Functions and Clinical Potential of Anti-microbial Host Defence Peptides

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Abstract- CHDP (cationic host defence peptides) commonly referred as anti-microbial peptides are naturally obtaining peptides that have the ability to fight infections either directly through their ability to be microbicidal or indirectly by their ability to affect the host's immunological responses. Interest in using these peptides capabilities to create novel treatments for the infectious illnesses, wound healing and chronic inflammatory diseases has grown as a result of CHDP's exceptional capacity to prevent infections and reduce detrimental inflammation. Several methods have employed for made optimized and synthetic peptide with little adverse effects. Now, we concentrate on advancements in our understanding of the range of Cationic host defence peptides (CHDP's) activities, developing potential and therapeutic uses of CHDP - based treatments.

Key words- Peptide, Antimicrobial, bacterial resistance, HDP

Introduction- The need of novel treatments to manage infections is highlighted by the global rise of bacteria that are multi-drug resistance and the continuous reduction in the development of new antibiotics. Corticosteroids, a frequent therapy for many chronic inflammatory conditions, can make people more vulnerable to infections, especially those caused by microorganisms that are resistant to antibiotics. Other tactics that can eliminate damaging inflammation as well as infections are therefore urgently needed. The discovery of cationic host defence peptides (CHDP), which have the ability to prevent increased inflammation and manage infections by their direct microbicidal activities and/or by modifying host immune responses, is one potential strategy.

Kiss and Michl originally identified CHDP, often referred to as antimicrobial peptides, in the speck-lead frog in the 1960s. It is currently recognised that CHDP are expressed in a wide

variety of taxa, including simpler amphibians and mammals as well as microbes, plants, and invertebrates.

CHDP generally have a net positive charge of +2 to +9 at physiological pH and are tiny, amphipathic peptides with fewer than 50 amino acids. However, these natural peptides differ significantly in sequence and structure.

There has been a lot of interest in using CHDP therapeutically during the past three decades, as evidenced by the more than 5,000 publications that have been published in this field of study since 2017. They include researching CHDP's potential therapeutic applications for conditions ranging from infections caused by bacteria that are multidrug resistant to chronic inflammatory illnesses including arthritis, asthma, and colitis, as well as certain malignancies. Clinically tested peptide-based therapeutics are largely used to treat infections such those of the respiratory tract, mouth, and catheters, as well as to promote wound healing.

This article will give an overview of the range of CHDP's activities as they are now understood, mostly from eukaryotes. These peptides' emerging medicinal uses, ongoing clinical studies, and the difficulties in the clinical development process will all be covered. A thorough examination of these strategies is outside the purview of this Review, despite the growing interest in the creation of non-peptide mimics of CHDP for therapeutic use, such as peptoid analogues.

Naturally occurring CHDP

Over 2,700 natural anti-microbial peptides, those marked as immunomodulatory, have been indexed in the antimicrobial peptide database. The next subsections provide an overview of the main eukaryotic families of CHDP that are relevant for drug development.

Vertebrate CHDP

The initial line of defence against microbial infections must include CHDP from vertebrates. When a pathogen is infected, CHDP can kill it by a variety of mechanisms (described below), either directly attacking the pathogen when it is present in high local concentrations or indirectly altering host defence components. Depending on the stage of the infection, these peptides' immunomodulatory effects might be pro- or anti-inflammatory (see below).

CHDP from vertebrates are amphipathic peptides that include amino acids on opposing sides of the molecules that have hydrophilic and hydrophobic side chains. These CHDP are capable of interacting with bacteria's negatively charged membranes. The defensins and cathelicidins, the two major families of CHDP in vertebrates (FIG. 1a), are generated as prepropeptides that are then cleaved to create mature active peptides.

Defensins are categorised into defensins based on the linking of cysteine residues and share a common -sheet core supported by three disulfide bridges between six conserved cysteine residues. -defensins are believed to have developed via gene duplication and are exclusively present in a small number of species, mostly primates and rodents. The genes encoding - and -defensins are contiguous on chromosome band 8p23.1 and likely share a common ancestor. Many human -defensins are generated and released by Paneth cells in the small intestine, and additional -defensins are also strongly expressed in neutrophils. All vertebrates have the ubiquitous and present -defensins. More than 30 genes in the human genome code for -defensins, and there are even more of these genes in mice. Epithelial cells are the primary producers of -defensins. The cyclic -defensins, which were isolated from the leukocytes of rhesus macaques and baboons, developed from -defensins when primates diverged. These compounds have antiviral properties and are the only cyclic peptides discovered in mammals. As a result, evidence supporting sequence divergence by both positive and negative selection of mammalian defensin genes following ancestral gene duplication varies amongst various species.

Fungal, Invertebrate & Plant CHDP

All eukaryotic species, including plants, fungi, and invertebrates without an adaptive immune system, depend on CHDP as an essential part of their innate immune systems. These CHDP are tiny amphipathic peptides with an overall positive net charge, just as those from vertebrates. These peptides may be divided into a variety of different structural categories, such as extended peptides, peptides with mixed helical and sheet structures, and peptides that are concentrated in certain amino acids (FIG. 1b). The quantity, distribution, and connection of cysteine residues, as well as the structure—which is often quite conserved within a CHDP family—are the main factors that determine how they are classified.

Within the same plant, gene duplication and fast evolution have created vast families of antimicrobial peptides. Plants like Arabidopsis and Medicago have up to 300 defensin or

defensin-like sequences, according to genome sequencing. They are incredibly varied, with the surface loops on conserved scaffolds serving as a visual representation of the sequence variety, aside from the cysteine and glycine residues that are necessary for the defensin fold. This may help to explain the variety of biological roles attributed to this protein family. Generally speaking, plant defensins are powerful antifungal compounds.

Synthetic innate defence regulator peptides:

The direct microcidal characteristics of these peptides were significantly optimised in early methods for developing CHDP as therapeutics. However it is now evident that methods used to improve in vitro microbicidal activities frequently produced peptides with higher degrees of cytotoxicity. The capacity of naturally occurring CHDP to cause mast cell degranulation, with the production of histamine and prostaglandin, as well as the activation of complement proteins, are additional properties that are undesired for drug development. As a result, in the past ten years, successful strategies have concentrated on creating synthetic peptides from CHDP sequences to maximise antimicrobial capabilities in vivo through the combination of some microbicidal activity and advantageous immunomodulatory functions, without lethal consequences.

IDR peptides, which are such tiny synthetic cationic peptides produced from natural CHDP, have been tested and refined for their immunomodulatory properties. By screening overlapping segments that represent the internal sequences of CHDP and by making single amino acid alterations, libraries of IDR peptides have been created. The majority of IDR peptides that have been identified thus far are bactenecin CHDP derivatives from cattle. IDR peptides are non-immunogenic and lack some of the possible negative consequences of some naturally occurring CHDP. IDR peptides can regulate infection in vivo and lower inflammation, as demonstrated in a number of infection models, while having a generally moderate direct effect on the pathogen.

