

# The Role of Human Beta-Defensin-2 in *Pseudomonas aeruginosa* Pulmonary Infection in Cystic Fibrosis Patients

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Received: August 26, 2013 / Published online: October 19, 2013

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## ABSTRACT

Cystic fibrosis (CF) is the most common genetic disease affecting the Caucasian population. Chronic *Pseudomonas aeruginosa* pulmonary infection is the major cause of morbidity and mortality in CF patients. Human beta-defensin-2 (hBD-2) is an inducible pulmonary antimicrobial peptide that exerts bacteriostatic activity in a concentration-dependent manner. The decreased expression and compromised function of hBD-2 contributes to the pathogenesis of *P. aeruginosa* infection in the CF lung. The purpose of this review is to outline the significance of hBD-2 in *P. aeruginosa* chronic pulmonary infection in CF patients.

**Keywords:** Antimicrobial peptides; Cathepsins; Cystic fibrosis; Human beta-defensin-2; Innate immunity; Neutrophil infiltration; *Pseudomonas aeruginosa*; Pulmonary infection; Toll-like receptor tolerance

## INTRODUCTION

The average human inhales ~10,000 L of air every day. Respiration is a portal of entry for not only atmospheric gases, but also for harmful particulate pervasive in the environment. The pulmonary epithelium is therefore continually exposed to microorganisms, but remains sterile under normal physiologic conditions. This remarkable phenomenon is a testament to the innate immune defenses that provide a silent mode of broad immune protection. The importance of the innate immune system in protecting the lungs from infection is clearly illustrated in the pathologic condition that arises in cystic fibrosis (CF) (mucoviscidosis), which severely damages the pulmonary innate immune defenses [1].

Cystic fibrosis is the most common lethal genetic disorder affecting the Caucasian population, with an incidence of 1 in 2,500

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births [2]. CF is caused by an autosomal recessive mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene within chromosome seven [3]. This mutation results in the functional defect in the cyclic adenosine monophosphate stimulated pulmonary chloride pump causing abnormal ion transport in epithelial cells [4, 5]. CF is therefore a disease of ion transport across the epithelium, affecting fluid secretion in exocrine glands and the epithelium of the respiratory, reproductive, and gastrointestinal tracts [6]. Although CF causes a multitude of pathophysiological effects, the most significant effect is the impaired ciliary clearance that results in the accumulation of mucus in the lung, creating a haven for bacteria. Moreover, the dehydrating conditions in the lung caused by the elevated levels of sodium chloride in the airway secretions severely weaken the host pulmonary innate defenses. The initial acute pulmonary infection of the CF lung is typically a result of colonization by *Haemophilus influenzae* and *Staphylococcus aureus*, while the ensuing chronic infection is caused by *Pseudomonas aeruginosa* [7, 8]. The chronic infection in the lungs of CF patients caused by *P. aeruginosa* is responsible for the high rate of morbidity and mortality associated with this genetic disease [9].

*Pseudomonas aeruginosa* is a ubiquitous, antibiotic resistant, Gram-negative opportunistic bacterium [10]. At 6.3 million base pairs, the PAO1 strain of *P. aeruginosa* has the largest genome sequenced [11]. This large genome provides the genetic machinery that enables *P. aeruginosa* to readily undergo significant genetic and phenotypic transformations in response to environmental changes, contributing to its versatility and antibiotic resistance potential. Although *P. aeruginosa* is pervasive in the environment, it

only causes infection in immunodeficient hosts, e.g., CF patients, patients with acquired immunodeficiency syndrome, burn victims, etc. Among the many clinical manifestations of *P. aeruginosa* infection, *P. aeruginosa*'s opportunistic mode of infection is most known in the chronic pulmonary infection that is the hallmark of CF [12]. Once acquired, *P. aeruginosa* almost always colonizes the lungs of CF patients for life [13].

### Human beta-defensin-2 (hBD-2) is a Major Effector of Innate Immunity

The innate immune system provides the first line of defense against microorganisms pervasive in the environment. Unlike the adaptive immune system, innate immunity is non-specific, lacks memory, and is not influenced by previous exposure. Antimicrobial peptides (AMPs) are cationic endogenous antibiotic proteins expressed throughout the epithelium that are effectors of the innate immune system. AMPs exert antimicrobial activity in a concentration-dependent manner, making their expression a critical factor in host defense [14]. The amphiphathic nature of AMPs contributes to their effectiveness at interacting with hydrophobic and anionic components of the bacterial membrane [15]. Cathelicidins,  $\alpha$ -defensins,  $\beta$ -defensins, and  $\theta$ -defensins are among the major classes of human AMPs [16].

