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Review

Defensins: Characteristics, mechanisms of action and viral infection

D. P. Rosete Olvera, C. Cabello Gutiérrez, J. I. Páramo Ramirez and M. E. Manjarrez Zavala*

Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas, Calzada de Tlalpan 4502, Sección XVI, Tlalpan, México D.F. CP 14080, Mexico

ABSTRACT

Defensins are a family antimicrobial peptides and play an important role in innate immunity. They are produced by leukocytes, macrophages, and epithelial cells. Some are produced constitutively, whereas others are induced by proinflammatory cytokines and infections caused by bacteria, fungi, parasites and viruses. Defensins are small cationic peptides containing six cysteines stabilized by three intramolecular disulfide bonds. Based on their size, structure and position of their disulfide bonds, they are classified in three groups: α, β and θ-defensins. The action mechanism is mainly based on the cationic and amphipathic properties which participate in the formation of pores in the cell membrane of infectious agents. They are also regulators of inflammation, cell proliferation inducers and damage repair. The abnormal expression of defensins has also been associated with human diseases. The effects of defensins on viral infections appear to be specific to the defensin, virus and target cell. The viral elimination is achieved by multiple mechanisms. Because of the ample spectrum of antiviral activity against different viruses and other microorganisms, defensins constitute attractive therapeutic candidates. In this review, we describe the general characteristics of the defensins as well as some action mechanisms in viral infections.

KEYWORDS: defensins, innate immunity, respiratory viruses, inflammation, antimicrobial peptides, β-defensins, α-defensins

*Corresponding author:
e_manjarrez@yahoo.com

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1. Introduction

Defensins are an important group of peptides with antimicrobial activity in humans. They are widely distributed in nature and constitute a part of the first line of defense against infections [1]. More than 50 years ago, peptides with antimicrobial activity in plants were reported [2]. In animals they were reported in the early 80's [3, 4]. It was in the mid 80's that Robert Lehrer *et al.* reported the structure of a new class of peptides stored by polymorphonuclear cells of humans and rabbits. These peptides were named *defensins* and showed activity against Gram-positive and Gram-negative bacteria [5, 6]. In 1987 Michael Zasloff discovered

that the skin of the frog *Xenopus laevis* contained glands rich in antimicrobial peptides and named them magainins. He observed that these peptides alter the permeability of the cell membranes of bacteria causing cell lysis [7]. Later came the description of the interaction of the peptides with bacterial membranes through their positive charges and the hydrophilic and hydrophobic aminoacids of their structure [8]. In the 90's there was a great surge in interest for the knowledge on antimicrobial peptides and there are several reports on their structure related to antimicrobial activity, expression sites, synthetic peptide synthesis associated to diseases and more recently on the action mechanisms against some viral infections in humans [9-17].

2. Classification and general features of defensins

Defensins constitute one of the most important antimicrobial peptides in humans. They are a part of the innate immunity and are produced by leucocytes, macrophages and epithelial cells throughout most of the human body (skin, respiratory, gastrointestinal and genitourinary tract). They are constitutively produced in response to infections caused by bacteria, fungi and some viruses. They are also produced during inflammatory processes [18, 19]. Defensins are cationic peptides, relatively rich in arginine, with molecular weights ranging from 3 to 4.5 kDa and approximately 20 to 45 aminoacids. In their structure they contain six cysteines residues that bind between them through disulfide bonds. On the basis of their size and pattern of disulfide bonding they are classified in three groups: α, β and θ-defensins [20, 21] (Figure 1).

α-defensins

They were discovered in 1985 and were first isolated from neutrophils, therefore they are also known as human neutrophil peptides (HNP). They are made up of 29 to 35 aminoacid residues and contain three disulfide bonds in positions 1-6, 2-4, 3-5. Six types have been identified, from the HNP1 to HNP4 are found stored mainly in the azurophilic granules of the neutrophils, but they have also been found in epithelia, macrophages, NK cells and some B and T cells [6, 22-24]. Defensins HD5 and HD6 are found mainly in Paneth cells of the small intestine and the epithelial cells of the female urogenital tract [23, 25].

