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Abstract

Cationic host defence peptides (CHDP), also known as antimicrobial peptides, are naturally-occurring peptides which can combat infections through their direct microbicidal properties and/or by influencing the host's immune responses. The unique ability of CHDP to control infections as well as resolve harmful inflammation has generated interest in harnessing the properties of these peptides to develop new therapies for infectious diseases, chronic inflammatory disorders and wound healing. Various strategies have been employed to design synthetic optimized peptides, with negligible toxicity. Here, we focus on the progress made in understanding the scope of functions of CHDP and the emerging potential clinical applications of CHDP-based therapies.

Introduction

The global increase in multi-drug resistant pathogens along with a steady decline in the discovery of new antibiotics, underscores the need for new therapies to control infections. In addition, common treatments used for many chronic inflammatory diseases such as corticosteroids can increase susceptibility to infections[1,2], including antibiotic recalcitrant pathogens[3]. There is therefore an urgent need for alternative strategies that can both kill pathogens as well as resolve harmful inflammation. One promising approach has emerged from the identification of Cationic Host Defence Peptides (CHDP), which can control infections either by their direct microbicidal properties and/or by modulating host immune responses, while also exhibiting the capacity to limit enhanced inflammation.

CHDP, also known as antimicrobial peptides, were first described in the 1960s by Kiss and Michl in the speckled frog[4]. Several seminal studies in the 1980s further defined antimicrobial peptides, notably cecropin isolated from moths by the Boman group[5], defensins from human neutrophils by Ganz and Lehrer[6], and magainins from amphibians by Zasloff et al[7]. CHDP are now known to be expressed across a diverse range of species, from microbes, plants, invertebrates to more complex amphibians and mammals.

CDHP are typically amphipathic small peptides with less than 50 amino acids and a net positive charge of +2 to +9 at physiological pH. However, these natural peptides differ significantly in sequence and structure, and are broadly classified into four structural groups based on conformation; α-helical linear peptides, those with β-sheet with disulphide bridges, cyclic peptides and peptides with extended flexible loop structures.

Initial research in the field was focused on the antimicrobial functions of this family of peptides, with a drive to discover new antibiotic-like therapies based on the naturally-occurring peptides. However, studies published in the 2000s established that the microbicidal action of some of these peptides e.g. the human cathelicidin LL-37 and β-defensins, were severely impaired under physiological salt concentrations (particularly by divalent cations) and in the presence of host factors such as anionic polysaccharides, apolipoproteins, DNA, f-actin and glycosaminoglycans[8,9]. The relatively poor direct microbicidal capabilities of these peptides under physiological conditions, at the low, naturally occurring concentrations observed *in vivo*, did not adequately explain their contribution in host defense against infections[10,11]. Subsequently, research over the last two decades has demonstrated that CHDP exhibit a wide range of functions beyond microbicidal activity, including the ability to influence immune responses, which contribute to their antimicrobial impact (reviewed in[12-15]).

This has led to the adoption of the current name for this family of peptides, Cationic Host Defence Peptides, which encompasses the wide range of described functions.

Over the last three decades there has been substantial interest in therapeutically harnessing CHDP, with more than 5000 papers published in this area of research since 2017 alone. These include the examination of potential clinical uses for CHDP ranging from infections including multidrug-resistant bacteria[16-19], to chronic inflammatory diseases such as arthritis[20], asthma[21] and colitis[22], as well as some cancers[23]. Peptide-based therapeutics currently in clinical trials are primarily for the treatment of infections such as respiratory, oral and catheter-related infections, and for wound healing (see http://dramp.cpu-bioinfor.org/browse/ClinicalTrialsData.php).

This review will provide an overview of current understanding of the scope of functions of CHDP, primarily from eukaryotes. Emerging therapeutic applications of these peptides, current clinical trials and the associated clinical developmental challenges will be discussed. Although there is increasing interest in the development of non-peptide mimics of CHDP for therapeutic application, such as peptoid analogs (reviewed in[24]), a comprehensive discussion of these approaches is beyond the scope of this review.

[H1] Naturally occurring CHDP

The antimicrobial peptide database has catalogued more than 2600 natural antimicrobial peptides, including those annotated as immunomodulatory[25]. The major families of CHDP from eukaryotes that are of interest from a drug discovery perspective are summarized below.

[H2] Vertebrate CHDP

CHDP from vertebrates have an essential role in the first line of defense against microbial pathogens. Upon infection, CHDP can kill pathogens through diverse mechanisms[26-31] (discussed below), acting rapidly and directly on the pathogen when present in high local concentrations, or indirectly to modify components of host defense. These peptides exhibit immunomodulatory activities that can be either pro- or anti-inflammatory depending on the phase of the infection (see below)[12-14,29]. CHDP from vertebrates are amphipathic peptides containing amino acids with hydrophilic and hydrophobic side chains at opposite sides of the molecules. These CHDP can interact with the negatively charged membranes of bacteria. The two main classes of CHDP in vertebrates, the defensins and cathelicidins (Fig. 1a), are produced as prepropeptides which are cleaved to yield mature active peptides.

Defensins have a common β-sheet core stabilized with three disulphide bridges between 6 conserved cysteine residues, and are subdivided into α-, β- and θ-defensins based on the linkage of cysteine residues. The genes encoding α- and β-defensins are adjacent on chromosome 8p23.1 and probably have common ancestry, with α-defensins thought to have evolved by gene duplication, and only found in some mammals, primarily primates and rodents[32]. Several of the human α-defensins are highly expressed in neutrophils, and other α-defensins are produced and secreted by Paneth cells in the small intestine[6,33,34]. The β-defensins are ubiquitous and present in all vertebrates. The human genome has >30 genes coding for β-defensins, and mice have even more genes coding for these peptides. These peptides are mainly produced in epithelial cells[33,35]. Finally, the cyclic θ-defensins arose from α-defensins after the divergence of primates and have been purified from the leukocytes of rhesus macaques and baboons. These molecules are the only cyclic peptides found in mammals and exhibit antiviral activity. Thus varying between different species, evidence exists for sequence divergence by both positive and negative selection of mammalian defensin genes following ancestral gene duplication[36-38].

Cathelicidins are produced as prepropeptides containing an N-terminal signal peptide, a cathelin-like domain, and the C-terminal mature peptide. The pro-cathelin-like domain is cleaved off primarily by serine proteases once the peptide is secreted[39,40]. These peptides are named after the conserved cathelin-like domain in the pro-peptide which is the common denominator of this family of peptides, as the active mature cathelicidins do not share sequence similarities. Most cathelicidins are α-helical (23-37 amino acids), amphipathic, cationic peptides with a hydrophobic surface enabling interaction and perturbation of membranes with anionic surfaces. The only human cathelicidin LL-37 is the most well studied peptide in this family. LL-37 is an α-helical peptide and is one of several cleavage products of hCAP18, the product of the only human cathelicidin gene *CAMP*[26]. Other α-helical cathelicidins include the sole mouse cathelicidin CRAMP[41] and chicken CATH-2[29]. Another class of cathelicidins comprises β-hairpin peptides (12-18 residues) such as the bovine bactenecin that have one intramolecular disulphide bond. Cathelicidins such as protegrins (16-18 residues) have two intramolecular disulphide bonds and adopt β-sheet structures. Finally, linear cathelicidins (13-39 residues) are enriched in specific amino acids e.g. tryptophan-enriched bovine indolicidin and proline-rich porcine PR-39. Cathelicidins are immunomodulatory antimicrobials with an important role in the regulation of the inflammatory response[29-31].

Several other vertebrate CHDP are also being explored as potential alternatives to antibiotics. Among these are histatins, which are histidine-rich CHDP constituents of saliva of mammals. Histatins

are less amphipathic than cathelicidins or defensins and may have different modes of action compared to other vertebrate CHDP as illustrated by their antifungal activity[42]. Finally, several CHDP produced by amphibian skin granular glands, such as magainin-2, have been used as prototypes for the development of novel antimicrobials[43].

[H2] Invertebrate, plant and fungal CHDP

CHDP are a vital component of the innate immune system of all eukaryotic organisms, including plants, fungi and invertebrates which lack an adaptive immune system. These CHDP, similar to those from vertebrates, are also small amphipathic peptides with an overall positive net charge[44,45]. These peptides fall into several diverse and distinct structural groups, including α-helical peptides, β-sheet peptides, peptides with mixed α-helical and β-sheet structures, extended peptides and peptides enriched in specific amino acids (Fig. 1b). Their classification is generally dependent on the number, spacing and connectivity of cysteine residues as well as structure which is normally highly conserved within a CHDP family.

In plants, gene duplication and rapid evolution has produced large families of antimicrobial peptides within the same organism[46]. Genome sequencing has revealed that plants such as Arabidopsis and Medicago have up to 300 defensin or defensin-like sequences. Apart from the cysteine residues and a glycine residue which are required for the defensin fold, these are highly diverse wherein the sequence diversity is displayed on the surface loops presented on conserved scaffolds. This may explain the diversity of biological functions defined for this family of proteins[47]. Plant defensins are generally potent antifungal molecules. However, plant defensins with antibacterial and insecticidal activity have also been described, along with those with roles in cell signalling, self- non-self-recognition during sexual reproduction, ion channel perturbation and enzyme inhibition[46,47]. Similar to the mammalian defensins, these also exhibit other functions, including roles in innate immunity, in addition to antimicrobial activity[33,48]. Although once considered an example of evolution of an ancient innate immunity molecule, it is now evident that mammalian and plant defensins evolved independently and are an excellent example of convergent evolution[46].

[H1] Synthetic peptides derived from CHDP

The vast repertoire of sequences and structures of natural antimicrobial peptides allows for the design and development of novel therapeutic peptide analogues or peptidomimetics. Various approaches are being applied in the design of new drug candidates based on CHDP; such as single amino acid

substitution, using internal segments of CHDP to derive smaller peptides, as well as *in silico* methods to predict new synthetic peptides based on the understanding of structure-function relationships[49-51].

[H2] Synthetic innate defence regulator (IDR) peptides

Early approaches to the development of CHDP as therapeutics focused heavily on optimizing the direct microbicidal properties of these peptides. However, it has become clear that strategies adopted to enhance *in vitro* microbicidal functions often resulted in peptides with increased levels of cytotoxicity. In addition, naturally occurring CHDP can also exhibit other functions undesirable for drug development such as an ability to induce mast cell degranulation, with release of histamine and prostaglandin, as well as activation of complement factors[52,53]. Therefore, in the last decade, effective strategies have focused on designing synthetic peptides from sequences of CHDP to optimize antimicrobial functions *in vivo*, through a combination of some microbicidal activity along with beneficial immunomodulatory functions, in the absence of cytotoxic effects.

