-1) and MCP-3 [42]. Moreover, in this experiment, the production of the anti-inflammatory mediator IL-10 was greatly enhanced, thus demonstrating the very dynamic nature of HDPs and their ability to balance both pro- and anti-inflammatory responses according to different cellular responses

Enhanced chemoattraction

Generally speaking, HDPs promote chemotaxis either in a direct and/or indirect manner. Enhanced recruitment of immune cells by HDPs has been observed indirectly by promoting the expression of well-known chemokines, CXCL8/IL8 and CCL2/MCP1, by monocytes/macrophages, epithelial cells, mast cells, and T cells [31,43]. *In vivo* models have confirmed these findings. For example, in mice infected with *Klebsiella pneumonia*, the marked reduction of

bacterial loads in response to neutrophil defensin HNP-1 was attributed to the increased accumulation of leukocytes at the site of infection [44]. Similar findings were observed using synthetic cationic peptides IDR-1 and IDR-1002 in *Staphylococcus aureus* and *Escherichia coli* invasive infection murine models, wherein protection was associated with leukocyte recruitment [22,23]. Further analyses were performed specifically on IDR-1002 to understand the molecular mechanisms leading to leukocyte recruitment. This process appears to be mediated at the molecular level through Gi-protein-coupled receptors and the PI3K, NF-κB, and MAPK pathways. At the cellular level, the enhanced protection observed seems to be associated with enhanced monocyte migration and adhesion to fibronectin [45].

At high concentrations, particular HDPs can act directly as chemokines themselves to recruit a variety of immune and epithelial cells, thus enhancing the clearance of infections and resolution of the affected site [30,46,47]. For example, *in vitro*, LL-37 directly attracts neutrophils and eosinophils, and this was found to be mediated by formyl-peptide receptors [30]. However, these effects were found only when using concentrations of LL-37 above physiological conditions (~1 μM), suggesting that this may not be the case *in vivo*.

Nonetheless, it is important to note that HDP and some chemokines have similarities because they are both amphipathic cations. In fact, shared functions and mechanisms of action have been noted between HDPs and more than 40 different human chemokines. For example, human β-defensin-2 shares the CCR6 receptor with the chemokine CCL-20 and can likewise (but less efficiently) induce chemotaxis of dendritic cells and T cells [14]. This might possibly demonstrate an evolutionary relationship between HDPs (especially defensins) and chemokines, which were developed to defend highly diverse microenvironments [48].

Enhanced extracellular and intracellular bacterial killing

Intriguingly, HDPs have also been suggested to kill microorganisms directly or indirectly through another innate immune mechanism known as neutrophil-derived extracellular traps (NETs) or mast cell derived extracellular traps (MCETs). NETs and MCETs are networks of extracellular components that include DNA and granular antimicrobial molecules such as LL-37 and HNP [49]. Extracellular trap formation is triggered in response to bacteria, activated platelets, as well as by a variety of inflammatory stimuli, whereby neutrophils expel their nuclear components and proteins from granules, such as myeloperoxidase, to entrap and disarm the pathogen [32]. The actual role of HDPs in NETs and MCETs has yet to be elucidated, but because HDPs have been more recently recognized for their antibiofilm activity, a hypothetical role for their presence in NETs is to selectively destroy biofilm cells, the likely growth state of bacteria within NETs [9,50].

In addition, enhanced intracellular bacterial killing has also been observed in neutrophils. Through *in vitro* bacterial killing assays, synthetic cationic peptides IDR-HH2, IDR-1002, and IDR0-1018 showed increased neutrophil-triggered killing of *E. coli*, and promoted the release of HDPs such as LL-37 and HNP [33].

Promotion of cellular differentiation

HDPs have been proposed to bridge the innate and adaptive immune responses by inducing the differentiation of dendritic cells and macrophages. When used in combination with GM-CSF and IL-4, LL-37 was found to be a potent modifier of human dendritic cell differentiation and activation *in vitro*. This was correlated with upregulation of endocytic activity, costimulatory molecule expression, and modified phagocytic receptor expression and function [34]. Likewise, Birayin *et al.* found that murine β-defensin 2 (mDF2β) can induce the activation of immature dendritic cells. Specifically, mDF2β acts as an endogenous ligand of TLR4, inducing the expression of co-stimulatory molecules and dendritic cell maturation *in vitro* [51]. Moreover, Van der Does *et al.* demonstrated that hLF1, a human lactoferrin-derived synthetic peptide, promotes the differentiation of dendritic cells while enhancing uptake and phagocytic functions, increasing reactive oxygen species (ROS) production, and enhancing Th17 polarization as well as the production of IL-17 and IL-10 cytokines [52].