β-defensins

β-defensins are composed of 36 to 42 aminoacid residues, and contain three disulfide bonds in positions 1-5, 2-4, 3-6 [23]. The first human β-defensin (HBD1) was isolated in large quantities from hemofiltrates from patients with renal diseases. It is constitutively expressed in several tissues and their expression is not increased by

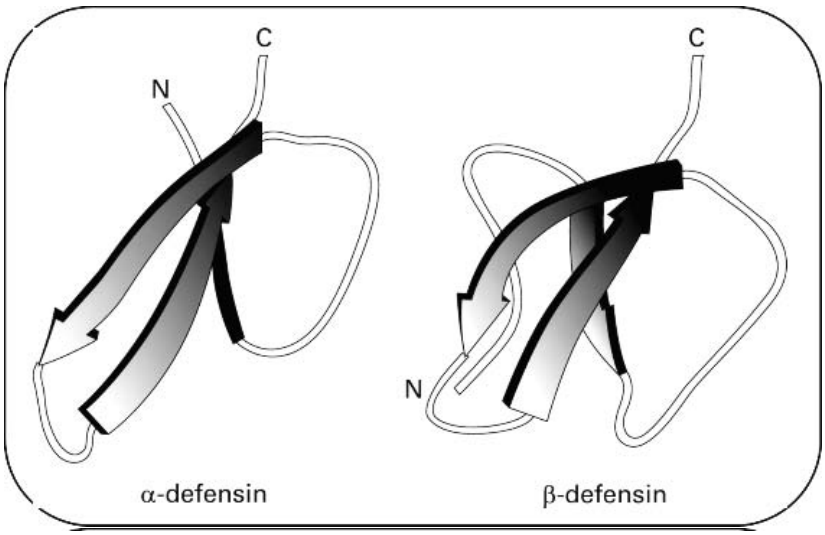

Figure 1. Structures representative of human defensins: α and β-defensins.

infectious stimuli or cytokines [26, 27]. HBD2 was purified from psoriatic lesions. It is expressed in large quantities in all the epithelia of the human body as a response to infections and inflammation [28-30]. HBD3 was also isolated from psoriatic lesions and human keratinocytes [31]. HBD4 was identified in several organs such as testicles, lung, stomach, kidney, uterus and neutrophils [32].

θ-defensins

They are composed of 18 aminoacid residues, six cysteines and three disulfide bonds. They have a circular configuration. They have been isolated from leucocytes and from the spinal cord of the Rhesus monkey. Three types have been identified (RTD1-3). They are not expressed in humans but they exhibit broad spectrum of properties against viruses, bacteria and fungi [33, 34].

3) Genetic structure of defensins

The genetic expression of defensins in man is perfectly regulated. The genes that code α and β defensins are in chromosome 8p23 [18, 35], except for those which are secreted in specific sites such as the epididimus, testicle, pancreas, kidney, skeletal muscle, that are located in chromosome 20 and have not been characterized [36]. Defensins are polymorphic in nature, each individual can have a variable number of genes that code them, and this explains in part the genetic susceptibility to different infections by pathogens [35, 37].

The genes that code for α-defensins (HNP1-4) are made up of three exons. The first exon codes for an untranslated 5' region. The α and the β-defensins formed by two exons that are the equivalent to exons 2 and 3 of the α-defensins (HNP1-4) [38]. In general, the structure of α and β defensins is the following: one gene is made up of a 5' and 3' untranslated region (UTR), two exons and an intron of variable size. Exon 1 codes for a signal sequence in the N-terminal region, and exon 2 codes for a propeptide and the mature peptide in the C-terminal region [39] (Figure 2).