Cryptic and synthetic peptides:

Histones and their fragments have a variety of antibacterial properties. They are not just located in nucleosomes; they may also be released during activation and are detected in the cytoplasm of cells. Histones and histone fragments make up much to 70% of the proteins in neutrophil extracellular traps (NETs). Another protein that breaks down by proteolysis in vivo and produces

anti-microbial peptides is lactoferrin. This protein is synthesised by a variety of tissues and is not just found in milk; it also has biological functions connected to host defence, such as antibacterial activity. Its digestive tract proteolysis results in pieces that are more energetic than the original protein. Lactoferricin, a peptide generated from the N-terminal portion of lactoferrin, is released after proteolytic digestion with pepsin. Lactoferrampin and lactoferricin 1-11 are two other active fragments mentioned. Similar to this, "retro-cyclins," which are synthetic compounds based on human -defensin pseudogenes, have been created as possible treatments, such as antivirals.

Antimicrobial actions of CHDP:

The development of these peptides as broad-spectrum antibiotics was spurred forward by CHDP's antibacterial properties on a variety of infections. The many associated modes of action seem to be reliant on the microbial pathogen.

Anti-bacterial activity:

The majority of cationic peptides primarily target the bacterial membrane. Due to the presence of anionic lipids, such as teichoic acids or lipo-polysaccharides (LPS; in Gram-negative bacteria), bacterial membranes are negatively charged (in Gram-positive bacteria). This electrostatic interaction between these negatively charged molecules and cationic chemicals explains why CHDP prefers the membranes of bacterial cells over those of plants, invertebrates, and vertebrates. The ability of these peptides to destabilise membranes depends on CHDP's amphipathic character.

When CHDP binds to the inner membrane of bacteria, the peptide can pass through and cause the bacterium to die (FIG. 2). The disruption of the outer membrane caused by CHDP and LPS interaction in Gram-negative bacteria has been identified as the main mechanism of action for CHDP's antimicrobial activity. The aggregate, toroidal pore, barrel-stave, and carpet models have been proposed as the four primary (inner) membrane perturbation models. As there is no specific target involved in CHDP's interaction with bacterial membranes, it has been hypothesised that the emergence of microbial resistance is improbable. Thus, CHDP can cause brief bacterial adaptations, unlike the processes that lead to the emergence of bacterial resistance to conventional antibiotics. For instance, a research revealed that when CHDP was removed

from the culture media, the bacteria went back to how they had been before; the adaptation made to fend against the effects of the CHDP was not preserved. Hence, it is unlikely that CHDPs would cause microbial "resistance," as reported for traditional antibiotics.

In addition to severely harming bacterial membranes, CHDP may also interfere with the production of cell walls. For instance, defensins like HNP1 and HBD3 function as antibacterial agents by docking on lipid II, a step in the formation of peptidoglycans. Furthermore, CHDPs, such as those that bind to ribosomes and are proline-rich peptides, can penetrate bacterial membranes to kill bacteria from within by binding to intracellular targets like nucleic acids or developing proteins. This has an impact on cellular functions like replication, transcription, translation, protein folding, and cell wall synthesis (FIG. 2). One important reason through which these peptides are so successful in vivo may be the simultaneous exposure of a pathogen to many distinct CHDP, maybe employing separate mechanisms of action.

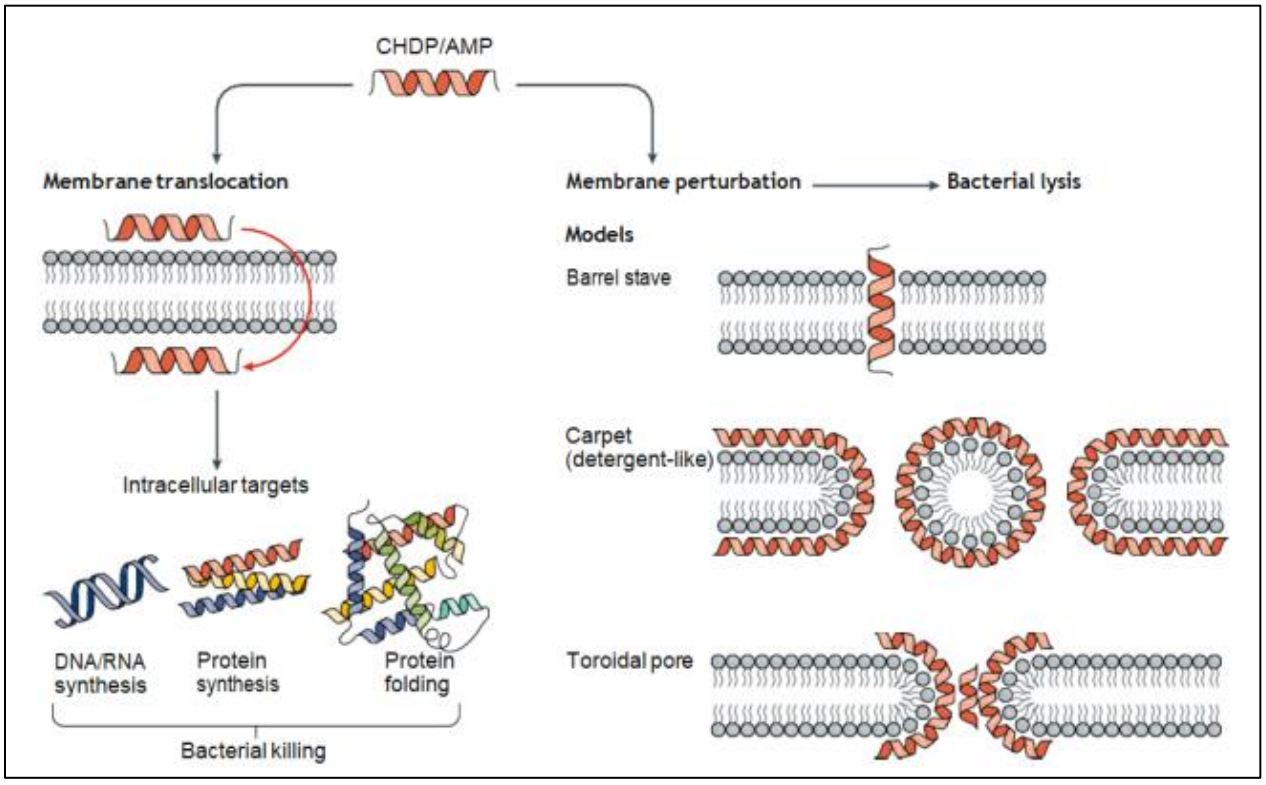

Fig. 2: CHDPs antibacterial mechanisms.

