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Chapter

Multifunctional Activity of the β-Defensin-2 during Respiratory Infections

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Abstract

Human β-defensin-2 is a small cationic peptide that is part of the innate and adaptive immunity. It is expressed mainly in the epithelium and has a broad spectrum of antimicrobial activity against bacteria, fungi and viruses. In addition to its antimicrobial activity, it has other biological functions. The alteration of the expression of β-defensin-2 in the respiratory epithelium has been associated with the pathogenesis of several respiratory diseases such as asthma, pulmonary fibrosis, pneumonia, tuberculosis, rhinitis, etc. The acute respiratory infections caused by viruses are the main cause of morbidity and mortality in the world; there are few studies and it is necessary to study this peptide to understand its role in the viral pathogenesis. In addition, it also becomes relevant in its potential to take advantage of its properties in the development of alternative therapies that allow the prevention or treatment of viral respiratory infections.

Keywords: β-defensin-2, pathogenesis, respiratory diseases, viral infections

1. Introduction

Defensins are a family of antimicrobial peptides that are part of the innate and adaptive immune system. They provide protection against too broad spectrum of pathogens, viruses, bacteria and fungi [1]. The defensins are small cationic peptides of 3–4 kb, and its structure is composed of beta sheets, six cysteines joined by disulfide bonds. In humans, it is classified in two groups: α- and β-defensins [2, 3]. The β-defensin-2 (HBD2) is found in the second group and it is a peptide of 41 amino acids; it is expressed mainly in the epithelial cells, mucous and skin [1, 4, 5]. It was isolated in 1997 from skin lesions in patients with psoriasis; later, it was detected in the epithelium of almost entire human body [6–14]. In recent years, HBD2 has been considered as a multifunctional peptide with antimicrobial activity and with immunomodulatory functions [15–17]. On the other hand, the HBD2 is expressed throughout the respiratory epithelium from the mouth to the lungs; it is believed that this defensin has a very important role in the defense against respiratory infections [2]. The alteration of the expression of the HBD2 in the respiratory epithelium has been associated with the pathogenesis of several respiratory diseases such as asthma, pulmonary fibrosis, pneumonia, tuberculosis, rhinitis, etc. [2, 9, 17–20].

The acute respiratory infections caused by viruses are the main cause of morbidity and mortality in the world, and HBD2 has got antiviral activity against some

respiratory viruses (influenza, respiratory syncytial virus, rhinovirus) [21–23]. The mechanisms of viral inactivation vary and include not only direct binding of the virus to the peptide but also indirect methods of inactivation via intracellular modulation of the viral replication, modulation of signaling pathways necessary for antiviral effects, and recruitment of immune cells that contribute to antiviral activity [2, 22]. This revision chapter focuses on the structural and general characteristics of HBD2, multifunctional activities and expression in respiratory diseases. We present some studies concerning the effect of respiratory viruses and their relationship with HBD2, mechanisms of action and their relevance as therapeutic agents.

2. General characteristics and classification

Defensins are a family of antimicrobial peptides that form part of the innate and adaptive immune system and constitute the first line of host defense against microorganisms. It has shown the broad antimicrobial activity spectrum against bacteria, fungi and viruses [1, 24].

The defensins are small cationic peptides of 28–42 amino acids, characterized by a β-sheet structure linked by tree disulfide bonds, which are formed by six cysteine residues [2, 25]. Based on the distribution of their cysteines and disulfide bonds, defensins are classified into two groups in humans: α- and β-defensins [1]. α-defensins have 29–35 amino acids and the positions of the cysteines are C1-C6, C2-C4 and C3-C5, while the β-defensins are composed of 38–42 amino acids with the positions of the cysteines of C1-C5, C2-C4 and C3-C6 [26].

The α-defensins are expressed mainly in neutrophils and called human neutrophil peptides with four types (HNP1–4). There are other α-defensins (HD5 and HD6), known as enteric defensins, that are expressed in Paneth cells in the small intestine [27]. The expression of HBD6 has also been confirmed in the female genitourinary system [1, 28].

β-defensins are mainly expressed in epithelial cells throughout the body, including mucous membranes and skin [29–32]. Four types (HBD1–4) have been identified in humans, but several analyses indicate that there may be approximately 31 types, of which HBD5 and 6 are expressed in epididymis with their importance in defense against infections. The other defensins are known only for their antimicrobial activity [33, 34]. The β-defensin-1 is expressed constitutively, while the other three (HBD2–4) are expressed by the effect of proinflammatory cytokines or during the infectious process [1, 28].

3. Molecular structure of β-defensin-2

The HBD2 is a small cationic peptide with a positive net charge (+6). It has 41 amino acid residues; the complete gene is approximately 4 kb. It consists of six cysteines in positions 1–5, 2–4 and 3–6, joined by three disulfide bonds. Its secondary structure consists of an N-terminal region linked to an alpha helix, three β-strands arranged in an antiparallel sheet and a C-terminal region [35–37] (**Figure 1**). Its structure has an amphipathic nature, with hydrophilic and hydrophobic amino acids on the surface of protein; it is stabilized by the disulfide bonds, which protect it from degradation by proteases [38, 39]. The alpha helix is also stabilized by the disulfide bonds (I and V) and by the first beta sheet that has a domain (Gly-X-CysIV), which is responsible for the native structure and the correct folding of the peptide [40, 41]. The N-terminal region binds to the membrane of microorganisms.