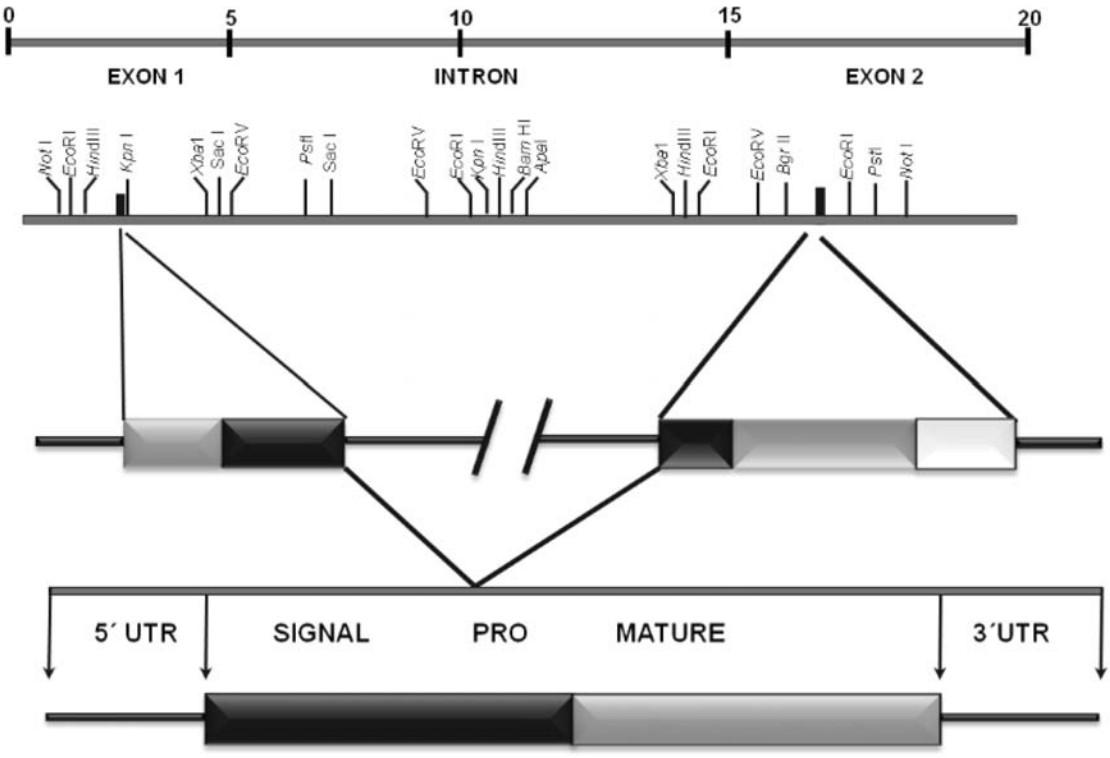

Figure 2. Structural organization of the defensins gene. It is represented with the following components: two exons surrounding an intron, 5' untranslated region (5' UTR), exon 1 which encodes the signal sequence, exon 2 which encodes for propeptide (PRO) and the mature peptide, and the 3' untranslated region (3' UTR).

4. Expression and synthesis regulation of defensins

α-defensins HNP1-4

These defensins were initially isolated from neutrophils, but they are also produced in minor quantities in epithelia, monocytes/macrophages, NK cells and some B and T cells [19, 40]. They are constitutively synthesized in bone marrow, in neutrophil precursor cells. These peptides are inactive and for their maturation they require a proteolytic processing (neutrophil proteases). α-defensins are stored in active and mature azurophilic granules. They are induced by infections and inflammatory processes, and in this stage they are ready for phagocytosis [35, 38, 41, 42].

Defensisns HD5 and 6 are produced by Paneth cells in the small intestine. Defensin HD5 has also been detected in reproductive tissue. In contrast with other α-defensins, they are stored in granules as inactive pro-defensins and they are activated through a proteolysis, generally by tripsin that is found in the digestive tissue. After exposure to a microorganism they are liberated to the small intestine lumen at the intestinal crypts level, though they can also be synthesized constitutively. The specific receptors and the translation routes that regulate their liberation have not been characterized [42-44].

β-defensins (HBD1-4)

These defensins are expressed in the epithelia and the skin of the human body. HBD1 is expressed constitutively, while HBD2 and HBD4 are induced by infections or inflammatory processes. HBD2 is expressed in dendritic cells and macrophages. HBD2 is also induced by inflammatory mediators such as interlukin 1 (IL-1α and IL-1β), alpha tumor necrosis factor (TNF-α, toll-like receptors (TLRs) and Pathogen Associated Molecular Patterns (PAMPs). It has been suggested that their expression is regulated by the synthesis of transcription factor NF-kB. However, the precise signaling route is not known. It has been observed that it activates the transcription of several genes involved in the immune and inflammatory response (IL-6, IL-8). HBD3 is induced by inflammation mediators, keratinocytes and epithelial cells. HBD4 does not activate NF-kB and its expression is not increased through stimulation by IL-1α, IL-6, interferon-γ or TNF- α [38, 42].

θ-defensins

These defensins, are circular peptides with 18 aminoacid, that are stabilized by 3 disulfide bonds. They originate by a splicing process and the circularization of two 9-aminoacid α-defensin precursor peptide segments. Three θ-defensins have been isolated only from leucocytes and bone marrow from the Rhesus monkey (RTD-1-3) and exhibit an ample spectrum of antibacterial, antifungal and antiviral properties. In humans they are only expressed as pseudogenes, a premature termination codon in the signal peptide impedes the translation [33, 34].

