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

Daniel Dalcin • Marina Ulanova

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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
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 pathophysiologic 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 amphiphatic nature of AMPs contributes to their effectiveness at interacting with hydrophobic and anionic components of the bacterial membrane [15]. Cathelicidins, α-defensins, β-defensins, and θ-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)-α, 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-α, interleukin (IL)-1β, 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 \textit{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 \textit{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, \textit{P. aeruginosa} undergoes significant genetic and phenotypic transformations in response to changes in the pulmonary milieu. \textit{P. aeruginosa} mutates to a mucoid, flagella-deficient phenotype over the course of chronic pulmonary infection [31, 32]. The changes in the expression of \textit{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 flagellin initiates an inflammatory response that includes the up-regulation of hBD-2 via a nuclear factor (NF)-κB 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-κB activation [37]. IRAK1 phosphorylation leads to the activation of NF-κB 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

![Diagram](https://example.com/diagram.png)

**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.

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**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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REFERENCES

1. Bals R, Weiner DJ, Wilson JM. The innate immune system in cystic fibrosis lung disease. J Clin Invest. 1999;103:303–7.
2. Dodge JA, Morison S, Lewis PA, et al. Incidence, population, and survival of cystic fibrosis in the UK, 1968–95. Arch Dis Child. 1997;77:493–6.
3. Rommens JM, Lannuzzi MC, Kerem B, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science. 1989; 245:1059–65.
4. Bobadilla JL, Macek M, Fine JP, Farrell PM. Cystic fibrosis: a worldwide analysis of CFTR mutations—correlation with incidence data and application to screening. Hum Mutat. 2002;19:575–606.
5. Zhou Y, Song K, Painter RG, et al. Cystic fibrosis transmembrane conductance regulatory recruitment to phagosomes in neutrophils. J Innate Immun. 2013;5:219–30.
6. Knowles M, Gatzy J, Boucher R. Increased bioelectric potential difference across respiratory
7. Rajan S, Saiman L. Pulmonary infections in patients with cystic fibrosis. Semin Respir Infect. 2002;17:47–56.

8. Hoiby N, Frederiksen B. Microbiology of cystic fibrosis. In: Hodson ME, Geddes DM, editors. Cystic Fibrosis. London: Arnold; 2000, p. 83–107.

9. Emerson J, Rosenfeld M, McNamara S, Ramsey B, Gibson RL. Pseudomonas aeruginosa and other predictors of mortality and morbidity in young children with cystic fibrosis. Pediatr Pulmonol. 2002;34:91–100.

10. Hancock RE. Resistance mechanisms in Pseudomonas aeruginosa and other nonfermentative gram-negative bacteria. Clin Infect Dis. 1998;27:93–9.

11. Stover CK, Pham XQ, Erwin AL, et al. Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen. Nature. 2000;406:959–64.

12. Oliver A, Canton R, Campo P, Baquero F, Blazquez J. High frequency of hypermutable Pseudomonas aeruginosa in cystic fibrosis lung infection. Science. 2000;288:1251–3.

13. Watt AP, Courtney J, Moore J, Ennis M, Elborn JS. Neutrophil cell death, activation and bacterial infection in cystic fibrosis. Thorax. 2005;60:659–64.

14. Guani-Guerra E, Santos-Mendoza T, Lugo-Reyes SO, Teran LM. Antimicrobial peptides: general overview and clinical implications in human health and disease. Clin Immunol. 2010;135:1–11.

15. Moskowitz SM, Ernst RK, Miller SL. PmrAB, a two-component regulatory system of Pseudomonas aeruginosa that modulates resistance to cationic antimicrobial peptides and addition of aminoarabinose to lipid A. J Bacteriol. 2004;186:545–79.

16. Pinheiro da Silva F, Machado MC. Antimicrobial peptides: clinical relevance and therapeutic implications. Peptides. 2012;36:308–14.

17. Yang D, Chertov O, Bykovskaiia SN, et al. Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science. 1999;286:525–8.

18. Bonito M, Jordan WJ, Eskdale J, et al. Human beta-defensin 2 induces a vigorous cytokine response in peripheral blood mononuclear cells. Antimicrob Agents Chemother. 2006;50:1433–41.

19. Tsustumi-Ishii Y, Nagaoka I. NF-kappa B-mediated transcriptional regulation of human beta-defensin-2 gene following lipopolysaccharide stimulation. J Leukoc Biol. 2002;71:154–62.

20. Bals H, Hiemstra PS. Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. Eur Respir J. 2004;23:327–33.

21. Zhang Z, Louboutin JP, Weiner DJ, Goldberg JB, Wilson JM. Human airway epithelial cells sense Pseudomonas aeruginosa infection via recognition of flagellin by Toll-like receptor 5. Infect Immun. 2005;73:7151–60.

22. Joly S, Maze C, McCray PB Jr, Guthmiller JM. Human beta-defensins 2 and 3 demonstrate strain-selective activity against oral microorganisms. J Clin Microbiol. 2004;42:1024–9.

23. Harder J, Meyer-Hoffert U, Teran LM, et al. Mucoid Pseudomonas aeruginosa, TNF-alpha, and IL-1 beta, but not IL-6, induce human beta-defensin-2 in respiratory epithelia. Am J Respir Cell Mol Biol. 2000;22:714–21.

24. Bals R, Wang X, Wu Z, et al. Human beta-defensin 2 is a salt-sensitive peptide antibiotic expressed in human lung. J Clin Invest. 1998;102:874–80.

25. Schroder JM, Harder J. Human beta-defensin-2. Int J Biochem Cell Biol. 1999;31:645–51.

26. Schibli DJ, Hunter HN, Aseyev V, et al. The solution structures of the human beta-defensins lead to a better understanding of the potent bactericidal activity of HBD3 against Staphylococcus aureus. J Biol Chem. 2002;277:8279–89.

27. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature. 2002;415:389–95.

28. Powers JP, Hancock RE. The relationship between peptide structure and antibacterial activity. Peptides. 2003;24:1681–91.

29. Corrales-Garcia LI, Possani LD, Corzo G. Expression systems of human β defensins: vectors, purification and biological activities. Amino Acids. 2011;40:13–14.

30. Taggart CC, Greene CM, Smith SG, et al. Inactivation of human beta-defensins 2 and 3 by elastolytic cathepsins. J Immunol. 2003;171:931–7.

31. Smith EE, Buckley DG, Wu Z, et al. Genetic adaptation by Pseudomonas aeruginosa to the airways of cystic fibrosis patients. Proc Natl Acad Sci USA. 2006;103:8487–92.

32. Jelsbak L, Johansen HK, Frost AL, et al. Molecular epidemiology and dynamics of Pseudomonas
aeruginosa populations in the lungs of cystic fibrosis patients. Infect Immun. 2007;75:2214–24.

33. Cobb LM, Mychaleckyj JC, Wozniak DJ, Lopez-Boado YS. *Pseudomonas aeruginosa* flagellin and alginate elicit very different gene expression patterns in airway epithelial cells: implications for cystic fibrosis disease. J Immunol. 2004;173:5659–70.

34. Soutourina OA, Bertin PN. Regulation cascade of flagellar expression in Gram-negative bacteria. FEMS Microbiol Rev. 2006;274:503–23.

35. Hayashi F, Smith KD, Ozinsky A, et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature. 2001;410:1099–103.

36. Chow JC, Young DW, Golenbock DT, Christ WJ, Gusovsky F. Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. J Biol Chem. 1999;288:10689–92.

37. Wu Q, Lu Z, Verghese MW, Randell SH. Airway epithelial cell tolerance to *Pseudomonas aeruginosa*. Respir Res. 2005;6:26.

38. Wehkamp J, Harder J, Wehkamp K, et al. NF-kappaB- and AP-1-mediated induction of human beta defensin-2 in intestinal epithelial cells by *Escherichia coli* Nissle 1917: a novel effect of a probiotic bacterium. Infect Immun. 2004;72:5750–8.

39. Chen CI, Schaller-Bals S, Paul KP, Wahn U, Bals R. Beta-defensins and LL-37 in bronchoalveolar lavage fluid of patients with cystic fibrosis. J Cyst Fibros. 2004;3:45–50.

40. MacRedmond R, Greene C, Taggart CC, McElvaney N, O’Neill S. Respiratory epithelial cells require Toll-like receptor 4 for induction of human beta-defensin 2 by lipopolysaccharide. Respir Res. 2005;6:1–11.

41. Greene CM, Carroll TP, Smith SG, et al. TLR-induced inflammation in cystic fibrosis and non-cystic fibrosis airway epithelial cells. J Immunol. 2005;174:1638–46.

42. Baggioni M, Dewald B. The neutrophil. Int Arch Allergy Immunol. 1985;76:13–20.

43. Doring G. The role of neutrophil elastase in chronic inflammation. Am J Respir Crit Care Med. 1994;150:S114–7.

44. Dunlevy FK, Martin SL, de Courcey F, Elborn JS, Ennis M. Anti-inflammatory effects of DX-890, a human neutrophil elastase inhibitor. J Cyst Fibros. 2012;11:300–4.

45. Jensen PO, Bjarnsholt T, Phipps R, et al. Rapid necrotic killing of polymorphonuclear leukocytes is caused by quorum-sensing-controlled production of rhamnolipid by *Pseudomonas aeruginosa*. Microbiology. 2007;153:1329–38.

46. Alhede M, Bjarnsholt T, Jensen PO, et al. *Pseudomonas aeruginosa* recognizes and responds aggressively to the presence of polymorphonuclear leukocytes. Microbiology. 2009;155:3500–8.

47. Van Gennip M, Christensen LD, Alhede M, et al. Inactivation of the rhlA gene in *Pseudomonas aeruginosa* prevents rhamnolipid production, disabling the protection against polymorphonuclear leukocytes. APMIS. 2009;117:537–46.

48. Wretlin B, Pavlovskis OR. *Pseudomonas aeruginosa* elastase and its role in pseudomonas infections. Rev Infect Dis. 1983;5(Suppl 5):S998–1004.

49. Tiouvanziam R. Neutrophilic inflammation as a major determinant in the progression of cystic fibrosis. Drug News Perspect. 2006;19:609–14.

50. Sonawane A, Jyot J, During R, Ramphal R. Neutrophil elastase, an innate immunity effector molecule, represses flagellin transcription in *Pseudomonas aeruginosa*. 2006. Infect Immun. 2006;74:6682–9.

51. Berger M. Inflammation in the lung in cystic fibrosis. A vicious cycle that does more harm than good? Clin Rev Allergy. 1991;9:119–42.

52. Wolters PJ, Chapman HA. Importance of lysosomal cysteine proteases in lung disease. Respir Res. 2000;1:170–7.

53. Ulrich M, Worlitzsch D, Viglio S, et al. Alveolar inflammation in cystic fibrosis. J Cyst Fibros. 2010;9:217–27.

54. Hoover DM, Rajashankar KR, Blumenthal R, et al. The structure of human beta-defensin-2 shows evidence of higher order oligomerization. J Biol Chem. 2000;275:32911–8.

55. Tate S, MacGregor G, Davis M, Innes JA, Greening AP. Airways in cystic fibrosis are acidified: detection by exhaled breath condensate. Thorax. 2002;57:926–9.