The cationic host defence peptides' (CHDP) direct antibacterial methods might involve membrane transfer of the peptides, followed by binding to intracellular targets such nucleic acids and/or proteins to destroy bacteria. The peptide and bacterial species may have an impact on the processes of translocation, which are unclear. Inner membrane transporters act as Trojan horses for proline-rich antimicrobial peptides (AMP), allowing them to enter and then bind within the ribosomal exit tunnel. Transient holes may be used for translocation by other CHDP. One of the main mechanisms of direct antibacterial activity has been identified as CHDP interaction with negatively charged bacterial membrane leading to membrane disruption. The barrel-stave, carpet, and toroidal pore models are the membrane perturbation theories put forward. The earliest permeabilization process to be postulated, the barrel-stave model, is regarded as the prototype for peptide-mediated transmembrane pore creation. According to this idea, peptides serve as staves and create barrel-shaped structures by inserting themselves vertically into the lipid bilayer. Based on electrostatic attraction, peptides that behave in accordance with the carpet model blanket the negatively charged membrane. The membrane bursts in a detergent-like way over a certain peptide threshold concentration, leading to the micelle synthesis of peptide with membrane lipids. In the toroidal pore model, which is a version of the aggregate model, the peptide disrupts the polar head group alignment of the lipids after concurrently attaching to the membrane. As a result, the acyl chain interactions of the lipids are disturbed, the curvature of the membrane changes, and the integrity of the membrane surface is compromised. The creation of temporary toroidal channels is induced when the peptides align themselves perpendicular to the membrane at specific peptide-to-lipid ratios.

Antiviral activity:

In addition to CHDP's antibacterial abilities, earlier research on its antiviral potential has been developed to show that it is effective against a variety of viruses. The majority of these research from recent years were carried out in vitro and described several mechanisms that underlie the antiviral actions, varying depending on which viruses were involved (TABLE 1).

The ability of CHDP (including defensins and cathelicidins) to destabilise the viral envelope on contact, harm the virions, and inhibit infectivity against many envel- oped viruses—such as influenza viruses, respiratory syncytial virus (RSV), Zika virus, vaccinia virus, and Kaposi's sarcoma-associated herpesvirus—is a common mechanism of action in vitro. This may occur upon contact with a solution or during viral exposure to CHDP linked with the plasma membrane during cell entrance. CHDP, however, has also been demonstrated to have antiviral activity against non-enveloped viruses, such as rhinoviruses, human papillomavirus 16 and adenoviruses.

This antiviral activity is exhibited by a reduction in viral replication and/or by binding viral capsid, which prevents the viral genome from being uncoated and entering the nucleus.

Virus	Peptide	Proposed mechanism of action in vitro
Influenza virus	Retrocyclins	Virus aggregation; blockade of increased virus uptake by professional phagocytes; RC2: haemagglutinin-mediated fusion of viral and endosomal membranes
	HNP	Virus aggregation; inhibition of PKC disrupts IAV endosomal trafficking; enhanced neutrophil phagocytosis of IAV
	LL-37	Disruption of viral envelope
	β-Defensins	Inhibition of IAV infectivity at higher concentrations applied before viral entry
	Urumin	Virion destruction, targeting H1 haemagglutinin
RSV	LL-37	Virion binding and destruction; prevention of infection and spread; function retained by core 22-mer
	HBD2	Viral envelope destabilization in solution or on exposure to plasma membrane-associated HBD2
Rhinovirus	Cathelicidins	Decreased infectivity and replication
Adenovirus	α-Defensins	Peptide binding to adenoviral capsid prevents uncoating and nuclear entry of the viral genome; dependent on optimal peptide hydrophobicity and charge
HPV-16	α-Defensins	Inhibition of uncoating and nuclear entry of the viral genome
HSV	α-Defensins, HBD3, retrocyclins	HSV binding to cellular receptors glycoprotein B and heparin sulfate inhibited; dependent on lectin-like properties rather than charge
HIV	Retrocyclins	Blockade of viral entry into cells by peptide binding to gp120 and CD4; dependent on lectin-like properties
	HNP	Disruption of cellular entry; inhibition of PKC activity, interfering with HIV replication

	LL-37	Suppression of HIV reverse transcriptase activity
	β-Defensins	Direct effects on virions; intracellular, postviral entry inhibitory functions
Vaccinia virus	Cathelicidins	Damage to integrity of the double-layered viral envelope
Zika virus	Cathelicidins	Direct inactivation of Zika virus; protective modulation of interferon signalling pathways
Kaposi's sarcoma-associated herpesvirus	Cathelicidin-derived peptide	Disruption of viral envelope

Table 1 | CHDPs Antiviral activities

The specific binding of CHDP to cellular receptors implicated in viral infection is one additional method of action against a variety of viruses, including the herpes simplex virus (HSV) and HIV. This mechanism is reliant on the lectin-like qualities of certain peptides. Additional antiviral effects could be brought on by the CHDP-mediated aggregation of viral particles, the inhibition of protein kinase C activity, and the immunomodulatory effects of the peptides on host immune cells (discussed in more detail later). These effects could include enhancing phagocyte function or altering cytokine responses. These antiviral mechanisms draw attention to the potential for baseline CHDP expression to function as a "antiviral shield" at mucosal surfaces, preventing virus reproduction and dissemination if it is elevated during first infection. Hence, treatments that encourage host CHDP expression or the administration of synthetic CHDP-derived peptides with well-defined, targeted characteristics may have both therapeutic and preventive benefits.

Antifungal activity:

Human fungus infections are becoming a more widespread issue. Non-life-threatening fungal infections of the skin and mouth are common in patients. Unfortunately, 1.5 million individuals every year pass away from invasive fungal infections caused by species including Aspergillus fumigatus, Cryptococcus neoformans, Candida albicans, and Histoplasma capsulatum. Life-threatening fungal infections can only be treated with a small selection of antifungals, and antifungal medication resistance is steadily growing. Due to the extensive usage of azoles in

agriculture, this is especially obvious in the case of azole resistance. Also, there are more and more immunocompromised patients across the world, which raises the risk of fungal infections. Both of these tendencies are quite unsettling and necessitate the creation of new, powerful antifungals that do not readily cause resistance. Additionally, in order to prevent the quick emergence of resistance in the environment, innovative antifungal chemicals must only be utilised in humans. Due of their various mechanisms of action, CHDP-derived compounds can serve as models for the creation of effective antifungals. There have been descriptions of antifungal CHDP from plants and animals. Plant defensins have mostly been used to describe their action against fungus. There have been reports of a number of CHDP-related death methods for yeasts and fungi, from cathelicidins and CHDP mimics to impacts on C. albicans' membrane functions and mitochondria. Note that fungus biofilms have a high level of antifungal resistance. Hence, testing for antibiofilm action in recently discovered CHDP-based antifungals is crucial.