5. Mechanisms of action

The action mechanisms of defensins have been described and several models have been proposed [45, 46]. The first step in the interaction between the positively charged defensin (cationic) and the negatively charged phospholipids of the bacterial membrane and that of other pathogens is formed through electrostatic attraction. Later, because of its amphipathic structure which contains apolar regions (with hydrophobic aminoacids) and regions with positive charges (cationic aminoacids: arginine, lysine and histidine) the defensins are incorporated in the cell membrane where they form pores that permeabilize it. The final consequence is cell lysis through osmotic shock [46] (Figure 3). Other biological processes have been described mainly for viruses, in which defensins participate, but they are not yet fully studied. These are related to protein synthesis, the damage to DNA or the activation of autolysins [45, 47, 48].

6. Importance in the immunity

Defensins were noted first for their participation on innate immunity, now the study of their immunoregulatory properties has made evident their importance in adaptive immunity. In infections or inflammatory processes the immune response of the host induces the production of defensins as a first line of defense, in which immune cells are activated (neutrophils, epithelial cells and keratinocytes) and induce the transcription of the genes of several cytokines, chemokines, growth factors, etc. Some of them are chemotactic for other types of cells such as monocytes, macrophages, dendritic cells and T cells. Through this interaction,

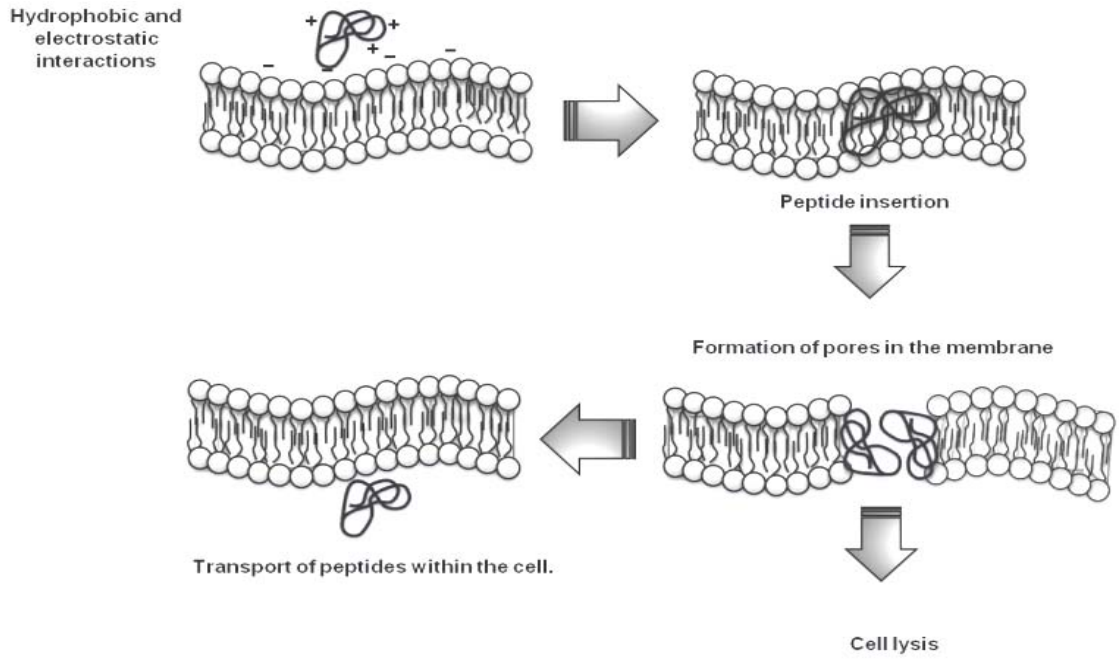

Figure 3. Schematic representation of how the defensins insert into lipid bilayers of the pathogens to form pores and to cause cell lysis.

the innate immunity serves as a bridge for the induction of adaptive immunity. In this way the action is reinforced and complemented to carryout in a more effective way its main function, elimination of microorganisms [22, 35, 47] (Figure 4).