Furthermore, macrophage differentiation was found to be induced by synthetic cationic peptides. For example, Pena *et al.* [12] observed *in vitro*, that IDR-1018 induces human macrophage differentiation towards an intermediate phenotype between proinflammatory M1 and anti-inflammatory wound-healing M2 macrophages. In this intermediate state induced by IDR-1018, macrophages developed a unique capability to maintain particular proinflammatory activities while producing anti-inflammatory and regulatory mediators such as CCL22 [12], a chemokine known to recruit Th2 cells and regulatory T cells [53,54]. In addition, this specific cellular state induced by IDR-1018 did not represent locked-in cellular reprogramming because IDR-1018-stimulated intermediate-state macrophages treated with IFN-γ could revert to an M1 phenotype, enabling normal inflammatory responses to environmental stimuli.

Direct effect on adaptive immunity

In addition to their effects on innate immunity and dendritic cells, of which both are crucial in influencing subsequent adaptive immune responses, HDPs can act directly on B and T cells. The murine homolog of human LL37, CRAMP (cathelin-related antimicrobial peptide), was shown to modulate immunoglobulin IgG1 production in B cells by suppressing the production of IL-4 by T cells [55]. Moreover, experiments performed in serum-containing media and whole blood demonstrated that LL-37 also promotes the rapid sensing of CpG oligodeoxynucleotides by B cells and plasmocytoid dendritic cells, but not by T cells [56]. In the same study, LL-37 was also shown to promote the rapid recognition of bacterial DNA, but not human DNA, by peripheral blood mononuclear cells (PBMCs), and this might explain the rapid recognition of peptide-associated microbial DNA in vaccine studies. All these attributes are consistent with adjuvant activity, which has been demonstrated for both HDPs and IDRs [57,58]. In fact, peptides are best presented in combination with other adjuvant components and optimally provide potent, long-lasting, protective immune responses even after a single dose. For example, synergistic interactions

between the bovine HDP indolicidin with CpG and polyphosphazene resulted in a long-term and well-balanced immune response in cattle [58], and a similar combination using the synthetic peptide IDR-1002 yielded a potent protective immune response in mice against *Bordetella pertussis* [58]. Cellular immune responses to *Chlamydia* in a prime–boost vaccine strategy were also promoted by a combination IDR-HH2/CpG adjuvant [59].

Promotion of wound-healing

HDPs, such as LL-37, have important roles in wound-healing by inducing chemotaxis of epithelial cells and the production of the metalloproteinases that are responsible for restructuring the extracellular matrix [60]. Additional evidence has also demonstrated that the lack of LL-37 impairs the re-epithelialization of chronic wounds such as ulcers [35]. Synthetic peptides have also shown wound-healing activity. For example, the synthetic peptide IDR-1018 has strong wound-healing properties *in vitro* and enhances cell viability and keratinocyte migration [26]. In a murine wound model, IDR-1018 demonstrated a therapeutic response in wound-healing and faster wound-closure compared to other peptides such as LL-37. Similar results were observed in a porcine wound model infected with S. *aureus* in which IDR-1018, as well as synthetic peptide SR-0379, enhanced wound-healing properties [26,61]. Although the mechanisms by which peptides promote wound-healing are not fully characterized, some studies have demonstrated that HDPs such as β-defensins promote keratinocyte migration through phosphorylation of the epidermal growth factor receptor (EGFR), which then activates STAT1 and STAT3 [62]. Moreover, LL-37 seems not only to act through EGFR, but also through formyl peptide receptor-like-1, FPRL-1, which leads to the activation of the PI3K/AKT and MAPK signaling pathways [63,64].

Modulation of autophagy

Autophagy is an important catabolic mechanism that involves the degradation and recycling of dysfunctional or unnecessary cellular components, allowing the preservation of cellular energy. In some instances it has been considered to be an innate defense mechanism that becomes activated in infections caused by intracellular microorganisms such as *M. tuberculosis*. It was demonstrated that the human cathelicidin LL-37 can induce autophagy in a vitamin D3-mediated manner, activating autophagy-controlled genes such as *Beclin-1* and *Atg5* [65]. Furthermore, Mayer *et al.* discovered that one of the causes of excessive inflammation in patients with cystic fibrosis is dysfunctional (stalled) autophagy, which could be corrected by the action of the synthetic peptide IDR-1018 [66].

