The immunology of host defence peptides: beyond antimicrobial activity

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Abstract | Host defence peptides (HDPs) are short, cationic amphipathic peptides with diverse sequences that are produced by various cells and tissues in all complex life forms. HDPs have important roles in the body's response to infection and inflammation. This Review focuses on human HDPs and explores the diverse immunomodulatory effects of HDPs from a systems biology perspective, which highlights the interconnected nature of the effect (or effects) of HDPs on the host. Studies have demonstrated that HDPs are expressed throughout the body and mediate a broad range of activities, which explains their association with various inflammatory diseases and autoimmune disorders. The diverse actions of HDPs, such as their roles in wound healing and in the maintenance of the microbiota, are also explored, in addition to potential therapeutic applications.

Host defence peptides (HDPs). Short (<50 amino acids) cationic amphipathic peptides with immunomodulatory and/or antimicrobial activities.

Antimicrobial peptides
Cationic peptides with an emphasis on their antimicrobial activities; traditional terminology for host defence peptides.

Immunomodulatory
The ability to modulate the immune response, including by influencing the production of chemokines and cytokines.

Innate defence regulator (IDR). Synthetic immunomodulatory peptide derived from natural host defence peptides.

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Host defence peptides (HDPs; also known as antimicrobial peptides) are produced by all complex animals, insects and plants, and generally have modest direct activity against a broad range of microorganisms[1,2]. Remarkably, there are more than 2,600 known natural peptides with very diverse sequences and structures[3]. Recent studies have demonstrated a wide range of activities in modulating the functions of host cells and tissues as part of natural host defences, which has led to the depiction of these peptides as HDPs — rather than antimicrobial peptides — to better define their multifaceted roles as immunomodulatory mediators and, under some circumstances, antimicrobial agents.

This shift in thinking derived originally from the observation that many HDPs lose their antimicrobial potency under physiological conditions, whereas their immunomodulatory activities can be detected both in tissue culture and *in vivo*[4]. In addition, a synthetic HDP modelled on bovine bactenecin, which completely lacked direct antibacterial activity *in vitro*, protected mice from infection *in vivo*[5], indicating that its anti-infective properties were due to its ability to modulate the immune response. Recently, the ability of HDPs to act as modulators of the immune response and immune cell signalling and/or function has been extensively studied, and their role in innate and adaptive immunity is becoming increasingly appreciated[4,6].

In this Review, we briefly outline what is currently known about HDPs and their role in modulating the immune response. We emphasize that HDPs are far more than simple immunomodulators acting through a single receptor or linear signalling pathway of the immune system. This complexity becomes evident by examining the protein–protein interaction network of the human cathelicidin LL-37 (also known as CAMP) (FIG. 1). LL-37 interacts with at least 16 proteins and receptors, which in turn interact with more than 1,000 secondary effector proteins[7,8]. This is consistent with our observations that the expression of more than 900 genes changes when human monocytes are stimulated with peptides such as LL-37 and other synthetic innate defence regulator (IDR) peptides (R.E.W.H., unpublished observations). Other human HDPs are also known to have pleiotropic effects on different cell types and signalling pathways throughout the body, highlighting the complexity of activities that are influenced by HDPs *in vivo*. In this Review, we discuss the findings that HDP functions go far beyond simply protecting the host from invading microorganisms and that HDPs indeed have essential roles in the complex signalling events that occur during immune responses.

Natural role of HDPs

The first insect HDP, cecropin from silk moths, was identified in 1980 (REF. 9). As insects lack an adaptive immune system similar to those in higher eukaryotes, it was suggested that they depended primarily on the induction of HDPs to protect against invading microorganisms. However, the identification of HDPs in animals with more complex immune systems dramatically altered our view of these molecules, and it is increasingly recognized that natural HDPs represent an important component of the immune response of virtually every living organism.

In humans, the epithelial cells in the skin and intestinal tract produce numerous HDPs and antimicrobial proteins to help to protect against the continuous exposure to environmental microorganisms, while

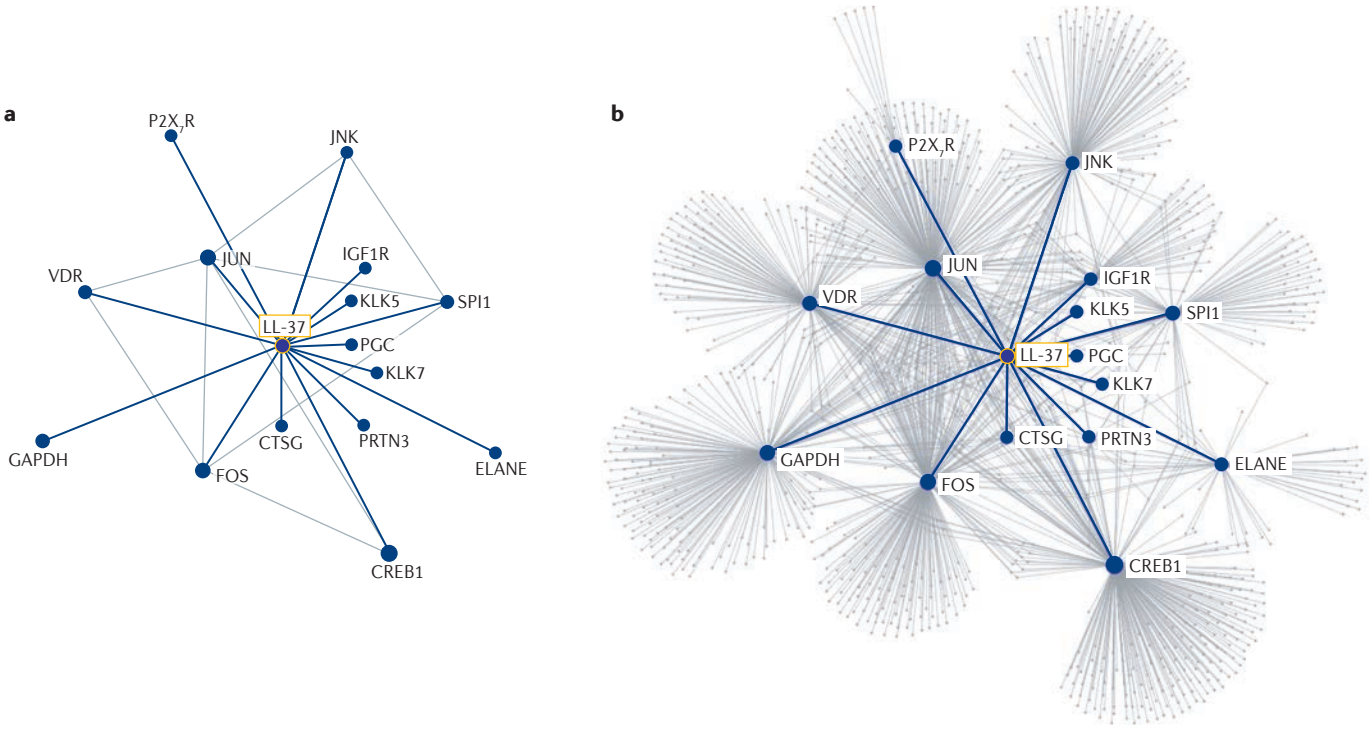


Figure 1 | **LL-37 interacts directly and indirectly with a broad range of genes and proteins. a** | A network interaction diagram showing zero-order interactions of the cathelicidin LL-37. These interacting proteins have diverse functions within the cell, including glucose metabolism and cytoskeletal dynamics (glyceraldehyde-3-phosphate dehydrogenase (GAPDH)), transcription (JUN, FOS, cAMP-responsive element-binding protein 1 (CREB1), vitamin D3 receptor (VDR) and SPI1)) and inflammation (cathepsin G (CTSG)). **b** | LL-37 has more than 1,000 first-order interaction partners (that is, direct interactors and proteins known to interact with the direct interactors (indicated by grey dots)), highlighting the complexity of the actions of the host defence peptide LL-37 within the cell. Network diagrams were created using NetworkAnalyst[8]. ELANE, neutrophil elastase; IGF1R, insulin-like growth factor 1 receptor; KLK, kallikrein; MAPK, mitogen-activated protein kinase; $P2X_7R$, $P2X_7$ purinergic receptor; PGC, PPAR-γ co-activator; PRTN3, proteinase 3.