7. Biological activities

In addition to antimicrobial properties, defensins can amplify the immune response of the host against multiple pathogens:

a. Chemotactic activity

Defensins have chemotactic activity for various types of cells. HNP1, 3 and 4 attract phagocytic cells such as neutrophils and monocytes. HBD2 attracts mastocytes and cells with receptor (CCR6) found in memory T cells, immature dendritic cells and some epithelial cells. They can act to directly recruit leukocytes or induce the expression of chemokines or cytokines. They also can indirectly promote recruitment of effector cells (neutrophils, monocytes, macrophages, immature dentritic cell, T cells) at the site of the infection or injury. These mechanims impede the dissemination of the infectious agent [35, 45].

b. Wound repair

The infection caused by a pathogen generally produces cell damage. Defensins besides eliminating the pathogen, stimulate tissue repair. They induce the liberation of growth factors that promote migration and proliferation of fibroblasts that deposit the extracellular matrix of epithelial cells to cover the damaged stratus. Recently HBD2 has been described as a potent wound repair promoter [35, 45, 49].

c. Antitumor activity

The composition of the membrane of tumor cells is different from that of untransformed cells, which makes them more permeable [50] and as a consequence, defensins easily induce cell lysis in tumoral cell lines.

d. Inflammatory mediator induction

Some α defensins have the capacity of inducing degranulation and liberation of histamine from mast cells, increasing the inflammatory response as well as vasodilation [51]. HBD3 and 4 also activate mast cells increasing the vascular permeability in the skin [52].