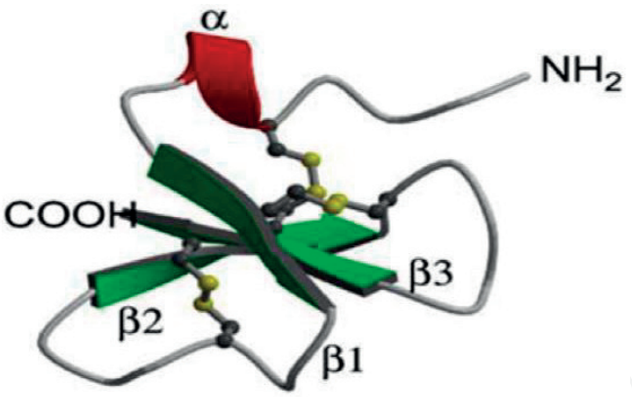


Figure 1.
Secondary structure of HBD-2 that consists of an N-terminal region linked to an alpha helix, three β-strands arranged in an antiparallel sheet and a C-terminal region [41].

The specific conformation of N-terminal region of β-defensins may be important for the biological properties of these proteins. [37].

The C-terminal region consists mainly of cationic amino acids (lysine and arginine) that are distributed asymmetrically with their positive charges and are important in antimicrobial activity [3, 38, 40].

4. Genetic structure of β-defensin-2

The gene codify for HBD2 is located on chromosome 8p23. The gene is approximately 4 kb and composed of a 5'and 3' non-translatable region, two exons separated by an intron. The first exon has 81 bp and codes for the signal peptide and the second exon has 238 bp and codes for a short anion segment called propeptide and the mature peptide [3, 25, 42]. The 5′ region of HBD2 has specific sites that bind to the transcription factor kB (NF-kB). In addition, there are sites that bind to other transcription factors such as C/EBP, and NF-IL-6, which are important for their expression [42–44].

The HBD2 have a polymorphic nature, and the number of copies of the gene varies in each individual [45]. It has been reported that there are 2–12 copies per diploid genome, and the number of copies is related to the level of expression. It has been suggested that these variations may have consequences on the function of immune system. Some infectious and inflammatory diseases are related to the number of copies of the HBD2 gene [44, 46] (**Figure 2**).

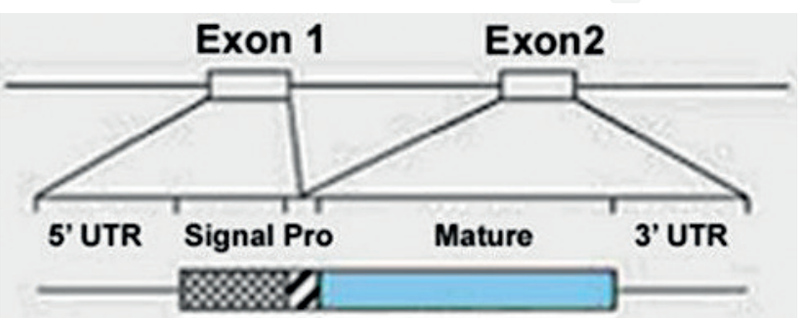


Figure 2.
Genetic structure of HBD2. It encompasses untranslated 5 'and 3' regions and two exons separated by an intron. The first exon codes for the signal peptide and the second exon codes for the propeptide and the mature peptide [3].

5. Expression and regulation of β-defensin-2

HBD2 is expressed mainly in all the epithelia of human body (respiratory, digestive, urogenital, conjunctive epithelium), mucous, peripheral blood and skin. In the respiratory system, HBD2 is expressed in mucous from the mouth to the epithelium of the lungs and is induced by bacteria, fungi and virus infections and by proinflammatory stimuli such as interleukin 1α (IL-1α), interleukin 1β (IL-1β), tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) and interleukin IL-6 (IL-6) [29, 47–51]. Recently, a study showed that the cytokines IL-17 and IL-22 are produced by Th17 cells, which are reportedly known to regulate the expression of HBD2 on mucosal surfaces [32]. HBD2 is produced as a functionally inactive peptide (prepro-defensin), and to achieve its biological activity, it must go through a post-translational modification process and form a mature peptide. Prepro-defensin is composed of a highly hydrophobic pre-peptide signal, a propeptide and the mature peptide. The prepropeptide is cut by proteases in the Golgi apparatus, and once removed, the mature peptide with antimicrobial activity is secreted on the surface of the epithelial cells [3, 52].

The regulation of the expression of the HBD2 in the respiratory epithelium involves multiple signaling pathways; most of these have been studied with bacterial infections. The bacterial proteins induce the expression of HBD2 in the respiratory epithelium through transcription factors (NF-kB) [53], the myeloid ELF-1-like factor (MEF) [54], the nuclear factor interleukin 6 (NF-IL6) [55] or the activated protein 1–3 (AP1–3) [56]. Signaling pathways involve mitogen-activated protein kinase (MAPKs) [49, 57, 58], phosphatidylinositol-3-kinase (PI3K) and protein kinase C (PKC) [59].

Another form to induce HBD2 expression is through cellular receptors, the respiratory epithelium expresses various receptors on its surface. Toll-like receptors (TLRs) 1–6 are expressed on the epithelial surface, and in the intracellular vesicles, the TLR3 and TLR7–9 are expressed in the endosomes or endoplasmic reticulum. These receptors recognize pathogens and have a cytoplasmic domain that is homologous to the IL-1 receptor and is responsible for initiating intracellular signaling pathways. This signaling cascade includes the activation of NF-kB. This transcription factor promotes the gene expression that contributes to the cytokines, chemokines, adhesion molecules, co-stimulatory molecules release as well as the expression of HBD2 [60].