Immunomodulatory actions of CHDP:

Early research on CHDP's non-microbic features focused on how these peptides affected immune cells, particularly how well they attracted leukocytes. Throughout the following two decades, research on CHDP's immune-related activities developed significantly, establishing a wide spectrum of roles. The activities of CHDP that are connected to immunity appear to depend on environmental factors, cell and tissue types, interactions with various cellular receptors, and peptide concentration. The molecular mechanism underlying CHDP's ability to selectively modulate immune responses is highly complex, according to studies to date. The actions of CHDP on modulating immunity and inflammation are outlined in the following subsections, with an emphasis on cathelicidins and defensins. To develop innovative treatment strategies based on CHDP-derived peptides, it is essential to comprehend the processes behind CHDP's capacity to modify immunity to guard against infection, alleviate inflammation, and contribute to immunological homeostasis.

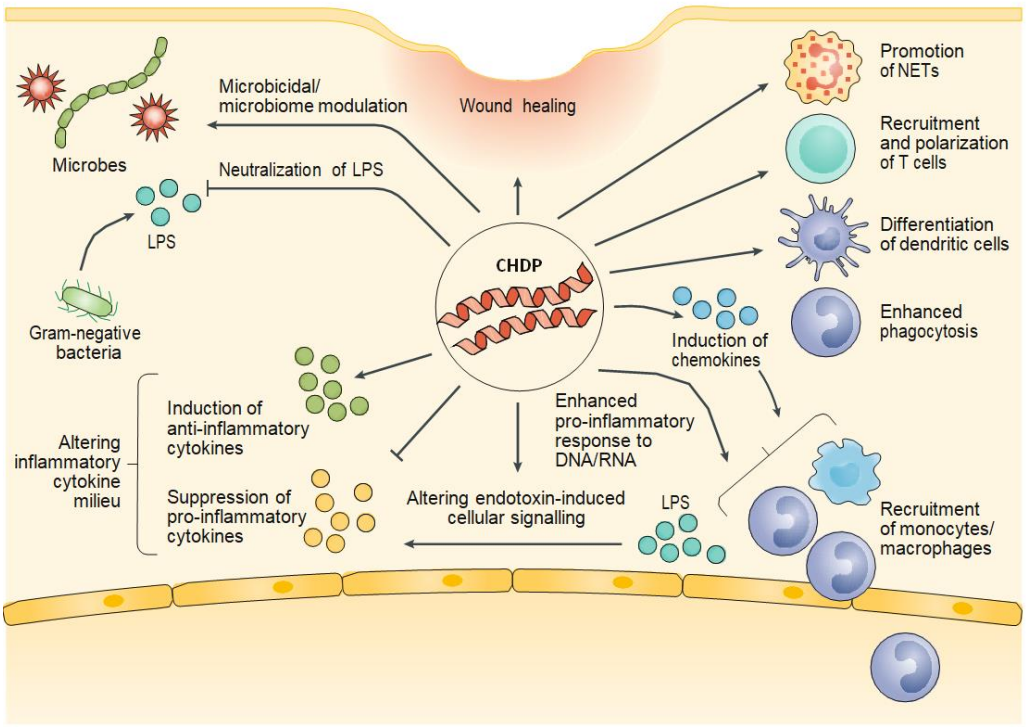

Fig. 3: **CHDP immunomodulatory mechanisms summary**

Immune activation:

One of the main mechanisms behind the capacity of these peptides to promote early clearance of infections has identified as protective stimulation of the innate immune system by CHDP. Leukocyte recruitment, neutrophil response control, and antigen-specific adaptive immunity are some of the functions of CHDP (discussed later). A rapidly developing field of study is the interplay of CHDP and the microbiota in mucosal immunity. While out of the purview of this review, this fascinating field of study raises the possibility of CHDP-mediated selectivity in microbiota management as well as the possibility of microbiota components contributing to the regulation of CHDP expression. Our understanding of the effects of microbial dysbiosis in disease states, ageing, and after treatment with broad-spectrum antibiotics may be improved by more study on CHDP-microbiota interaction.

Leukocyte recruitment:

Direct chemotactic activity is seen by immune cells, such as neutrophils, macrophages, mast cells, and T cells, towards CHDP and its IDR derivatives. By causing the release of chemokines, CHDP also indirectly promotes the recruitment of leukocytes. A main immuno-modulatory mechanism associated with these peptides' capacity to fight infections has been identified as their capacity to stimulate chemokine secretion and improve leukocyte recruitment. Many cellular receptors, including chemokine receptors (like CCR6 and CCR2), GPCRs, including the formyl peptide receptors, and Toll-like receptors (TLRs), as well as contact with intracellular proteins like GAPDH and p62, are involved in the underlying processes. Yet, it has been demonstrated that LL-37 can regulate the internalisation of chemokine receptor CXCR2 on monocytes and neutrophils, hence reducing chemo- taxis. As a result, these actions appear to be dependent on the stage of infection and inflammation. Uncertain molecular mechanisms govern the dichotomy of CHDP, which selectively induces chemokine production and boosts leukocyte recruitment without affecting the peptide's anti-inflammatory properties (addressed in the following section).

Neutrophil function:

Neutrophil activity may be affected by CHDP, changing how an infection develops. For instance, these peptides can increase the inflow of neutrophils both directly through their chemotactic function and indirectly by encouraging the release of neutrophil chemokines (such IL-8 and GRO-) in a way that is MAPK-dependent, which helps to control infections. Similar to this, a recent study revealed that the CHDP hepcidin produced in the skin after bacterial infections can promote neutrophil responses and increase chemokine production. Moreover, CHDP are found on NETs, which may aid in NET-mediated anti-bacterial actions. According to reports for the influenza A virus, LL-37 can promote NET development and aid in antiviral action (IAV). Recent research has demonstrated that CHDP post-translational changes, particularly citrullination, may change how NET-associated CHDP like cathelicidins operate. In vivo bacterial clearance can be promoted by LL-37 by boosting early neutrophil responses. Moreover, the ability of LL-37 to prime and increase neutrophil antimicrobial capabilities is demonstrated by its ability to promote intracellular calcium mobilisation, the creation of reactive oxygen species (ROS), as well as augment ROS production mediated by other inflammatory stimuli. The effects of CHDP on neutrophils are only one aspect of this peptide's potential to influence the

host cellular response to infections; other innate and adaptive cellular responses can also be affected.

Antigen presentation and adaptive immunity:

Due to their ability to draw antigen-presenting cells like monocytes/macrophages and dendritic cells (DCs) to the site of infections, CHDP act as a bridge between innate and adaptive immunity (as dis- cussed earlier). These peptides can also improve macrophage phagocytosis, which speeds up immunological activity and aids in bacterial removal. CHDP can influence the production and polarisation of lymphocyte responses, which in turn shapes the adaptive immune response, in addition to activating antigen-presenting cells. For instance, the human defensins HBD2 and HBD3 may both stimulate interferon- production from plasmacytoid DCs, which in turn affects the beginning of T cell responses. By activating TLRs, exposure to HBD3 also increases the expression of the co-stimulatory molecules CD80, CD86, and CD40 on myeloid cells, promoting the development of an adaptive immune response. By modifying DC development and function both in vitro and in vivo, LL-37 permits greater production of co-stimulatory molecules and the promotion of a modified adaptive response. It also encourages DC activation and boosts B cell activation and proliferation by stimulating follicular DCs. Since CHDP affects adaptive immunity, it has been investigated whether these peptides—mostly cathelicidins—can be used as adjuvants to boost systemic and mucosal antigen-specific immune responses.