also maintaining homeostasis of the host microbiota[10] (BOX 1). Some HDPs can be found at high concentrations (mg per ml) within the body, such as in the granules of leukocytes[11] (and most likely in the immediate vicinity of degranulating phagocytes) or at the bottom of intestinal crypts[12]. In these circumstances, it is possible that the direct antimicrobial activity of HDPs is responsible for creating environments that are highly toxic to invading bacteria, whereas such functions are sufficiently diluted at the mucosa so the commensal microorganisms are maintained. However, high concentrations of HDPs are exceptions; their biological concentration is generally much lower (ranging from ng per ml to μg per ml), and their antimicrobial activity is inhibited by the presence of physiological concentrations of salt, serum proteins and/or lipoproteins and glycosaminoglycans[13]. It is now well established that HDPs have multiple effects on various different cell types throughout the body, including a stimulatory effect on immune cells (TABLE 1). Thus, despite their effects on bacterial cells, we hypothesize that the primary role of HDPs is to function as important signalling molecules that modulate cellular functions, including those involved in the immune response. Several other human proteins and peptides are produced at mucosal surfaces or are present in secreted fluids such as saliva and tears, including lysozyme, lactoferrin, lipocalins, calprotectin and histatins[8,12], but these do not fit well into the definition of cationic amphipathic peptides such as HDPs. Many articles have been published on each of these molecules but, for the purposes of this Review, we have chosen to focus our attention on the two primary classes of human cationic amphipathic HDPs: LL-37 and defensins.

Pleiotropic
Having more than one effect on the biological system.

Diapedesis
The migration of leukocytes across the endothelium, which occurs by leukocytes squeezing through the junctions between adjacent endothelial cells.

Human HDPs

Expression and immunomodulatory activity of LL-37. The human cathelicidin LL-37 is transcribed from the *CAMP* gene in various cell types, including epithelial cells and many cells of the immune system[14,15]. The effects of LL-37 are widespread, as this HDP is known to elicit a wide range of responses in a broad assortment of cell types (TABLE 1). These include both pro- and anti-inflammatory activities that vary depending on the cell type and inflammatory stimuli that are present. Additional effects include: anti-infective immune modulation (for example, induced expression of several chemokines), direct chemoattractant activity towards various immune cells, anti-inflammatory activity, wound healing, pro-angiogenic activity, pro-apoptotic activity in some cell types such as epithelial cells and regulatory T cells, anti-apoptotic activity in neutrophils, mast cell degranulation to enhance diapedesis and adjuvant

Box 1 | **Maintenance of the host microbiota**

Interest in the human microbiome and the influence of the commensal microbiota on health and disease has dramatically increased in recent years. Host defence peptides (HDPs) possess immunomodulatory and antibacterial activities, which suggest that these molecules might have a direct effect on the composition and health of the host microbiota. For example, the HDPs produced by Paneth cells help to maintain the microbial community in the intestine through direct antibacterial activity, as well as by coordinating immune responses in intestinal epithelial cells[118]. Defects in HDP production in the gut have been linked to inflammatory bowel diseases, such as Crohn disease and colitis[10]. It was recently discovered that many commensal species of the gut express an enzyme (LpxF) that dephosphorylates the lipid A portion of lipopolysaccharide (LPS), which leads to a decreased overall negative charge on the cell surface and increased resistance to the antibacterial activity of HDPs in the commensal microbiota[119]. This is analogous to the mechanism by which bacteria neutralize the LPS lipid A phosphate with an aminoarabinose group, resulting in enhanced resistance to antimicrobial peptides[120].

Any dysregulation in HDP production could negatively affect the microbial distribution at sites within the body and contribute to disease. For example, low copy numbers of β-defensin genes are reported to influence the microbial community of the nasopharynx and allow for the growth of bacteria that contribute to otitis media[121]. Thus, synthetic HDPs might promote the growth of specific bacterial species within the microbiome, and a recent study identified a synthetic HDP that selectively killed pathogenic *Streptococcus mutans* in an oral multispecies bacterial community, leading to reconstruction of the entire oral microbial community[122]. Our understanding of the relationship between HDPs, the immune response and the healthy microbiome is still in its infancy, but it is possible that synthetic HDPs could be used in the future to promote healthy microbial communities.

activity that demonstrates bias towards T helper 1 (T_H1) cell responses, in addition to weak antimicrobial activity and independently determined activity against bacterial biofilms[4,13–15]. Many of these activities have been demonstrated in mouse models[4]; mice express a distinct cathelicidin peptide, encoded by *Camp*, which shares 67% homology with LL-37.

Although LL-37 is expressed constitutively in many cell types, epithelial cell expression is modulated by inflammatory triggers such as wounding or infections[14,15]. In addition, vitamin D3 has a role in the expression of LL-37, as there are several vitamin D3 response elements that are located in the promoter of *CAMP*[16]. Indeed, one of the purposes of vitamin D3 supplementation trials in Asia is to treat tuberculosis by inducing LL-37 expression and benefiting from its antimycobacterial immunomodulatory effects[17]. Vitamin D3 modulates LL-37 expression in various cell types, including monocytes and keratinocytes, and acts in a synergistic manner with lipopolysaccharide (LPS) to promote LL-37 production in neutrophils[16]. In addition, vitamin D3 is essential for the induction of LL-37 in monocytes and keratinocytes through the Toll-like receptor 1 (TLR1) and TLR2 pathways in response to bacterial infection and injury[15].

LL-37 exerts its effects by binding to or transactivating various extracellular and intracellular receptors[4,13], and it is capable of translocating into cells[15]. Inside cells, its activity is extremely complex as it induces hundreds of genes[18]. This is a consequence of its ability to modulate NF-κB inhibitor-α (IκBα), mitogen-activated protein kinases (MAPKs) p38, extracellular signal-regulated kinase 1 (ERK1; also known as MAPK3) and ERK2 (also known as MAPK1), JUN N-terminal kinase (JNK; also known as MAPK8) and phosphoinositide 3-kinase (PI3K) pathways[18], as well as several other pathways[4]. Downstream of these pathways, at least a dozen transcription factors are activated, including most subunits of NF-κB (transiently), cAMP-responsive element-binding protein 1 (CREB1), hypoxia-inducible factor 1α (HIF1α), activator protein 1 (AP-1), AP-2 and early growth response protein 1 (EGR1)[4,18,19]. This very complex action of LL-37 is probably responsible for its many properties and is further modulated depending on the presence of a co-stimulus such as LPS or granulocyte–macrophage colony-stimulating factor (GM-CSF).

In peripheral blood mononuclear cells, LL-37 enhances the interleukin-1β (IL-1β)-induced production of cytokines, such as IL-6 and IL-10, and chemokines, such as CC-chemokine ligand 2 (CCL2) and CCL7, by a mechanism that is repressed by interferon-γ (IFNγ), IL-12 and IL-4 (REF. 20). LL-37 also enhances pro-inflammatory signalling through TLR3 in response to double-stranded viral RNA in epithelial cells[21], flagellin-mediated activation of TLR5 in keratinocytes[22] and CpG-induced activation through TLR9 in B cells and plasmacytoid dendritic cells (pDCs)[23]. Synergy has also been observed between LL-37, IL-17 and IL-22 in keratinocytes to increase the expression of CXC-chemokine ligand 8 (CXCL8; also known as IL-8) and IL-6, which suggests a possible role for LL-37 in skin inflammation[24].