Modulation of apoptosis and pyroptosis

HDPs seem to also be involved in the modulation of apoptosis (programmed cell death). Apoptosis, a non-inflammatory cellular death mechanism, is promoted in epithelial cells and delayed in neutrophils by LL-37. For example LL-37 preferentially promotes cellular apoptosis in airway epithelium infected with *Pseudomonas aeruginosa*, and this may represent a mechanism to promote pathogen clearance [36] through the activation of caspases 3 and 9. By contrast, LL-37 can inhibit apoptosis of neutrophils, increasing their usually short half-life. This process, which seems to be regulated through purinoceptor 7 (P2X7) and G protein-coupled receptors, can be considered as a mechanism to enhance innate host responses [36].

Another type of cell death, pyroptosis, which is induced by pathogen- and damage-associated molecules, promotes proinflammatory cytokine production and is considered to have detrimental effects on the host. LL-37 was able to inhibit LPS/ATP-induced pyroptosis in macrophages through the inhibition of P2X7 association with ATP and consequent caspase-1 activation as well as direct LPS binding [37], thereby reducing the possible harmful effects of excessive inflammation.

Opportunities: limitations and potential solutions

The findings described above demonstrate that HDP and their synthetic cousins modulate pro- and anti-inflammatory responses in a variety of inflammatory settings, and utilize different cellular mechanisms to destroy the invading pathogen to return the immune system to homeostasis. As we stride towards the post-antibiotic era there is considerable interest in the pharmaceutical exploitation of HDPs. The diverse immunomodulatory features of HDPs make them intriguing candidates for treating infectious diseases as well as a variety of immune disorders. However, presumably due to issues regarding toxicity, the majority of antimicrobial peptide therapies that have entered clinical trials were designed for topical applications only [67]. Moreover, the cost of manufacturing HDPs and the risk of proteolytic degradation may make therapy with such peptides expensive. Below, and in Table 2, we outline the potential limitations of HDPs as therapeutics and propose prospective solutions.

Cost of synthesis

The cost of synthesis represents one hurdle in developing HDPs as therapies although, arguably, immune modulators might only need to be applied once and might also be effective in smaller amounts than antimicrobial peptides. Chemical synthesis using standard fluorenylmethoxycarbonyl (F-moc) chemistry, even adopting new strategies such as solution-phase chemistry, can be expensive ($50–$400 per gram) [15,68]. Genetically engineering prokaryotic systems to produce recombinant fusion peptides is

Table 2. Threats impeding clinical use of HDPs and proposed solutions.

Threats	Possible solutions
Cost of synthesis	Recombinant fusion peptides derived from prokaryotic systems Small peptidomimetics Synergistic formulas
Toxicity	Designing new mimetics and screening for lowered toxicity Using InnateDB to predict undesired interactions with molecules of the innate immune response
Instability	D-peptides Non-natural amino acids Liposomal nanoparticle formulations for sustained delivery

a potentially cheaper alternative, although this assumes the use of natural amino acids [69]. Conversely, the high cost of production can be tackled by creating shorter synthetic peptides based on natural HDPs, but with improved activity, as has been demonstrated for the 12 amino acid IDR peptides. Systematic single amino acid substitutions of a short HDP backbone template (methods described in detail in Fjell *et al.* [8]) can be used to create large peptide libraries. After testing key peptide properties (e.g., chemokine induction or inhibition of LPS-stimulated TNF-α expression), this enables the application of computer-aided quantitative structure–activity relationship (QSAR) analysis which uses artificial intelligence to correlate biological activity to the molecular structure of a compound (based on the amino acid sequence and physical properties termed descriptors), thereby streamlining the development of optimized peptides. Indeed, such approaches led to the design of IDR peptides that have shown protective effects in several different animal infection models [22,23,26,28,58,59]. Moreover, HDPs have shown to act synergistically with other cationic peptides (i.e., when combined, bovine indolicidin and LL-37 produced a greater-than-additive effect in the reduction of LPS-induced production of TNF-α in the human monocyte-like cell line THP-1) [70]. Because less peptide is required to get the same desired effect, synergistic formulas may help to reduce the overall cost of therapy.

Toxicity and unforeseen consequences

Another potential impediment for the development of immunomodulatory peptides is unknown toxicities that have bedeviled the antimicrobial peptides. For example, LL-37 causes histamine release from mast cells [71]. Moreover, in humans, abnormally high levels of LL-37 have been associated with psoriasis [72] and rosacea [73]. When high concentrations are injected in mice, LL-37 induces erythema and increased neutrophil infiltration, resulting in the inflammatory hallmarks of rosacea [73]. Nevertheless, such toxicities are not always observed with IDR peptides, many of which act systemically [22,23], and may be further prevented through judicious formulation. In addition, cationic amphipathic peptides have the potential to aggregate, although questions remain whether this feature is responsible for the observed immunomodulatory effects and/or whether aggregation of these peptides is instead the cause of toxicity [74].