Regulation of inflammation-

As was already mentioned, CHDP perform a variety of immune system-stimulating activities that may be categorised as pro-inflammatory reactions that facilitate the clearance of infections. However, in the context of an inflammatory and/or pathogenic stimulus, strong CHDP-mediated anti-inflammatory actions have been shown, indicating that CHDP are also regulatory molecules that restrict heightened inflammation. Therefore, it may be more fair to characterise CHDP as molecules that can balance inflammation to promote immunological homeostasis rather than categorising them as either pro-inflammatory or anti-inflammatory molecules. Numerous studies that show that a lack of these peptides causes enhanced inflammatory responses and that cathelicidin-deficient animals display a more severe inflammatory phenotype than wild-type

mice support the anti-inflammatory role of CHDP. Similar to this, lower levels of -defensin expression in human enterocytes have been linked to Crohn's disease. In fact, it is well known that defences play a crucial role in preserving the integrity of the intestinal mucosa and immunological homeostasis. It's noteworthy that exogenous administration of CHDP (such as LL-37, CATH-2, BMAP-28, and HBD2) and synthetic peptides (such as IDR-1 and IDR-1002) has been demonstrated to reduce inflammation in a variety of animal models of infection and septic shock. Similar to how IDR-1002 successfully reduced airway inflammation in vivo, an LL-37-derived peptide regulated the disease process in a mouse model of inflammatory arthritis. In contrast, despite higher expression of pro-inflammatory genes, cathelicidin-deficient animals show enhanced survival in a caecal ligation and puncture model of sepsis. As a result, the outcome of CHDP-mediated immunomodulatory reactions appears to be context- and cell-environment-dependent. This has been shown in research looking at the interaction between TLRs and CHDP, as will be detailed later.

Cell death:

The majority of endogenous peptides are comparatively less poisonous to eukaryotic cells than CHDP, despite the fact that it may quickly permeabilize prokaryotic membranes. By allowing nucleic acids and DNA dyes to enter eukaryotic cells without causing cell lysis, CHDP like LL-37 and CATH-2 may temporarily break the membrane or open a hole. However, both in vitro and in vivo eukaryotic cell death is induced by exposure to high doses of LL-37. The vitality of primary human lymphocytes and monocytes is largely unaffected by exposure to high concentrations of the peptide in this phenomena, which is cell type dependent. The effects of in vivo apo- ptosis production by CHDP on innate or adaptive responses are still unclear. However, it has been discovered that high concentrations of LL-37 selectively cause the death of infected epithelial cells, suggesting a further host defensive mechanism. Additionally, several forms of cell death play crucial roles in preserving immunological homeostasis as well as in increasing or dampening, and subsequently resolving, inflammatory reactions. The regulation of neutrophil death and the anti-inflammatory abilities of apoptotic neutrophils are particularly significant in this situation. The anti-inflammatory effects of LL-37 on activated macrophages are linked to the LL-37-mediated release of granule contents from the apoptotic cells, possibly as a result of the release of LL-37 and -defensins from these apoptotic neutrophils. LL-37 can induce rapid

secondary necrosis of apoptotic neutrophils. Therefore, the magnitude and resolution of inflammatory responses to infection may be influenced by CHDP-mediated regulation of cell death.

Antimicrobial therapies:

Preclinical studies- Studies using mouse models have shown that CHDP is essential for the management of infections. For instance, cathelicidin-deficient mice show reduced host defence against streptococcal skin infections, impaired clearance of Pseudomonas aeruginosa infections from the lung and cornea, more severe pox skin lesions upon vaccination with the vaccine virus, more severe infection with RSV upon challenge, and increased susceptibility to infection with Citrobacter rodentium in the gastrointestinal tract, Escherichia coli in the urinary tract, and Klebsiella pneumonia. Although the observed effects might potentially be the consequence of altered immunomodulation in the knockout mice, the decreased antimicro- bial activity seen in these cathelicidin-deficient mice may be explained by the lack of direct actions of the mouse cathelicidin CRAMP on these pathogens.

Numerous animal infection models for bacterial, viral, and fungal infections have shown exogenous delivery of several CHDP to be beneficial. However, it is still unclear whether the observed effectiveness is largely attributable to the injected CHDP's direct anti-microbial effects on the pathogen, to the influence of the peptides on human immunity, or to a mix of the two. Therefore, a thorough in vivo examination of the peptides' method of action is required.

In preclinical trials, the administration of CHDP orally or systemically has been unsuccessful due to a number of reasons, including

(1) high local concentrations of CHDP

(2) coordinated activities of various CHDP

(3) synergism with other molecules at the site of infection.

However, several in vivo studies have demonstrated the effectiveness of CHDP-derived peptides when applied topically. In a methicillin-resistant Staphylococcus aureus mouse model, for

instance, a recent study revealed that topical treatment of a pep- tide created utilising LL-37 and tachyplesin 1 as chemical markers proved protective. Similarly, it was discovered that localised delivery of a CHDP-derived peptide reduced bacterial load more effectively than rifampicin therapy for TB. Furthermore, nanostructure-based technology has recently demonstrated promise as a reliable method of peptide administration for in vivo infection control.

The potential application of CHDP with implanted medical equipment, prosthetic joints, and other implants for the prevention of nosocomial infections is a significant field of investigation. Biomaterial-associated infections, a significant issue in clinical practise, are caused by pathogens resistant to antibiotics that build bacterial biofilm on medical equipment. To prevent bacterial adherence, CHDP may be immobilised on the surfaces of biomaterials. For instance, it has been demonstrated that synthetic peptides derived from LL-37 and a trombocidin-derived peptide are efficient in preventing the development of biofilm by a clinical isolate of S. aureus that is associated with biomaterials. Additionally, once the surface has been coated, the antibacterial activity of CHDP may still be present depending on the chemical tethering process. This method's drawback is that it only kills germs that are right next to the surface. Application of CHDP-releasing biomaterials may be a more effective strategy for preventing implant-related infections. Numerous hydrogels, nanotubes, and microporous calcium phosphate coatings have been found to suppress bacterial growth in vivo.