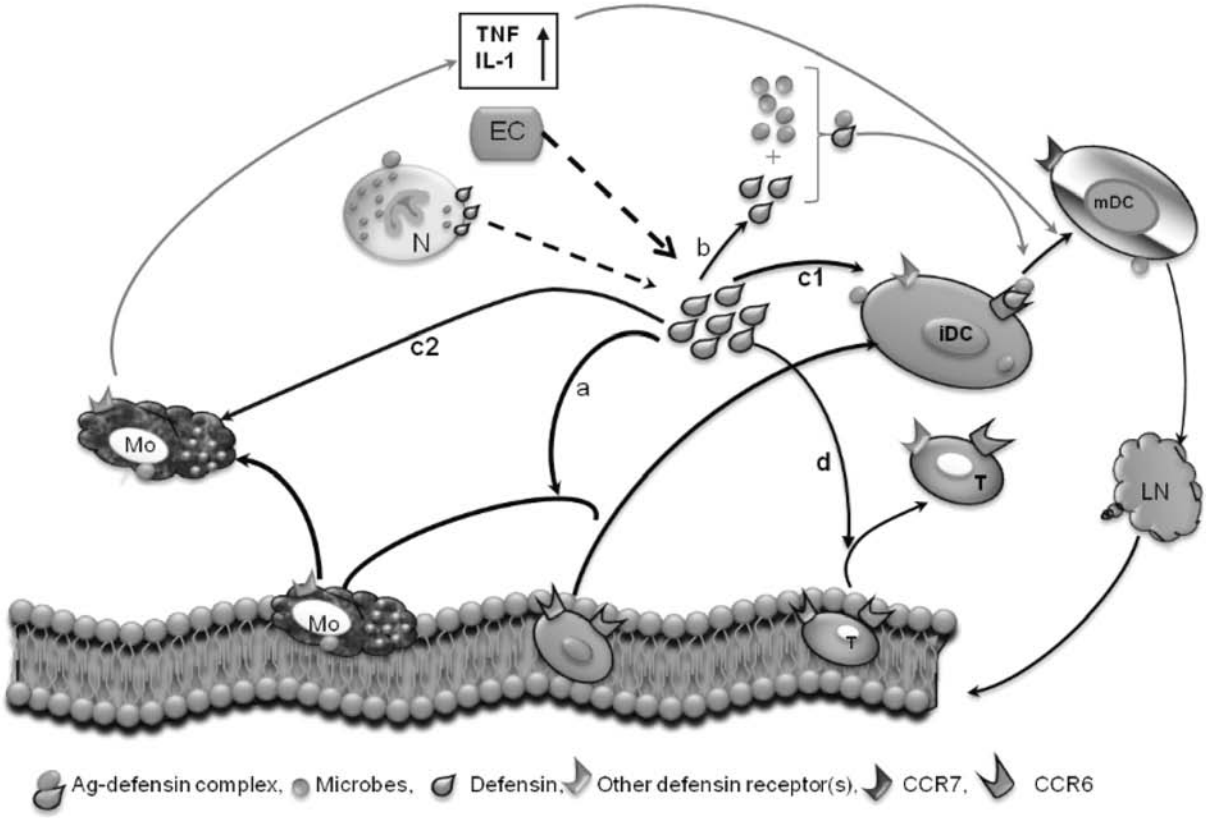

Figure 4. Effect of alpha and beta defensins in adaptive immunity. Alpha and beta defensisns are produced by epithelial cells (EC, keratinocytes) and neutrophils (N) respectively. **(a)** recruitment of immature dendritic cells (iDCs) at the infection site. **(b)** defensin-Ag (Ag) complex formation and the receptor-mediated internalization to iDCs. **(c1)** maturation of iDCs directly or indirectly mediated by TNFα and IL-1. **(d)** recruitment of T cells.

e. Phagocytosis and complement system activation

The α defensins are capable of enhancing phagocytosis. They can directly activate phagocytic cells and also induce the production of antimicrobial peptides (catelicidins), proteins (lisozyme, lactoferrin) and inorganic substances (hydrogen peroxide and nitric oxide), that are present simultaneously to fight infection in a more efficient way [35, 47]. The α-defensins can enhance or suppress the activation of the classical pathway of complement *in vitro* by binding to C1q [47, 53].

f. Immunomodulator properties

Defensins modulate the immune response through various mechanisms. Epithelial cells stimulate defensins production (HNP1-3); these induce the transcription and production of IL-8. It is a strong neutrophil chemotactic factor. By degranulation, neutrophils liberate more defensins and as a consequence more IL-8, both of which result in a positive-feedback [35, 47]. HBD2 activates immature dendritic cells by TLR4 dependent mechanisms and induces a Th1 response [54].

8) Diseases associated with defensins

As described previously, the defensins are multifunctional peptides that can regulate many events related to the immune response of the host. They can confer protection against microbial infections and inflammation. However when the expression of defensins is decreased, there can be an increased susceptibility to infectious diseases. Recent studies have shown that the expression of some defensins is associated with the pathogenesis of several diseases, though the action mechanism is not well known. The list of diseases in which defensins are involved has increased. Some of them are:

Crohn's disease

An inflammatory disease of the small intestine and/or colon. The pathogenesis is associated to the reduced expression of α-defensins (HNP2, HNP3, HD5 and HD6) and β-defensins (HBD2, HBD3, HBD4) [42]. Patients tend to have few copies of the genes of α and β-defensins, the pathogens invade the mucosa and trigger the inflammatory process [23, 35, 55].

Psoriasis and atopic dermatitis

These are diseases of the skin that are very common. The patients with atopic dermatitis are more susceptible to skin infections than patients with psoriasis. Patients with atopic dermatitis have insufficient amounts of HBD2. They are frequently subject to skin infections by several pathogens. The difference between the diseases is due to the action of cytokines IL-4 and IL-13, which are elevated in atopic dermatitis and they can block the production of HBD2 [23, 35, 45, 56].

Asthma

Rhinovirus causes common cold and asthma exacerbations. One of the mechanisms proposed for the explanation of exacerbation periods is the relationship of defensins that stimulate the liberation of histamine and prostaglandins by mast cells, increasing inflammation in this way [57-59].

Other diseases

In pulmonary diseases such as diffuse bronchiolitis, bronchiolitis obliterans and bacterial pneumonitis, HBD2 levels are elevated in pulmonary fluid and blood, and are related to a high degree of inflammation [13, 60-62]. The bacteria *H. pylori* is responsible for a variety of gastrointestinal infections as well as peptic ulcer and gastric cancers. The mechanism responsible for disease induction is not known well. However, *H. pylori* induces the expression of HBD2 y 3, which have strong bactericidal activity against this bacteria [13, 23].

In systemic eritematosus lupus there are high levels of α-defensins [63] and colon cancer, HPN1-3 is related to the progression of the disease. This can be used as molecular markers [64]. Other diseases are related to bone [65] and central nervous system [66] and even with infections by parasites [67].

9. Viral infections and defensins

Initially defensins were described by their antimicrobial activity. In 1986 the first report on their antiviral activity appeared [68] and it was thought that enveloped viruses were the main target and that their mechanism of action was similar to that of bacteria. Recently it has been shown that defensins modulate viral infections through several mechanisms, with both enveloped and non-enveloped viruses and include DNA, RNA, and retroviruses [14, 69-71].

