Airway epithelial innate host defence in chronic obstructive pulmonary disease

Amatngalim, G.D.

CHAPTER 2

Antimicrobial Peptides in Chronic Obstructive Pulmonary Disease.

Gimano D. Amatngalim and Pieter S. Hiemstra

Department of Pulmonology, Leiden University Medical Center, Leiden, The Netherlands

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is a frequent, chronic lung disease associated with significant morbidity and mortality. Respiratory infections play a central role in the disease, not only during exacerbations but also in the stable phase of the disease. These infections contribute to the development and progression of the disease, and many patients are colonized by respiratory pathogens. The pathogens are present in the lung, despite the presence of large numbers of neutrophils, especially during acute states of inflammation. These neutrophils may release antimicrobial peptides (AMPs) that may not only serve to kill these pathogens but also contribute to tissue injury and inflammation. In addition, smoke affects many elements of the host immune system, including the expression of epithelial AMPs. Furthermore, the activity of AMPs may be decreased in the purulent airway secretions often present in COPD patients. Possibly vitamin D treatment may contribute to restoring local AMP deficiency and thereby to reducing exacerbations in COPD.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a frequent respiratory tract disorder characterized by persistent airflow limitation (1). It is mainly caused by extensive exposure and an abnormal response toward harmful environmental substances and gases, most prominently, the exposure to cigarette smoke. Only ~20 % of the smoking population develops the disease, indicating the additional importance of genetics and other environmental predispositions. The progressive decrease in lung function in COPD is caused by airflow obstruction resulting from a variety of structural changes in the lung, including destruction of the alveoli and alveolar attachments (emphysema), mucus hypersecretion, and subsequent airway plugging especially in small airways, and changes in the airway wall. These structural changes are accompanied by a chronic inflammatory process in which a variety of cell types play a role. Small airway pathology is characterized by increased accumulation of neutrophils, macrophages, and T-cells during disease progression and the occurrence of B-cell lymphoid follicles in severe disease stages (2, 3). Neutrophils are regarded as one of the driving forces of emphysema, causing excessive tissue damage by an imbalance between neutrophil-derived proteases and protease inhibitors. This imbalance is also observed in genetically predisposed patients with alpha-1 antitrypsin deficiency development (4), a condition associated with liver disease and early onset emphysema.

RESPIRATORY INFECTIONS IN COPD

Chronic inflammation in stable COPD is frequently accompanied by bacterial and/or viral infections (5, 6). These infections contribute to persistence of airway inflammation and are thought to contribute to the etiology, pathogenesis, and clinical course of COPD. During acute exacerbations of COPD, a sudden decrease in airflow and an accompanying increase in airway inflammation is associated with the acquisition of new bacterial strains, most notable non-typeable *Haemophilus influenzae* (NTHi), *Moraxella catarrhalis*, and *Streptococcus pneumoniae* (7). Interactions between bacterial and viral pathogens may contribute to the intensity of exacerbations. This is illustrated by a study from Wilkinson *et al.*, who showed that simultaneous detection of bacterial (mostly NTHi) and viral (mostly rhinovirus) pathogens during an exacerbation is associated with an increased bacterial load, inflammation, and symptoms and with decreased lung function (8). It is now clear that infections not only contribute to increased inflammation during exacerbations but also in the stable phase of the disease. This is illustrated by a study by Bresser *et al.*, showing that NTHi colonization may contribute to airway inflammation and airflow obstruction in COPD (9). Various mechanisms may explain the observed bacterial persistence in COPD, including antigenic variation, acquisition of new strains, tissue invasion, and biofilm formation. The observation that COPD patients are frequently unable to eradicate bacteria from their airways despite the presence of antibacterial antibodies and the abundant presence of neutrophils suggests that tissue invasion and biofilm formation are important processes. Adherence to airway epithelial cells is an important process in bacterial persistence. NTHi may penetrate between epithelial cells (10) and even into epithelial cells (11), which may protect them from host defense systems in the lung as well as from antibiotics. This protection from host immunity and antibiotics is also achieved by formation of biofilms, in which bacterial adhesion is the first