The presence of LL-37 during the differentiation of macrophages from monocytes results in macrophage polarization to a pro-inflammatory M1 phenotype[25], whereas LL-37 alters the differentiation of DCs such that they promote T_H1 cell responses and thereby promotes enhanced adaptive immunity[26]. Self DNA in combination with LL-37 has a pro-inflammatory effect on pDCs and monocytes. This response is mediated through the TLR9 pathway[27] in pDCs and by cytosolic DNA-sensing mechanisms in monocytes[28]. LL-37 in combination with self DNA also promotes pDC cellular maturation[29]. Furthermore, LL-37 can activate the inflammasome through the $P2X_7$ purinergic receptor ($P2X_7R$) in both macrophages and monocytes[30]. Co-stimulatory molecule expression, endocytic function and secretion of cytokines inducing T_H1 cell responses are all increased in the presence of LL-37 (REF. 26). LL-37 also induces the migration of keratinocytes, neutrophils and eosinophils[15].

Conversely, various anti-inflammatory effects of LL-37 have also been described. These effects are also complex, with LL-37 acting at several stages of monocyte activation[31], one of which is direct binding to CC-chemokine receptor 2 (CCR2)[32,54]. For example, stimulation with LL-37 causes pDCs, myeloid DCs (mDCs), monocytes, B cells and T cells to produce higher levels of anti-inflammatory IL-10 than untreated cells[18]. LL-37 can inhibit the activation of DCs that occurs in response to the TLR ligands LPS, flagellin and lipoteichoic acid, which is determined by decreased levels of IL-6, IL-12p70 and tumour necrosis factor (TNF), and decreased surface expression of HLA-DR,

Table 1 | **HDP expression patterns, targets and functions**

HDP-expressing cells	Target	HDP activity
Cathelicidin LL-37		
• Epithelial cells • Macrophages • Monocytes • DCs • Neutrophils • NK cells • Mast cells	Neutrophils	Chemotactic through FPRs and anti-apoptotic
	Monocytes	Induces chemokine (such as CXCL8, CCL2 and CCL7) and anti-inflammatory cytokine expression, and promotes differentiation to pro-inflammatory macrophages
	B cells	Induces chemokine expression
	T cells	Induces chemokine expression
	Eosinophils	Chemotactic through FPRs
	Macrophages	Suppresses pro-inflammatory TNF response
	Airway epithelium	Pro-apoptotic
	pDCs	Activated by self DNA–LL-37 complexes, promotes CpG detection and induces anti-inflammatory cytokine expression
	mDCs	Induces chemokine and anti-inflammatory cytokine expression
	Keratinocytes	• Induces chemokine production, cell migration and wound healing • Anti-apoptotic
	Mast cells	Induces chemotaxis and histamine release
	Cancer cells	• Promotes cancer by acting as a growth factor • Required for NK cell antitumour activity • Tumour suppression
	Bacterial cells	Antibacterial
	Bacterial cells growing in biofilms	Antibiofilm
	Fungi	Antifungal
	Parasites	Antiparasitic
α-defensins — HNP1, HNP2, HNP3 and HNP4		
• Neutrophils (primary source) • Monocytes • Lymphocytes • Gastrointestinal epithelium	T cells	Chemoattractant
	Immature DCs	Chemoattractant
	Macrophages	Chemoattractant
	Mast cells	Chemoattractant
	Lung epithelial cells	• Stimulates cytokine release (such as CXCL8, CCL2 and GM-CSF) • Promotes cell proliferation
	Dermal fibroblasts	• Promotes collagen expression and wound healing • Suppresses collagenase production
	Endothelial cells	• Anti-angiogenic • Apoptotic
	Platelets	• Activates platelets • Promotes apoptosis
	Cancer cells	Antitumour (HNP1)
	Bacterial cells	Antibiotic
	Candida	Antifungal
	Viruses	Antiviral
α-defensins — HD5 and HD6		
• Paneth cells (primary source) • Female reproductive epithelium (HD5) • Airway epithelium (HD5)	Macrophages	Chemoattractant (HD5)
	Mast cells	Chemoattractant (HD5)
	Intestinal epithelial cells (HD5)	• Activates NF-κB, pro-apoptotic • Induces chemokine production (such as CXCL8) • Maintains gut homeostasis
	$CD4^+$ T cells (HD5)	Induces chemokine and cytokine production (such as CXCL8 and IL-2)
	Skin epithelial cells (HD5)	Promotes stem cell migration and wound healing
	Bacterial cells	• Formation of nanonets (HD6) • Antibiotic (HD5)
	Viruses	Enhances HIV infectivity and inhibits HIV infection (HD5)

Table 1 (cont.) | **HDP expression patterns, targets and functions**

HDP-expressing cells	Target	HDP activity
β-defensins — HBD1, HBD2, HBD3 and HBD4		
• Epithelial cells (primary source) • Monocytes • Macrophages • DCs	Peripheral blood mononuclear cells	Upregulates cytokine production (HBD2)
	Immature DCs	Chemotactic
	T cells	Chemotactic
	Neutrophils	Chemoattractant (HBD2)
	Macrophages and mDCs	Induces expression of CD80, CD86 and CD40
	Intestinal epithelial cells	Promotes cell migration and wound healing
	Epidermal keratinocytes	• Stimulates chemokine and cytokine production (such as IL-6, IL-10, CCL2 and CCL20) • Increases migration and proliferation
	Cancer cells	• Tumour suppression (HBD1) • Chemoattractant for tumour-associated macrophages (HBD3) • Promotes cancer cell proliferation (HBD4)
	Lymphatic endothelial cells	Chemotactic
	Oral epithelial cells	Reduces infectivity of HIV
	Vascular tissue	Angiogenic
	Bacterial cells	Antimicrobial
	Viruses	Antiviral, inhibits HIV replication

CCL, CC-chemokine ligand; CXCL8, CXC-chemokine ligand 8; DC, dendritic cell; FPR, *N*-formyl peptide receptor; GM-CSF, granulocyte–macrophage colony-stimulating factor; HBD, human β-defensin; HD, human α-defensin; HDP, host defence peptide; HNP; human neutrophil peptide; IL, interleukin; mDC, myeloid DC; NF-κB, nuclear factor-κB; NK cell, natural killer cell; pDC, plasmacytoid DC; TNF, tumour necrosis factor. Table compiled from recent reviews on LL-37 (REFS 14,15) and human defensins[35,36,127]. See Supplementary information S1 (table) for references to the original studies.

CD80, CD83, CD86 and CCR7. Hence, LL-37 decreases inflammation in response to these TLR agonists[33]. After an initial transient burst of NF-κB activation that is required for LL-37 induction of chemokines[18,31], LL-37 also suppresses TLR4 signalling by diverse mechanisms, including by the prevention of further degradation of IκB[31,34]. Production of the chemokines CCL5 and CXCL10 is abrogated by the interaction of double-stranded RNA with LL-37 in keratinocytes, which has been shown to reduce TLR3 signalling[24].

Expression and immunomodulatory activity of human defensins. Human defensins are separated into two major classes according to their cysteine disulfide connectivities, α-defensins and β-defensins[35]. There are six members of the α-defensin family of HDPs that are further separated into the human neutrophil peptides (HNPs), which are the α-defensins 1–4, and human α-defensin 5 (HD5) and HD6. The cell types that express and are affected by defensins, as well as the known functions of defensins, are summarized in TABLE 1. HNPs are produced by neutrophils as well as monocytes, lymphocytes and natural killer (NK) cells[35,36]. HD5 and HD6 are expressed in the Paneth cells of the small intestine, as well as the epithelial cells of the airway, gastrointestinal tract and female reproductive tract[35,36]. Human β-defensins (HBDs) are widely distributed throughout the body: they are expressed in epithelial cells and are readily produced by monocytes, macrophages and DCs[35,36]. HNP production can be stimulated by pro-inflammatory cytokines in immature monocyte-derived DCs[37], whereas nucleotide-binding oligomerization domain-containing protein 2 (NOD2) induces the expression of HNP1 (also known as neutrophil defensin 1) and HD5 in gut epithelial cells[38,39]. HBD1 is constitutively expressed in epithelial cells, whereas the expression of HBD2 is inducible by NF-κB activation[35,36]. TNF stimulates HBD3 production, and the presence of bacteria stimulates the production of both HBD3 and HBD4 (REFS 40,41).