Clinical trials- The majority of CHDP used in clinical trials up to this point have been created for topical use or as inhalants to treat infections (see Dramp Database and TABLE 2). Pexiganan, an analogue of the magainin peptide, was one of the most advanced of these and was examined in phase III clinical trials as a topical cream for the treatment of infected diabetic foot ulcers. However, because it did not outperform conventional therapies, development was stopped. Omiganan, an antibacterial drug developed from CHDP, is undergoing numerous studies (see TABLE 2). For instance, a phase III trial is now being conducted to assess the long-term safety of topical omiganan for the treatment of rosacea. Additionally, an inpatient phase II clinical study demonstrated that localised application of a hydrogel containing the human lactoferricin-derived peptide PXL01 was safe, well-tolerated, and effective as an antiadhesion therapy

postoperatively following tendon restoration surgery. Additionally, LL-37 topical studies for the treatment of venous leg ulcers are still being conducted (TABLE 2).

A modest number of clinical trials examining the toxicity and effectiveness of CHDP administered by oral and intravenous methods have also been carried out or are in progress (TABLE 2). In a phase III clinical study, for instance, iseganan, an equivalent of the peptide protegrin, was used as an oral solution to treat oral mucositis but did not demonstrate appreciable effectiveness (TABLE 2). Surotomycin and iseganan also underwent phase III trials but were disqualified from further research due to either insufficient efficacy or efficacy that was not better than that of existing medications (TABLE 2). Brilacdin, a synthetic defence mimic, is being delivered intravenously in phase III clinical research to treat skin infections. Murepavadin phase III studies for intravenous bacterial pneumonia have recently been stopped because to patients' elevated blood creatinine levels, a sign of severe kidney impairment. This is reminiscent of nephrotoxicity problems with polymyxins, cationic nonribosomal peptides that were formerly used to treat Gram-negative bacterial infections and are now the last-resort antibiotics.

Immunomodulatory therapies-

Preclinical studies: Early clinical experiments using synthetic analogues of CHDP that were intended to maximise their microbicidal activity were only moderately effective, possibly as a result of a failure to appreciate the significance of these peptides' immune-related actions. CHDP are unquestionably necessary for the management of infections in vivo, despite problems with concentration at mucosal surfaces and antagonistic factors at sites of inflammation. In vivo, administration of LL-37 protects against P. aeruginosa, influenza, and RSV infection. This protection is caused by the drug's ability to increase protective early neutrophil responses, rather than by the pathogen's ability to directly cause microbic activity in the neutrophils.

Table 2: Selected antimicrobial peptides that are in clinical trials or approved

Peptide	Origin	Indication	Status	Company	Clinical trial identifiers
Topical					

D2A21, Demegel	Synthetic cecropin peptide	Burn wound infections	Phase III	Demegen	Not listed
Pexiganan (Locilex, MSI-78)	Analogue of magainin, isolated from African clawed frog Xenopus laevis	Infected diabetic foot ulcers	Phase III complete; rejected, efficacy not superior	PLx Pharma Inc. (formerly Dipexium Pharmaceuticals Inc.)	NCT00563394, NCT00563433, NCT01590758, NCT01594762
CLS001	Omiganan	Local catheter site infections	Phase III complete (discontinued)	Mallinckrodt, BioWest Therapeutics Inc., Cadence Pharmaceuticals Inc.	NCT00231153, NCT00027248, 2005-003194-24
(omiganan,	pentahydrochloride,				
MBI-226)	synthetic cationic				
	indolicidin derivative				
		Papulopustular	Phase III current	Cutanea Life	NCT02576860, NCT02547441,
		Topical skin antisepsis	Phase III complete	Mallinckrodt	NCT00608959
		rosacea		Sciences Inc.	NCT02576847, 2015-002921-20,
					2015-002919-15, 2015-002920-23
		Atopic dermatitis	Phase II complete	Cutanea Life Sciences Inc.	NCT03091426, NCT02456480, 2016-003849-28, 2014-003689-26
		Acne vulgaris	Phase II complete	Cutanea Life Sciences Inc., BioWest Therapeutics Inc.	NCT02571998, NCT02066545, NCT00211497, NCT00211523

		Condylomata	Phase II complete	Cutanea Life	NCT02849262, 2015-005553-13
		acuminata (external		Sciences Inc.	
		Vulvar intraepithelial	Phase II complete	Cutanea Life	NCT02596074, 2015-002724-16
		neoplasia		Sciences Inc.	
		genital warts)			
Iseganan (IB-367)	Analogue of protegrin 1	Ventilator-associated pneumonia	Phase II/III; rejected, no efficacy	IntraBiotics Pharmaceuticals	NCT00118781
		Facial seborrhoeic dermatitis	Phase II current	Cutanea Life Sciences Inc., Maruho Co. Ltd	NCT03688971, 2017-003106-41
NVXT (Novexatin NP213)	Cyclic arginine-based heptamer	Fungal nail infection (onychomycosis)	Phase IIb complete	NovaBiotics	NCT02933879, NCT02343627
PXL01	Synthetic macrocyclic 25-amino acid peptide derived from human lactoferricin	Prevention of postsurgical adhesions and scar prevention	Phase IIb complete; phase III trials planned	Promore Pharma (formerly Pergamum AB)	NCT01022242, 2009-012703-25
LL-37	Human cathelicidin subunit	Venous leg ulcers	Phase IIb current	Promore Pharma (formerly Pergamum AB)	2018-000536-10
PAC-113, P-113	Histatin 5 derivative (12 amino acids)	Oral candidiasis	Phase IIb complete	Demegen, Pacgen Biopharmaceuticals Corporation;	NCT00659971

				sold over the counter in Taiwan by General Biologicals Corporation	
HXP124	Plant defensin	Fungal nail infection (onychomycosis)	Phase IIa complete	Hexima	ACTRN12618000131257
(PMX-30063)	mimetic	proctitis/ulcerative proctosigmoiditis	phase III planned		
Brilacidin	Synthetic defensin	Ulcerative	Phase II complete;	Alfasigma S.p.A.	Not listed
		Oral mucositis in	Phase II complete;	Innovation	NCT02324335, NCT01211470
		patients with head	FDA fast track	Pharmaceuticals	
		and neck cancer	designation	(formerly Cellceutix)	
LTX-109 (Lytixar)	Synthetic cationic	Atopic dermatitis,	Phase II complete	Lytix Biopharma	NCT01223222, 2010-021438-68
	tripeptide	skin infection			
		Nasal infections by	Phase I/II complete	Lytix Biopharma	NCT01158235, 2010-019254-40
		Impetigo	Phase II complete	Lytix Biopharma	NCT01803035
		methicillin-			

		resistant/			
		methicillin-sensitive			
		Staphylococcus aureus			
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
(CKPV)2, CZEN-002	Derivative of α-melanocyte stimulating hormone	Vulvovaginal candidiasis	Phase II complete	Zengen, Abiogen Pharma	2005-001360-31
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	Acute external otitis	Phase II complete	DermaGen AB and Promore Pharma (formerly Pergamum AB)	NCT01447017, 2011-004356-20
	kininogen, cationic				
	random-coil peptide				
		Atopic dermatitis	Phase I/II complete	DermaGen AB and Promore Pharma (formerly Pergamum AB)	NCT01522391