Innate defence regulator (IDR) peptides are such small synthetic cationic peptides derived from natural CHDP, which have been screened and optimized for immunomodulatory functions[12,54]. Libraries of IDR peptides have been generated by screening overlapping fragments representing internal sequences of CHDP, and by single amino acid substitutions[49-51]. Most of the IDR peptides published to date are derivatives of the bovine CHDP bactenecin[21,49,54]. IDR peptides are non-immunogenic and do not have the potentially adverse effects associated with some natural CHDP. In general, IDR peptides have a modest direct effect on the pathogen, yet can control infection *in vivo* and reduce inflammation, as shown in a variety of infection models[54-56]. The beneficial use of IDR peptides for the control of infections is now well established in preclinical models of a wide range of pathogens, including multidrug resistant bacteria, viruses, parasites, and antibiotic-recalcitrant bacterial biofilms[54,56-60]. IDR peptides also exhibit adjuvant functions to enhance mucosal immunity and antigen-specific humoral response[61-64], and are effective in controlling inflammation and related physiological outcomes in models of sterile inflammation[65] and chronic inflammatory diseases[21]. A distinct advantage of IDR peptide-based therapy, compared to current anti-inflammatory therapeutics, is the potential to resolve infections along with the ability to control inflammation[54,57].

[H2] Cryptic and synthetic peptides

Several mammalian cationic proteins like histones, lactoferrin, thrombin and their cleavage products have antimicrobial activities. Histones and fragments thereof have a wide range of antimicrobial

activities[66]. These are not restricted to nucleosomes but are also found in the cytoplasm of cells and can be released upon activation. Indeed, up to 70% of the proteins of neutrophil extracellular traps (NETs) comprise histones and histone fragments[67]. Lactoferrin is another example of a protein that yields antimicrobial peptides upon proteolytic degradation *in vivo*. This protein is not confined to milk, it is produced by several tissues and has several biological activities related to host defense, including antimicrobial activity. Its proteolysis in the gastrointestinal tract produces fragments that are more active than the native protein[68]. Proteolytic digestion with pepsin releases lactoferricin, a peptide derived from the N-terminal region of lactoferrin. Other active fragments described are Lf(1-11) and lactoferrampin[69]. Similarly, synthetic molecules based on human θ-defensin pseudogenes, termed retrocyclins, have been developed as potential therapeutics for example as antivirals[70]. These observations that antimicrobial and/or immunomodulatory peptides can be released from larger proteins during inflammation or infection has led to the exciting concept of developing synthetic CHDP as prodrugs in which the N-terminus of the peptide is modified by a linker and a negatively charged promoiety. Such prodrugs can be selectively cleaved by a disease-associated enzyme, thereby targeting peptide activity to pathologically affected parts of the body[71]. In addition, these discoveries have led to bioinformatics approaches to identify segments of secreted proteins that could be developed as novel antimicrobial peptides of human origin. Bioinformatic tools have been developed to find those cryptic sequences and the peptides thus defined are known as cryptic peptides[72]. Among the identified and tested cryptic peptides are those from two apolipoproteins[73]. Other researchers have developed entirely novel antimicrobial peptides by high throughput screening of libraries of peptides that have been synthesized semi-randomly[74,75] or by rational design[51].

[H1] Antimicrobial actions of CHDP

The antimicrobial effects of CHDP on a wide range of pathogens have provided the impetus for the development of these peptides as broad-spectrum antimicrobials. Related mechanisms of action are diverse and appear to be dependent on the microbial pathogen.

[H2] Antibacterial activity

The bacterial membrane is the main target for most cationic peptides. Bacterial membranes are negatively charged because of the presence of anionic lipids, lipopolysaccharides (LPS; in Gram-negative bacteria) or teichoic acids (in Gram-positive bacteria). These negatively charged molecules initiate an electrostatic interaction with cationic compounds which explains the preference of CHDP for

bacterial membranes compared to the membranes of cells from plants, invertebrates and vertebrates. The amphipathic nature of CHDP is important for the membrane-destabilizing properties of these peptides. CHDP can bind to the bacterial inner membrane leading to penetration of the peptide, leakage of bacterial cell contents and cell death[27,28] (Fig. 2). In Gram-negative bacteria, interaction of CHDP with LPS results in perturbation of the outer membrane and this has been defined as a primary mode of action for the antimicrobial activity of CHDP[76]. Four main models of (inner) membrane perturbation have been proposed: aggregate, toroidal, barrel-stave and carpet model[76] (Fig. 2). It should be cautioned that these proposed mechanisms of action stem largely from experiments with model membranes. As the interaction of CHDP with bacterial membranes does not involve a specific target, it has been speculated that the development of microbial resistance is unlikely. Indeed, CHDP can elicit transient bacterial adaptations, the mechanisms of which are different from those involved in the development of bacterial resistance to conventional antibiotics. For example, a study has shown that removal of CHDP from the culture medium resulted in the bacteria reverting to their original state: the adaptation to counter the effect of the CHDP was not maintained[77]. Therefore, microbial 'resistance', as described for conventional antibiotics, is unlikely to develop for CHDPs.

In addition to inducing gross damage of bacterial membranes, CHDP may also affect cell wall synthesis. For example, defensins such as HNP-1 and hBD3 exert their antibacterial activity by docking on lipid II, the intermediate in peptidoglycan synthesis[78-80]. In addition, CHDP such as the ribosome-binding proline-rich peptides can cross the bacterial membrane and kill bacteria from within, by binding to intracellular targets such as nucleic acids or nascent proteins and subsequently affect cell processes such as replication, transcription, translation, protein folding and cell wall synthesis[28,76,81,82] (Fig. 2). Simultaneous exposure of a pathogen to multiple different CHDP, potentially utilizing different modes of action, may be a critical mechanism by which these peptides are so effective *in vivo*. An exciting recent study in drosophila utilized CRISPR gene editing to delete all known immune-inducible CHDP and demonstrated both additive and synergistic antimicrobial functions of the peptides, as well as highly specific CHDP-pathogen interactions *in vivo*[83].

It should be noted that the antibacterial effects of CHDP are measured under non-physiological conditions in most *in vitro* studies. This is a problem as the direct microbicidal activities of many CHDP are antagonized under physiological *in vivo* conditions, such as higher ionic strength, presence of divalent cations and host lipids and proteins. Thus, it may be argued that CHDP may not function *in vivo* as antimicrobials with direct microbicidal properties[11,84]. However, some CHDP (and derived synthetic peptides) with compromised direct antimicrobial activity *in vitro* exhibit the capacity to

actively control infections *in vivo*, which may be due to local concentrations of CHDP released by neutrophil degranulation at the site of infection being higher than *in vitro* MIC values. A more plausible explanation is that CHDP-mediated immunomodulatory functions and/or the concerted action of CHDP with other immunity-related factors are critical in the resolution of infections *in vivo* (discussed below). However, the relationship between antibacterial potency of CHDP *in vitro* and its immunomodulatory functions is not understood. There is no evidence of an inverse correlation between antibacterial potency and the ability of CHDP to induce an immune response[84].

[H2] Antiviral Activity

In addition to their antibacterial properties, early observations of the antiviral potential of CHDP[85] have been expanded to demonstrate activity against a range of viruses. The majority of these studies in recent years have been performed *in vitro* and have described a variety of mechanisms that underpin the antiviral effects, differing in the context of specific viruses (Table I).

A common mechanism of action against many enveloped viruses (such as Influenza, Respiratory Syncytial virus, Zika, Vaccinia virus, and Kaposi's sarcoma-associated herpesvirus) *in vitro* is the capacity of CDHP (including defensins and cathelicidins) to destabilise the viral envelope upon contact, damaging the virions and inhibiting infectivity[86-94]. This may happen upon contact in solution or upon viral exposure to plasma membrane-associated CHDP during cell entry[88]. However, CHDP have also been shown to have antiviral activity against non-enveloped viruses (such as Rhinovirus, Human Papillomavirus 16 and Adenovirus), by decreasing viral replication[95,96] and/or via binding viral capsid, thereby preventing uncoating and nuclear entry of the viral genome[97-101]. An additional mechanism of action against a number of viruses (such as Herpes Simplex Virus and HIV) relates to specific CHDP binding to cellular receptors involved in viral infection, that is dependent upon the lectin-like properties of some peptides[102-110]. Further antiviral effects may also result from CHDP-mediated aggregation of viral particles[111], inhibition of PKC activity[112,113], and immunomodulatory effects (discussed in detail below) of the peptides on host immune cells such as enhancing phagocyte function[111,114] or by modification of cytokine responses[115,116]. These antiviral mechanisms highlight the possibility that baseline expression of CHDP could create an “antiviral shield” at mucosal surfaces and prevent replication and spread of virus, if upregulated after initial infection. Therapies aimed at inducing host CHDP expression, or the application of synthetic CHDP-derived peptides with defined, selective properties, could therefore have both preventative and/or therapeutic potential.

[H2] Antifungal activity

Fungal infections in humans are a growing problem world-wide. Many patients suffer from non-life-threatening fungal infections of the skin and oral cavity. However, invasive infections by fungal species such as *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Candida albicans* and *Histoplasma capsulatum* are responsible for the death of 1.5 million people annually[117]. Only a limited set of antifungals is available for the treatment of life-threatening fungal infections and the development of antifungal drug resistance continues to increase[118]. This is particularly evident for azole-resistance because of the wide-spread use of azoles in agriculture[119]. In addition, the world-wide number of immunocompromised patients is increasing which leads to more fungal infections. Both trends are very disturbing and demand the development of new potent antifungals that do not easily elicit resistance. Furthermore, it is important that novel antifungal compounds be only used in humans to prevent rapid resistance development in the environment. Because of their diverse modes of action CHDP-derived molecules may be used as paradigms for the development of potent antifungals. Antifungal CHDP from plants and vertebrates have been described[48]. The activity of plant defensins has primarily been described against fungi[120]. Various killing mechanisms of CHDP on yeast and fungi have been reported, ranging from effects on mitochondrial functions of *Candida albicans* by histatin 5 to membrane effects on this yeast by cathelicidins and CHDP mimics[121-123]. It should be noted that fungal biofilms are highly resistant to antifungals[124]. It is thus important to screen for the anti-biofilm activity of newly developed CHDP-based antifungals[124].

[H1] Immunomodulatory actions of CHDP

The earliest studies into the non-microbicidal properties of CHDP were on their effects on immune cells, primarily related to the ability of these peptides to recruit leukocytes[125-127]. Following this, research on immunity-related functions of CHDP increased exponentially over the next two decades, defining a diverse range of functions. Immunity-related functions of CHDP seem to be dependent on the environmental stimuli, cell and tissue type, interaction with different cellular receptors, and the concentration of the peptides. Studies to date indicate that the molecular mechanism underpinning the ability of CHDP to selectively modulate immune responses is highly complex involving intracellular uptake of the peptides, which may or may not be mediated by membrane-associated GPCR, interaction with several intracellular interacting protein partners or receptors (e.g. GAPDH and p62), alteration of several signaling pathways (NFκB, p38 and JNK MAPK, MKP-1, and PI3K), engagement of different transcription factors, all of which seem to be dependent on factors such as the peptide concentration,

kinetic of response and the environmental stimuli (reviewed in[12,15,128,129]). The pleiotropic immunomodulatory functions of CHDP raise questions regarding the primary biological role of these peptides. Below, we summarize the activities of CHDP on modulation of immunity and inflammation (Fig. 3), primarily focusing on cathelicidins and defensins. Understanding mechanisms that underpin the ability of CHDP to modulate immunity to protect against infection, resolve inflammation and contribute to immune homeostasis, is critical to the development of novel therapeutic approaches based on CHDP-derived peptides.