In a study with lung epithelial cells infected with *L. pneumophila*, it was observed that the infection induces the release of HBD2, and its expression is mediated by the receptors TLR2 and TLR5 and activation of MAPKs (p38, JNK) and transcription factors NF-kB and AP-1 [50, 57, 58, 61].

Another way to regulate the expression of HBD2 is through the interaction with receptors; its chemotactic property was initially discovered in different types of cells such as monocytes, T cells and immature dendritic cells. The activity is carried through the CCR6 receptor binding [32].

Other receptors involved in the regulation of the HBD2 expression are the receptors for vitamin D and protease-coupled receptors (PARs) [62–65].

Nowadays, HBD2 is considered a multifunctional molecule; the ability to bind to several ligands suggests that there may be more receptors and signaling pathways still to be discovered and that may have important biological activities in the immune system.

6. Mechanisms of action of β-defensin-2

Several mechanisms of action have been proposed for defensins; however, the main mechanism of action of the HBD2 is to eliminate microorganisms

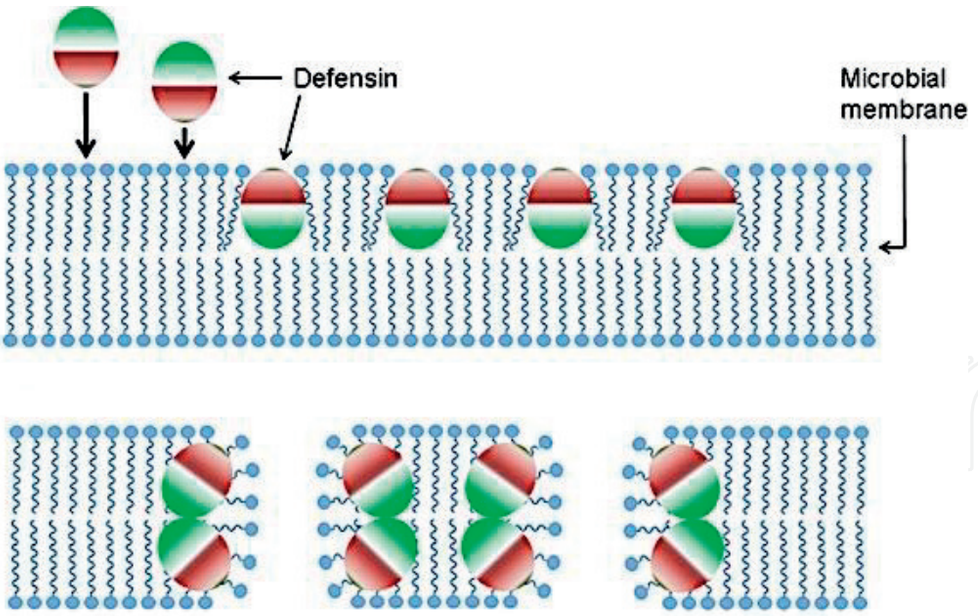


Figure 3.
Mechanisms of action of HBD2. The defensin with its positive charge is attached by electrostatic attraction to the membrane of the pathogen forming pores [3].

directly through the interaction with the microorganism membrane. The first step is given by the electrostatic attraction between the cationic defensin with positive charge and the microorganism's membrane components with negative charges [37].

Components of the bacteria membrane have been identified as targets for the HBD2. Lipopolysaccharides are targets for Gram-negative bacteria, teichoic acid for Gram-positive bacteria and phospholipids for both bacteria. In Gram-negative bacteria, the peptides are inserted into the membrane by hydrophobic interactions. It is thought that it possibly involves a folding of the peptide within the structure of the membrane [38, 66, 67]. After the electrostatic interaction of the peptide with the membrane and the displacement of the lipids, the defensin is added to the surface of membrane. There are several modes of action that have been proposed to describe how defensins are oriented to form pores and how the structure of the membrane is altered, becomes permeable, such as cell lysis and finally results in the death of the microorganism (**Figure 3**) [3, 37, 68].

The high content of negatively charged amino acids in the membranes of bacteria is the main factor that makes them more susceptible to being targeted by defensins. The membrane of the eukaryotic cells predominates lipids with neutral charge without net charge, they have a high level of cholesterol, and bacteria do not contain cholesterol in their membranes. Cholesterol causes the membrane to condense and prevents the peptide from penetrating; this also has an asymmetric distribution of phospholipids contributing to the resistance against defensing. These mechanisms explain why these peptides are not toxic in eukaryotic cells [69, 70].

7. Multifunctional activities of β-defensin-2

HBD2 was isolated and characterized by its antimicrobial activity. Currently, several studies have described different biological activities of HBD2, and it is considered as a multifunctional protein. Some of the account on its biological activities is given below.

7.1 Antimicrobial activities

HBD2 has a broad spectrum of activity against a wide variety of bacteria, fungi and viruses. The mechanism of action of this defensin begins with the interaction of the negative charges of the pathogen membrane, the formation of pores and finally the lysis of the microorganism. The variability of the composition of the membranes of the different pathogens explains in part the different antimicrobial effects [2, 22].

7.2 Innate and adaptive immunity

HBD2 is important in innate immunity and constitutes the first line of host defense against infections by microorganisms. Its role in adaptive immunity is attributed mainly to its chemotactic activity in immature dendritic cells and memory T lymphocytes through the CCR6 receptor [26].