Conversely, there may be other unforeseen consequences to modulating the immune response, especially in the face of genetic or disease-related immune dysfunctions, and such possibilities must be investigated in depth. Perhaps the most comforting thought in this regard is that such peptides have co-evolved with animals, and are part of most immune responses, and might therefore be expected to be benign or at least not overtly harmful. To assist in understanding the molecular interactions of HDPs or synthetic peptides with cells, novel systems biology approaches involving high-throughput omics platforms and databases, and analysis tools such as Innate DB, can be implemented [75]. The publicly available database and analysis platform, Innate DB, allows network and correlation analyses based on molecular interactions, and such analyses are being used to probe the complexity of immune responses and immune modulation [75]. This information can also be leveraged to reveal prospective undesirable interactions of new peptide therapies.

Susceptibility to degradation

Another potentially noteworthy issue is proteolytic degradation of HDPs and IDRs composed of natural amino acids. Peptides are susceptible to degradation by enzymes secreted by bacteria such as *Staphylococcus aureus* which produces the metalloproteinase aureolysin, abolishing the activity of LL-37 [76]. Moreover, many host proteases, such as the digestive enzymes trypsin and chymotrypsin, cleave proteins and peptides at basic and hydrophobic residues respectively; all of which are important for HDP structure and function [77]. However, recent studies on the pharmacokinetics of IDR-1018 in the context of protection of infant mice from LPS–hypoxia–ischemia-mediated brain injury have shown a more complex picture [28]. Radiolabeled peptide injected intraperitoneally enters the blood, liver, and spleen within 2 minutes, and thereafter there is an initial rapid decrease, achieving steady-state concentrations in the blood, liver, spleen, and brain of 2–6 μg peptide per g tissue (equivalent to 2–3 μg/ml in the blood).

The initial rapid decline in peptide concentrations in the blood especially (half-life of ~2 min) might be counteracted by the use of D- or non-natural- amino acids as well as altered backbones (peptidomimetics), making the peptides protease-resistant [78], although there would be a trade-off between the increased cost of such manipulations and the possible ability to reduce the dosage. Nevertheless, in the context of immunomodulation, little is known about the interactions of D-peptides with their protein targets and whether substitution of D-amino acids would compromise receptor–ligand interactions. Furthermore, judicious formulation, for example in liposomal nanoparticles, may allow more sustained delivery of the peptide [79].

Concluding remarks

Although most clinical trials to date have focused on the antimicrobial properties of HDPs, they display a wide range of immunomodulatory activities including modulating pro- and anti-inflammatory responses, enhancing chemoattraction, killing of extracellular and intracellular bacteria, promoting cellular differentiation, activating the innate and adaptive compartments, promoting wound-healing, and modulating autophagy as well as apoptosis and pyroptosis. Indeed, the effectiveness of HDPs and IDRs in several different animal disease models serves as a proof of concept that exploiting host defenses can serve as a promising new paradigm for the treatment of infectious diseases and inflammatory disorders. Regardless, there are several issues that must be investigated to enable the development of HDPs for clinical use – including the high cost of the peptides, potential toxicities, and biological stability. However, these limitations can be tackled by applying new strategies for deriving peptides, such as developing smaller peptides or mimetics based on HDP backbones with improved activity, as well as the implementation of D-peptides that resist proteolytic degradation. Moreover, systems biology approaches such as QSAR modeling and curation platforms

such as Innate DB can support the development of new therapies by streamlining the development of improved therapies and clarifying potential toxicities, as well as shedding light on the immune interactome of a new agent. These promising solutions bring hope that HDPs and IDRs can serve as a potential pharmaceutical approach to be used to target infectious and inflammatory diseases.

Acknowledgments

The authors own peptide research has been generously supported by the Canadian Institutes for Health Research (CIHR) and the Grand Challenges in Global Health Research Program through the Foundation for the National Institutes for Health, the Gates Foundation, and CIHR. R.E.W.H. holds a Canada Research Chair, and O.M.P. was supported by a Vanier–CIHR Doctoral award.