[H2] Immune activation

Protective activation of the innate immune system by CHDP has emerged as one of the key mechanisms underpinning the ability of these peptides to promote early clearance of infections. The actions of CHDP include recruiting leukocytes, modulating neutrophil responses and influencing antigen-specific adaptive immunity (discussed below). In addition, the interaction of CHDP and the microbiome in mucosal immunity is a rapidly emerging area of research. Although beyond the scope of this review, this exciting area of research suggests the potential for CHDP-mediated selectivity in control of the microbiome[130-133], but also the potential for components of the microbiome to contribute to the regulation of CHDP expression. Further research on CHDP-microbiome interaction may enhance understanding of the consequences of microbial dysbiosis in disease states, aging and following treatment with broad-spectrum antibiotics.

[H3] Leukocyte recruitment

Immune cells e.g. neutrophils, macrophages, mast cells and T-cells exhibit direct chemotactic activity towards CHDP and their IDR derivatives[54,57,127,134-136]. CHDP also indirectly promote recruitment of leukocytes by inducing the release of chemokines[10,128,137-140]. The ability of these peptides to induce chemokine release and enhance recruitment of leukocytes has been defined as a primary immunomodulatory mechanism related to their ability to protect against infections[54,57,141]. Underlying mechanisms involve several cellular receptors such as chemokine receptors (e.g. CCR6 and CCR2), G-protein coupled receptors (GPCR) including the formyl peptide receptors (reviewed in [12,142]), and Toll-like receptors (TLR)[135,143,144], as well as interaction with intracellular proteins such as GAPDH and p62[145,146]. However, these functions appear to be dependent on the phase of infection and inflammation, as it has been shown that LL-37 can mediate the internalization of chemokine receptor CXCR2 on monocytes and neutrophils, consequently dampening chemotaxis[147]. Molecular processes

that control the dichotomy of CHDP to selectively induce chemokine secretion and enhance leukocyte recruitment, without altering the anti-inflammatory functions of the peptide (discussed in the next section) are not completely understood.

[H3] Neutrophil function

Neutrophils are the major innate immune effector cells of the early phase response to infections, and are a primary source of both defensins and cathelicidins which are stored pre-formed in the secondary and primary granules respectively, and released upon neutrophil degranulation. CHDP can influence the function of neutrophils to modify infection outcomes. For example, these peptides can enhance influx of neutrophils both by direct chemotactic function[127,136,144] and indirectly by promoting the secretion of neutrophil chemokines e.g. IL-8 and Gro-α in a MAPK-dependent manner[137,148], to promote control of infections. Similarly, a recent study has shown that CHDP hepcidin induced in the skin during bacterial infections can enhance chemokine production and promote neutrophil responses[149]. CHDP are also located on neutrophil NETs, potentially contributing to NET-mediated antibacterial effects[150]. LL-37 can facilitate NET formation and contribute to antiviral activity, as reported for influenza A virus[86]. It should be noted that recent studies have shown that post-translational modifications of CHDP, in particular citrullination, may alter the functions of NET associated CHDP such as cathelicidins[151-153]. LL-37 can also promote bacterial clearance *in vivo* by enhancing early neutrophil responses[154]. In addition, LL-37 can induce intracellular calcium mobilisation and the generation of reactive oxygen species (ROS)[147] as well as enhance ROS production mediated by other inflammatory stimuli[155], indicating the capacity to prime and enhance neutrophil antimicrobial functions. The ability of CHDP to modulate the host cellular response to infections is not restricted to effects on neutrophils, these peptides can also modify other innate and adaptive cellular responses (reviewed in[12,14,29,33]).

[H3] Antigen presentation and adaptive immunity

CHDP serve as a link between innate and adaptive immunity, as a consequence of their capacity to recruit antigen presenting cells (APCs) such as monocytes / macrophages and dendritic cells (DCs) to the site of infections (as discussed above). These peptides can also enhance phagocytosis in macrophages, facilitating clearance of bacteria by boosting immune activation[156-159]. CHDP can not only activate APCs, but also influence the generation and polarization of lymphocyte responses, thus shaping the adaptive immune response. For example, human defensins hBD2 and hBD3 can both

induce production of IFN-α from plasmacytoid DCs and consequently influence the initiation of T-cell responses[160]. The expression of co-stimulatory molecules CD80, CD86 and CD40 on myeloid cells are also upregulated by exposure to hBD3, facilitating the promotion of adaptive immune response via the engagement of TLRs[161]. LL-37 can modulate the adaptive immune response by multiple effector functions; LL-37 facilitates enhanced expression of co-stimulatory molecules and promotion of a modified adaptive response via modulation of dendritic cell differentiation and function *in vitro*[159,162] and *in vivo*[163], promotes DC activation[164,165] and enhances the activation/ proliferation of B-cells via activating follicular DCs[166]. The influence of CHDP on adaptive immunity has led to the examination of application of these peptides, primarily cathelicidins, as adjuvants to enhance systemic and mucosal antigen-specific immune responses[144,160,166].

[H2] Regulation of inflammation

As discussed above, CHDP exhibit multiple functions to activate the immune system which can be classified as pro-inflammatory responses that aid in the clearance of pathogens. In contrast, potent CHDP-mediated anti-inflammatory effects have been demonstrated in the presence of an inflammatory and/or pathogenic challenge, thus suggesting that CHDP are also regulatory molecules that limit enhanced inflammation. Therefore, rather than being described as either pro- or anti-inflammatory molecules, it may be appropriate to define CHDP as molecules that can balance inflammation to promote immune homeostasis. The anti-inflammatory function of CHDP is reinforced by several studies that have demonstrated that the deficiency of these peptides results in increased inflammatory responses; cathelicidin-deficient mice exhibit a more severe inflammatory phenotype compared to wild type[155,167,168]. Similarly, reduced expression of β-defensin in enterocytes of humans has been associated with Crohn's disease[169]. Indeed the critical role of defensins in maintaining the integrity of intestinal mucosa and immune homeostasis is well established (reviewed in[34]). Notably, exogenous application of CHDP e.g. LL-37, CATH-2 and BMAP-28, hBD2, and synthetic peptides e.g. IDR-1 and IDR-1002, has been shown to control inflammation in various animal models of infections and sepsis[55,57,59,60,170-173]. Similarly, an LL-37-derived peptide controlled the disease process in a murine model of inflammatory arthritis[20], and IDR-1002 effectively alleviated airway inflammation *in vivo*[21]. In contrast, cathelicidin-KO mice demonstrate increased survival rate in a cecal ligation and puncture model of sepsis, despite increased expression of pro-inflammatory genes[167]. Therefore, the outcome of CHDP-mediated immunomodulatory responses seems to be context dependent and reliant on the

cellular environment. This has been demonstrated in studies examining the cross talk between TLR and CHDP, as discussed below.

[H3] Crosstalk of CHDP with TLRs

Several studies have demonstrated that cathelicidins exhibit potent anti-endotoxin properties *in vitro* and *in vivo*[10,138,170,174,175], both by binding bacterial lipopolysaccharide (LPS)[174,175] and by intervening in TLR signaling mechanisms[138]. The ability of cathelicidins such as LL-37 to modulate TLR-to-NFκB signaling is not restricted to its effect on TLR4, as LL-37 also dampens responses to TLR2/1 agonists and whole bacteria[10,176]. Downstream outcomes of CHDP-mediated modulation of the TLR-to-NFκB pathway in the presence of inflammatory stimuli results in the selective suppression of specific pro-inflammatory responses such as production of TNF and ROS, without the neutralization of innate immune functions such as chemokine production, and concurrent enhancement of anti-inflammatory mediators such as IL-10, IL-1RA, A20 and NFκBIA[138,177-180]. A caveat is that the effect of endogenous CHDP to limit inflammation may be impeded by peptide modifications that occur under inflammatory conditions. A recent study demonstrated that the enzyme peptidyl arginine deaminase released by inflammatory cells can mediate citrullination of LL-37, which compromises the ability of the peptide to intervene in TLR-signaling to dampen pro-inflammatory responses in macrophages, and to control sepsis *in vivo*[151]. Therefore, it is essential to consider the composition of the inflammatory milieu in studies that aim to define or optimize the anti-inflammatory functions of cationic peptides.

It should be noted that the modulation of TLR-mediated signalling by CHDP is not necessarily "anti-inflammatory", as these peptides can also induce chemokines to attract leukocytes such as IL-8 and MCP-1 (pro-inflammatory response) at the same time as inhibiting LPS-induced TNF[10,138,181]. Similarly, cathelicidins can also exhibit both pro- or anti-inflammatory activities depending on the phase of the infection. For example, CATH-2 enhances the sensing of bacterial DNA[182] and is not anti-inflammatory if the bacterial infection has not been completely resolved[182,183], while LL-37 promotes acute protective pro-inflammatory responses primarily in the context of an infection, including via activation of the inflammasome, but not in the absence of pathogens[154,184]. However, in instances where the bacteria are killed, both LL-37 and CATH-2 exhibit anti-inflammatory activity and can prevent activation via TLR2 and TLR4[183]. This is one of the mechanisms described for cathelicidin-mediated dampening of inflammatory responses induced by bacterial products, which may be especially important in pulmonary infections to protect the respiratory epithelium[173].

Similarly, human defensins e.g. hBD3 exhibit anti-endotoxic properties *in vitro* and *in vivo,* via modulation of TLR signalling pathways[185,186], but also induces the production of pro-inflammatory cytokines by monocytes[161], increases TLR9-dependent responses to bacterial DNA[187], and modifies MDA- and TLR-mediated responses to Poly I:C[188]. In addition, hBD3 can induce the maturation of dendritic cells (DC)[161], while hBD2 and 3 can induce TLR9-dependent plasmacytoid DC IFN-α release, secondary to interaction between the peptides and otherwise non-inflammatory DNA to mediate *in vivo* adjuvant properties[189]. This is similar to the capacity to mediate TLR7-, TLR8- and TLR9-dependent immunogenic responses to self-DNA/RNA, which was first described for LL-37[164,165,190]. However, LL-37 enhances TLR3-mediated responses[191,192] and promotes release of IL-1β[193]. These observations demonstrate the potential of CHDP to modulate innate and downstream adaptive response via their impact upon pattern recognition signalling pathways.

[H3] Modulation of cytokine-mediated responses

Limited studies have demonstrated the ability of CHDP, especially LL-37, to modulate cytokine-mediated responses in various cell types. LL-37 induces the expression of the IL-1 family of genes including Th17/Th1-related genes such as *IL-6* and *IL23A* in keratinocytes[194]. Consistent with this, another study demonstrated that LL-37 can synergize with cytokines IL-1β and GM-CSF to enhance chemokine production, but not with cytokines such as IL-4 or IL-12, in human PBMC[195]. In contrast, LL-37 suppresses cytokine IL-32-induced pro-inflammatory cytokines such as TNF, IL-6 and IL-1β, by activating the dual phosphatase MKP-1 which is a known negative regulator of inflammation, without altering its ability to induce chemokine production[177]. Mechanisms that control the ability of CHDP such as LL-37 to limit inflammation without altering chemokine production are not completely understood. To that end, a recent study has demonstrated that LL-37-induced chemokine release and subsequent leukocyte recruitment is selectively mediated by GPCR via the CdC42 RhoGTPase pathway, without impacting LL-37-induced anti-inflammatory cytokine release[137].