In a similar manner to LL-37, human defensins have both pro- and anti-inflammatory roles in the immune system, although they tend to be less potent. Defensins are found in very modest concentrations in most normal tissues, but at higher concentration at sites where neutrophils degranulate and in intestinal crypts[35,36]. For example, neutrophils dying from apoptosis or necrosis release HNPs into the surrounding milieu[42]. The presence of HNPs can limit a pro-inflammatory response by disrupting the release of nitric oxide and inflammatory cytokines from macrophages[42]. Conversely, HNPs released from neutrophils can also increase bacterial phagocytosis by macrophages by stimulating the production of TNF and IFNγ by macrophages, which in turn leads to increased expression of CD32 (also known as FcγRIIB) and CD64 (also known as FcγRI)[43]. Other activities of HNPs include: chemoattractant activity for multiple cell types; induction of cytokine and chemokine production; anti-inflammatory activity; pro- or anti-angiogenic activity; wound-healing activity; promotion of gut homeostasis; pro-apoptotic activity for some cell types; formation of nanonets; adjuvant effects such as

Box 2 | **Studying HDP responses in complex immune responses**

The immune system is a popular target for emerging therapies, including host defence peptides (HDPs) and synthetic variants, to treat infectious and inflammatory diseases. However, the immune system is very complex, involving more than 1,800 different genes that are integrated into dozens of different pathways and sub-networks and expressed in many different cell types, and is further integrated and overlapping with the nervous, lymphatic, circulatory and hormonal systems[123]. Thus, it is important to consider the intricate nature of the immune system from a systems perspective. This is especially important in understanding how peptides work and in developing HDP mimetics for immunomodulatory therapies, as a small perturbation of the immune response can have drastic ramifications for the organism such as sepsis, chronic inflammation and metabolic distress. This can be done by using the 365,000 known protein–protein interactions in humans (see the InnateDB database) as a framework for understanding functional interconnectedness. For example, JUN, which is one of the interaction partners of the cathelicidin LL-37 (FIG. 1), activates the transcription of interleukin-2, which has an integral role in the cell's response to viruses and bacteria. In addition, JUN interacts with 516 other genes and proteins within the cell and has roles in angiogenesis, apoptosis, cell cycle regulation, axon regeneration, liver development and DNA replication, to name a few. Therefore, it is impossible to separate the activity of this protein or any of its interaction partners into a single biological role. To develop more effective immunomodulatory therapies, it is crucial to understand the diversity and interconnectedness of immune system pathways and the proteins that they contain. Such an understanding will allow us to minimize the unwanted effects and maximize the desirable effects of therapeutics.

activation of antigen-presenting cells and promotion of DC maturation; tumour suppression; and anticancer, antifungal, antiviral and generally weak antimicrobial activity (TABLE 1).

Defensins function as chemoattractants to promote the chemotaxis of neutrophils, monocytes, T cells, DCs and mast cells[35,36]. In addition, keratinocytes display increased migration and proliferation, as well as upregulation of pro-inflammatory cytokines and chemokines, upon stimulation with HBD2, HBD3 and HBD4 (REF. 44). HBD1, HBD2 and HBD3 can also cause upregulation of the chemokines CXCL8 and CCL2 in peripheral blood mononuclear cells. In addition, each of these defensins modulates the expression of its own unique set of cytokines and chemokines, implying that each HBD has a unique role in innate immunity[45]. Some of these properties can be ascribed to interaction with specific receptors on the surface of various cells; for example, HBD3 can activate monocytes via TLR1- and TLR2-mediated signalling[46]. Conversely, HNP1 enhances the expression of the cytokines TNF and IL-1β in monocytes stimulated with *Staphylococcus aureus*[47]. Like LL-37, HBD2 and HBD3 can enhance TLR9-mediated signalling that is initiated by CpG oligonucleotides[48]. HNPs have been shown to disrupt angiogenesis and increase apoptosis of endothelial cells[49,50], and HD5 exposure can lead to inflammation and apoptosis of intestinal epithelial cells and T cells[51].

Defensins can also have opposite properties; for example, HBDs display anti-apoptotic properties towards neutrophils[52]. HBD3 has anti-inflammatory properties: it can suppress LPS-induced TNF production in macrophages via both NOD-like receptor (NLR) and TLR pathways[53,54]. HBD1, HBD2 and HBD4 enhance angiogenesis through endothelial cell recruitment[55], whereas HBD2 specifically leads to the augmentation of blood endothelial cell division[56]. HBD3 has also demonstrated angiogenic properties through upregulation of CXCL8 in macrophages[32].

In summary, naturally occurring HDPs are produced by a wide range of cells throughout the body, and they can influence multiple signalling pathways that are involved in inflammation and immunity. In the next section, we examine the complexity of these pathways and discuss how the use of systems biology approaches is necessary to fully comprehend the biological activities of HDPs.

An integrated perspective on HDP activity. As mentioned above, HDPs have various effects on many systems and signalling pathways throughout the body, and small differences in their expression and distribution can potentially have large effects on the observed biological activities that they influence. Here, we discuss this complexity in terms of the immune response (BOX 2) and emphasize how each of these activities, and the underlying pathways and gene products, needs to be appreciated when considering the profound biological role of HDPs. Importantly, most studies of cathelicidin and defensin activity have focused on the effects of a single HDP on one or a few cytokines and biological pathways. This results in a fragmented view of the effects of HDPs and belies the manner in which these peptides influence interconnected cellular processes. A handful of studies have conducted global transcriptional profiling on cells exposed to HDPs and various immune system agonists. For example, the response of $CD14^+$ monocytes to LL-37 was investigated using microarrays, which showed that HDP treatment altered the expression of 475 genes[18]. These genes are involved in diverse pathways, including MAPK signalling, insulin signalling, cell–cell adhesion and eicosanoid metabolism. In addition, binding sites for 32 different transcription factors were enriched in promoters of genes that had altered expression in response to LL-37. This suggests that the effects of LL-37 are quite broad and not just confined to a few biological pathways.

As another example of the complex effects of an HDP on the immune response, we have re-analysed the data of a study in which macrophages stimulated with the TLR4 agonist KDO_2–lipid A (KLA) were exposed to HBD3 (REF. 54). There was a substantial effect of HBD3 on TLR4 activation — 5,494 genes compared with 1,779 genes were differentially expressed between macrophages stimulated with KLA or KLA and HBD3, respectively. To understand the effects of HBD3 in more detail, we performed a pathway overrepresentation analysis using InnateDB[7]. There were a total of 7 upregulated pathways and 22 downregulated pathways in response to treatment of KLA-stimulated macrophages with HBD3 (BOX 3). The upregulated pathways seemed to be very diverse and included interleukin signalling, lipid metabolism and Fcγ receptor-dependent phagocytosis. However, a protein–protein interaction network built using the differentially expressed genes as seeds in each of these pathways, as well as proteins with which these differentially expressed proteins are

Global transcriptional profiling
Measurement of the entire gene expression profile to obtain an overall picture of the activity of genes in a cell or biological system.

known to interact (FIG. 2a), revealed that these pathways were indeed interconnected and shared proteins (purple nodes within the network). Genes that were stimulated by KLA and suppressed by HBD3 were even more numerous than those stimulated by KLA alone (915 genes compared with 817 genes), with 22 pathways overrepresented (BOX 3). Intriguingly, more than 30 genes that are involved in cytokine signalling were differentially expressed between these two conditions (FIG. 2b), but both upregulated and downregulated genes were observed[54], indicating that the cellular response to this defensin is much more complex than originally thought.