Disclaimer statement

R.E.W.H. is developing innate defense regulator (IDR) peptides and has filed several patents for these peptides which have been assigned to his employer, the University of British Columbia. Two of these patented IDR peptides have been licensed to Elanco Animal Health for use in animal infection models. One IDR peptide, which is funded by the Cystic Fibrosis Canada Translational Initiative, is being developed as a treatment for hyperinflammatory lung disease in cystic fibrosis. Other peptides have been licensed to the Pan-Provincial Vaccine Enterprise for development as components of an adjuvant formulation for vaccines used against RSV infections in animals. A provisional patent application on the use of cationic anti-biofilm peptides has been filed (U.S. Patent Application No. 61/870,655).

References

1 Parker, M.T. and Hewitt, J.H. (1970) Methicillin resistance in *Staphylococcus aureus*. *Lancet* 1, 800–804
2 Pendleton, J.N. *et al.* (2013) Clinical relevance of the ESKAPE pathogens. *Expert Rev. Anti Infect. Ther.* 11, 297–308
3 Zasloff, M. (2002) Antimicrobial peptides of multicellular organisms. *Nature* 415, 389–395
4 Phoenix, D.A. *et al.* (2013) *Antimicrobial Peptides: Their History, Evolution, and Functional Promiscuity*, Wiley
5 Hancock, R.E. *et al.* (1998) *Trends Biotechnol.* 16, 82–88
6 Hale, J.D. and Hancock, R.E. (2007) Alternative mechanisms of action of cationic antimicrobial peptides on bacteria. *Expert Rev. Anti Infect. Ther.* 5, 951–959
7 Spindler, E.C. *et al.* (2012) Deciphering the mode of action of the synthetic antimicrobial peptide Bac8c. *Antimicrob. Agents Chemother.* 55, 1706–1716
8 Fjell, C.D. *et al.* (2012) Designing antimicrobial peptides: form follows function. *Nat. Rev. Drug Discov.* 11, 37–51
9 de la Fuente-Nunez, C. *et al.* (2014) Broad-spectrum anti-biofilm peptide that targets a cellular stress response. *PLoS Pathog.* 10, e1004152
10 Bowdish, D.M. *et al.* (2005) Impact of LL-37 on anti-infective immunity. *J. Leukoc. Biol.* 77, 451–459
11 Herasimenka, Y. *et al.* (2005) Interaction of antimicrobial peptides with bacterial polysaccharides from lung pathogens. *Peptides* 26, 1127–1132
12 Pena, O.M. *et al.* (2013) Synthetic cationic peptide IDR-1018 modulates human macrophage differentiation. *PLoS ONE* 8, e52449
13 Koczulla, R. *et al.* (2003) An angiogenic role for the human peptide antibiotic LL-37/hCAP-18. *J. Clin. Invest.* 111, 1665–1672
14 Yang, D. *et al.* (1999) Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. *Science* 286, 525–528
15 Hilchie, A.L. *et al.* (2013) Immune modulation by multifaceted cationic host defense (antimicrobial) peptides. *Nat. Chem. Biol.* 9, 761–768
16 Amid, C. *et al.* (2009) Manual annotation and analysis of the defensin gene cluster in the C57BL/6J mouse reference genome. *BMC Genomics* 10, 606
17 Arnett, E. and Seveau, S. (2011) The multifaceted activities of mammalian defensins. *Curr. Pharm. Des.* 17, 4254–4269
18 Sorensen, O.E. *et al.* (2001) Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. *Blood* 97, 3951–3959
19 Fukumoto, K. *et al.* (2005) Effect of antibacterial cathelicidin peptide CAP18/LL-37 on sepsis in neonatal rats. *Pediatr. Surg. Int.* 21, 20–24
20 De, Y. *et al.* (2000) 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.* 192, 1069–1074
21 Putsep, K. *et al.* (2002) Deficiency of antibacterial peptides in patients with morbus Kostmann: an observation study. *Lancet* 360, 1144–1149
22 Nijnik, A. *et al.* (2010) Synthetic cationic peptide IDR-1002 provides protection against bacterial infections through chemokine induction and enhanced leukocyte recruitment. *J. Immunol.* 184, 2539–2550