Besides, it was proven that the effects of defensins on viral infections depend mainly on three factors: a) type of defensin (α, β y θ), b) type of virus (enveloped or naked) and c) type of target cell. Also, two other main mechanisms were described for viral inactivation: 1) Direct: when the defensin joins the viral membrane by electrostatic attraction, the membrane is permeabilized and the defensin penetrates causing the formation of pores and finally, viral lysis (Figure 2). 2) Indirect: by affecting some step in the viral replication cycle or modulating the signaling pathways necessary for antiviral effects, or, by recruiting immune cells that contribute to antiviral activity [14, 69, 71,72].

10. Mechanisms of action of defensins on viral infections

Defensin can have multiple mechanisms of action against viruses.

a. Blocking of viral entry by interaction with heparan sulfate

Heparan sulfate is the most important glycosaminoglycan molecule, found in most human cells, intracellular granules and extracellular matrix. It serves as a receptor for several viruses (HSV1, cytomegalovirus, HIV, B3 coxsackievirus, B-hepatitis). Its negative charge interacts with the defensin, blocking the union of the virus with the cellular membrane [73, 74].

b. Blocking of cellular spread

Some viruses induce the formation of syncytia and this property is used to propagate from cell to cell. Some defensins can inhibit this type of propagation by avoiding the formation of syncytia. Rabbit α- defensin (NP1) has been reported to inhibit the propagation of HSV in this way [75, 76].

c. Blocking of viral entry by interaction with viral glycoproteins

Enveloped viruses use some of their glycoproteins to enter into the host cell. Defensins can interact with these glycoproteins, blocking the viral entry. Retrocyclin 2 (RTC2) interacts with protein B of HSV2 with high affinity protecting the cell from the infection. RTC1 binds a gp120 from HIV with the same effect [73, 76].

d. Other mechanisms

Other mechanisms have been described related to genetic material, enzymes, replication, translation, virus transcription, and it has been suggested that the mechanism of action of defensins is similar to that reported for bacteria. One of the few examples is retrocyclins that inhibit the formation of proviral DNA *in vitro*, protecting CD4+ T lymphocytes from infection by HIV1. Recently it has been demonstrated that HNP1 inhibits the activation of the C kinase protein in various types of cells, affecting the translation signaling pathways of the host cell [14, 77].

11. Action mechanisms of defensins in some viral infections

Influenza virus

The influenza virus belongs to the *Orthomixoviridae* family. It produces acute respiratory infections and affects mainly small children and adults over 60 years old [78]. It is an RNA virus with envelope, with the capacity to mutate; it produces epidemics and pandemics. The α-defensins HNP1-3 inhibit the virus by several mechanisms: a) by a direct effect in a moderate way; b) interfering with signaling pathways of the cell; c) favoring the aggregation of viral particles, and promoting their elimination by neutrophils, and d) modulating viral activity by means of binding to effector cells through proteins such as surfactant D, that inhibits hemaglutination and reduces the production of H_2O_2 by neutrophils. The β-defensin, HBD2 destroys the membrane of the virus in epithelial cells and HBD3 inhibits influenza virus hemagglutinin fusion with the cellular membrane of respiratory epithelial cell. The θ-defensin RTC2 blocks the fusion of hemaglutinin with the cellular membrane [14, 79-81].

Respiratory syncytial virus

This virus belongs to the *Paramixoviridae* family. It affects mainly young children producing bronchiolitis and pneumonia with high mortality rates [82]. HBD2 inhibits the entrance to the cell and destabilizes the viral membrane. The activity of this defensin in epithelial cells of the lung is through the activation of NF-kB and the production of TNF [72, 83].

Herpes virus

Herpes virus causes lesions in the oral mucosa (HSV1) and genital mucosa (HSV2). Some infections can become complicated producing encephalitis, meningitis or keratitis, generally with type 1. They establish latent infections with periodic reactivations and are common worldwide [78]. The α-defensins have antiviral properties through different mechanisms. HNP1 in the absence of serum has a direct effect in HSV1. HNP1-3 inhibits the attachment and entry of HSV2. HNP4 and HD6 also prevent the binding and penetration of the virus in the cell. All of the α-defensins with the exception of HNP4 interact with the O- and N-linked glycans of HSV2. They act as lectins, preventing the interaction of glycoprotein B (gB) with the receptor. HNP1-3 and HD5 bind to gB from HSV1 with high affinity. HNP4 and HD6 bind to heparan sulfate, but not to gB. The β-defensin HBD3 prevent binding and entry of the HSV2 to the target cell. HBD3 binds gB and heparan sulfate. The θ-defensins interact with gB of HSV2 impeding the interaction with the receptor [15, 72, 75, 84, 85].