More recently, the effects of a synthetic HDP (IDR-1018) on macrophage differentiation were assessed by RNA sequencing[57]. IDR-1018 is a 12-residue peptide with similar sequence characteristics to natural HDPs, as it is composed of cationic and hydrophobic residues[58]. Exposure to IDR-1018 caused macrophages to adopt a phenotype that was intermediate between the M1 (pro-inflammatory) and M2 (anti-inflammatory) states and, concurrently, 876 genes changed expression. In addition to modulating genes involved in the immune response (for example, 71 genes that changed expression had interferon regulatory factor 4 (IRF4)-binding sites), IDR-1018 induced the expression of genes that are involved in organization and degradation of the extracellular matrix and pyruvate metabolism.

These examples highlight that HDPs can exert multiple effects on diverse signalling pathways and help to explain the substantial complexity of HDP action. In addition, it is important to consider that any given immune cell might be concordantly influenced by multiple HDPs, which may have complementary or antagonistic effects on the immune response of effector cells. For example, a recent report described the synergistic effects of HD5 and HD6, and demonstrated that HD6 affected the host response that is induced by HD5 while having no effect on the antibacterial activity[59]. The potential *in vitro* and *in vivo* effects of stimulation with multiple HDPs have yet to be explored in detail, but determining these interrelated pathways could be crucial for our understanding of immune system functionality. The wide-reaching effects of HDPs might also explain the observation that impaired or uncontrolled production of these peptides has been implicated in several disease states, as discussed in the following section.

Box 3 | **Mouse macrophage pathways regulated by HBD3 in KLA-treated cells**

Pathway overrepresentation analysis was conducted on data described in REF. 54, using the Reactome[128] ontology system within the InnateDB[7] data analysis suite. Duplicate pathways have been removed.

Downregulated pathways

- Cytokine-induced signalling
- Chemokine receptor activation
- TGFβ-activated kinase 1 (TAK1)-mediated activation of nuclear factor-κB (NF-κB) by phosphorylation and activation of IκB kinase (IKK) complexes
- MYD88–MYD88 adaptor-like protein (MAL) pathways initiated at the plasma membrane
- Induction of type I interferons (IFNs) by the retinoic acid-inducible gene I (RIG-I)–melanoma differentiation-associated protein 5 (MDA5) pathway
- Toll-like receptor (TLR) signalling pathways
- TNF receptor-associated factor 6 (TRAF6)-mediated induction of NF-κB and mitogen-activated protein kinase (MAPK) by TLR7, TLR8 and/or TLR9 activation
- Receptor-interacting protein (RIP)-mediated activation of NF-κB by Z-binding protein 1 (ZBP1)
- ZBP1-mediated induction of type I IFNs
- MYD88-independent signalling pathways
- Downstream T cell receptor (TCR) signalling pathways
- Downstream B cell receptor (BCR) signalling pathways
- Cytosolic sensing of pathogen-associated DNA
- CD28-dependent phosphoinositide 3-kinase (PI3K)–AKT signalling
- Cyclin D-associated events in the G1 phase
- FcεRI-mediated NF-κB activation

Upregulated pathways

- General metabolism
- Metabolism of lipids and lipoproteins
- Interleukin-induced signalling
- Classical antibody-mediated complement activation
- Polyamine oxidase-mediated conversion of polyamines to amines
- FcγR-dependent phagocytosis
- MAPK activation in TLR cascades

HBD3, human β-defensin 3; KLA, KDO_2–lipid A.

Relationship between HDPs and disease

Several diseases have been shown to be characterized by a dysregulation in the levels of HDPs. Many of these diseases are disorders related to the immune system, whereas others are influenced by HDPs themselves or by effector molecules made by cells that are directly affected by HDPs. Although this is consistent with the suggestion that these molecules have a role in disease progression, the relationship between HDP levels and disease is often observational rather than established cause-and-effect. In the following section, we describe a range of human disorders in which a dysregulation of natural HDPs has been observed and is known to correlate with disease onset and progression.

Cancer. The influence of natural HDPs on the growth of tumours is proving to be exceedingly complex. Xenografted tumours grow faster in *Camp*$^{-/-}$ mice (which lack cathelicidin), and *in vitro* analysis of NK cells from *Camp*$^{-/-}$ mice shows that they display impaired cytotoxicity towards tumours compared with NK cells from wild-type mice[60]. Genetic analysis revealed lower copy numbers of defensin genes in patients with pancreatic adenocarcinoma compared with healthy controls[61]. In addition, HBD1 has been shown to act as a tumour suppressor against oral squamous cell carcinoma[62]. Taken together, these studies suggest that one function of HDPs is to prevent tumour growth and promote an immune response to tumours. However, the actual situation is more complex, and several studies have indicated that some HDPs promote neoplastic growth. For example, in contrast to HBD1, HBD2 and HBD3 actually promote cell growth of oral