23 Scott, M.G. *et al.* (2007) An anti-infective peptide that selectively modulates the innate immune response. *Nat. Biotechnol.* 25, 465–472
24 Achtman, A.H. *et al.* (2012) Effective adjunctive therapy by an innate defense regulatory peptide in a preclinical model of severe malaria. *Sci. Transl. Med.* 4, 135ra64
25 Rivas-Santiago, B. *et al.* (2013) Ability of innate defence regulator peptides IDR-1002, IDR-HH2 and IDR-1018 to protect against *Mycobacterium tuberculosis* infections in animal models. *PLoS ONE* 8, e59119
26 Steinstraesser, L. *et al.* (2012) Innate defense regulator peptide 1018 in wound healing and wound infection. *PLoS ONE* 7, e39373
27 Shestakov, A. *et al.* (2013) Synthetic analogues of bovine bactenecin dodecapeptide reduce herpes simplex virus type 2 infectivity in mice. *Antiviral Res.* 100, 455–459
28 Bolouri, H. *et al.* (2014) Innate defense regulator peptide 1018 protects against perinatal brain injury. *Ann. Neurol.* 75, 395–3410
29 Mookherjee, N. *et al.* (2006) Modulation of the TLR-mediated inflammatory response by the endogenous human host defense peptide LL-37. *J. Immunol.* 176, 2455–2464
30 Tjabringa, G.S. *et al.* (2006) Human cathelicidin LL-37 is a chemoattractant for eosinophils and neutrophils that acts via formyl-peptide receptors. *Int. Arch. Allergy Immunol.* 140, 103–112
31 Nijnik, A. *et al.* (2012) Signaling pathways mediating chemokine induction in keratinocytes by cathelicidin LL-37 and flagellin. *J. Innate Immun.* 4, 377–386
32 Mantovani, A. *et al.* (2011) Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat. Rev. Immunol.* 11, 519–531
33 Niyonsaba, F. *et al.* (2013) The innate defense regulator peptides IDR-HH2, IDR-1002, and IDR-1018 modulate human neutrophil functions. *J. Leukoc. Biol.* 94, 159–170
34 Davidson, D.J. *et al.* (2004) The cationic antimicrobial peptide LL-37 modulates dendritic cell differentiation and dendritic cell-induced T cell polarization. *J. Immunol.* 172, 1146–1156
35 Heilborn, J.D. *et al.* (2003) The cathelicidin anti-microbial peptide LL-37 is involved in re-epithelialization of human skin wounds and is lacking in chronic ulcer epithelium. *J. Invest. Dermatol.* 120, 379–389
36 Barlow, P.G. *et al.* (2010) The human cathelicidin LL-37 preferentially promotes apoptosis of infected airway epithelium. *Am. J. Respir. Cell Mol. Biol.* 43, 692–702
37 Hu, Z. *et al.* (2014) Antimicrobial cathelicidin peptide LL-37 inhibits the LPS/ATP-induced pyroptosis of macrophages by dual mechanism. *PLoS ONE* 9, e85765
38 Rosenfeld, Y. *et al.* (2006) Endotoxin (lipopolysaccharide) neutralization by innate immunity host-defense peptides. Peptide properties and plausible modes of action. *J. Biol. Chem.* 281, 1636–1643
39 Scott, M.G. *et al.* (2000) Cutting edge: cationic antimicrobial peptides block the binding of lipopolysaccharide (LPS) to LPS binding protein. *J. Immunol.* 164, 549–553
40 Mookherjee, N. *et al.* (2009) Intracellular receptor for human host defense peptide LL-37 in monocytes. *J. Immunol.* 183, 2688–2696
41 Nijnik, A. *et al.* (2009) Human cathelicidin peptide LL-37 modulates the effects of IFN-gamma on APCs. *J. Immunol.* 183, 5788–5798
42 Yu, J. *et al.* (2007) Host defense peptide LL-37, in synergy with inflammatory mediator IL-1beta, augments immune responses by multiple pathways. *J. Immunol.* 179, 7684–7691
43 Mookherjee, N. and Hancock, R.E. (2007) Cationic host defence peptides: innate immune regulatory peptides as a novel approach for treating infections. *Cell. Mol. Life Sci.* 64, 922–933
44 Welling, M.M. *et al.* (1998) Antibacterial activity of human neutrophil defensins in experimental infections in mice is accompanied by increased leukocyte accumulation. *J. Clin. Invest.* 102, 1583–1590