Several mechanisms have been proposed for HBD2 that contributes to the adaptive immunity: (a) Increase in the recruitment of immature dendritic cells. Immature dendritic cells are recruited from circulating blood or tissue near the site of inflammation by chemoattractants that interact with their corresponding receptors (CCR1, CCR5, CCR6). (b) Formation of defensin-antigen complexes. HBD2 forms defensin-antigen complexes facilitating the presentation to dendritic cells. (c) Maturation of dendritic cells. HBD2 induced the maturation of dendritic cells for direct production of IL-2 or indirect production of TNF and IL-1 by monocytes and macrophages. (d) Recruitment of memory T cells. It facilitates the recruitment of memory T cells that are the effector cells of adaptive immunity [15, 26].

HBD2 contributes to unite the innate and adaptive immune response, and this property has been applied for the development of vaccine adjuvants, since it promotes adaptive immunity when it is administered together with antigens [15, 26].

7.3 Inflammation

Inflammation is a protective reaction by the host to eliminate injurious stimuli (microorganism, damage cell or irritants). Some viral infections cause severe inflammation, and the tissue can be damaged and then it must be repaired. The mechanisms include the production of anti-inflammatory cytokines, lipid mediators, glucocorticoids, immune cell apoptosis, etc. [3].

HBD2 plays a critical role in regulating inflammation processes in the respiratory system and modulates the production of inflammatory cytokines and chemokines. An increase in local expression has been observed; but in severe cases, the HBD2 can be detected systemically [71].

HBD-2 can promote histamine release and prostaglandin D2 production in mast cells, suggesting a role in allergic reactions [1, 26, 72].

7.4 Anti-inflammatory activity

HBD2 and the complement system are two important innate immune mechanisms against a broad range of microorganisms. The complement is composed of more than 30 proteins found in the human serum, and it is activated by three different pathways (classical, alternative and lectin) [73, 74]. It has been described that HBD2 binds to C1q (first component of the complement system) and inhibits the classical complement pathway. HBD2 have a dual protective role not only as an antimicrobial agent but also to provide protection against uncontrolled activation of complement system [73, 75, 76].

7.5 Immunomodulatory properties

Recently, the study of the HBD2 has focused on the modulation of the immune response. It has been suggested that multiple mechanisms of action may be involved from direct binding to the membrane of the microorganism to the union with different types of cellular receptors that can induce transduction signals, gene transcription and various signaling pathways [77]. This mechanism paves the way for the development of new therapies for infectious respiratory diseases.

7.5.1 Moxifloxacin/HBD2

In a study with epithelial lung cells (A549) stimulated with LPS, it was demonstrated that the association of moxifloxacin/HBD2 has an anti-inflammatory effect. Moxifloxacin is a fluoroquinolone against Gram-positive and Gram-negative bacteria, which may have affected the immune system. The treatment induced a reduction of proinflammatory cytokines (IL-1 and IL-6). These data support the hypothesis of its immunomodulatory capacity of HBD2 to neutralize the components of bacteria that induce the activation of cytokines [78].

7.5.2 Vitamin D (VitD)

VitD plays an important role for the calcium homeostasis in the bones. Currently, the interest has focused on the modulation of the immune response in fighting viral respiratory infections. Some studies show that patients with deficiencies in VitD are at higher risk of suffering respiratory infections in the upper respiratory tract [79]. One study showed the association of polymorphisms in the VitD receptor and severe bronchiolitis in patients infected by the respiratory syncytial virus [80].

The immunomodulatory effect has been of great importance in some viral infections; several types of cells including epithelial cells treated with VitD induce the expression of the receptor and the production of antimicrobial peptides such as the HBD2. In the patients infected with HIV, the high levels of VitD and its receptor increase the amount of IL-10 and HBD2, which are associated with a natural resistance to HIV infection [62].

The VitD receptor is expressed in several types of cells (monocytes, B cells, T cells and NK), is an endogenous immunomodulator and induces the transcription of the HBD2. This work shows that the expression of HBD2 is important to render tolerance to viral infections [62, 64].

7.5.3 PARs (receptors coupled to proteases)

It is a family of receptors coupled to the G-protein, which is formed by seven transmembrane domains with an amino-terminal extracellular domain and a C-terminal domain. PARs are activated by proteolytic cleavage in the N-terminal domain by serine proteases. N-terminal serves as a ligand to carry out intracellular signaling. They are expressed in epithelial, endothelial and immune cells such as leukocytes, mast cells, eosinophils, neutrophils and mastoid cells. Four types of receptors have been described (PAR-1–4). PAR-1, 3 and 4 are activated mainly by thrombin and are involved in the aggregation of platelets. PAR-2 is activated by trypsin, tryptase from mastoid cells, protease 3 from neutrophils and tissue factor, factor VIIa and Xa [81]. Recently, PARs have been implicated in the regulation of the expression of antimicrobial peptides found in epithelial cells such as defensins [65, 82]. The researchers identified the expression of the HBD2 in human

gingiva by *P. gingivalis* infection. Further, proteases of the bacteria induced the expression of the defensin through PAR2. The authors suggest that this signaling pathway can lead to the development of preventive therapies in mucosal infections [65].

7.5.4 Triptolide

This is an immunosuppressive and anti-inflammatory agent that was extracted from an herb of Chinese origin (*Tripterygium wilfordii*). It decreases the expression of the NF-kB and genes related to inflammatory processes. This review chapter shows that this agent suppresses the expression of HBD2 induced by IL-1β in A549 cells and the suppression is associated with the inhibition of NF-kB [83].

7.5.5 Neutrophilic elastase

It is a serine protease that is expressed in neutrophils and stored in their granules. It has been reported that neutrophilic elastase in the bronchial epithelium has a direct effect against bacteria; in addition, it can regulate the increased expression of the HBD2 [84].