Beta-defensins are at the interface between the adaptive and innate immune systems; beta-defensins exhibit chemotactic function towards immature dendritic cells, memory T cells expressing the chemokine receptor CCR6, neutrophils primed with tumor necrosis factor (TNF)- $\alpha$ , and mast cells [17, 18]. Individual beta-defensins have specific antimicrobial activity. Among the various types of defensin

AMPs, only the expression of human beta-defensin-2 (hBD-2) and human beta-defensin-3 (hBD-3) is increased following stimulation by pro-inflammatory cytokines; all other defensin AMPs are continuously expressed [19]. However, although the expression of hBD-2 and hBD-3 can be stimulated by pro-inflammatory cytokines, e.g., TNF- $\alpha$ , interleukin (IL)-1 $\beta$ , IL-17, and IL-22, these antimicrobial peptides are still expressed in unstimulated cells in basal amounts [20, 21]. An additional difference between these two AMPs that are induced by humoral stimulation is that hBD-2 primarily targets Gram-negative bacteria, such as *P. aeruginosa*, while hBD-3 exerts broad bacteriostatic activity against both Gram-positive and Gram-negative bacteria [22]. hBD-2, like all defensins, is found throughout the epithelium of mammals. However, hBD-2 is most concentrated in the epithelia of the lung, tonsils, and trachea, and therefore plays a critical role in the prevention of pulmonary infection [23, 24]. The inducible properties of hBD-2 suggest it plays a significant role in innate immune defense.

Human beta-defensin-2 is a cationic, 41 amino acid, 4 kDa, AMP intricately involved in the innate immune response of vertebrates that works synergistically with other antimicrobial molecules, such as lactoferrin and lysozyme [24, 25]. Like other beta-defensins, hBD-2 is a monomeric protein containing six conserved cysteine residues forming three core disulfide bonds [26]. The initial contact between hBD-2 and invading microorganisms is an electrostatic amphipathic attraction between the cationic AMP and the negatively charged phospholipid groups of the bacterium's phospholipid bilayer [27, 28]. Following initial electrostatic attraction, hBD-2 exerts its antimicrobial effects through insertion within the phospholipid bilayer disrupting the membrane

integrity of the invading bacteria resulting in the collapse of membrane potential and death of the invading pathogen [29]. Nuclear magnetic resonance (NMR) analysis of the crystal structures of hBD-2 suggests that the formation of a hBD-2 octamer is a prerequisite to the binding of the bacteria cell surface and subsequent increases in membrane permeability [30].

### Decreased hBD-2 Expression Occurs in Chronic *P. aeruginosa* Infection

A common theme in pathogen—host interactions is the selection against virulence factors required for the establishment of infection, as the stage the infection shifts from acute to chronic. Genetic variants are selected that promote long-term survivability and clonal expansion, while variants that no longer provide a survival advantage are selected against. In the CF lung, *P. aeruginosa* undergoes significant genetic and phenotypic transformations in response to changes in the pulmonary milieu. *P. aeruginosa* mutates to a mucoid, flagella-deficient phenotype over the course of chronic pulmonary infection [31, 32]. The changes in the expression of *P. aeruginosa* virulence factors affect the expression of hBD-2 in the pulmonary epithelium that weakens the innate immune defense of the lung [33].

Flagellum is a structure common to most Gram-negative bacteria derived from flagellin monomers that confers motility, promotes adhesion, and consequently is a significant bacterial virulence factor [34]. Flagellum is a bacterial ligand that is detected by toll-like receptor (TLR) 5 [35]. The activation of TLR5 by flagellum initiates an inflammatory response that includes the up-regulation of hBD-2 via a nuclear factor (NF)- $\kappa$ B dependent pathway in airway epithelial cells [21]. The loss of flagella

expression during the transition to the mucoid phenotype allows *P. aeruginosa* to evade the antimicrobial activity of hBD-2 through decreased TLR5 stimulation, contributing to *P. aeruginosa*'s pathogenesis in the CF lung [21, 35].