essential step (12). Biofilms are microbial communities that adhere to a surface and in which the microorganisms are embedded in a self-produced matrix. Importantly, microorganisms present in biofilms are not always readily detected by conventional culture techniques. Therefore, molecular techniques including microbiome sequencing are important to gain insight into the lung microbiome in COPD. Several recent studies have used such techniques and provided important insight in the microbial population composition in COPD. Hilty *et al.* demonstrated in bronchial brushings and bronchoalveolar lavage (BAL) specimens of COPD patients an increase in *Proteobacteria*, mainly *Haemophilus*, *Moraxella*, and *Neisseria* species, and a decrease in the phylum *Bacteroidetes* compared to healthy controls (13). Erb-Downward *et al.* furthermore showed in severe COPD that the diversity of the microbiome is reduced (14). These initial findings show some differences with other studies which may have been caused by differences in study design, sampling techniques, and small sample sizes (15). Nevertheless, the overall conclusions are the same, showing that the lung microbiome in COPD differs from that in healthy subjects and smokers with normal lung function. The COPD patient lung microbiome also differs from the microbiome in patients with cystic fibrosis. Further microbiome studies in COPD are needed to explore a wide range of topics, including changes in the composition of the microbiome over time, its relation to disease severity and response to therapy, the influence of antibiotic therapy on the microbiome compositions, its role in development and progression of the disease, as well as regional heterogeneity.

ANTIMICROBIAL PEPTIDES IN COPD

The prominent role of respiratory infections despite intense inflammation in the COPD lung suggests that the pulmonary immune system does not function optimally in COPD. Antimicrobial peptides (AMPs) play a central role in host defense against infection in the lung (16), which is also supported by, e.g., *in vivo* mouse studies showing that gene deletion or overexpression of AMP genes affects pulmonary host defense against NTHi (17) and *Pseudomonas aeruginosa* (18). Based on this role of AMPs, several studies have investigated expression and activity of AMPs in COPD in search for an explanation for the increased susceptibility to infection and increased inflammation in COPD.

Neutrophil-derived antimicrobial peptides

The excessive number of neutrophils in the lung consequently leads to high detectable quantities of neutrophil-derived AMPs. A-defensins (human neutrophil peptides; HNP) are abundantly present in sputum samples of COPD patients and found elevated in more severe disease stages compared to mild-to-moderate COPD(19). Analysis of sputum and bronchoalveolar lavage fluid (BAL) from COPD patients using a proteomic approach specifically demonstrated an increase of HNP1 and HNP2 levels, while HNP3 levels were similar to those of healthy controls (20, 21). In addition, also in α-1 antitrypsin-deficient patients, higher levels of HNPs were observed (22, 23). Similar to HNPs, concentrations of the cathelicidin antimicrobial peptide LL-37 are elevated in induced sputum samples of mild-to-very severe COPD patients (24). Compared to nonsmokers, LL-37 was already increased to some extent in smokers with a normal lung function, an observation not noticed for HNPs (21). This suggests that cigarette smoking plays an important role in increasing LL-37 levels in the airway. Moreover, a study examining the relation of LL-37 with bacterial colonization during acute exacerbations

revealed that increased levels of LL-37 in sputum samples correlated with the acquisition of NTHi and *M. catarrhalis* (25). The usually protective role of neutrophils in host defense against pathogens may be dysfunctional in COPD. High levels of neutrophil-derived HNPs and LL-37 are suggested to contribute to the chronic inflammatory state (Figure 1) (26), and HNPs were found to increase bacterial adherence to epithelial cells (27). The combined release of AMPs with reactive oxygen species and other neutrophil granule–derived proteins, such as cathepsin G, elastase, and S100 proteins, causes extensive tissue damage that contributes