PL-5	α-Helical peptide	Bacterial skin infections	Approval by State Food and Drug Administration of China for clinical trial	Changchun ProteLight Pharmaceutical & Biotechnology Co.	Not listed
Lotilibcin (WAP-8294A2)	Lipodepsipeptide	Methicillin-resistant S. aureus	Phase I complete	aRigen Pharmaceuticals, Green Cross Corporation	Not listed
Oral					
Iseganan (IB-367)	Analogue of protegrin 1	Oral mucositis in patients with head and neck cancer	Phase III complete; no efficacy	National Cancer Institute, IntraBiotics Pharmaceuticals	NCT00022373
Surotomycin (CB-183, 315)	Cyclic lipopeptide, analogue of daptamycin	Diarrhoea caused by Clostridioides difficile	Phase III complete; rejected, efficacy not superior	Cubist Pharmaceuticals, Merck & Co. Inc.	NCT01597505, NCT01598311, 2012-000252-3
NVB-302	Synthetic type B lantibiotic	C. difficile infection	Phase I complete	Novacta	ISRCTN40071144
RDP58, Delmitide acetate, allotrap 1258	d-Amino acid decapeptide	Ulcerative colitis	Phase II complete	Genzyme, Procter & Gamble	2004-004077-29
Intravenous					
Xydalba)		Osteomyelitis and septic arthritis	Phase IV current	Therapeutics)	NCT03426761
Dalbavancin (BI397,	Semisynthetic lipoglycopeptid	Acute bacterial skin infections	Approved	Allergan (formerly Actavis and	NCT03233438

Dalvance,	e			Durata	
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
AB103 (p2TA)	Synthetic anionic CD28 dimer mimetic peptide	Necrotizing soft tissue infections	Phase III current	Atox Bio Ltd	NCT02469857, 2018-001125-15
Peptide	Origin	Indication	Status	Company	Clinical trial identifiers
Murepavadin (POL7080)	Synthetic cyclic β-hairpin peptidomimetic based on the cationic antimicrobial peptide protegrin 1	Ventilator-associated bacterial pneumonia caused by Pseudomonas aeruginosa	Phase III; suspended, adverse events	Polyphor Ltd	NCT03409679, NCT03582007
Neuprex,	BPI-derived peptide	Burns	Phase II complete	University of Texas Southwestern Medical Center	NCT00462904
opebacan,					
BPI rBPI21					
		Myeloablative	Phase I/II;	Xoma LLC	NCT00454155
		allogeneic	terminated, lack		
		haematopoietic stem	of enrolment		
		cell			

		transplantation			
Brilacidin (PMX-30063)	Synthetic defensin mimetic	Acute bacterial skin and skin structure infections	Phase II complete; phase III planned; FDA fast track designation	Innovation Pharmaceuticals (formerly Cellceutix)	NCT02052388
hLF1-11	First cationic domain	Infections during	Phase I/II complete;	AM-Pharma	NCT00509938, NCT00430469
EA-360	Linear tetrapeptide, derived from human chorionic gonadotropin	Systemic inflammatory response and renal function	Phase IIa/b current	Exponential Biotherapies	NCT03145220, 2014-002481-78
	of human lactoferrin	haematopoietic stem	withdrawn		
	(11 residues)	cell transplantations			
		Candidaemia	Phase I/II; withdrawn	AM-Pharma	NCT00509834
		Bacteraemia due	Phase I/II;	AM-Pharma	NCT00509847
		to Staphylococcus	withdrawn		
		epidermidis			
Friulimicin B	Cyclic lipopeptide	Pneumonia, staphylococcal skin infections	Phase I; rejected, unfavourable pharmacokinetics	MerLion Pharmaceuticals	NCT00492271

CHDP-based drug development considerations:

Due to the relatively high concentrations required for direct antimicrobial effects, as well as the cytotoxic effects these peptides exhibit at those concentrations (mast cell degranulation, completion activation, apoptosis of mammalian cells, and induction of pro-inflammatory cytokine production), the use of natural CHDP as effective therapeutics is not particularly viable. As a result, peptides discovered using semi-random high-throughput screening, synthetic peptides created from natural CHDP, and synthetic peptides are currently emerging as prospective lead drugs. Recent research has also concentrated on the development of molecules incorporating CHDP on tiny abiotic scaffolds for therapeutic uses as well as non-peptide CHDP mimics such peptoid analogues.

The formulation and distribution of peptide-based drugs, as well as their expensive manufacture, are major obstacles. It is important to take into account biological factors that affect peptide stability and bioavailability, such as mucosal pH, the presence of host or microbial proteases that can break down candidate peptides, and a number of other elements that can reduce peptide activity, like physiological salt concentration, mucus, DNA, and microbial saccharides. The use of d-amino acid peptides, modification of peptides by amidation or acetylation of the terminal sections, or targeted substitution of tryptophan or histidine with a non-natural amino acid are a few strategies being investigated to increase peptide stability.

CHDP often has less antibacterial action than traditional antibiotics. Even if the novel compounds do not cause antimicrobial resistance, regulatory authorities expect new antimicrobials to be non-inferior to current antibiotics. More physiologically appropriate modified methodologies may be essential since CHDP may not be suitable for current in vitro antimicrobial susceptibility test methods to predict in vivo efficacy, even for topical use. With all of this in mind, it is likely to be challenging to utilise CHDP orally or systemically in a way that will directly kill bacteria. The innovative approach of using immunomodulatory CHDP as an adjuvant to antibiotics, as well as discoveries of CHDP displaying actions against antibiotic-resistant pathogens, do, however, offer promise. This is because it has been discovered that CHDP and conventional antibiotics work together synergistically.

Development of antimicrobial resistance to CHDP:

Understanding how frequently infections acquire resistance to peptide-based treatments will be crucial for deciding whether or not to use cationic host defence peptides (CHDP) as new-generation antibiotics. Because CHDP interact with targets in the pathogen on wider surfaces than small-molecule antibiotics do, bacteria are less likely to evolve to reduce CHDP activity as a result of single amino acid alterations. Furthermore, CHDP have complex modes of action, frequently interacting with several targets in microorganisms, so that "resistance" to the peptides requires many mutations within the pathogen. In fact, a recent study demonstrated that it is difficult for bacteria to evolve to withstand the effects of CHDP. In addition to the direct microbicidal activities, the indirect CHDP-mediated effects of improving host immune responses to control infections offer a crucial complement to these actions, offering a complex attack on pathogens throughout infection. However, recent research has shown that both bacterial and fungal pathogens are capable of creating defences against CHDP's effects. The processes through which infections adapt to the human CHDP have been thoroughly investigated and evaluated. Repulsion, sequestration, removal, and degradation are frequently used by bacteria to combat CHDP effects (FIG. 4). Both the alteration of the pentapeptide on lipid II, a key CHDP target, and the acylation of lipid A, which changes the stiffness of the membrane, are additional ways of bacterial adaptation. An innovative new method of treating atopic dermatitis that is now being researched in human clinical trials is focused on utilising this kind of selectivity, which would have obvious therapeutic advantages over broad-spectrum antibiotics.