Some bacterial virulence factors remain expressed throughout different stages of infection. Although *P. aeruginosa* isolates from the chronic stage of pulmonary infection are flagella-deficient, other virulence factors, which are TLR agonists and stimulate hBD-2 expression, remain expressed. For example, lipopolysaccharide (LPS) is an endotoxin attached to the outer membrane of Gram-negative bacteria that is an agonist of TLR 4 [36]. Although LPS expression does not decrease as pulmonary infection shifts from the acute to chronic stage, the cellular responsiveness to LPS decreases.

A study involving the exposure of airway epithelial cells to a regime of two discrete bacterial infections demonstrated reduced TLR responsiveness in the second bacterial challenge due to down-regulation of the IRAK1 signaling protein, which is involved in NF- $\kappa$ B activation [37]. IRAK1 phosphorylation leads to the activation of NF- $\kappa$ B and AP-1, which are two transcription factors that induce the up-regulation of IL-8 and hBD-2 in airway epithelial cells [38]. Although this in vitro model only measured the production of IL-8, not hBD-2, these results provide a mechanistic explanation for the reduced levels of hBD-2 expression in the chronic stage of pulmonary infection in CF patients [39]. Furthermore, the reduced expression of hBD-2 in the lung in advanced chronic pulmonary infection (owing to decreased TLR responsiveness) provides further insight as to why *P. aeruginosa* only colonizes the lung post-*S. aureus* and *H. influenzae* infection. Moreover, this

underscores the potential influence of hBD-2 in the progression of chronic pulmonary infection in CF patients. The down-regulation of TLR4 expression in the airway epithelia in response to acute infection may result in reduced hBD-2 expression, promoting *P. aeruginosa* colonization [40].

### Neutrophil and Macrophage Infiltration Contribute to Degradation of hBD-2 in the CF Lung

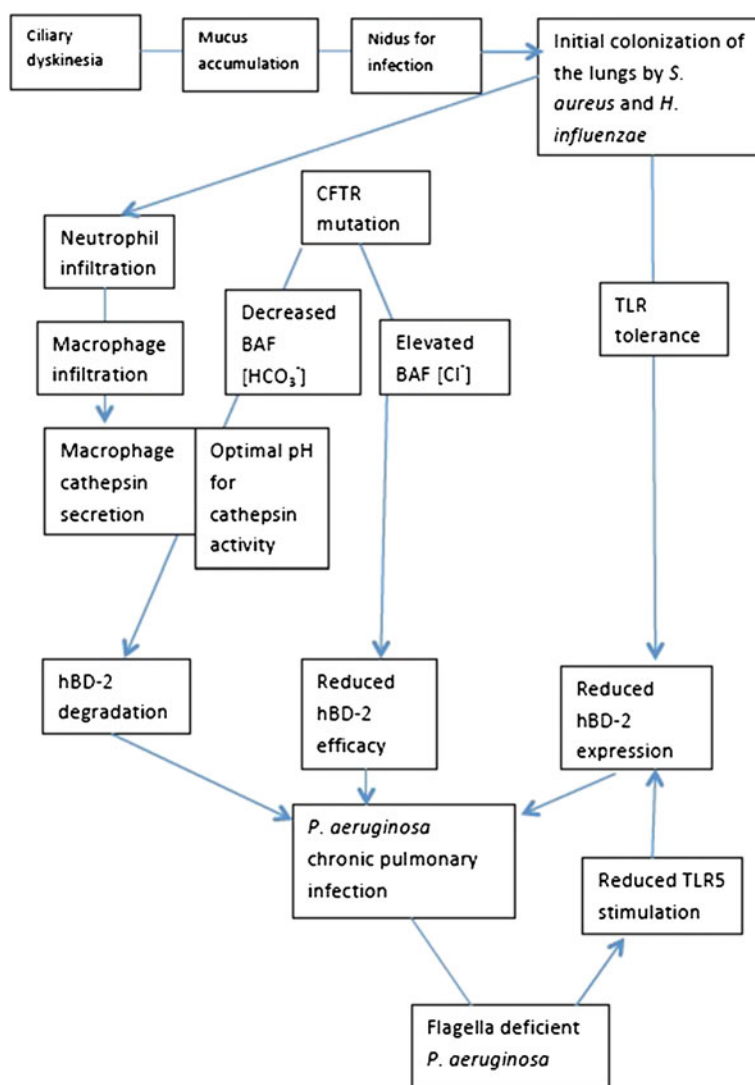
Inflammation is a protective tissue response to infection or injury. In the context of the CF lung, the inflammatory responses induced by *P. aeruginosa* severely damage the pulmonary epithelium. Exposure of the airway epithelium to *P. aeruginosa* induces the expression of the potent neutrophil chemokine IL-8, initiating neutrophil infiltration [41].