7.5.6 Dexamethasone

It is a synthetic glucocorticoid that is used in the treatment of respiratory, allergic or autoimmune diseases. Its effect is to decrease the expression of proinflammatory cytokine genes through NF-kB. The clinical use of glucocorticoids can increase the susceptibility to infections by decreasing the expression of antimicrobial peptides such as the HBD2. In this study, they investigated the molecular mechanism by which dexamethasone modulated HBD2 expression in response to IL-1b in A549 cells and, the role of MAPKs, MKP-1, AKT, and NF-kB transcription factor. They demonstrated that dexamethasone suppresses the expression of HBD2 for this signaling pathway [83].

7.5.7 Isoleucine

Isoleucine is an essential amino acid that can induce the expression of the HBD2 in the epithelium. Its expression involves the activation of NF-kB/rel family of trans-activating factors. The authors suggest that isoleucine or analogues may have clinical utility as immunostimulants that could bolster the defense of the respiratory epithelium and mucosae [85].

7.5.8 Hyaluronic acid

When the skin epithelium suffers damage, the hyaluronic acid, which is found in the extracellular matrix, is fragmented and activates keratinocytes which in turn stimulate the HBD2 production. The induction is mediated by toll receptors (TLR2 and TLR4) as well as other signaling pathways such as c-Fos and protein kinase C; thus, the epithelium is protected from infections [86].

7.5.9 Sirtuin1 (SIRT1)

It is a nicotinamide adenine dinucleotide-dependent histone deacetylase, which regulates several processes of the innate and adaptive immune system.

The anti-inflammatory properties of SIRT1 are reportedly known to show [87] that the infection with *S. pneumoniae* in alveolar epithelial cells (A549) induces HBD2 production involving the SIRT1. Furthermore, HBD2 production induced by *S pneumoniae* is mediated by the MAPK activation (p38), and the expression of IL-8 is regulated by the phosphorylation of ERK.

7.6 Wound repair

This process has been described in the epithelium of skin and can be achieved by various means, which includes the modulation of cytokines production, cell proliferation and migration and in some cases angiogenesis [88, 89]. It has been demonstrated that HBD2 is expressed in normal skin [90], and its expression increases when the skin is damaged or during the chronic infection [91].

Patients with diabetes mellitus suffer from skin ulcers, and the expression of HBD2 does not increase when compared to normal skin. The scarce expression of HBD2 is seen during the chronic disease. The authors supposed that high glucose levels inhibit the expression of HBD2 in human keratinocytes [92].

HBD2 is reportedly seen to elicit intracellular Ca+2 mobilization and increased keratinocyte migration and proliferation [93]. Besides, this peptide induced phosphorylation of EGFR, signal transducer and activator of transcription STAT1 and STAT3. These are intracellular signaling molecules involved in keratinocyte migration and proliferation.

7.7 Angiogenesis

It is a process by which endothelial cells proliferate and migrate towards the angiogenic stimulus to form new blood vessels. This process is part of the repair of damaged tissues and severe inflammatory processes. The present paper demonstrates that HBD2 stimulates the migration, proliferation and formation of capillary tubes of endothelial cells [94].

7.8 Cytokines and chemokines

Peripheral blood mononuclear cells when stimulated by the HBD2 induce a strong cytokine response. The cytokines that were detected in the highest concentration were interleukin 6 (IL-6), interleukin 8 (IL-8) and interleukin 10 (IL10). It was also found that monocyte chemotactic protein 1 (MCP-1) induces a strong response and also induces RANTES, IL-1β, ENA-78 and GRO, so that the induction patterns of cytokines/chemokines can be crucial in the development and amplification of the immune response against pathogenic microorganisms [95].

7.9 Hypertension

It has been proven that HBD2 can regulate blood pressure and provide a new mechanism for the treatment of hypertension [96].

8. Respiratory diseases associated with the expression of β-defensin-2

The respiratory epithelium is the largest surface of the human body in contact with external medium, and it exposed to a large number of pathogens.

The respiratory epithelium counts upon many defense effectors and one of them is the production of defensins. They act as a first line of host defense against invading microorganism. The cells of respiratory epithelium produce four types of defensins (HBD1–4). HBD1 is expressed constitutively and HBD2–4 are expressed during infectious or inflammatory processes. HBD2 is the most expressed in all respiratory epithelium from the oral cavity, pharynx, larynx, trachea, serous cells and submucosal glands to the lung [1, 29, 97]. Several authors have reported expression of the HBD2 in these tissues and also detected the protein in healthy and diseased people in different secretions of the body such as bronchoalveolar fluid, saliva, blood, plasma, milk, sputum, nasal secretions, etc. [32, 98–102].

Several studies have suggested that there is a relationship between HBD2 and the pathogenesis of several respiratory diseases. The increase or decrease of its expression can result in the protection or amplification of the disease.

8.1 Lung cancer

This type of disease has a high mortality rate and the treatments are very expensive. It is difficult to detect early stages of the disease or if the tumor is benign or malignant, especially in the preliminary condition. The higher concentrations of HBD2 in the serum of patients did not show any relation with the histopathological classification [103].