[H2] Cell death

Although CHDP can rapidly permeabilize prokaryotic membranes, most natural peptides are relatively less toxic to eukaryotic cells. CHDP such as LL-37 and CATH-2 can enter eukaryotic cells[182,196] and facilitate the entry of nucleic acids and DNA dyes[182,193,197], without inducing cell lysis, suggesting a temporary membrane disruption or pore opening. However, exposure to high concentrations of LL-37 induces eukaryotic cell apoptosis *in vitro* and *in vivo* [198-201]. This phenomenon is cell type-dependent

with the viability of primary human lymphocytes and monocytes relatively unaffected by exposure to high concentrations of the peptide[159,202]. The impact of CHDP-mediated induction of apoptosis on innate or adaptive responses *in vivo* remains unknown. However, high concentrations of LL-37 have been found to preferentially induce death in infected epithelial cells and represent a potential additional host defence mechanism[184,199]. Furthermore, different modes of cell death have important roles in maintaining immune homeostasis, and in amplifying or dampening, and later resolving, inflammatory responses. In this context, the control of neutrophil death and anti-inflammatory properties of apoptotic neutrophils is particularly important[203]. Interestingly, LL-37 can induce rapid secondary necrosis of apoptotic neutrophils[178,204], with anti-inflammatory effects upon activated macrophages being associated with LL-37-mediated release of granule contents from the apoptotic cells, perhaps resulting from release of LL-37 and α-defensins from these apoptotic neutrophils[205]. Thus, CHDP-mediated regulation of cell death has the potential to affect the magnitude and the resolution of inflammatory responses to infection.

[H1] Development of CHDP-based therapies

Based on the properties discussed above, many CHDP from either prokaryotes or eukaryotes and their derivatives are currently being investigated for a variety of indications including their use as antimicrobials and anti-inflammatories, as well as their application in wound healing,

[H2] Antimicrobial therapies

[H3] Preclinical studies

Studies in murine models have demonstrated the critical role of CHDP in the control of infections. For example, cathelicidin-deficient mice[206] exhibit less effective host defense against Streptococcal skin infections[206], impaired clearance of *Pseudomonas aeruginosa* infections from the lung[207] and the cornea[208], develop more severe pox skin lesions upon infection with Vaccinia virus[92], develop more severe infection with RSV upon challenge[90], and exhibit increased susceptibility to infection with *Citrobacter rodentium* in the intestinal tract[209], *Escherichia coli* in the urinary tract[210] and *Klebsiella pneumoniae* in the lung[211]. The impaired antimicrobial activities observed in these cathelicidin-deficient mice may be explained by the absence of direct effects of the mouse cathelicidin, CRAMP, on these pathogens, although the observed effects may also be the result of altered immune modulation in the knock-out mice.

Exogenous administration of many CHDP has been found to be effective in various animal infection models for bacterial, viral, and fungal infections (reviewed in[12,212]). However, it remains to be determined if the observed efficacy is primarily due to the direct antimicrobial activities of the administered CHDP on the pathogen or due to the effect of the peptides on host immunity, or a combination of both. Therefore, the mode of action of the peptides needs to be carefully examined *in vivo*.

Several factors have limited the success of oral or systemic use of CHDP in preclinical studies, including (i) high local concentrations of CHDP, (ii) concerted actions of different CHDP and (iii) synergism with other molecules at the site of infection. However, many *in vivo* studies have shown efficacy of CHDP-derived peptides when these are administered topically. For example, a recent study showed that topical application of a peptide designed using LL-37 and Tachyplesin 1 as chemical benchmarks was protective in a MRSA murine model[213]. Similarly, localized intra-tracheal administration of a CHDP-derived peptide was shown to be better in lowering bacterial load in the lungs compared to rifampicin treatment in a murine model of tuberculosis[214]. Moreover, nanostructure-based technology has recently shown promise as an effective delivery system for slow release of peptides for infection control *in vivo*[215].

A growing area of interest is the potential use of CHDP with indwelling medical devices, prosthetic joints and other implants for the prevention of nosocomial infections[216,217]. Bacterial biofilm formation on medical devices include pathogens recalcitrant to antibiotics which results in biomaterial-associated infections, a major problem in clinical practice. CHDP may be immobilized on the surfaces of biomaterials to prevent adhesion of bacteria. For example, synthetic peptides designed from LL-37 and a trombocidin-derived peptide was shown to be effective in inhibiting biofilm formation by a biomaterial-associated clinical isolate of *S. aureus*[216]. In addition, depending on the chemical tethering procedure, it is possible to retain antibacterial activity of CHDP after coating the surface. The disadvantage of this approach is that only bacteria in the immediate vicinity of the surface are killed. Application of CHDP-releasing biomaterials may be a better approach to prevent infections from implants. It has been reported that several hydrogels and also nanotubes and microporous calcium phosphate coatings inhibit bacterial growth *in vivo*[217].

Another potential application of CHDP is their development as potential first line antiviral treatments for use during pandemics, where there is insufficient time to produce vaccines (such as new influenza (IAV) pandemics), or for viral infections for which vaccines are not available (such as Respiratory Syncytial Virus (RSV)), and more broadly for other viral pathogens. An early proof of

concept study in mice demonstrated that gel-based application of HD5 protected against HSV infection[102]. More recently, intravaginal instillation of a synthetic defensin, identified by HD5 mutant screening, showed prophylactic and/or therapeutic efficacy in a lethal HSV-2 infection model[103]. In another example, prophylactic application of RC-2 in a murine HSV-mediated ocular keratitis model modestly reduced viral titres and reduced disease[105], but had no effect on disease pathology when applied post-infection. Intranasal human and murine cathelicidins both have shown *in vivo* antiviral activity equivalent to current first line neuraminidase inhibitors in a lethal murine IAV model, when applied concomitantly with virus and daily thereafter, dramatically improving survival despite modest effects on viral load[116]. Similarly, intranasal Urumin[87], intranasal mBD2 (optimal when premixed with virus before infection[218]), intravenous delivery of recombinant mBD3[219] or intramuscular delivery of mBD1-mBD3 fusion genes[220] were all protective in murine lethal IAV infection models. These studies all demonstrate the therapeutic potential of CHDP as antivirals *in vivo*, although further investigation is needed. Many *in vivo* studies suggest that while early direct contact of virus and CHDP may be protective due to direct damage to the virions, later stage modulation of host immune / inflammatory responses by the peptides may also be critical in the antiviral activity of a CHDP[115,221]. Such observations highlight the importance of studies that address both the direct microbicidal activities and the immunomodulatory properties of these peptides.

[H3] Clinical trials

Most CHDP in clinical trials so far have been formulated for topical applications or as inhalants for the treatment of infections (see http://dramp.cpu-bioinfor.org/browse/ClinicalTrialsData.php and Table II[222]). One of the most advanced of these was Pexiganan, an analogue of the magainin peptide, which was tested as a topical cream for the treatment of infected diabetic foot ulcers in Phase III clinical trials. However, development was terminated because it did not perform better than current treatments. There are several trials in progress using Omiganan, a CHDP-derived antimicrobial compound (detailed in Table II). For example, a Phase III trial evaluating the long-term safety for topical application of Omiganan as a treatment for rosacea is ongoing. In addition, localized application of a human lactoferricin-derived peptide PXL01-containing hydrogel was shown to be safe, well tolerated and effective as anti-adhesion treatment postoperatively after tendon repair surgery, in an in-patient Phase II clinical trial[223]. Also, clinical trials are ongoing for topical application of LL-37 for treatment of venous leg ulcers (Table II)[224].

A small number of clinical trials investigating the toxicity and efficacy of CHDP using oral and intravenous administration routes have also been conducted or are ongoing (Table II). For example, Iseganan, an analog of the peptide protegrin was used as an oral solution for oral mucositis in Phase III clinical trial, but did not show significant efficacy (Table II). Similarly, Surotomycin and Isegan completed Phase III trials but were rejected for ongoing development either due to poor efficacy or efficacy that was not superior to current drugs (Table II). Phase III clinical studies using intravenous Brilacidin, a synthetic defensin mimetic), for skin infections are starting soon. Recently, Phase III trials of Murepavadin as an intravenous treatment for bacterial pneumonia were terminated due to increased serum creatinine levels in patients, indicative of acute kidney injury. This is reminiscent of nephrotoxicity issues with polymyxins, the cationic nonribosomal peptides which were used for treatment of Gram-negative bacterial infections and are currently used as the antibiotics of last resort.

Another strategy being evaluated and tested in clinical trials is to enhance the expression/production of endogenous CHDP for chronic inflammatory and infectious disease. A multicentre, double-blind, randomized clinical trial demonstrated that supplementation with vitamin D was beneficial to control exacerbations in COPD, especially for patients deficient in vitamin D[225]. Vitamin D3 results in the induction of LL-37 in macrophages which has been also associated with intracellular killing of *Mycobacterium tuberculosis* in a human trial[226]. Similarly, oral phenylbutyrate, with or without vitamin D supplementation, leads to the induction of LL-37 in macrophages and lymphocytes, and has been evaluated in the treatment of adults with active pulmonary tuberculosis[226]. Therefore, strategies to enhance endogenous CHDP production may be valuable for antimicrobial therapies to counter challenges associated with peptide delivery, stability and bioavailability.

In summary, only a handful of peptide-derived treatments have made it to market, they include PAC-113, a histatin analog that is being sold in Taiwan as a topical treatment for oral candidiasis and Dalbavancin, a semisynthetic lipoglycopeptide that has been approved in the US for intravenous treatment of acute skin infections. Although the failure rate has so far been high, the number of CHDP in clinical trials has grown rapidly and is likely to lead to success in the future.

[H2] Immunomodulatory therapies

[H3] Preclinical studies

Early clinical trials using synthetic analogues of CHDP that had been designed to maximize microbicidal activity achieved only moderate efficacy[227], perhaps due to failure to recognize the importance of immunity-related functions of these peptides. Despite issues of concentration at mucosal

surfaces and antagonizing factors at sites of inflammation, CHDP are clearly essential for the control of infections *in vivo*[90,92,206-211]. Application of LL-37 is protective against infection with *P. aeruginosa*, influenza and RSV *in vivo* [89,116,228], with mode of action involving enhanced, protective early neutrophil responses, rather than by direct microbicidal activity against the pathogen[154]. Comparable results were also obtained after *in ovo* application of CATH-2 in chickens to induce a long-lasting protection against respiratory *E. coli* infections[229]. Similarly, CHDP-derived synthetic peptides such as IDR peptides were also shown to be protective in various infection models wherein IDR peptides protect the host from the pathogen by modulating the host immune response, and in parallel suppress the release of inflammatory cytokines such as TNF and IL-6, suppress reactive oxygen species (ROS), and dampen neutrophil degranulation[54,57-59]. These studies demonstrate that CHDP or related synthetic peptides can provide protection against infections by modulating host immune response rather than by directly targeting the pathogen.