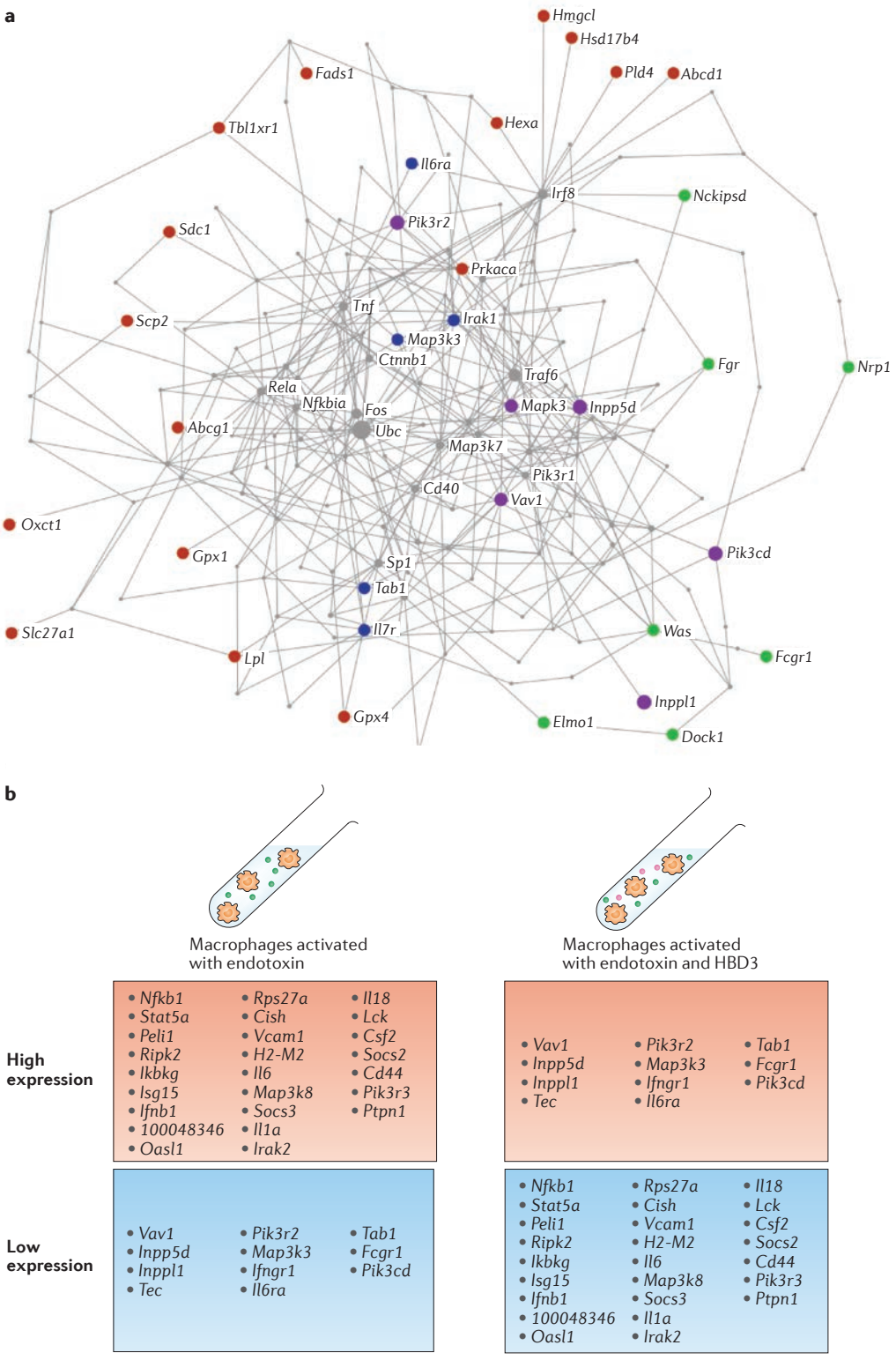


Figure 2 | **The complex response of KLA-stimulated mouse macrophages to HBD3. a** | A network interaction diagram that shows proteins belonging to the Fcγ receptor-dependent phagocytosis pathway (green), interleukin signalling pathway (blue) and lipid and lipoprotein metabolism pathway (red). These are a subset of the genes that changed expression after treatment with human β-defensin 3 (HBD3), as determined using GEO2R[126]. Although these pathways accomplish divergent functions within the cell, they are interconnected and share several common proteins (purple). **b** | Summary of the expression patterns of cytokine signalling genes in macrophages activated with endotoxin alone or endotoxin together with HBD3. Although cytokine signalling is thought to be dampened by the presence of the cathelicidin LL-37, many genes belonging to this pathway are upregulated by HBD3. The network diagram and expression data were generated using NetworkAnalyst[8]. KLA, KDO_2–lipid A.

squamous cell carcinoma[63]. The role of α-defensins in cancer progression has not been studied in great detail, although increased levels of HNPs have been observed in patients with cancer[64]. These data have been interpreted to suggest that many defensins are tumour promoting, although conversely they might reflect an aberrant host response. Consistent with tumour promotion, defensins promote cell growth and proliferation *in vitro*, and/or influence the tumour microenvironment by promoting angiogenesis or anti-apoptotic signalling[36].

The role of LL-37 in cancer has been widely studied, and various roles have been proposed for this HDP in tumour growth and progression. To prevent cancer in healthy individuals, LL-37 interacts with NK cells and promotes their toxicity towards tumour cells[60]. In addition, the combined stimulation of CpG oligonucleotides and LL-37 further enhances the proliferation and activation of NK cells[65], indicating that LL-37 can suppress tumours through the activation of innate immunity. Synthetic peptide fragments derived from LL-37 have also been identified as potential anticancer drugs (recently reviewed in REF. 66). The anticancer activity of other HDPs deriving from either direct lytic activity or promotion of cancer cell apoptosis is actively being investigated[67].

The dysregulation of LL-37 in different cells and tissues seems to contribute to the promotion of cancer and increased tumorigenesis. LL-37 is overexpressed in tumours and, coincidently, there is stimulated growth and proliferation of these cancer cells *in vitro*[68]. Consistent with this, LL-37 stimulates pancreatic cancer stem cells through *N*-formyl peptide receptor 2 (FPR2) and $P2X_7R$, leading to enhanced growth and proliferation[69]. Tumour-associated macrophages are also stimulated by the secretion of Nodal homologue and/or activin A and TGFβ1 by pancreatic ductal adenocarcinoma cells, leading to increased expression of LL-37 by tumour-associated macrophages and creating a positive feedback loop that further promotes tumour growth[69]. These results collectively suggest that LL-37 might promote tumour growth by activating cancer cells. Consistent with this, blocking the interaction between LL-37 and the receptors FPR2 and $P2X_7R$ impaired pancreatic tumour cell growth[69], demonstrating that such interactions might potentially be targeted by designing synthetic HDPs. On the basis of these examples, there is clearly much to be learned about the role of HDPs in the growth and progression of cancer cells. There is growing interest in this area of research, as improving our understanding of these processes could potentially identify novel targets for cancer therapeutics.

Respiratory diseases. The lungs and respiratory tract are constantly exposed to potential pathogens from the air that is breathed. The respiratory system uses mucocilliary clearance, phagocytic cells, cytokines and HDPs to act as a first line of defence against pathogens[70]. Dysregulated production of HDPs from respiratory tract epithelial cells seems to contribute to several pulmonary diseases, including cystic fibrosis, chronic obstructive pulmonary disease (COPD) and asthma.

Cystic fibrosis is an autosomal recessive disorder that is caused by a mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. This mutation causes a disruption in the ionic balance within the airway and is characterized by a build-up of thick mucus in the lungs[71]. The lungs of patients with cystic fibrosis are persistently colonized by bacteria, particularly *Pseudomonas aeruginosa* and *S. aureus*. Interestingly, HDPs are still secreted and are often present at much higher concentrations in the lungs of patients with cystic fibrosis than in the lungs of healthy individuals[72]. However, the antibacterial activity of these HDPs is compromised[73], probably as a result of the high salt concentrations present in the cystic fibrosis airway. Synthetic HDPs have demonstrated promise as potential treatments for cystic fibrosis. For example, IDR-1018 is a potent immunomodulatory anti-infective HDP that can dampen hyperinflammatory responses of cystic fibrosis epithelial cells by correcting dysfunctional autophagy[74], and it also possesses broad-spectrum antibiofilm activity[75] that could directly target the biofilm-forming activity of chronic pathogens in the lungs of individuals with cystic fibrosis.

COPD is a chronic lung disease characterized by poor airflow in the lungs, and patients often suffer from shortness of breath, coughing and increased mucus production. The rates of COPD are increasing and, unfortunately, there is no treatment currently available. COPD is characterized by a low level of chronic inflammation and recurrent bacterial infections. As with cystic fibrosis, the levels of most HDPs in the lungs are elevated in patients with COPD[76], and increased HNP concentrations correlate with the severity of lung obstruction[77].

Asthma is an airway inflammatory disease that is caused by constriction of the bronchi within the lungs, which causes airway obstruction and difficulty in breathing[78]. The roles of HDPs in asthma are poorly understood, but some data indicate that HDPs have a role in this disease. The levels of LL-37 in the lungs of patients with asthma are lower than in the lungs of patients suffering from cystic fibrosis or COPD[76], which is consistent with distinct differences in the inflammatory changes in asthma. LL-37 was recently shown to activate eosinophils from patients with asthma and to promote the release of inflammatory mediators that are known to contribute to asthmatic inflammation[79]. Infection with rhinovirus is known to trigger asthma exacerbations, and it has recently been shown that rhinovirus infection leads to increased levels of CXCL8 and α-defensins in the bronchoalveolar lavage fluid of individuals with asthma[80]. This increase in HDP production was attributed to infiltrating neutrophils that were attracted to the airways owing to the increased concentration of CXCL8 (REF. 80).