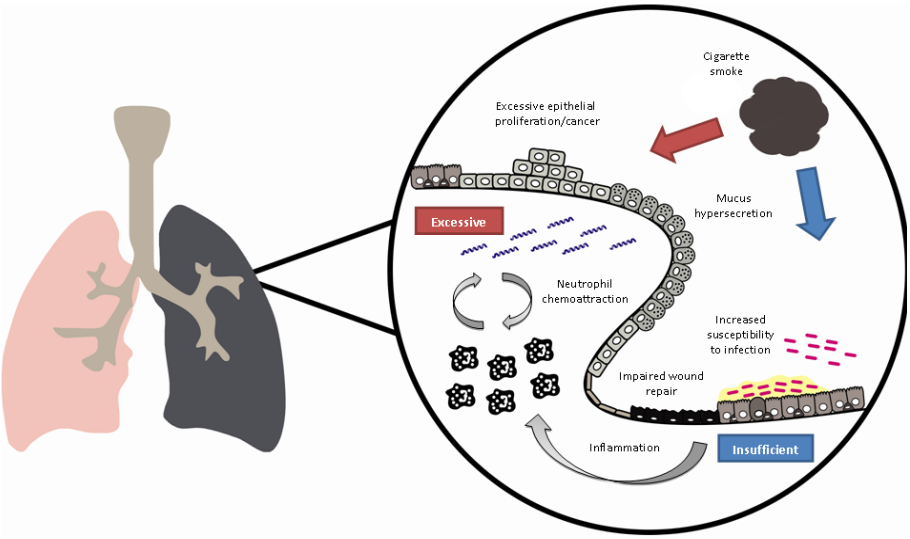

Figure 1. Dysregulation of antimicrobial peptide expression and activity by cigarette smoke. Cigarette smoke-induced chemoattraction of neutrophils causes excessive levels of neutrophil-derived AMPs in the lung, which may contribute to airway epithelial remodeling (including proliferation and mucus hypersecretion) and possibly carcinoma development, and further enhancement of neutrophilic inflammation. This mechanism may be increased by posttranslational modification of AMPs such as HNPs and LL-37, which may be increased in smokers and alter the activity of these peptides. Cigarette smoke furthermore not only impairs expression of AMPs by airway epithelial cells exposed to microbial stimuli but also decreases ciliary activity and increases mucus hypersecretion. These mechanisms contribute to an increased susceptibility to respiratory tract infections and may impair proper wound healing. Moreover, these respiratory infections lead to a further increase in airway inflammation.

to airway remodeling and the maintenance of inflammation (28). The release of neutrophil extracellular traps (NETs), consisting of complexes of DNA including high levels of LL-37 and HNPs, has been shown to contribute to autoimmune diseases via the development of autoantibodies (29). However, although various studies have provided evidence for an autoimmune response in COPD [e.g. (30), it is unclear whether autoimmunity contributes to COPD development and progression or may be a response to tissue injury that in itself is not pathogenic. In vitro experimental data support the increased cytotoxic activity and

immunomodulatory properties of HNPs and LL-37 at high concentrations. Exposure of airway epithelial cells to HNPs may result in cell death, whereas lower concentrations increase the secretion of pro-inflammatory cytokines, including the neutrophil chemoattractant IL-8 (31, 32). Furthermore, HNPs also increase the transcription and secretion of the mucin MUC5AC by an airway epithelial cell line (33, 34). This finding indicates a potential role in promoting mucus hypersecretion. A further role in decreased mucociliary clearance was suggested by the observation of an association between increased HNP levels and squamous metaplastic epithelium (35). This finding combined with the mitogenic activity of neutrophil defensins toward airway epithelial cells (33, 36) suggests a possible role in airway epithelial remodeling. HNPs can also contribute to the increased bacterial colonization in acute exacerbations as it has been demonstrated that the peptides increase the adhesion of NTHi, *M. catarrhalis*, and *S. pneumoniae* toward the airway epithelial surface (27, 37). Adhesion may be the first step in biofilm formation, or facilitate intra- or intercellular localization, mechanisms that may provide protection against the host immune system. Similar to HNPs, LL-37 can contribute to the increased neutrophilic infiltration in the lung by inducing the expression of IL-8 by airway epithelial cells and airway smooth muscle cells (38, 39) or via a direct chemotactic activity (40). Furthermore, it has been shown that LL-37 can drive macrophage differentiation toward a pro-inflammatory phenotype, thereby potentially increasing the inflammatory state in COPD (41). Similar to HNPs, it has been shown that LL-37 also increases airway epithelial cell proliferation (42), which may contribute to epithelial remodeling. The switch in function from antimicrobial effectors toward harmful mediators is not a unique property of HNPs and LL-37 at high concentrations. Several studies report that cigarette smoke-related posttranslational modifications of both AMPs may affect their function. The converting enzyme responsible for ADP-ribosylation of HNP1, arginine-specific ADP-ribosyltransferase 1, is increased in smoking individuals, and levels of ADP-ribosylated HNP1 are increased in BAL fluid from smokers 43. This may have important functional consequences, since ADP-ribosylation of HNP1 decreases the antimicrobial effect of the peptide, while increasing the cytotoxic and IL-8 inducing properties (43, 44). Recently, citrullination was described as a novel mechanism for posttranslational modification of LL-37 that may also be increased in smokers, and it was demonstrated that citrullination of LL-37 decreases antimicrobial activity and increases chemotactic activity (45). Citrullination of proteins is mediated by members of the peptidylarginine deiminase (PADI) family and expression of PADI2, and presence of citrullinated proteins was found to be increased in the lungs of smokers (45, 46). This points toward a potential mechanism by which modification of HNPs and LL-37 alters the activity of these peptides, which may contribute to the development and progression of COPD.