Neutrophils are granulocytic polymorphonuclear leukocytes that play a key role in innate defense [42]. However, in the CF lung the abnormal accumulation and persistence of neutrophils produces an inflammatory response that severely damages the lung [43, 44]. The quorum-sensing controlled production of rhamnolipid by *P. aeruginosa* induces rapid necrotic killing of invading neutrophils, which explains why the neutrophils do not significantly contribute to the elimination of *P. aeruginosa* in the CF lung [45–47]. In the CF lung, infiltrating neutrophils and most *P. aeruginosa* strains secrete elastase—a serine protease that exerts diverse biological effects that contribute significantly to the progression of pulmonary CF disease [48, 49]. Elastase is a potent protease that exerts antimicrobial activity against most Gram-negative bacteria, but not against *P. aeruginosa* [50]. The viability and morphology of *P. aeruginosa* remains unaltered even when

exposed to neutrophil elastase (NE) concentrations as high as 25 μM, which is commonly present in the CF lung [51]. After a short life span, neutrophils succumb to apoptosis and subsequent phagocytotic clearance by macrophages [13].

Cathepsins are cysteine proteases secreted by macrophages that are involved in the remodeling of the extracellular matrix [52]. Pulmonary macrophage influx occurs in response to the elevated levels of apoptotic neutrophils in the

lungs of CF patients resulting in cathepsin secretion into the bronchoalveolar fluid (BAF) of the CF lung [51, 53]. Beta-defensins have a conserved core structure of three disulfide bridges, which are susceptible to proteolytic cleavage by cathepsins present in the BAF [54]. Specifically, cathepsins B, L, and S have been found to cleave the disulfide bonds of hBD-2 and hBD-3 resulting in their degradation and loss of antimicrobial activity [30]. In addition to the high concentrations of cathepsins in the BAF of



**Fig. 1** Compromised hBD-2 function in the CF lung promotes chronic pulmonary infection by the opportunistic pathogen *P. aeruginosa*

the CF lung, the low pH of the CF BAF promotes optimal enzymatic activity for cathepsin proteolytic activity; most cathepsins have optimal proteolytic function in acidic pH and lose their proteolytic properties at physiologic pH [52]. The BAF of CF patients is acidic because of impaired bicarbonate transport across the pulmonary epithelium caused by the CFTR mutation [55]. Furthermore, the elevated  $[Cl^-]$  present in the BAF resulting from the functional CFTR defect reduces the efficacy of hBD-2 due to the reduced electrostatic interaction between the cationic hBD-2 peptide and the anionic resting membrane potential of invading microorganisms [24]. The overexpression of cathepsins during chronic pulmonary infection may cause increased degradation of hBD-2, promoting bacterial colonization and infection [30].

## CONCLUSION

Many factors contribute to the pathogenesis of *P. aeruginosa* in the lungs of CF patients (Fig. 1). It is becoming increasingly evident that the regulation of hBD-2 expression and degradation has profound implications in pulmonary infections. hBD-2 is an indicator of inflammation and an essential component of the innate immune system. The regulation of hBD-2 activity, expression, and prevention of degradation (cathepsin inhibitors) are potential therapeutic options for CF patients that may decrease the high rates of morbidity and mortality associated with this common genetic disease.

## ACKNOWLEDGMENTS

This project was funded by an Undergraduate Student Research Award from the Natural

Sciences and Engineering Research Council of Canada. Dalcin is the guarantor for this article, and takes responsibility for the integrity of the work as a whole.

**Conflict of interest.** Dalcin and Dr Ulanova declare no conflict of interest.

**Compliance with ethics.** The analysis in this article is based on previously conducted studies, and does not involve any new studies of human or animal subjects performed by any of the authors.

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