Synthetic IDR peptides are primarily being developed as immunomodulatory therapies to control infections, with particular focus on antibiotic resistant infections. IDR-1 was the first such peptide shown to be protective against both Gram-positive and Gram-negative bacteria, including antibiotic-resistant infections such as methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus (VRE) and Salmonella in murine preclinical models[54]. Subsequently, an analog of IDR-1, peptide SGX94, showed broad-spectrum activity against bacterial infections solely by modulating the host immune response to control infections in pre-clinical studies[230]. Other IDR peptides such as IDR-1002 and IDR-1018 have also been shown to be beneficial in preclinical models of a wide range of pathogens, including multidrug resistant bacteria, viruses, parasites, and antibiotic-recalcitrant bacterial biofilms[56-59]. In general, the mechanism of action of IDR peptides is primarily mediated by influencing host innate immune responses to enhance immune cell recruitment to the site of infection to promote bacterial clearance, rather than by directly targeting the pathogen. IDR peptides also influence neutrophil functions to augment neutrophil-mediated killing of bacteria and the release of natural CHDP from neutrophils[231].

In addition to the potential application of CDHPs in infections, the immunomodulatory functions of these peptides are being examined for use in other indications. Cathelicidin-deficient mice develop more severe colitis in a non-infectious DSS-induced model with greater pro-inflammatory cytokine expression and cell death compared to wild type controls, and with impaired mucus production[232]. In contrast, therapeutic intrarectal administration of the murine cathelicidin mCRAMP (or mCRAMP-expressing *Lactococcus lactis*) is protective in the DSS-induced colitis murine model[233,234]. Similarly,

therapeutic administration of exogenous LL-37 or mCRAMP can modulate *C. difficile* colitis by inhibiting toxin-A-associated intestinal inflammation[235]. These studies suggest that CHDP and/or derived synthetic peptides have the potential to control chronic inflammatory diseases. Consistent with this, limited preclinical studies have demonstrated the beneficial effects of CHDP-derived synthetic peptides for chronic inflammatory diseases such as in inflammatory arthritis[20], asthma[21], and in colitis[22] models. For example, exogenous administration of a LL-37-derived synthetic peptide prevented the development of arthritis, suppressed autoantibodies and prevented cartilage degradation of the joints, in a collagen-induced arthritis murine model[20]. Elafin delivered by adenovirus ameliorated colitis, suppressed inflammatory cytokines and related NF-κB activation in non-infectious DSS-induced colitis murine model[22]. In addition, the peptide IDR-1002, a bactenecin derivative, improved allergen-induced airway hyperresponsiveness, controlled airway inflammation and suppressed inflammatory cytokine IL-33 production, in a murine model of asthma[21]. These preclinical studies suggest that CHDP and their derivative peptides can be developed for non-infectious inflammatory diseases.

Due to the ability of CHDP to regulate inflammation, many studies have also explored the effects of these peptides on cancers. However, the therapeutic use of CHDP-based peptides for cancers remains controversial, as the effect of these peptides on cancer pathology seems to be dependent on the specific type of cancer[236]. Discussing the nuances of pharmaceutical relevance of CHDP in cancer pathology is beyond the scope of this review[237,238]. Nevertheless, a gene therapy study using hDB2 showed that application of this peptide resulted in enhanced local anti-tumor effects in preclinical murine models using CT26, MethA and LL/2 tumor cells, the mechanism of which was associated with immunomodulatory functions of the peptide, namely its ability to activate endogenous dendritic cells[23].

Another promising avenue for harnessing the immunomodulatory functions of IDR peptides is their application as adjuvants for new vaccine formulations, as IDR peptides enhance mucosal immunity and antigen-specific humoral responses[61-64]. Adjuvant formulations with IDR peptides have shown promise in various preclinical studies for vaccines against mycoplasma[63], influenza HINI strain[239], and pertussis[240]. In these studies, IDR peptides with modest antimicrobial activity used in different vaccine formulations showed profound immunomodulatory effects. For example, a bactenecin derived-IDR peptide used in a vaccine formulation with *M. bovis* proteins elicited a balanced humoral IgG1/IgG2 response for use in cattle[63]. Intranasal administration of a nanoparticle-based vaccine formulation containing an immunomodulatory IDR peptide as an adjuvant resulted in a strong cellular and humoral response against H1N1 influenza strain[239]. The potential use of IDR peptide as an adjuvant in vaccines is strongly supported by studies demonstrating that the presence of IDR peptide in these formulations is

effective in eliciting a balanced Th1/Th2 immune response along with facilitating the reduction of antigen dose required for immunity[241].

The application of immunomodulatory CHDP in wound healing is also being explored, driven by studies showing that growth factors associated with stimulation of regeneration of tissues also induce the production of endogenous CHDP such as LL-37, β-defenisn and lipocalin in keratinocytes[242]. Aligned with this, *in vitro* studies have demonstrated that CHDP such as defensins and LL-37 promote angiogenesis and wound healing[243-246], prevent protease-mediated skin barrier damage[247] and promote re-epithelialisation of wounds[248]. These phenotypic changes are driven by peptide-mediated activation of signaling intermediates and transcription factors that activate EGFR through the induction of a G-protein-coupled receptor, in particular for the peptide LL-37[244]. Accordingly, application of LL-37 was shown to be effective in a murine model of excisional wound[244].

[H3] Clinical trials

Despite many preclinical studies describing the immunomodulatory therapeutic potential of CHDP-derived peptides, there have been very few successful clinical trials. The human cathelicidin LL-37 has been applied in a first-in-man randomized, placebo-controlled clinical trial, and shown to be both safe with no local or systemic adverse reactions and to enhance healing of hard-to-heal venous leg ulcers (Table II)[224]. A recent notable study in current clinical development is the use of silicone hydrogel contact lenses coated with a synthetic immunomodulatory peptide Mel4 to reduce contact lens-associated infections and inflammation[249]. Notably, the potential therapeutic and prophylactic success of immunomodulatory CHDP-derived peptides as alternatives to antibiotics in veterinary medicine may pave the way for clinical trials in humans (One Health approach)[250].

[H1] Considerations in CHDP-based drug development

The use of natural CHDP as effective therapeutics is not particularly viable as concentrations that exhibit direct antimicrobial effects are relatively high, and at that concentration range these peptides exhibit cytotoxic effects such as mast cell degranulation, complement activation and apoptosis of mammalian cells, and induce pro-inflammatory cytokine production[11,12,15,251] . Thus, synthetic peptides derived from natural CHDP, synthetic designed peptides and peptides found by semi-random high throughput screening are now emerging as putative lead compounds. Recent studies have also focused on non-peptide CHDP mimics such as peptoid analogs and developing compounds using CHDP on small abiotic scaffolds, for therapeutic applications (reviewed in[24]).

Major challenges facing peptide-based drug development include formulation and delivery, as well as high production cost. Biological factors that affect peptide stability and bioavailability must be taken into consideration, for example mucosal pH and the presence of host or microbial proteases that can degrade candidate peptides[252-254], as well as several other factors which can impair peptide activity such as physiological salt concentration, mucus, DNA and microbial saccharides[252,254]. Several approaches are therefore being explored to enhance peptide stability, such as using D-amino acid peptides and modification of peptides by amidation or acetylation of the terminal regions or by targeted substitutions of tryptophan or histidine with a non-natural amino acid (reviewed in[212]).

The antimicrobial activity of CHDP is generally less than that of conventional antibiotics. Regulatory authorities require new antimicrobials to be non-inferior to existing antibiotics, even if the new compounds do not elicit antimicrobial resistance. CHDP may be unsuitable for standard *in vitro* antimicrobial susceptibility test methodologies to predict *in vivo* efficacy, even for topical application, and more physiologically relevant modified approaches may be vital[255]. Taking all this into consideration, the oral or systemic use of CHDP, with the aim to directly kill microorganisms, will likely be difficult to achieve. However, discoveries of CHDP demonstrating effects against antibiotic resistant pathogens do hold promise[217], as does the new direction of applying immunomodulatory CHDP as an adjunct to antibiotics, due to the observed synergy of CHDP with conventional antibiotics[256-259].

Additional approaches that are being considered to counter the challenges associated with CHDP-based therapy include the use of nutritional supplements such as vitamin D or phenylbutyrate or other products based on short chain fatty acids to enhance the levels of endogenous CHDP[226]. In addition, topical application of analogs of vitamin D was shown to enhance local expression of cathelicidin in psoriatic skin, which would circumvent any challenges that may be related to high systemic levels of vitamin D[260,261]. Also formulations using nanoparticles or liposomes for slow release and targeted delivery of CHDP[262-264], as well as the use of shorter synthetic peptides such as IDR or cryptic peptides are being examined (discussed above). In preclinical studies[56-59], shorter synthetic peptides such as IDR peptides have demonstrated negligible toxicity, no immunogenicity, and cost considerably less to produce than most CHDP, thus making these valuable candidates to investigate for clinical application. In addition, considerable less peptide is required if CHDP are used as immunomodulatory agents compared to their use as direct antimicrobial agents. Furthermore, new production methods such as the efficient usage of expression systems rather than chemical synthesis reduce the costs of production[217,265,266].

Although CHDP offer a promising approach to treat infections, a key challenge for the use of CHDP or related synthetic analogues as antimicrobials is the development of pathogen-associated resistance mechanisms. Indeed resistance of pathogens to human CHDP has been demonstrated with multiple mechanisms adapted by bacteria to evade the direct antimicrobial effects of the peptides[267-269] (see Box. 1 and Fig. 4). However, bacteria have a limited number of ways to resist CHDP, and this resistance is often costly. An important recent study of *in vivo* survival and pathogenicity of a CHDP-resistant *S. aureus* (evolved *in vitro* in the presence of cationic peptides) showed that resistance to the peptides provided no survival advantage to the bacteria in an insect host environment that is dominated by antimicrobial peptides, bacterial clearance was at least as efficient as for the sensitive strains[270]. Nevertheless, harnessing the immunomodulatory actions of CHDP to selectively boost the host immune response rather than directly targeting the pathogen may be the path forward in the development of CHDP-based anti-infective therapies.

[H1] Outlook

The repertoire of functions exhibited by CHDP ranges from direct antimicrobial activity to a wide range of effects on host defense mechanisms, highlighting the critical role of these molecules in infection and immunity. Although, research in this field was initially focused on the development of new 'antibiotics' based on cationic antimicrobial peptides, it is now well appreciated that CHDP have a critical role in immunity; from activation of innate immunity, enhancement of antigen presentation and phagocytosis, to influencing adaptive immunity and memory functions, along with potent anti-inflammatory functions. It is thus not surprising that research in this field has intensified in the context of drug development for a variety of clinical applications ranging from the control of antibiotic-resistant pathogens, alleviation of inflammation in chronic disease, their use as antibiotic adjuvants, to the targeting of specific cancers. However, there are many challenges associated with CHDP-based drug development, notably those associated with formulation and delivery, the potential for drug resistance, as well as the lack of solid pharmacokinetic data. Nevertheless, the wide range of CHDP functions defined to date provides a diverse range of natural molecules for the design and optimization of new drugs. Despite many associated challenges and the limited understanding of structure-function relationships, the potential of CHDP-based therapies remains a promising new clinical direction.