45 Madera, L. and Hancock, R.E. (2012) Synthetic immunomodulatory peptide IDR-1002 enhances monocyte migration and adhesion on fibronectin. *J. Innate Immun.* 4, 553–568
46 Durr, M. and Peschel, A. (2002) Chemokines meet defensins: the merging concepts of chemoattractants and antimicrobial peptides in host defense. *Infect. Immun.* 70, 6515–6517
47 Yang, D. *et al.* (2001) Participation of mammalian defensins and cathelicidins in anti-microbial immunity: receptors and activities of human defensins and cathelicidin (LL-37). *J. Leukoc. Biol.* 69, 691–697
48 Yount, N.Y. and Yeaman, M.R. (2006) Structural congruence among membrane-active host defense polypeptides of diverse phylogeny. *Biochim. Biophys. Acta* 1758, 1373–1386
49 von Kockritz-Blickwede, M. *et al.* (2008) Phagocytosis-independent antimicrobial activity of mast cells by means of extracellular trap formation. *Blood* 111, 3070–3080
50 Overhage, J. *et al.* (2008) Human host defense peptide LL-37 prevents bacterial biofilm formation. *Infect. Immun.* 76, 4176–4182
51 Biragyn, A. *et al.* (2002) Toll-like receptor 4-dependent activation of dendritic cells by beta-defensin 2. *Science* 298, 1025–1029
52 van der Does, A.M. *et al.* (2012) The antimicrobial peptide hLF1-11 drives monocyte-dendritic cell differentiation toward dendritic cells that promote antifungal responses and enhance Th17 polarization. *J. Innate Immun.* 4, 284–292
53 Mantovani, A. *et al.* (2004) The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.* 25, 677–686
54 Qin, X.J. *et al.* (2009) CCL22 recruits CD4-positive CD25-positive regulatory T cells into malignant pleural effusion. *Clin. Cancer Res.* 15, 2231–2237
55 Kin, N.W. *et al.* (2011) Cathelin-related antimicrobial peptide differentially regulates T- and B-cell function. *Eur. J. Immunol.* 41, 3006–3016
56 Hurtado, P. and Peh, C.A. (2010) LL-37 promotes rapid sensing of CpG oligodeoxynucleotides by B lymphocytes and plasmacytoid dendritic cells. *J. Immunol.* 184, 1425–1435
57 Nicholls, E.F. *et al.* (2010) Immunomodulators as adjuvants for vaccines and antimicrobial therapy. *Ann. N. Y. Acad. Sci.* 1213, 46–61
58 Kovacs-Nolan, J. *et al.* (2009) CpG oligonucleotide, host defense peptide and polyphosphazene act synergistically, inducing long-lasting, balanced immune responses in cattle. *Vaccine* 27, 2048–2054
59 Brown, T.H. *et al.* (2012) Comparison of immune responses and protective efficacy of intranasal prime-boost immunization regimens using adenovirus-based and CpG/HH2 adjuvanted-subunit vaccines against genital *Chlamydia muridarum* infection. *Vaccine* 30, 350–360
60 Shaykhiev, R. *et al.* (2010) The antimicrobial peptide cathelicidin enhances activation of lung epithelial cells by LPS. *FASEB J.* 24, 4756–4766
61 Tomioka, H. *et al.* (2014) Novel anti-microbial peptide SR-0379 accelerates wound healing via the PI3 kinase/Akt/mTOR pathway. *PLoS ONE* 9, e92597
62 Niyonsaba, F. *et al.* (2007) Antimicrobial peptides human beta-defensins stimulate epidermal keratinocyte migration, proliferation and production of proinflammatory cytokines and chemokines. *J. Invest. Dermatol.* 127, 594–604
63 Carretero, M. *et al.* (2008) In vitro and in vivo wound healing-promoting activities of human cathelicidin LL-37. *J. Invest. Dermatol.* 128, 223–236
64 Yin, J. and Yu, F.S. (2010) LL-37 via EGFR transactivation to promote high glucose-attenuated epithelial wound healing in organ-cultured corneas. *Invest. Ophthalmol. Vis. Sci.* 51, 1891–1897
65 Yuk, J.M. *et al.* (2009) Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. *Cell Host Microbe* 6, 231–243
66 Mayer, M.L. *et al.* (2013) Rescue of dysfunctional autophagy attenuates hyperinflammatory responses from cystic fibrosis cells. *J. Immunol.* 190, 1227–1238
67 Kindrachuk, J. *et al.* (2011) Stability, toxicity, and biological activity of host defense peptide BMAP28 and its inversed and retro-inversed isomers. *Biopolymers* 96, 14–24
68 Afacan, N.J. *et al.* (2012) Therapeutic potential of host defense peptides in antibiotic-resistant infections. *Curr. Pharm. Des.* 18, 807–819