Autoimmune disorders. There is increasing evidence that HDPs have important roles in the progression and severity of autoimmune disorders, which is consistent with significant involvement of HDPs in the immune response. For example, type 1 diabetes mellitus (T1DM) is an autoimmune disease wherein the

Box 4 | **Development of synthetic HDPs for therapeutic applications**

Natural host defence peptides (HDPs) have served as templates for the design of synthetic polypeptide sequences that are optimized for a specific biological purpose. This strategy has proved useful for the design of HDPs with enhanced antibacterial activity, many of which are currently being evaluated in clinical trials[2]. As the diverse activities of HDPs continue to be appreciated, a tremendous opportunity has emerged to design synthetic HDPs to address many inflammatory diseases. Unfortunately, the sequence requirements governing the immunological and anti-inflammatory activities of HDPs are currently poorly understood. Therefore, any sequence manipulation to alter certain physicochemical properties (hydrophobicity or positive charge) are made without any knowledge of the effect that they will have on the biological activity. As a result, any observed improvement in activity has been largely due to serendipity, and it is possible that any changes that are made might unintentionally enhance unfavourable characteristics such as cytotoxicity. This makes the task of optimizing HDPs difficult, time consuming and, considering the cost of synthetic peptides, quite expensive. Peptide arrays (so-called SPOT synthesis) are making peptide screening more cost effective and can be combined with improved screening methods to assess the biological activity of many peptides simultaneously[124]. Such methods are informing our understanding of the sequence requirements that contribute to the immunomodulatory and cytotoxic activities of synthetic HDPs. These larger data sets can be used to relate the observed biological activities to specific HDP sequences using molecular descriptors that mathematically describe the chemical information of each polypeptide and serve as a surrogate for structural analysis. Quantitative structure activity relationship (QSAR) models can be generated on the basis of this information, and the best QSAR models are used to accurately predict the activity of virtual peptides. This strategy has been successfully applied to the generation of novel antimicrobial peptides with enhanced antibacterial activity[125] and is relevant to the optimization of any synthetic HDP sequence with a measurable biological activity.

immune cells of the body attack the insulin-producing β-cells of the pancreas. Interestingly, LL-37 and HBD1 levels are reduced in patients with T1DM compared with healthy individuals[81], suggesting that HDP dysregulation might influence this disease state. However, in mouse studies, DNA from β-cells can form a complex with neutrophil-derived CAMP that is capable of interacting with pancreatic pDCs through TLR9 (REF. 82). This leads to the production of IFNα[82], which would promote the progression of T1DM[83] and suggests that HDP activity might directly influence this disease state. In addition, short-chain fatty acids produced by specific gut microbiota influence CAMP production from pancreatic β-cells and protect against diabetes in non-obese diabetic mice[84], indicating that the gut microbiota and diet have an important influence on HDP production.

T2DM occurs when the body develops resistance to the insulin derived from β-cells. HDP production is also affected by T2DM, as patients exhibit lower levels of HDP expression compared with healthy individuals, which might contribute to an increased risk of developing tuberculosis[85]. HDP production in the diabetic foot ulcers of patients with T2DM is also altered compared with that in healthy skin, which might contribute to improper wound healing and prolonged infection[86].

The dysregulation of HDPs has emerged as a contributing factor in other autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus[87] and psoriasis (see below). It is believed that this is primarily due to a pro-inflammatory response that is triggered by HDPs, particularly the production of type I IFN, which is an important contributor to autoimmune diseases[88], although alternatively it could be a response to inflammation.

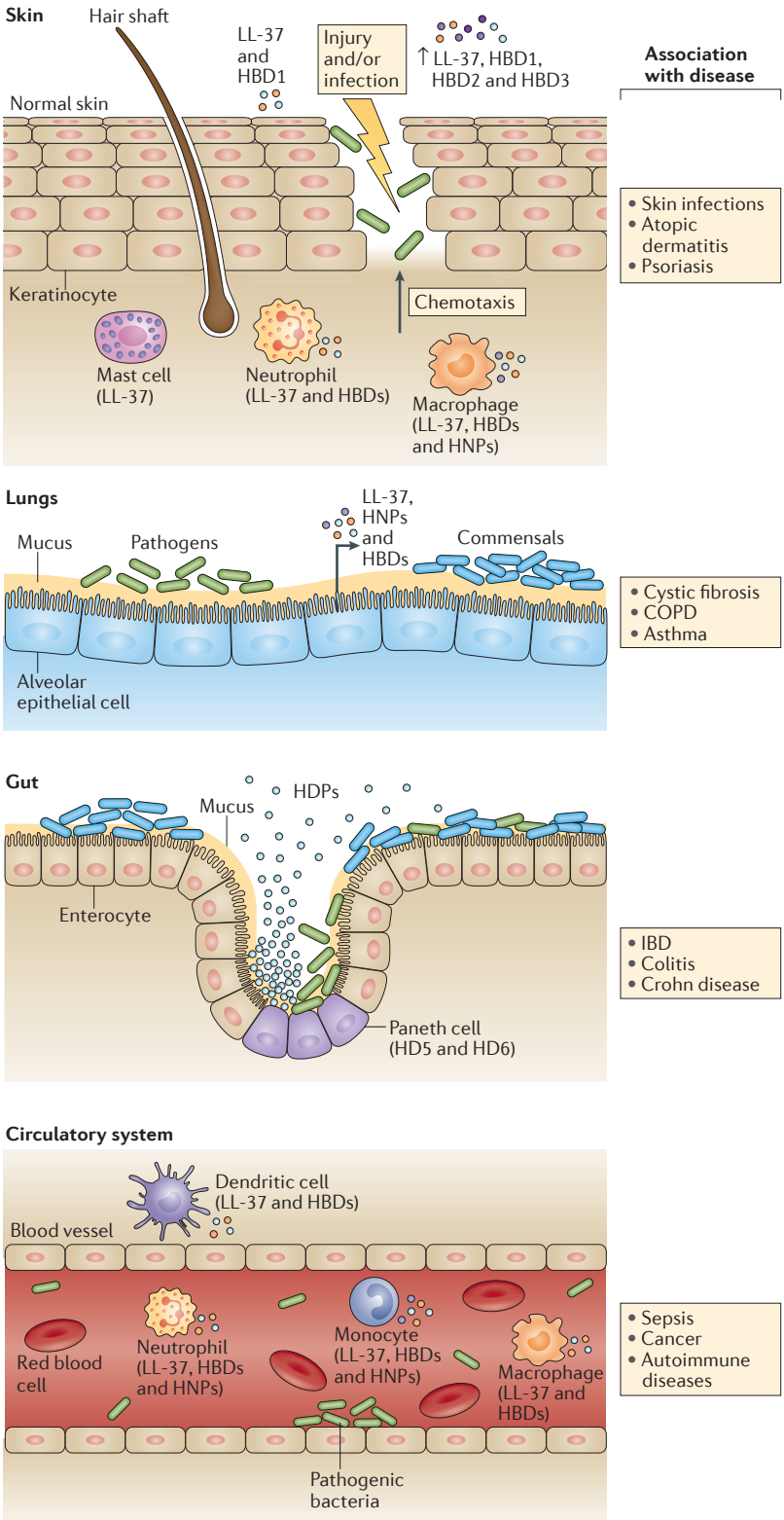
Skin diseases. HDPs are important components of the defence mechanisms of skin[10]. Skin keratinocytes produce many HDPs, including constitutive expression of HBD1 and strong induction of HBD2 and HBD3 upon bacterial challenge or inflammation[35,36]. LL-37 is normally produced in the sweat[89] and mast cells[90] of healthy skin but is strongly induced in inflamed skin[91]. As many HDPs are readily produced by healthy skin, it is understandable that some skin conditions, such as atopic dermatitis and psoriasis, are associated with a dysregulation of the HDPs that are produced by skin cells.

Atopic dermatitis is an inflammatory condition of the skin that is characterized by itchy, red and swollen skin that occasionally cracks and causes open wounds and sores. The role of HDPs in atopic dermatitis is emerging and remains controversial[92]. In early studies, the expression of HDPs in patients with atopic dermatitis was found to be significantly reduced compared with patients with psoriasis[93]. This led some researchers to conclude that the decreased expression of HDPs in the skin made patients with atopic dermatitis susceptible to skin infections[92]. However, there is growing evidence that HDPs are actually highly expressed in atopic dermatitis skin lesions compared with healthy skin, although HDPs levels in non-lesional skin are unchanged[94,95]. In addition, several studies have evaluated the immunomodulatory role of HDPs in relation to the chronic inflammation that is seen in atopic dermatitis[96]; thus, further work is needed to fully understand the role of HDPs in the pathogenesis of atopic dermatitis.