8.2 Pneumonia

It is a disease of the lower respiratory system that mainly affects young children and older adults and has a high mortality rate and a great social and economic impact due to long periods of hospitalization and resistance to antibiotics. The alteration of the immune function of the mucosa of the respiratory system in these age groups constitutes a risk factor for contracting pneumonia. Bacterial infections are frequent, such infections lead to a serious deterioration of lung function, and are usually associated with persistent colonization by bacteria such as *Pseudomonas aeruginosa, Haemophilus influenzae, Streptococcus pneumonia* and *Legionella pneumophila.* Patients with acute pneumonia caused by bacterial infections have shown that the HBD2 is expressed in lung tissue and the concentration of the protein increases in blood and alveolar fluid [98, 101]. When the HBD2 levels in plasma are below 12.5 mg/ml, the patients with pneumonia have a high possibility of needing mechanical ventilation and development of new complications or death [104].

In an in vitro model, *L. pneumophila* induces the release of hBD-2 in A549 cells in a manner dependent on TLR2 and TLR5. The activation of p38 MAPK and JNK, as well as NF-B and AP-1, is involved in the production of hBD-2 induced by *L. pneumophila*. Therefore, regulation of the release of hBD-2 by *L. pneumophila* in A549 cells appears to be determined by multiple signaling molecules and may contribute to host defense in Legionnaires disease. [57].

8.3 Cystic fibrosis

Cystic fibrosis is a chronic lung disease with high morbidity and mortality rates. It is caused by a recessive mutation in the transmembrane conductance regulator gene (CFTR), which is located on chromosome 7 on the apical surface of epithelial cells [19, 24, 105]. This mutation causes abnormal transport of chlorine ions, which induce an increase in the salinity of the alveolar fluid. The high salt concentration abrogates the antimicrobial activity of HBD2; this might explain the recurrent bacterial infections in the lungs of these patients [32, 105].

Bacterial infections in patients with cystic fibrosis are very common; in the acute phase, the patients are infected mainly with *Haemophilus influenzae* and *Staphylococcus aureus*. While in the chronic phase, the infection is always caused by *Pseudomonas aeruginosa*, a Gram-negative bacterium, opportunistic and resistant to antibiotics [32].

The decrease in the expression and degradation of HBD2 plays an important role in the pathogenesis of pulmonary infection for *P. aeruginosa* in patients with cystic fibrosis [105]. They explain that in the chronic phase of infection, the phenotype of the bacteria changes and it loses the flagella. The flagella are composed of flagellin, which is a virulence factor that promotes mobility and adhesion. The flagellum is the ligand that triggers the signaling pathway for the expression of HBD2. When losing the flagella, the bacterium does not recognize the receptor (TLR5) of the epithelium and transcription of the gene is not executed through NF-kB. The bacterium causes damage to the epithelium of the lung producing a severe inflammatory process, with production of IL-8. IL-8 causes the accumulation of neutrophils; later, they enter apoptosis and phagocytosed by macrophages. Many macrophages accumulate in the lung and secrete cathepsin, a cysteine protease that remodels the extracellular matrix found in large quantities in bronchoalveolar lavage. This enzyme degrades the disulfide bonds of the defensins by inactivating them, thus losing their antimicrobial activity [9].

The patients with cystic fibrosis and polyps showed high levels of HBD2 and TLR2 expression with an increase in IL-8 [106]. In nasal epithelium of patients with CF, HBD2 is not upregulated in response to inflammatory stimuli. The higher levels of inflammatory markers were seen but without any correlation with the expression of HBD2. They explain that the epithelium of patients with cystic fibrosis is chronically exposed to inflammatory stimuli and lost the capacity to upregulate defensin synthesis. The inflammatory stimuli may not be the sole inducers of defense expression [107].

8.4 Chronic obstructive pulmonary disease (COPD)

COPD is an inflammatory disease of the respiratory tract that is characterized by recurrent infections, severe inflammation and is associated with a reduction of airflow and a decrease in lung function [108]. The main environmental risk factor is smoking; some authors suggest that tobacco smoke inhibits the activation of the host's innate immune system. The *in vitro* studies with respiratory epithelium exposed to tobacco smoke are reported to inhibit the expression of HBD2 when infected with bacterium [109]. COPD patients who smoke have lowered basal hBD-2 expression in the epithelial cells of the central airways, which correlates with the amount of cigarette pack years. The decreased HBD2 expression is associated with current or former smoking, which makes the population more susceptible towards infections by microorganisms [108, 110].

Moreover, genetic factors can contribute to the progression of the disease. The variation in the number of copies of HBD2 gene in epithelial cells is associated with the pathogenesis of the disease [110]. Moreover, genetic factors can contribute to the progression of the disease. The variation in the number of copies of HBD2 gene in epithelial cells is associated with the pathogenesis of COPD. In this study showed a significantly higher proportion of the patients with severe COPD had high diploid β-defensin copy numbers (five or more) compared with the control sample.

In another study, the expression of HBD2 in peripheral lung tissue in patients with COPD is elevated and associated with the habit of smoking and with the high levels of IL-8 as well as severity of the disease [111].

8.5 Allergic rhinitis (AR)

AR is an inflammatory disorder that occurs in the nasal mucosa and triggered by the exposure to allergens (mites, pets, insects, pollen, latex items, tobacco particles, ozone, nitrogen oxide, sulfur dioxide, aspirin, etc.) producing inflammation mediated by IgE. Clinically, it is characterized by symptoms such as rhinorrhea, sneezing, nasal congestion and itching. These symptoms worsen a person's productivity and quality of life and cause sleep disturbances, fatigue or depression. Although it occurs more frequently in young children, adults are affected as well [18].

Studies with tonsil tissue (lymphocytes) and pharyngeal epithelial cells from patients with allergic rhinitis found a reduction in the expression of HBD2. When cells are exposed to Th2 cytokines (IL-4, IL-5 and IL-13) and histamine *in vitro*, they also cause a decrease in the expression of HBD2. Therefore, patients with allergic rhinitis are more susceptible to respiratory infections and severe exacerbations [112–114].