69 Bommarius, B. *et al.* (2010) Cost-effective expression and purification of antimicrobial and host defense peptides in *Escherichia coli*. *Peptides* 31, 1957–1965
70 Bowdish, D.M. *et al.* (2005) Immunomodulatory activities of small host defense peptides. *Antimicrob. Agents Chemother.* 49, 1727–1732
71 Niyonsaba, F. *et al.* (2001) Evaluation of the effects of peptide antibiotics human beta-defensins-1/-2 and LL-37 on histamine release and prostaglandin D(2) production from mast cells. *Eur. J. Immunol.* 31, 1066–1075
72 Morizane, S. *et al.* (2012) Cathelicidin antimicrobial peptide LL-37 in psoriasis enables keratinocyte reactivity against TLR9 ligands. *J. Invest. Dermatol.* 132, 135–143
73 Yamasaki, K. *et al.* (2007) Increased serine protease activity and cathelicidin promotes skin inflammation in rosacea. *Nat. Med.* 13, 975–980
74 Yin, L.M. *et al.* (2012) Roles of hydrophobicity and charge distribution of cationic antimicrobial peptides in peptide-membrane interactions. *J. Biol. Chem.* 287, 7738–7745
75 Mookherjee, N. *et al.* (2009) Systems biology evaluation of immune responses induced by human host defence peptide LL-37 in mononuclear cells. *Mol. Biosyst.* 5, 483–496
76 Sieprawska-Lupa, M. *et al.* (2004) Degradation of human antimicrobial peptide LL-37 by *Staphylococcus aureus*-derived proteinases. *Antimicrob. Agents Chemother.* 48, 4673–4679
77 Kim, H. *et al.* (2014) De novo generation of short antimicrobial peptides with enhanced stability and cell specificity. *J. Antimicrob. Chemother.* 69, 121–132
78 Molhoek, E.M. *et al.* (2011) Improved proteolytic stability of chicken cathelicidin-2 derived peptides by D-amino acid substitutions and cyclization. *Peptides* 32, 875–880
79 Sobczak, M. *et al.* (2013) Polymeric systems of antimicrobial peptides – strategies and potential applications. *Molecules* 18, 14122–14137
80 Bevins, C.L. (2013) Innate immune functions of alpha-defensins in the small intestine. *Dig. Dis.* 31, 299–304
81 Ouellette, A.J. (2011) Paneth cell alpha-defensins in enteric innate immunity. *Cell. Mol. Life Sci.* 68, 2215–2229
82 Gursoy, U.K. *et al.* (2013) Human neutrophil defensins and their effect on epithelial cells. *J. Periodontol.* 84, 126–133
83 Rupec, R.A. *et al.* (2010) What is really in control of skin immunity: lymphocytes, dendritic cells, or keratinocytes? facts and controversies. *Clin. Dermatol.* 28, 62–66
84 Ferris, L.K. *et al.* (2013) Human beta-defensin 3 induces maturation of human Langerhans cell-like dendritic cells: an antimicrobial peptide that functions as an endogenous adjuvant. *J. Invest. Dermatol.* 133, 460–468
85 Navid, F. *et al.* (2012) Induction of regulatory T cells by a murine beta-defensin. *J. Immunol.* 188, 735–743
86 Semple, F. and Dorin, J.R. (2012) Beta-defensins: multifunctional modulators of infection, inflammation and more? *J. Innate Immun.* 4, 337–348
87 Vandamme, D. *et al.* (2012) A comprehensive summary of LL-37, the factotum human cathelicidin peptide. *Cell. Immunol.* 280, 22–35
88 Salvado, M.D. *et al.* (2013) Cathelicidin LL-37 induces angiogenesis via PGE2-EP3 signaling in endothelial cells, in vivo inhibition by aspirin. *Arterioscler. Thromb. Vasc. Biol.* 33, 1965–1972
89 Chen, X. *et al.* (2013) Human antimicrobial peptide LL-37 modulates proinflammatory responses induced by cytokine milieus and double-stranded RNA in human keratinocytes. *Biochem. Biophys. Res. Commun.* 433, 532–537
90 Coffelt, S.B. *et al.* (2009) The pro-inflammatory peptide LL-37 promotes ovarian tumor progression through recruitment of multipotent mesenchymal stromal cells. *Proc. Natl. Acad. Sci. U.S.A.* 106, 3806–3811
91 Krasnodembskaya, A. *et al.* (2010) Antibacterial effect of human mesenchymal stem cells is mediated in part from secretion of the antimicrobial peptide LL-37. *Stem Cells* 28, 2229–2238
92 Mader, J.S. *et al.* (2011) The human cathelicidin, LL-37, induces granzyme-mediated apoptosis in regulatory T cells. *J. Immunother.* 34, 229–235