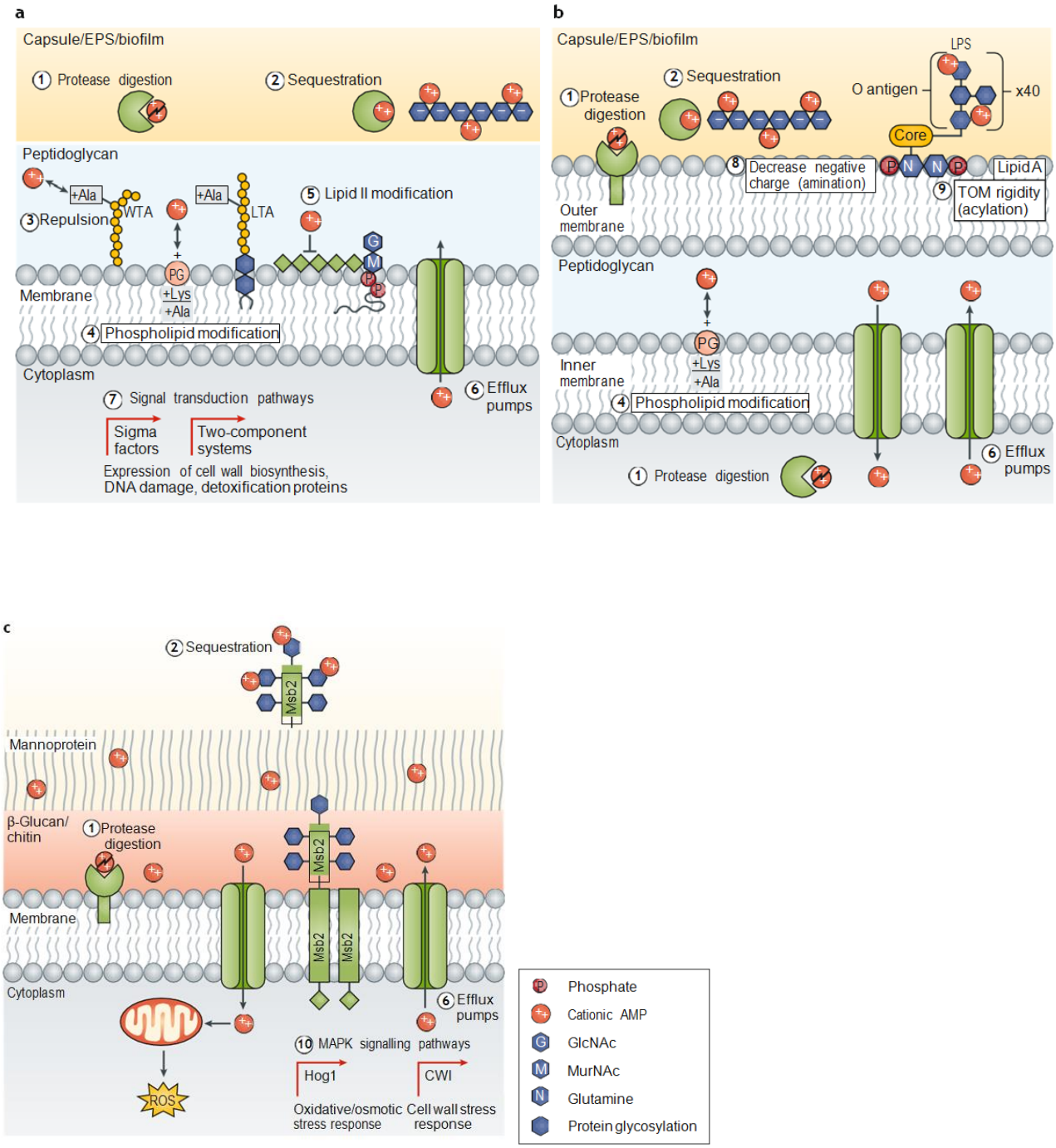

Fig. 4: Common resistance mechanisms to CHDP in bacterial and fungal pathogens

C- Gram-positive bacteria. B- Gram-negative bacteria. C- Yeasts/fungi.

Mechanism 1: Degradation by secretory, cytosolic, or outer membrane (OM) proteases. Mechanism 2: Proteins released by the body, anionic polysaccharides, or mannosylphosphate side chains on glycoproteins (found in fungi) or O antigen (found in Gram-negative bacteria)

sequester substances. Mechanism 3: Wall teichoic acid (WTA) or alanylated lipoteichoic acid (LTA) electrostatic repulsion. Mechanism 4: Aminoacylated phospatidylglycerol (PG) electrostatic repulsion. Mechanism 5: Modification of the pentapeptide on lipid II to inhibit the binding of cationic host defence peptides (CHDP). Mechanism 6: CHDP export through efflux pumps. Mechanism 7: Signal transduction pathways are activated, causing the production of genes that either strengthen the wall or detoxify CHDP activity byproducts. System 8, lipid an alteration caused by amine substances. Mechanism 9, increased membrane stiffness by acylation of lipid A. Mechanism 10: To protect fungi from oxidative, osmotic, or cell wall stress, mitogen-activated protein kinase (MAPK) signalling pathways are triggered. EPS, extracellular polysaccharide; GlcNAc, N-acetylglucosamine; LPS, lipopolysaccharide; MurNAc, N-acetylmuramic acid; ROS, reactive oxygen species; AMP, antimicrobial peptide.

Outlook-

The diverse spectrum of impacts on host defence systems and direct antibacterial activity displayed by CHDP emphasise the crucial role that these molecules play in infection and immunity. Although the initial focus of research in this area was on the creation of novel "antibiotics" based on cationic antimicrobial peptides, it is now widely acknowledged that CHDP play a crucial role in immunity, influencing everything from innate immunity to memory and adaptive immunity to powerful anti-inflammatory functions. Thus, it is not surprising that research in this area has gotten more attention in the context of developing drugs for a range of clinical applications, from the management of antibiotic-resistant pathogens, the reduction of inflammation in chronic disease, and their use as antibiotic adjuvants, to the targeting of particular cancers. The development of CHDP-based drugs is not without its difficulties, particularly those related to formulation and distribution, the possibility of drug resistance, and the paucity of reliable pharmacokinetic data. However, the broad range of CHDP activities that have been identified so far offer a variety of natural compounds for the development and improvement of novel medications. The possibility of CHDP-based therapeutics continues to be an exciting new clinical avenue despite several related difficulties and the inadequate understanding of structure-function correlations.

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