8.6 Rhinosinusitis

Rhinosinusitis is characterized by the presence of at least two respiratory symptoms, nasal obstruction and nasal discharge, wherein the presence or absence of nasal polyps is seen. In one study, the sinonasal epithelial cells of patients with rhinosinusitis with nasal polyps showed a decrease in the expression of β-defensin-2 in response to the presence of IL-4 and IL-13 [114–116].

8.7 Otitis media

It is a very common disease mainly affecting the young children under 3 years of age having episode of otitis media. The pathogenesis of the disease is multifactorial, and among the most important factors are viral infections (respiratory syncytial virus, rhinovirus, influenza A, adenovirus) and bacterial infections (Streptococcus pneumoniae, nontypeable *Haemophilus influenzae* (NTHI), and Moraxella catarrhalis). The acute illness resolves quickly but the chronic or recurrent disease can cause hearing loss [39, 117].

The epithelial cells of the middle ear express HBD2, and two signaling pathways have been described. HBD2 expression is induced by pro-inflammatory stimuli such as interleukin 1 alpha (IL-1a), tumor necrosis factor alpha (TNF-a), and lipopolysaccharide (LPS). Their transcriptional activation is mediated through an Src-dependent Raf-MEK1/2-ERK signaling pathway [119]. The other known route is when the bacterium is recognized by the TLR2 of epithelial cells through the MyD88-IRAKI-TRAF6-MKK3/6-p38 MAP kinase signal transduction pathway [117, 118].

8.8 Asthma

It is a common chronic obstructive disease characterized by obstruction, hyperreactivity and inflammation. It affects all age groups throughout the world and has high morbidity and mortality rates [119]. Epidemiological studies indicate that the pathogenesis of asthma is multifactorial. The viral respiratory infections are the main cause of exacerbations or asthma attacks; influenza viruses, parainfluenza, rhinovirus and respiratory syncytial are those that have been identified more frequently [120, 121].

The mechanisms that explain how viruses cause or exacerbate asthma are diverse and some are not yet fully characterized. Recent studies indicate that HBD2 may

play an important role in the pathogenesis of asthma. Some mechanisms have been suggested to explain the exacerbations of asthma such as (a) patients with asthma develop a TH2 type response; it induced the production of cytokines (IL-4, IL-5, IL-13) that inhibit the production of HBD2, causing susceptibility to infections [122]. (b) HBD2 induces the activation and degranulation of mastoid cells that release histamine and prostaglandins, substances that increase asthma exacerbations [26].

8.9 Recurrent respiratory papillomatosis

It is a disease in the respiratory tract caused by infection with human papilloma virus. The papilloma virus belongs to the Papovaviridae family. It is a circular double-stranded DNA virus and has no envelope, and its genome is 7200–8000 bp. There are more than 100 serotypes, which due to their oncogenic capacity are divided into high risk and low risk [123].

The disease characterized by abnormal proliferation of epithelial keratinocytes forming a papilloma. It occurs more frequently in the larynx but might spread to the trachea or even lungs. It is common in children 2–4 years of age and in children over 12 years of age and in young adults. The first symptom is progressive dysphonia, with symptoms of variable respiratory obstruction, dyspnea and stridor. Some patients have spontaneous remission and others may have a very rapid growth that may require multiple surgical procedures or cause obstruction resulting in death. Papilloma viruses have a high morbidity rate because of its recurrence, and there are no satisfactory treatments [124, 125].

The serotypes of papilloma that have been found with higher frequency are the serotypes of low risk 6 and 11; nevertheless, a 2% can be infected with other subtypes like the 16 and 18 of high risk, but these are associated with transformation of cells causing carcinoma [123].

The expression of HBD2 in samples of papillomavirus induced lesions in patients with recurrent respiratory papillomatosis, and its association with IL-8 [126] was studied. The lesions showed high levels of HBD2 expression, and the inflammatory process was not significant. Despite the higher expression of HBD2 in the deep-seated tissues, the persistence of the virus leads to the disease progression. They suggest that HBD2 can serve as a signaling molecule to induce the adaptive immune response against viral infection with acceptable inflammatory events.

8.10 Diffuse panbronchiolitis (DP)

DP is a disease with chronic inflammation in the bronchioles of respiratory system. The immune cells are activated, and neutrophils are found in large quantities and activate T lymphocytes. There are often bacterial infections (*P. aeruginosa* and *H influenzae*). The pathogenesis of this disease is not clear; but recently, HBD2 has been implicated. In a study with patients with DP, high levels of HBD2 in bronchoalveolar fluid and plasma were found, suggesting that it could play an important role in the defense against infections. The levels of HBD2 in BAL fluid may be a useful marker of airway inflammation in patients with DPB [127].

8.11 Tuberculosis

Tuberculosis is one of the most frequent infectious diseases that cause more deaths; its incidence is still a global health problem. *Mycobacterium tuberculosis* is the causative agent of tuberculosis, and it can cause a progressive disease or a latent infection. It has been reported that a third part of the population is latently infected

and 10% have active disease. There are many difficulties due to highly resistant strains and fewer therapeutic agents [128].

However, HBD2 is suggested to play an important role in the control of the disease by direct inactivation of the bacteria or as an immunostimulant in vaccines. This bacterium mainly infects macrophages and lung epithelial cells. The *in vitro* studies have shown that *M. tuberculosis* induces the expression of HBD2 in human lung epithelial cells (A549) and is associated with the destruction of bacteria [129, 130].

