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MULTI-AUTHOR REVIEW

Host-defense peptides: from biology to therapeutic strategies

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Abstract Primitive innate defense mechanisms in the form of gene-encoded antimicrobial peptides are now considered as potential candidates for the development of new therapeutics. They are well known for their function as the first protective barrier of all organisms against microbial infections. In addition, emerging studies reveal that they assist in modulating the host immune system.The biological properties of these host-defense peptides, their role in human health, their cell selectivity and related molecular mechanisms are discussed in this multi-author review along with the strategies to transform them or their peptidomimetics into clinically usable drugs.

Keywords Host-defense peptides · Innate immunity · Anti-infective drugs · Antibiotic resistance · Immunomodulatory · Mode of action · Endotoxin · Therapeutics

Ribosomally made cationic antimicrobial peptides (AMPs, 8–50 amino acids long) are the main elements of the innate immune system; a weapon that all multicellular organisms are "born with" to rapidly protect themselves against microbial invasion. Innate immunity is the oldest type of immunity in evolution and refers to defense events that do not require prior exposure to the pathogen in order to identify danger from an infection [1]. Importantly, peptide-mediated innate immunity is recognized as the first host-protective barrier. It comes into action at a rate compatible with the generation time of microbes and much faster than the activation of adaptive immunity (involving antibodies and recruitment of cellular elements) in vertebrates [2]. AMPs were discovered 30 years ago when Boman isolated cecropins from pupae of the moth *Hyalophora cecropia* [3], Ganz and Lehrer characterized the defensins from mammalian neutrophils [4] and Zasloff identified magainins in skin extracts of the African clawed frog *Xenopus laevis* [5]. In the following years, particular attention was devoted to elucidating the role of AMPs in human health and how their functional failure might cause disease. Despite the fact that AMPs were initially discovered on the basis of their antibiotic activity (to date, more than 1,000 native AMPs are known), they have been found to have additional biological properties that assist in modulating the host immune system: stimulation of chemotaxis, suppression of proinflammatory cytokine production, promotion of angiogenesis and wound healing. Because of these properties, these peptides are now referred to more properly as host-defense peptides (HDPs).

Presently, the widespread and often empirical use of conventional antibiotics has led to a drastic reduction in their therapeutic efficacy and to the appearance of multi-drug-resistant strains. This represents a serious threat to life especially in hospitals, where treatment of infectious diseases has become a worldwide concern. Therefore, alternative antibiotics/antimycotics are urgently needed. HDPs are able to distinguish microorganisms from mammalian cells. The former are endowed with anionic components in their cell wall (e.g., endotoxin or lipopolysaccharide, LPS, in gram-negative bacteria, and lipoteichoic acid, LTA, in gram-positive bacteria) and with negatively charged phospholipids in their membranes, whereas the membranes of the latter contain mostly neutral phospholipids. These differences are believed to be one of the reasons underlying the preferential activity of cationic

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HDPs towards microbial cells [6]. In contrast to traditional antibiotics, a large number of HDPs kill microbes by perturbing their membrane and causing irreversible damage that reduces the development of resistance. In addition, several HDPs can also neutralize the toxic effects of LPS and LTA, which are frequently released from bacteria during antibiotic therapy [7]. It is worth noting that LPS and LTA activate immune cells, resulting in the release of cytokines, such as TNF-α, whose over-secretion can lead to septic shock syndrome and death of the host. Thus, having a natural compound with both microbial killing capacity and LPS-/LTA-neutralizing activity is particularly advantageous. HDPs are currently considered as promising templates for the generation of novel anti-infective weapons with new mode(s) of action [8]. Importantly, nonpeptide antimicrobial molecules that mimic the activity of AMPs have been recently designed. Their low cost of synthesis and resistance to protease degradation have encouraged studies aimed at bringing them into clinical use and commercial production.

The aim of this multiauthor review is to provide an overview of our current understanding of the biological function(s) of HDPs and the feasibility for their use as new therapeutic agents.