Psoriasis is a common autoimmune inflammatory disease of the skin that is characterized by red, scaly and raised plaques on the skin. LL-37 is overexpressed in the skin of patients with psoriasis[97]. LL-37 can bind to self DNA to form an immunogenic complex that is capable of activating pDCs through TLR9 (REF. 27). It was recently reported that other HDPs, such as HBD2 and HBD3, could also activate pDCs by forming complexes with self DNA[98]. These activated pDCs can influence T cell polarization, which is believed to have an important role in the pathogenesis of psoriasis[99]. LL-37 itself might potentially have an exacerbating influence on psoriasis progression, as it has been shown to induce increased TLR9 expression in keratinocytes, leading to increased type I IFN production by keratinocytes[100]. In addition, LL-37 was recently identified as an autoantigen for circulating T cells in patients with psoriasis[101].

Other diseases. There are various known disorders that influence HDP production and lead to increased inflammation or increased susceptibility to infections, such as Kostmann syndrome, specific granule deficiency, chronic oral inflammation[102], Crohn disease and colitis[10]. In many cases, it is still unclear whether the dysregulation of natural HDPs causes disease onset or whether the progression of disease leads to alterations in HDP levels. Continued study of the complexity of

the immune response in specific disease contexts and a deeper understanding of the effect of HDPs on diverse signalling pathways might lead to the development of new therapeutics and treatment options for these immune-associated disorders.



Other activities of HDPs

Use of synthetic mimetics of HDPs as therapeutics. HDPs are amphipathic cationic peptides, and mimetics can be easily designed that capture, and indeed enhance, the immunomodulatory activities of these peptides (BOX 4). The most advanced synthetic peptides, such as IDR-1018 (REF. 58), demonstrate activity in animal models of cerebral malaria, multidrug-resistant *Mycobacterium tuberculosis*, *Escherichia coli*, *S. aureus* (including methicillin-resistant *S. aureus*) and herpes virus, as well as inducing wound healing and being efficacious in sterile inflammation models such as a preterm birth brain damage (that is, LPS–hypoxia ischaemia) model[4,6]. Thus, these peptides are being pursued clinically and preclinically to prevent infections and inflammation.

Induction of HDPs as therapeutics. Modulation of HDP levels has been explored as a potential therapeutic approach for treating various diseases, and much of this work has focused on the stimulation of HDP production by vitamin D. Vitamin D is known to induce production of several HDPs, such as HBD2 and LL-37 (REF. 16). Vitamin D treatment of primary cell cultures that were prepared from skin biopsy samples of patients with diabetic foot ulcers led to increased production of HBD2 and LL-37 in the cell supernatant and improved wound healing *in vitro*[103]. A clinical study examined the effect of vitamin D supplementation in Crohn disease and found that patients receiving vitamin D showed increased levels of LL-37, as well as higher quality of life scores[104], although the underlying mechanism of this

Figure 3 | **Diversity of HDP activities within the body and relationship with disease states.** Host defence peptides (HDPs) are produced by various different cell types throughout the body. Highlighted here are HDPs that are produced by the epithelial cells of the skin, lungs and gut, as well as the immune cells of the circulatory system. On the skin, certain HDPs, such as the cathelicidin LL-37, human β-defensins (HBDs) and human neutrophil peptides (HNPs), are constitutively expressed by keratinocytes and mast cells, whereas the expression of others can be strongly induced in response to injury or infection, which attracts immune cells to the area surrounding the damaged tissue. In the lungs, HDPs produced by airway epithelial cells help to protect from invading microorganisms. In the gut, Paneth cells produce large amounts of human α-defensin 5 (HD5) and HD6 at the base of intestinal crypts, helping to prevent infection from pathogenic bacteria and maintain homeostasis of the commensal microorganism community. In the circulatory system, many immune cell types express HDPs in response to infection and inflammation. These HDPs can have multiple effects throughout the body, depending on the location where they are produced and the types of cells that are present. A dysregulation of HDP expression at any of these sites can contribute to various disease states. Examples of diseases associated with each body site in which altered production of HDPs has been observed are shown on the right. COPD, chronic obstructive pulmonary disease; IBD, inflammatory bowel disease.

Neurotropic
Localization to nerve tissue.

correlation remains unclear. Similarly, phenylbutyrate enhances cathelicidin production, even when given orally, and works through the vitamin D receptor[105]. Like vitamin D3, phenylbutyrate had a substantial effect on resistance to *M. tuberculosis* infection in a randomized controlled clinical trial[17]. Although many of these studies have focused on inducing HDPs *in vivo*, it is worth noting that inhibiting HDP production may be a potential treatment for various inflammatory diseases. For instance, treatment with etanercept, which is a TNF inhibitor, of patients with psoriasis resulted in significant improvement in psoriatic lesions and a significant decrease in the expression levels of HDPs, including HBD2, LL-37 and psoriasin (also known as S100A7)[106].

Wound healing. Early clinical observations revealed that LL-37 levels in chronic ulcers were much lower than in surgical wounds[107], suggesting that decreased levels of LL-37 might contribute to an inability of wounds to heal. Consistent with this, LL-37 possesses substantial wound-healing properties in mice and promotes wound healing in airway epithelial cells[108] and keratinocyte migration[109], which has also been shown for HBDs[110]. In a Phase I/II clinical trial, synthetic LL-37 proved to be an effective topical treatment for hard-to-treat venous leg ulcers[111], whereas HBD3 application to infected wounds accelerated wound closure in animal models[112]. Other synthetic peptides with immunomodulatory properties have also been shown to promote wound healing in mice and pigs[113], indicating that HDPs hold promise as topical treatments and ointments that promote re-epithelialization, skin healing and wound closure.

HDPs as biomarkers for disease. As the dysregulation of HDPs is often associated with disease states, it stands to reason that these molecules could serve as biomarkers for specific disorders. This has proved to be particularly useful for identifying patients with underlying bacterial infections that would otherwise take a long time to culture and diagnose. For example, urinary levels of HNP1, HNP2, HNP3 (also known as neutrophil defensin 3), HD5 and HBD2 were found to be substantially increased in patients presenting with a urinary tract infection in the emergency department[114]. HDPs have also been identified as biomarkers in cancer, as HNP levels are elevated in the serum of patients with colon cancer and might serve as a blood marker for colon cancer[115].

New activities of HDPs. New activities for HDPs continue to be identified in the literature, demonstrating that interest in these molecules continues to grow. A recent example described an HDP isolated from earthworms that exhibited neurotropic activity in mouse neural stem cells and was protective in a mouse model of Parkinson disease[116]. Another interesting study described a novel bone-forming cell phenotype that was generated by differentiating blood-derived monocytes in the presence of LL-37. The authors suggest that these new cells, termed monoosteophils, could potentially be used to enhance the repair of broken bones or even treat osteoporosis[117]. Evidently, further studies are needed to demonstrate the effectiveness of both treatments but are likely to reflect the broad regulatory properties of HDPs. Improved understanding of these processes should reveal further interesting applications for HDPs.

Conclusions and future directions

HDPs form an integral part of the immune system and contribute to the complexity of signalling events that accompany infection and inflammation. They act on a diverse range of cell types and are an important regulatory component of the innate immune response at various surfaces of the body, including the skin, lungs, intestine and circulatory system (FIG. 3), as well as influencing subsequent adaptive immune responses. Substantial work has gone into elucidating the specific pathways that are affected by HDPs, and our understanding of how the dysregulation of HDP production contributes to diseases continues to grow as we seek to understand their relationship with inflammation and inflammatory diseases. It is important to emphasize that most HDPs do not act on specific signalling pathways in their effector cells. Instead, the pleiotropic effects of HDPs occur simultaneously within an organism, and it is imperative that we examine these interactions at a systems level to fully appreciate the breadth of cell signalling that is influenced by HDP production. This information could reveal important pathways that are associated with disorders of the immune system and identify novel targets for therapeutic intervention.

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Competing interests statement
The authors declare competing interests: see Web version for details.

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ERRATUM

The immunology of host defence peptides: beyond antimicrobial activity

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In the original version of this article, the first subheading of Table 1 was incorrect. It should read Cathelicidin LL-37. This has now been corrected online and for the print version. We apologize for this error.