The transfection of monocytes derived from macrophages with the HBD2 gene increases the ability to control the growth of *M tuberculosis* when compared with non-transfected cells [131]. Children infected with *M. tuberculosis* present high concentrations of HBD2 in bronchoalveolar lavage suggesting its involvement in the pathogenesis of disease.

Results are favorable in animal model studies in mice, mice were vaccinated with DNA vaccines (gene encoding β-defensin-2 and antigens of *M tuberculoisis*) and months later were challenged with *M tuberculoisis* strains. The level of protection was evaluated by survival, and tissue damage. DNA vaccines showed protection with significant higher survival and less tissue damage in the mice [132]. When a person is infected with *M. tuberculosis*, the microenvironment is reduced in oxygen; this triggers the expression of the vitamin D receptor and HBD2 inhibits the growth bacteria. The lack of local oxygen benefits the macrophage for its elimination.

Other signaling pathways that have been discovered are in monocytes through the CD40L and IFN receptors that converge in the activation of vitamin D. HBD2 is expressed and acts against the bacterium *M. tuberculosis* [134].

8.12 Sepsis

The infectious diseases can cause severe sepsis and the patient's immune system to suffer drastic changes. This study investigated the concentration and the expression of HBD2 in peripheral whole blood cells from patients with severe sepsis. They detected that the peripheral blood cells expressed a decrease in the expression of HBD2. The patient serum showed higher concentrations of HBD2. The patients with severe sepsis have severe inflammatory processes and the proinflammatory cytokines are at high concentrations (IL-1 and TNF); these cytokines also induce the expression of HBD2. It is suggested that these cytokines may be involved in the overproduction of HBD2 in the blood of patients with sepsis. The decreased HBD2 induced in peripheral blood cells was not associated with decreased plasma levels, suggesting that peripheral blood cells do not represent the exclusive source of released protein [135].

9. Antiviral activity of β-defensin-2 in respiratory infections

The HBD2 was initially studied as an antibacterial peptide, and its antiviral activity has been recently demonstrated. The notion was that it only acted in enveloped viruses, but other studies have proven that they also act against naked viruses and any type of genome DNA, RNA and retroviruses. The mechanisms of antiviral inactivation have been classified as direct and indirect. The direct ones occur when HBD2 binds to the viral membrane by electrostatic attraction, causing pore formation and lysis of microorganism. The indirect mechanism occurs when it inactivates an intracellular pathway of the virus replication cycle or when there is recruitment of immune cells that contribute to their antiviral activity [2].

Acute respiratory infections caused by viruses are very common, causing high rates of mobility and mortality. There are few studies that relate to HBD2 and viral infection, and some of them are presented in the following paragraphs.

9.1 Influenza virus

The influenza virus belongs to the Orthomyxoviridae family and renders acute respiratory infection affecting mainly children and adults. It is an RNA virus, enveloped and has the ability to mutate rapidly, causing epidemics and pandemics [136].

Influenza virus induces the production of HBD2 in the epithelial cells of the respiratory tract *in vivo* and *in vitro* [137]. In the *in vitro* study with infected MDCK cells with influenza virus, recombinant murine β-defensin-2 prevents infection by blocking the entry of the virus. The lungs of murine model infected with influenza virus showed an increased expression of β-defensin and therefore confer protection against infection [21, 138].

9.2 Respiratory syncytial virus (VSR)

This virus belongs to the family Paramyxoviridae and affects principally infants, young children and older adults causing bronchiolitis and pneumonia. VSR has a high rate of morbidity and mortality having RNA enveloped virus of negative sense. There are no vaccines available and only fewer antivirals available which have been seen ineffective [22, 139].

The human lung cells (A549) infected with RSV expressed HBD2 [140]. The expression of the peptide depends on the activation of NF-kB and the action of TNF produced by the virus. The elimination of the virus is due to damage to the membrane.

9.3 Adenovirus

The adenoviruses belong to the Adenoviridae family; 51 serotypes divided into six species have been recognized (HAd A-F). Species B, C and E produce respiratory infections. Adenoviruses are double-stranded linear DNA viruses, lack envelope and replicate in the nucleus, and their genome has a size of 36 kb. These viruses spread rapidly in closed environments such as military camps, orphanages, boarding schools and prisons. The acute respiratory infections are transmitted mainly by aerosols and by direct inoculation through fingers. Although this disease is benign and with little severity, the infection might be severe in immunosuppressed patients afflicted with HIV and with kidney transplants. The HBD2 inactivates the infection for adenovirus *in vitro*; the mechanisms of action are not yet known due to lack of *in vivo* studies [140].

9.4 Rhinovirus

The rhinovirus is the main cause of the common cold belonging to Picornaviridae family. They are small, single-chained, naked RNA viruses. Respiratory infections caused by rhinoviruses are associated with asthma exacerbations in children and adults. Rhinovirus infection in the A549 cells (human lung cells) and bronchial epithelial cells induces the expression of HBD2. The virus replication appears essential for the expression of peptide [141].

10. Conclusions

Initially, β-defensin-2 was considered an antimicrobial peptide. The advance in the study of these molecules has made it possible to know that β-defensin-2 is a peptide with multiple functions. However, its role in the pathology of respiratory

diseases is unclear. During the infectious processes of the respiratory epithelium, HBD2 is expressed by the effect of the infection, and it has been described that it may be closely associated with the severity of the disease. The study of HBD2 during viral respiratory infections is unclear, so it is necessary to continue investigating the role of this molecule in the immune response activated by viral infections.

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