The first review by Conlon shows the structural diversity and distribution of HDPs in frog skin secretions and their function against environmental pathogens. The skin is our primary shield against potential pathogens and has evolved innate and adaptive mechanisms to enhance immunity in response to their attack. This happens also in mammals where these molecules (in particular cathelicidins and β-defensin peptides, well-described by Bernard and Gallo) play a sentinel role in protecting the skin and in regulating skin disorders. The review by Kolar and McDermott explains in depth how the eye and its associated tissues encompassing the lacrimal system and lids have elaborated peptide-based mechanisms to ward off infections. These authors also discuss the possibility of using HDPs or their derivatives to prevent/manage eye disease. All animals must also defend themselves against microbes that can easily penetrate the epithelium of the respiratory and gastrointestinal tracts. As comprehensively summarized by Ouellette, in the small intestine of most mammals, Paneth cells at the base of the crypts of Lieberkühn secrete α-defensin HDPs at high levels in response to cholinergic stimulation and when exposed to bacterial antigens. The author focuses on the crucial function of these peptides in the enteric innate immunity of mammals and how their deficiency is associated with gastrointestinal diseases. Unlike α-defensins, β-defensin HDPs are expressed by a large variety of epithelial cell types and at many more sites than α-defensins, including human kidney, skin, pancreas, nasal and oral mucosa, cornea and airway epithelium. While α- and β-defensins have been isolated and characterized from most mammals tested to date, θ-defensins are another group of defensin HDPs which are restricted to nonhuman primates. As reported by Cole and coworkers θ-defensins are physically distinguished as the only known cyclic peptides of animal origin. However, humans do not produce them due to a premature stop codon in the signal sequence of their pseudogenes. The production of θ-defensins can be restored using synthetic approaches and these peptides exhibit an exceptional therapeutic index both as inhibitors of HIV-1 entry and as bactericidal agents.

Among the numerous families of natural HDPs, most of which have a membrane-perturbing effect, the review by Gennaro and collaborators explores the peculiarities of proline-rich peptides. These constitute a straightforward example of HDPs which penetrate susceptible cells and may act as key molecules for internalizing membrane-impermeant drugs. In agreement with the above, Hancock and colleagues emphasize that although cationic HDPs were originally discussed in terms of their direct microbicidal activity against bacteria, fungi, parasites and viruses, nowadays they are increasingly recognized as multifunctional mediators, with both antimicrobial and immunomodulatory properties that make them an exciting and novel approach to fighting infection. As with all new antimicrobials, the issue of resistance is a central theme in their processing and clinical application. During evolution, microorganisms have devised a variety of mechanisms to evade deleterious injury from HDPs, including the ability to degrade/inactivate them, to expel them using multidrug efflux pumps or to repel them by alteration of the pathogen's surface charge. However, despite the finding that resistance can be induced in vitro, it is very modest compared to that manifested towards conventional antibiotics. Moreover, a general mechanism by which bacteria can resist every single AMP does not seem to exist. This aspect is widely discussed by Koprivnjak and Peschel.

In past years, much effort has been directed towards gaining an insight into the mode of action of HDPs by means of biophysical techniques. The review by Stella and coworkers analyzes in depth how spectroscopic techniques and molecular dynamic simulation have been employed to obtain a wealth of information on the interactions and structures of HDPs in membrane environments. Nevertheless, it is not yet completely understood how these peptides act on their target intact cells. Moreover, low-cost development for therapeutic and industrial purposes requires that HDPs are small and structurally simple. Therefore, considerable research effort has been directed towards optimizing peptide length and amino acid sequence. In the review by Shai and the current author differences in the in vitro and in vivo mode(s) of action and target cell specificity of two types of short peptides of native or engineered

origin are addressed. Interestingly, conjugation of fatty acid chains with linear native peptides is discussed as an alternative strategy to designing successful novel microbicides. In order to overcome drawbacks that limit the development of HDPs into valuable therapeutic agents (poor bioavailability, poor proteolytic stability), researchers have developed mimics or peptidomimetics endowed with better properties while retaining the basic features of membrane-active natural AMPs (such as a cationic charge and an amphipathic design). Multimeric peptides, cationic steroids, peptoids, and oligoacyllysines (OAKs) have been investigated, as discussed by Giuliani and Rinaldi. Finally, Epand and coauthors deal with the role of membrane lipids in the activity of cationic peptides and OAKs. One mechanism is the clustering of anionic lipids by cationic antimicrobials, resulting in some species of bacteria being more susceptible than others. The review emphasizes structures in which lipid mixtures can assemble to be utilized as antimicrobial delivery systems, increasing the effectiveness of antimicrobial substances upon in vivo administration.

To summarize, although HDPs have been known for more than two decades, interest in them continues to grow. They are studied from the standpoint of basic science research, with the aim of expanding our knowledge of the innate immune system, and as medical tools with the aim of better diagnosing and treating human disease. Taking into consideration the 12 studies discussed in this review, we can expect that some native HDPs or synthetic compounds mimicking HDPs will find their appropriate place in the market as new anti-infective agents as well as for other applications including wound healing, vaccine adjuvants, and antiendotoxic and anticancer drugs.

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