Boxes

Box 1: Development of antimicrobial resistance to CHDP:

A major consideration for the potential application of CHDP as new generation antibiotics will have to include a thorough understanding of the frequency of resistance development of pathogens to peptide-based therapies. CHDP have promise over small molecule antibiotics because the surfaces with which they interact with targets in the pathogen are larger[271] and hence single amino acid substitutions are unlikely to lead to adaptations of bacteria to mitigate CHDP activity. Furthermore, CHDP have complicated mechanisms of action, often interacting with more than one target in microbes, such that multiple mutations within the pathogen are needed for 'resistance' to the peptides. Indeed, a recent study showed that bacterial adaptations to resist CHDP action do not develop easily[272]. The additional, indirect CHDP-mediated effects of enhancing host immune responses to control infections provide an important complementation to the direct microbicidal activities, providing a multi-faceted attack on pathogens during infection. Nevertheless, recent studies have revealed that bacterial and fungal pathogens are capable of developing mechanisms to resist the effects of CHDP. The mechanisms of adaptation of pathogens to human CHDP have been studied extensively and reviewed[267-269]. Common mechanisms in bacteria to counter the effects of CHDP are repulsion, sequestration, removal and degradation (Fig. 4). Additional mechanisms of bacterial adaptations are modification of the pentapeptide on Lipid II, a prominent CHDP target, and by altering the rigidity of the membrane by acylation of Lipid A. Mechanisms that fungi employ to enhance tolerance to CHDP have mainly focused on Candida species, which also employ repulsion, sequestration, removal by efflux pumps and proteolytic degradation against peptides such as LL-37, histatin 5, HNP-1, hBD-3 and lactoferrin[273]. Commensal bacteria of the host microbiome must be able to survive the CHDP presented by epithelial and mucosal surfaces. This may in part be due to the relatively low concentrations of CHDP at mucosal surfaces (except for specific niches such as the intestinal crypts) and somewhat inhibitory environments in which secreted CHDP are present normally in the absence of inflammation. In addition, proteases that cleave endogenous peptides to generate the active form of mature CHDP are also either absent or inactive in the absence of an inflammatory response. In contrast, CHDP in the phagolysosome of a neutrophil, for example, are at a high concentration in a controlled environment optimized for pathogen killing. Relative bacterial resistance to host CHDP is a prerequisite for effective commensal colonisation[131], a property which may be most critical for stability through periods of inflammation where increased levels of CHDP may be capable of preferentially removing pathogens without totally decimating the healthy microbiome. Harnessing this sort of selectivity would have clear therapeutic advantages over broad spectrum antibiotics, and is indeed the focus of an exciting new approach to treating atopic dermatitis[274], now being developed in human clinical trials.

Figure legends

Figure 1: Structures of CHDP

Part a illustrates examples of structures of CHDP from vertebrates. Two cathelicidins are depicted: human LL-37 and chicken CATH-2 (with proline-induced kink). Two human defensins are shown: the α-defensin HD-5 and the β-defensin HBD-2. The pairing and positioning of the six conserved cysteine residues is as follows: α-defensins: Cys1-Cys6, Cys2-Cys4, Cys3-Cys5; β-defensins: Cys1-Cys5, Cys2-Cys4, Cys3-Cys6. Magainin-2, a peptide from Xenopus laevis; its analogue, pexiganan, was developed as a topical agent. Amino acid side chains: red, hydrophobic; blue, basic; green, acidic. Part b provides examples of the diversity of CHDP structures**.** Tertiary structures of selected peptides from plants, fungi and invertebrates, arranged by secondary structure content. Beta strands shown in blue, alpha helices in red, and disulphide bonds in yellow (PDBs: 1MR4, 1NB1, 2RNG, 1BHP, 1GD3, 2L2R, 1JBL, 1HEV, 2MAL, 5E5Q, 5OQS).

Figure 2: Models of antibacterial mechanisms of CHDP. Direct antimicrobial mechanisms of CHDP can be mediated by membrane translocation of the peptides followed by binding to intracellular targets such as nucleic acids and/or proteins to kill bacteria. The mechanisms of translocation are not clear and may depend on the peptide and bacterial species. Proline-rich antimicrobial peptides use inner membrane transporters as Trojan horses to gain entry and subsequently bind within the ribosomal exit tunnel. Other CHDP may use transient pores for translocation. Interaction of CHDP with negatively charged bacterial membrane resulting in membrane perturbation has been defined as a primary mode of direct antimicrobial action. The models of membrane perturbation proposed are barrel-stave, carpet and toroidal pore models. The barrel-stave model was the first permeabilization mechanism proposed and considered to be the prototype in peptide-mediated transmembrane pore formation. In this model, peptides act as staves and vertically insert into the lipid bilayer forming barrels. Peptides which act according to the carpet model cover the negatively charged membrane based on electrostatic attraction. Above a certain peptide threshold concentration, the membrane ruptures in a detergent-like manner resulting in micelle formation of peptide with membrane lipids. The toroidal model is a variation of the aggregate model, where after parallel binding of the peptide to the membrane, the peptide distorts the alignment of the polar head groups of the lipids. This results in perturbation of the acyl chain interactions of the lipids, changes in membrane curvature and destabilization of membrane surface

integrity. At certain peptide to lipid ratios, the peptides orient perpendicularly to the membrane and induce the formation of transient toroidal channels.

Figure 3: Summary of immunomodulatory mechanisms of CHDP. Immunomodulatory functions exhibited by CHDPs include but are not limited to; recruitment of antigen presenting cells to site of infections either directly or indirectly by induction of chemokines to enhance antimicrobial effects, facilitating the activation of NETs, altering endotoxin-mediated signaling pathways, suppression of pro-inflammatory cytokines, enhancing phagocytosis and pro-inflammatory responses to nucleic acids, induction of anti-inflammatory cytokines, influencing differentiation of dendritic cell and polarization of T-cells. Adapted from van der Does A, Hiemstra P and Mookherjee N, *Adv Exp Med Biol* 2019[15].

Figure 4: Diagram of common resistance mechanisms to CHDP in bacterial and fungal pathogens. (A) Gram positive bacteria (B) Gram negative bacteria (C) yeast/fungi. 1. Degradation by secreted proteases, outer membrane proteases or cytosolic proteases. 2. Sequestration by secreted proteins, anionic polysaccharides, mannosylphosphate side chains on glycoproteins (fungi) or O-antigen (Gram negative bacteria). 3. Electrostatic repulsion by alanylated lipoteichoic acid (LTA) or wall teichoic acid (WTA). 4. Electrostatic repulsion by aminoacylated phospatidyl glycerol (PG). 5. Blocking CHDP binding by altering the pentapeptide on Lipid II. 6. Export of CHDP by efflux pumps. 7. Activation of signal transduction pathways that induce expression of genes that reinforce the wall or detoxify products of CHDP activity. 8. Lipid A modification by amine compounds. 9. Enhanced membrane rigidity by lipid A acylation. 10. Activation of MAPK signaling pathways in fungi for protection against oxidative, osmotic or cell wall stress. Adapted from Joo et al., 2016, and Swidergall and Ernst, 2014.

1 **Tables**

2 **Table I: Antiviral activities of cationic host defence antimicrobial peptides**

Virus	Peptide	Proposed mechanism of action *in vitro*	References
Influenza	HNP	• Virus aggregation • Inhibition of PKC disrupts IAV endosomal trafficking • Enhanced neutrophil phagocytosis of IAV	85,111,112
	Retrocyclins	• Virus aggregation • Increased virus uptake by professional phagocytes • RC2: haemagglutinin-mediated fusion of viral and endosomal membranes blocked	113,275,276
	β-defensins	• Inhibition of IAV infectivity at higher concentrations applied before viral entry	114,115,219
	LL-37	• Disruption of viral envelope	116,218,277
	Urumin	• Virion destruction, targeting H1 hemagglutinin	87
RSV	hBD2	• Viral envelope destabilisation in solution or upon exposure to plasma membrane-associated hBD2	88,278
	LL-37	• Virion binding and destruction • Prevention of infection and spread • Function retained by core 22-mer	89,90
Rhinovirus	Cathelicidins	• Decreased infectivity and replication	91,95
Adenovirus	α-defensins	• Peptide binding to adenoviral capsid prevents uncoating and nuclear entry of the viral genome • Dependent upon optimal peptide hydrophobicity and charge	97-99,279
HPV 16	α-defensins	• Uncoating and nuclear entry of the viral genome inhibited	101
HSV	α-defensins, HBD3, retrocyclins	• HSV binding to cellular receptors glycoprotein B and heparin sulphate inhibited • Dependent upon lectin-like properties,	100,102,103,105

		rather than charge	
HIV	HNP	• Disruption of cellular entry • Inhibition of PKC activity, interfering with HIV replication	106,280-282
	Retrocyclins	• Viral entry into cells blocked by peptide binding to gp120 and CD4 • Dependent upon lectin-like properties	107-110,283-285
	β-defensins	• Direct effects on virions • Intracellular, post-viral entry inhibitory functions	286-288
	LL-37	• Suppression of HIV reverse transcriptase activity	289,290
Vaccinia virus	Cathelicidins	• Integrity of the double layered viral envelope damaged	92,291
Zika virus	Cathelicidins	• Direct inactivation of Zika virus • Protective modulation of interferon signalling pathways	93
Kaposi's sarcoma-associated herpesvirus		• Disruption of viral envelope	292

Table 2. Antimicrobial peptides under clinical trials.

Peptide	Origin	Indication	Status	Company	Clinical trial identifier
Topical					
Pexiganan (Locilex®, MSI-78)	Analog of magainin, isolated from African clawed frog *Xenopus laevis*	Infected diabetic foot ulcers	Phase III complete. ***Rejected, efficacy not superior***	PLx Pharma Inc. (formally Dipexium Pharmaceuticals Inc.)	NCT00563394, NCT00563433, NCT01590758, NCT01594762
D2A21, Demegel	Synthetic cecropin peptide	Burn wound infections	Phase III	Demegen	Not listed
CLS001 (Omiganan, MBI-226)	Omiganan pentahydrochloride. Synthetic cationic indolicidin derivative.	Local catheter site infections	Phase III complete (discontinued)	Mallinckrodt, BioWest Therapeutics Inc, Cadence Pharmaceuticals, Inc.	NCT00231153, NCT00027248, 2005-003194-24
		Topical skin antisepsis	Phase III complete	Mallinckrodt	NCT00608959
		Papulopustular rosacea	Phase III current	Cutanea Life Sciences, Inc.	NCT02576860, NCT02547441, NCT02576847, 2015-002921-20, 2015-002919-15, 2015-002920-23
		Acne vulgaris	Phase II complete	Cutanea Life Sciences, Inc., BioWest Therapeutics Inc	NCT02571998, NCT02066545, NCT00211497, NCT00211523
		Atopic dermatitis	Phase II complete	Cutanea Life Sciences, Inc.	NCT03091426, NCT02456480, 2016-003849-28, 2014-003689-26
		Vulvar intraepithelial neoplasia	Phase II complete	Cutanea Life Sciences, Inc.	NCT02596074, 2015-002724-16
		Condylomata acuminata (external genital warts)	Phase II complete	Cutanea Life Sciences, Inc.	NCT02849262, 2015-005553-13
		Facial seborrheic dermatitis	Phase II current	Cutanea Life Sciences, Inc./Maruho Co., Ltd	NCT03688971, 2017-003106-41
Iseganan (IB-367)	Analog of protegrin-1	Ventilator-associated pneumonia	Phase II/III. ***Rejected, no efficacy***	IntraBiotics Pharmaceuticals	NCT00118781
PXL01	Synthetic macrocyclic 25 amino acid peptide derived from human lactoferricin	Prevention of post-surgical adhesions and scar prevention	Phase IIb complete. Phase III trials planned	Promore Pharma (formally Pergamum AB)	NCT01022242, 2009-012703-25