Human immunodeficiency virus

It belongs to the *Retroviridae* family. It has a reverse transcriptase enzyme, when the virus is transcribed from RNA to DNA. It can stay as a provirus and become integrated to the host cell. It infects CD4 T lymphocytes, B lymphocytes, monocytes, macrophages, dendritic cells, etc. The action mechanisms of defensins in HIV infections are diverse and complex in contrast with other viral infections. The secretion of some types of defensins can inhibit or exacerbate the infection. The α-defensins HNP1-3 inhibit the replication by the direct interaction with the virus. Because of their lectin like properties, they block the fusion with the infected cell by binding a viral gp120 or

a CD4 viral receptor. HNP1 in the absence of serum, directly inactivates the virus and HNP2 decreases the expression of CD4. In macrophages, HNP1 and 2 increase the expression of type CC chemokines that compete with the receptors inhibiting the infection by the virus. HNP4 inhibits viral replication more efficiently than the other defensins. It acts independently from lectins by binding a gp120 from HIV and CD4 with low affinity [86-94]. The β-defensin HBD2 does not affect viral fusion, but it inhibits DNA retrotranscription. The θ-defensins 1-3 (RTC1-3) act as lectins and inhibit the entrance of HIV by binding a gp120 and CD4 through interactions with sugars bound to O and N. RTC1 directly binds to the gp41protein of the virus envelope. It blocks the formation of the 6 helix loop necessary for fusion [83, 84, 86-91].

Other viruses

Defensins can also block some infections caused by naked viruses. *In vitro* α and β-defensins inhibit the infectivity of adenovirus and of adenoassociated viruses. HNP1 and HD5 inhibit the infection by adenovirus of epithelial cells of the conjunctive and lung. Another mechanism is by the direct interaction of defensins (HNP1, HD5) with the viral capsid, specifically with protein V1. It blocks the disassembling of the capsid in the endosome, preventing the liberation of the virus to the cell membrane [92-95]. A similar mechanism occurs with the papilloma virus [96]. The β-defensin HBD1also inhibits the infection by adenovirus of conjunctive and lung epithelial cells. Rhinovirus induce the expression of HBD2 y 3, only when there is viral replication [97].

12. Synthetic peptides

To study the antimicrobial activities and the clinical use of defensins, synthetic peptides have been designed analogous to those that are synthesized naturally, or with variations in their structure to improve antimicrobial activity and reduce toxicity. Studies have shown some important characteristics of the structure of defensins in preserving or increasing antimicrobial activity: the disulfide bonds are important in the folding of the molecule; the preservation of the cystein in position V maintains antimicrobial activity; the substitution of the arginine in comparison with other aminoacids, conserves the antimicrobial activity; the charge of the aminoacids and their distribution in the molecule in the C-terminal region are also important. These studies have focused on bacteria and few have been done with virus. Some mechanisms of action have been determined, but those on signaling pathways and the effects on animals are unknown. There is little progress in the development of efficient methods for the large-scale elaboration of peptides, as well as in the activity in different media and types of cells [98-105].

13. Clinical applications and perspectives

In the market there are few antiviral agents for viral infections. This, together with new diseases that appear and the large amount of immuno-supressed subjects, as well as cancer of different tissues and organs, has led to the search for new methods and therapeutic alternatives. Defensins are considered as the ideal option, because it is believed that there is very little possibility that microorganisms can develop resistance to them [13, 14, 106]. Due to the broad spectrum of antimicrobial activities, their multiple functions and their role of innate and adaptive immunity of the host, the production of new synthetic peptides has been proposed.

14. Conclusion

Defensins play an important role in innate and adaptive immunity of man against an ample variety of microorganisms including viruses and inflammatory processes, and they have the capacity to modulate the immune response. Their study has allowed the association of defensins to various diseases, however, the pathogenesis has been insufficiently studied. It has been suggested that they can be used as prophylactic as well as therapeutic agents in numerous human diseases. However, much research is needed and many problems must be solved, such as their efficiency against viral infections. There is also little advance in the matter of their mass production, their stability in different media, etc. But great achievements are expected in the near future.

Conflict of interests

None of the authors has a conflict of interest.

15. Acknowledgement

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