NVXT (Novexatin® NP213)	Cyclic arginine-based heptamer	Fungal nail infection (onychomycosis)	Phase IIb complete	NovaBiotics	NCT02933879, NCT02343627
PAC-113, P-113	Histatin 5 derivative (12 amino acids)	Oral candidiasis	Phase IIb complete	Demegen/Pacgen Biopharmaceuticals Co. **Sold over the counter in Taiwan by General Biologicals Corporation**	NCT00659971
LL-37	Human cathelicidin subunit	Venous leg ulcers	Phase IIb current	Promore Pharma (formally Pergamum AB)	2018-000536-10
HXP124	Plant defensin	Fungal nail infection (onychomycosis)	Phase IIa complete	Hexima	ACTRN12618000131257
Brilacidin (PMX-30063)	Synthetic defensin mimetic	Ulcerative proctitis / ulcerative proctosigmoiditis	Phase II complete. Phase III planned	Alfasigma S.p.A	Not listed
		Oral mucositis in patients with head and neck cancer	Phase II complete FDA Fast track designation	Innovation Pharmaceuticals (formally Cellceutix)	NCT02324335, NCT01211470
LTX-109 (Lytixar™)	Synthetic cationic tripeptide	Atopic dermatitis, skin infection	Phase II complete	Lytix Biopharma	NCT01223222, 2010-021438-68
		Impetigo	Phase II complete	Lytix Biopharma	NCT01803035
		Nasal infections by methicillin-resistant/-sensitive *Staphylococcus aureus* (MRSA/MSSA)	Phase I/II complete	Lytix Biopharma	NCT01158235, 2010-019254-40
(CKPV)2, CZEN-002	Derivative of α-melanocyte stimulating hormone	Vulvovaginal candidiasis	Phase II complete	Zengen/Abiogen Pharma	2005-001360-31
OP-145 (AMP60.4Ac)	Cathelicidin family (LL-37 derivative)	Chronic suppurative otitis media (middle ear infections)	Phase II complete	OctoPlus BV/Dr Reddy's Research and Development BV	ISRCTN12149720
C16G2	Synthetic peptide	Prevention of dental caries due to *Streptococcus mutans*	Phase II complete	Armata Pharmaceuticals	NCT03052842, NCT03004365, NCT02594254, NCT02509845, NCT02254993, NCT02044081, NCT03196219
DPK 060	Derived from kininogen, cationic random coil peptide	Acute external otitis	Phase II complete	DermaGen AB and Promore Pharma (formally Pergamum AB)	NCT01447017, 2011-004356-20

		Atopic dermatitis	Phase I/II complete	DermaGen AB and Promore Pharma (formally Pergamum AB)	NCT01522391
Lotilibcin (WAP-8294A$_2$)	Lipodepsipeptide	Methicillin-resistant *S. aureus* (MRSA)	Phase I complete	aRigen Pharmaceuticals/ Green Cross Corporation	Not listed
PL-5	Alpha helical peptide	Bacterial skin infections	Approval by State Food and Drug Administration of China (SFDA) for clinical trial	Changchun ProteLight Pharmaceutical & Biotechnology Co	n/a
Oral					
Surotomycin (CB-183, 315)	Cyclic lipopeptide, analog of daptamycin	Diarrhea caused by *Clostridium difficile*	Phase III complete. ***Rejected, efficacy not superior***	Cubist Pharmaceuticals/Merck & Co., Inc.	NCT01597505, NCT01598311, 2012-000252-3
Iseganan (IB-367)	Analog of protegrin-1	Oral mucositis in patients with head and neck cancer	Phase III complete. ***No efficacy***	National Cancer Institute (NCI)/ IntraBiotics Pharmaceuticals	NCT00022373
RDP58, Delmitide acetate, allotrap 1258	d-amino acid decapeptide	Ulcerative colitis	Phase II complete	Genzyme/Procter & Gamble	2004-004077-29
NVB-302	Synthetic type B lantibiotic	*Clostridium difficile* infection	Phase I complete	Novacta	ISRCTN40071144
Intravenous					
Dalbavancin (BI397, Dalvance®, Xydalba™)	Semisynthetic lipoglycopeptide	Acute bacterial skin infections	Approved	Allergan (formally Actavis and Durata Therapeutics)	n/a
		Osteomyelitis and septic arthritis	Phase IV current		NCT03426761
AB103 (p*2TA*)	Synthetic anionic CD28 dimer mimetic peptide	Necrotizing soft tissue infections	Phase III current	Atox Bio Ltd	NCT02469857, 2018-001125-15
Dusquetide (SGX942)	Synthetic 5 amino acid peptide derived from indolizidine, immunomodulator	Oral mucositis in patients with head and neck cancer	Phase III current. FDA Fast track designation	Soligenix	NCT03237325, 2017-003702-41
Murepavadin (POL7080)	Synthetic cyclic beta hairpin peptidomimetic based on the cationic antimicrobial peptide protegrin I	Ventilator-associated bacterial pneumonia by *Pseudomonas aeruginosa*	Phase III ***suspended, adverse events***	Polyphor Ltd	NCT03409679, NCT03582007
Neuprex®, opebacan, bactericidal/permeability-	BPI-derived peptide	Burns	Phase II complete	University of Texas Southwestern Medical Center	NCT00462904

increasing protein $rBPI_{21}$		Myeloablative allogeneic hematopoietic stem cell transplantation	Phase I/II. ***Terminated, lack of enrollment***	Xoma LLC	NCT00454155
Brilacidin (PMX-30063)	Synthetic defensin mimetic	Acute Bacterial Skin and Skin Structure Infections (ABSSSI)	Phase II complete. Phase III planned FDA Fast track designation	Innovation Pharmaceuticals (formally Cellceutix)	NCT02052388
EA-360	Linear tetrapeptide, derived from human chorionic gonadotropin hormone (hCG)	Systemic inflammatory response and renal function	Phase IIa/b current	Exponential Biotherapies	NCT03145220, 2014-002481-78
hLF1-11 (human lactoferrin 1-11)	First cationic domain of human lactoferrin hLF (11 residues)	Infections during haematopoietic stem cell transplantations	Phase I/II complete. ***withdrawn***	AM-Pharma	NCT00509938, NCT00430469
		Candidaemia	Phase I/II ***withdrawn***	AM-Pharma	NCT00509834
		Bacteremia due to Staphylococcus epidermidis	Phase I/II ***withdrawn***	AM-Pharma	NCT00509847
Friulimicin B	Cyclic lipopeptide	Pneumonia, Staphylococcal skin infections	Phase I. ***Rejected, unfavourable pharmakokinetics***	MerLion Pharmaceuticals	NCT00492271

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Conflict of interest

NM is listed as an inventor on patents related to immunomodulatory aspects of host defence peptides and IDR peptides. MA is the Chief Scientific Officer and Director of the start-up company Hexima which has a cationic antimicrobial peptide in clinical trials for treatment of onychomycosis. HH owns stock in start-up company Celestial Therapeutics Inc, and has patents on antimicrobial peptide therapeutics licensed to Zoetis. DD declares no conflict of interest.

Publisher's note

TOC

Naturally-occurring cationic host defence peptides (CHDP), also known as antimicrobial peptides, can control infections by their direct microbicidal properties and by modulating host's immune responses. In addition, certain CHDP can resolve harmful inflammation. Here, Mookherjee et al. assess the emerging potential to therapeutically harness these peptides to treat infectious diseases, chronic inflammatory disorders and wound healing, highlighting current preclinical studies and clinical trials.

Subject categories

Biological sciences / Drug discovery [URI /631/154]

Health sciences / Anatomy / Haemic and immune systems / Immune system [URI/692/698/1543/1565]

Biological sciences / Immunology / Infectious diseases [URI/631/250/255]

Health sciences / Diseases / Immunological disorders / Inflammatory diseases [URI/692/699/249/2510]

Fig 1

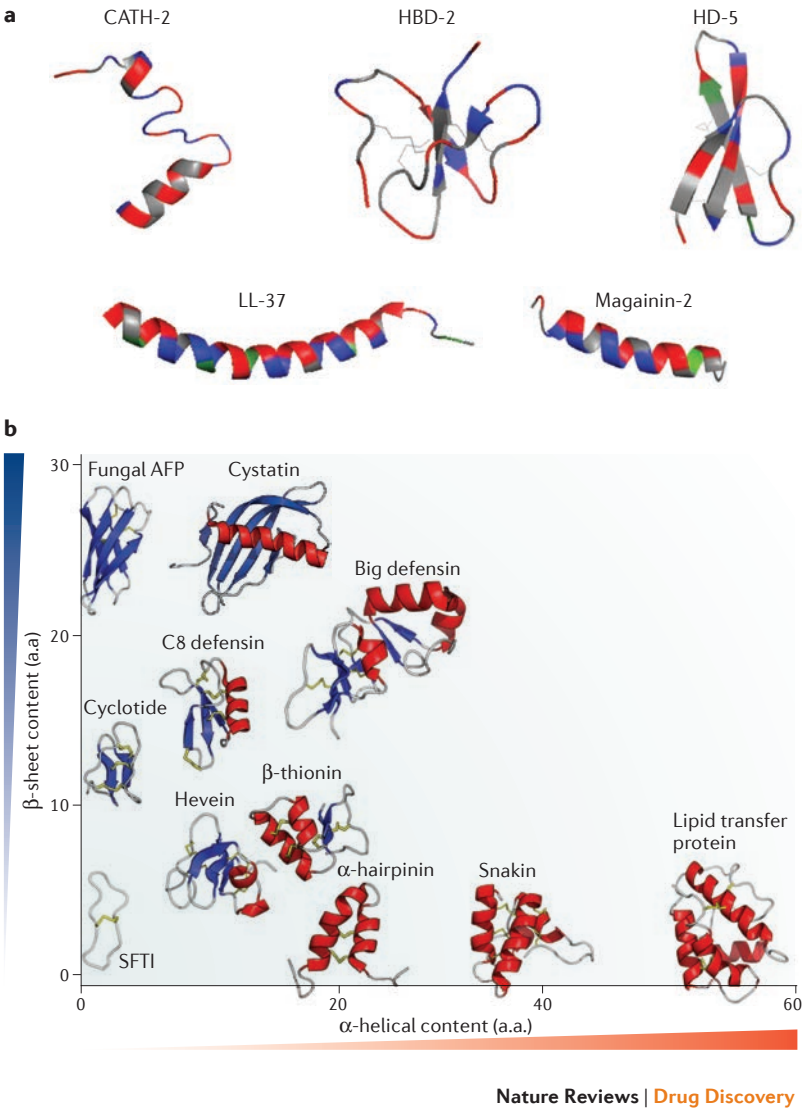
a
CATH-2
HBD-2
HD-5
LL-37
Magainin-2
b
Fungal AFP
Cystatin
Big defensin
C8 defensin
Cyclotide
β-thionin
Hevein
α-hairpinin
Snakin
Lipid transfer protein
SFTI
β-sheet content (a.a)
α-helical content (a.a.)
0
10
20
30
0
20
40
60

Fig 2

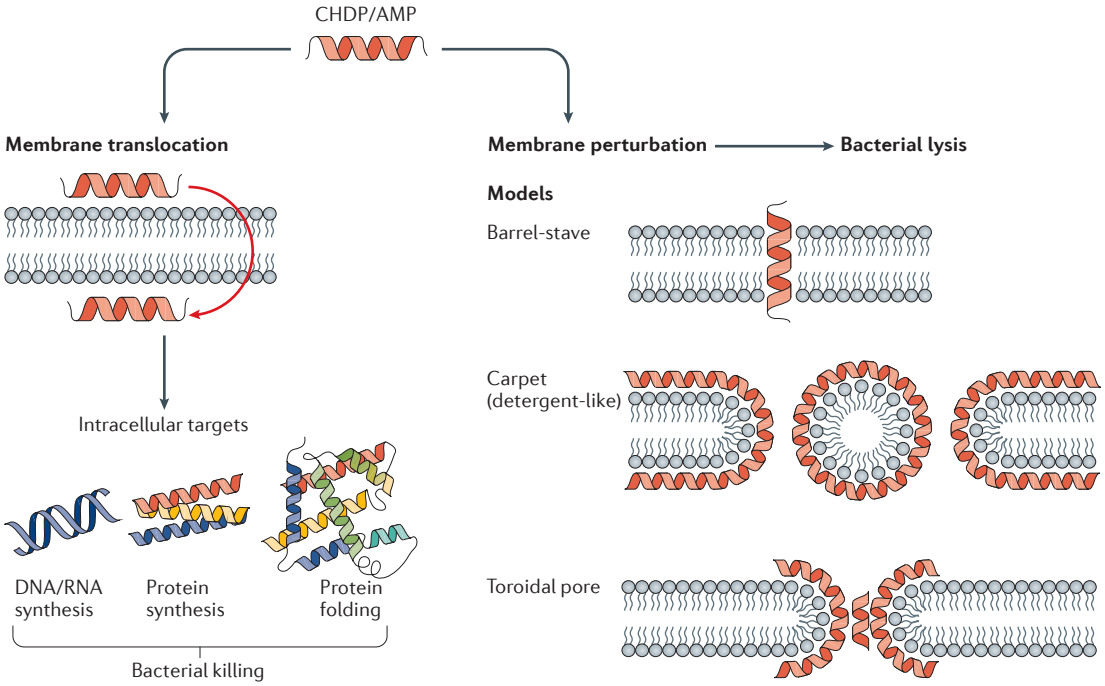

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Fig 3

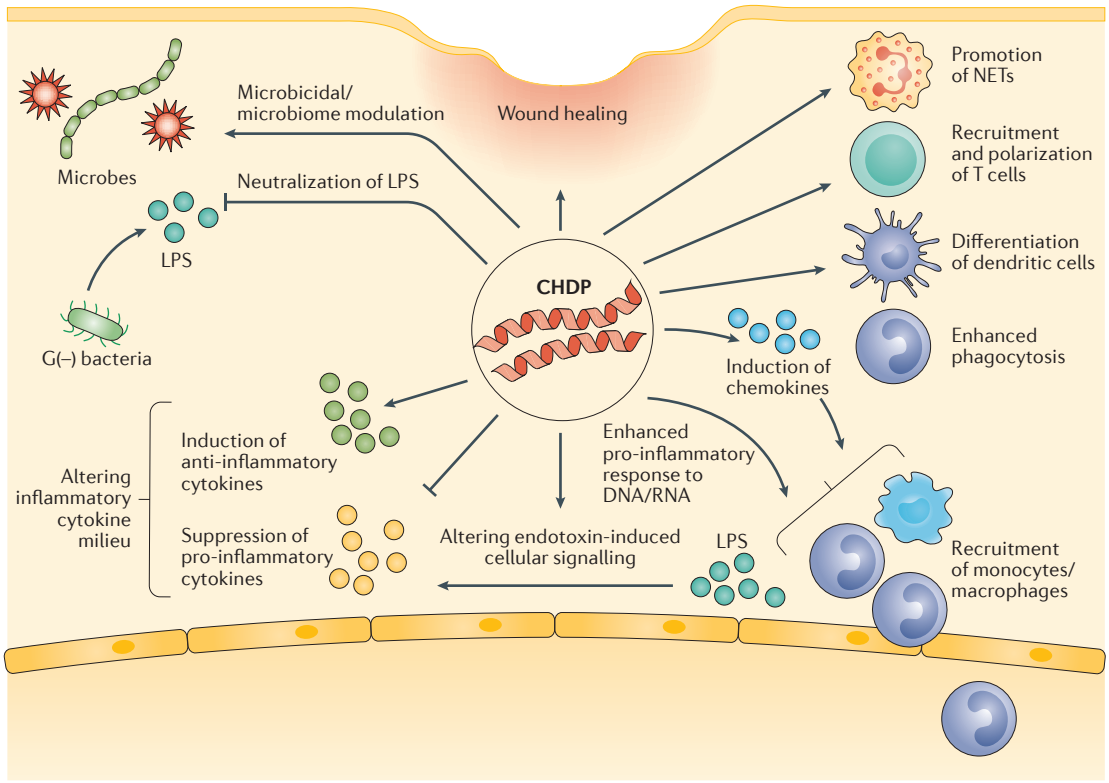
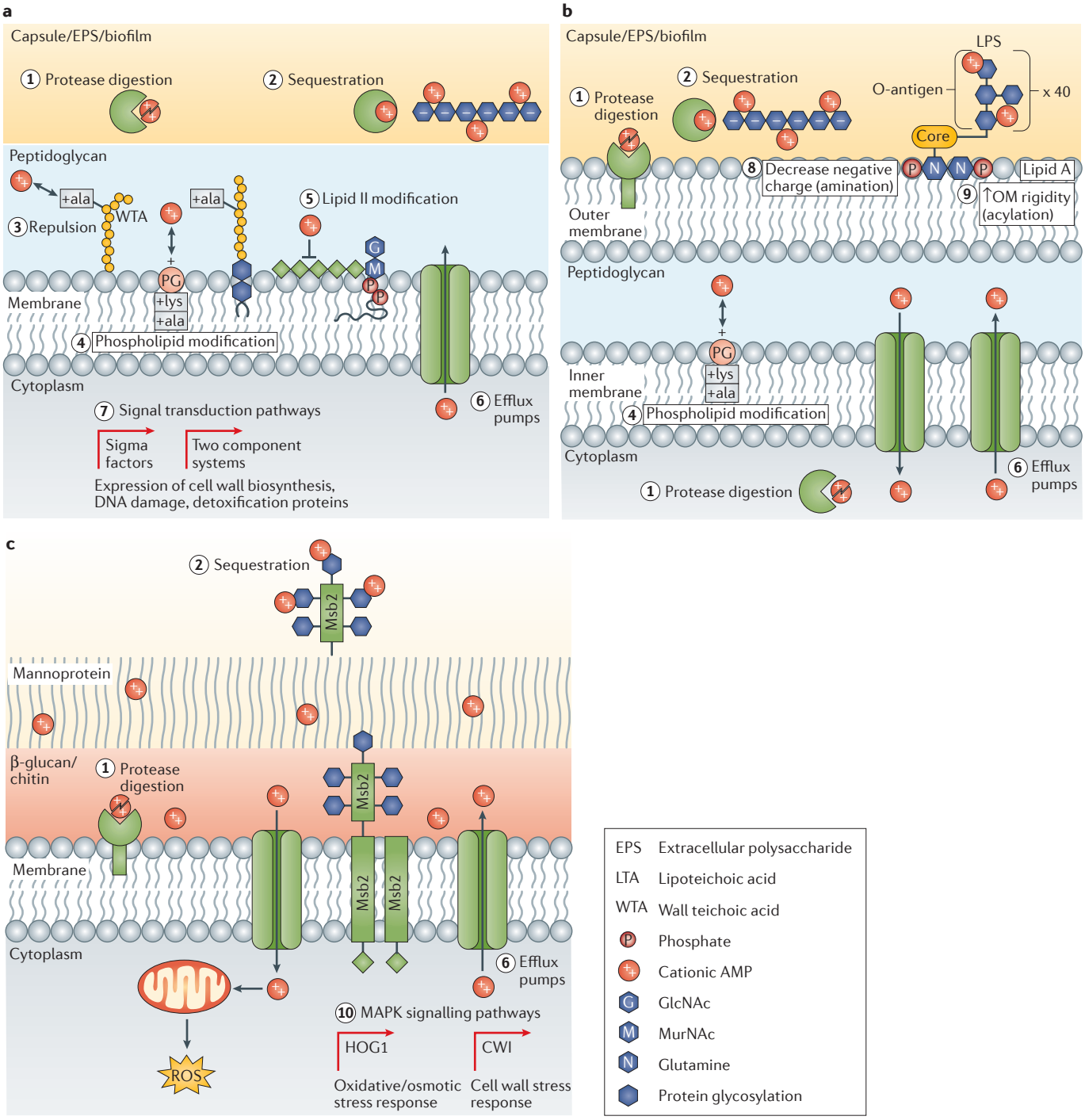

Microbes
Microbicidal/
microbiome modulation
Wound healing
Promotion
of NETs
Recruitment
and polarization
of T cells
Neutralization of LPS
LPS
CHDP
Differentiation
of dendritic cells
G(–) bacteria
Induction of
chemokines
Enhanced
phagocytosis
Induction of
anti-inflammatory
cytokines
Enhanced
pro-inflammatory
response to
DNA/RNA
Altering
inflammatory
cytokine
milieu
Suppression of
pro-inflammatory
cytokines
Altering endotoxin-induced
cellular signalling
LPS
Recruitment
of monocytes/
macrophages

Fig 4


a
Capsule/EPS/biofilm
1 Protease digestion
2 Sequestration
Peptidoglycan
+ala
WTA
3 Repulsion
+ala
5 Lipid II modification
PG
+lys
+ala
Membrane
4 Phospholipid modification
Cytoplasm
7 Signal transduction pathways
Sigma factors
Two component systems
Expression of cell wall biosynthesis, DNA damage, detoxification proteins
6 Efflux pumps
b
Capsule/EPS/biofilm
LPS
1 Protease digestion
2 Sequestration
O-antigen
x 40
Core
8 Decrease negative charge (amination)
P N N P
Lipid A
9 ↑OM rigidity (acylation)
Outer membrane
Peptidoglycan
PG
+lys
+ala
Inner membrane
4 Phospholipid modification
Cytoplasm
1 Protease digestion
6 Efflux pumps
c
2 Sequestration
Msb2
Mannoprotein
β-glucan/ chitin
1 Protease digestion
Msb2
Msb2
Msb2
Membrane
Cytoplasm
6 Efflux pumps
ROS
10 MAPK signalling pathways
HOG1
CWI
Oxidative/osmotic stress response
Cell wall stress response
EPS Extracellular polysaccharide
LTA Lipoteichoic acid
WTA Wall teichoic acid
P Phosphate
Cationic AMP
G GlcNAc
M MurNAc
N Glutamine
Protein glycosylation