Epithelial expression of antimicrobial peptides

In contrast to HNPs and LL-37, levels of human β-defensin-2 (hBD-2) are decreased in induced sputum samples and BAL of COPD patients (47). Furthermore, Herr *et al.* found an association between decreased hBD-2 levels and cigarette smoking in patients hospitalized with an acute pneumonia (48). The cigarette smoke–mediated suppression of hBD-2 expression seems to be persistent, as 1 year smoking cessation did not result in an increase in hBD-2 sputum levels in asymptomatic smokers (49). In COPD patients, hBD-2 expression is predominantly decreased in the central airways, while in the distal airways the expression was increased compared to controls (50). A previous report showed that hBD-2

depletion in BAL supernatants increased the number of apoptotic neutrophils, suggesting a role of hBD-2 in protection of cells from apoptosis (51). In contrast to HNPs, hBD-2 does not induce IL-8 expression in airway epithelial cells (52), so it remains to be further investigated if hBD-2 contributes to inflammation in COPD. In vitro antibacterial assays demonstrate efficient killing activity of hBD-2 toward acute exacerbation-associated bacteria (53). This suggests that the observed suppression of hBD-2 expression in COPD in central airways contributes to the increased bacterial colonization during acute exacerbations. As the airway epithelium is regarded as the main cellular source of hBD-2 in the airways (54), it is hypothesized that airway epithelial cells display an impaired host defense activity (Figure. 1). *In vitro* experiments demonstrate that prior cigarette smoke exposure of air–liquid interface-cultured airway epithelial cells inhibits *P. aeruginosa*- and *M. catarrhalis*-induced expression of hBD-2 (48, 55). Furthermore, it was shown that apical surface fluid derived from these cells displayed decreased antimicrobial activity. Stimulation of epithelial cells with the cigarette smoke–derived compound acrolein similarly showed an inhibition of hBD-2 expression (56). In contrast to *in vitro* experiments, cigarette smoke exposure in murine models showed increased mouse β-defensin-2 expression in the lung (57), which is not in line with the findings in these *in vitro* studies, and observations in central airways and airway secretions in human studies (47, 48, 50). Recent studies have highlighted a role for vitamin D in the regulation of expression of antimicrobial peptides in epithelial cells and macrophages (58, 59). Furthermore, vitamin D deficiency is frequent in COPD and correlates with disease activity (60). This would suggest that vitamin D supplementation may be beneficial in COPD and may be a good strategy to prevent exacerbations that are so frequently associated with infections (61). A recent intervention study showed that high-dose vitamin D supplementation may indeed reduce exacerbations in those COPD patients with severe vitamin D deficiency (62).

Genetic and Epigenetic Mechanisms

Several studies have investigated the association between genetic and epigenetic differences of AMPs with COPD development. Both α- and β-defensin-coding genes are localized at highly polymorphic regions (63). Genetic association studies assessing the role of single nucleotide polymorphisms (SNPs) in the gene encoding human β-defensin-1 (hBD-1) with COPD development revealed population-dependent outcomes. The hBD-1 gene contains polymorphisms in both the promoter region and the two coding exons (64). In a Japanese population study, the nucleotide polymorphism in exon 2, resulting in a change of valine to isoleucine at position 38, was more frequent in COPD patients compared to controls (65). A study in a Chinese Han population describes an association between a polymorphism localized at exon 1 (44 C/G) with COPD susceptibility (66). In Caucasians, an association between SNPs in the hBD-1 gene and COPD could not be found (67, 68). In addition, also polymorphisms in HNP1/3 were not associated with COPD development in such populations (67). This indicates that the relation between polymorphisms and disease may differ between populations and that further studies on the functional consequences of these polymorphisms are needed. Janssens *et al.* examined the association of copy-number variations of the hBD-2 gene with COPD development (69). Using *in vitro* cultured epithelial cells, it was shown in this study that five and higher diploid copy numbers of the hBD-2 gene was significantly more often present in COPD patients compared to controls. Moreover, it was shown that epithelial cells with high diploid copy number displayed a higher expression of hBD-2 induced by TNF-α and furthermore have a higher bacterial killing activity. These results are

in contrast with earlier mentioned observations of a decrease in hBD-2 levels in BAL and induced sputum and inhibition of hBD-2 expression by airway epithelial cells after cigarette smoke exposure. Therefore, the additional effects of environmental factors on the induced expression of hBD-2 in COPD patients with high copy numbers of the hBD-2 gene should be taken into consideration. Andresen *et al.* investigated the role of epigenetics in hBD-1 expression in COPD (70). Using airway epithelium and cells derived from BAL, it was shown that mRNA levels of hBD-1 were higher in cells of COPD patients with mild to very severe disease compared to cells of healthy controls. The difference in mRNA levels was not due to a difference in DNA methylation of the hBD-1 gene promoter, but rather correlated with histone H3 lysine 4 methylation. These studies highlight that copy-number variations and epigenetic mechanisms may contribute to the control of expression levels of antimicrobial peptides in COPD.

Activity of Antimicrobial Peptides in COPD

During airway inflammation and infection, the local environment in which AMPs are active undergoes dramatic changes. Increased production of mucus as a result of smoking, inflammation, and infection may impact on local host defense against infection. Whereas the mucus layer that is positioned on top of the periciliary layer (Figure. 2) normally acts to trap and remove inhaled particles and pathogens, decreased mucociliary clearance in COPD

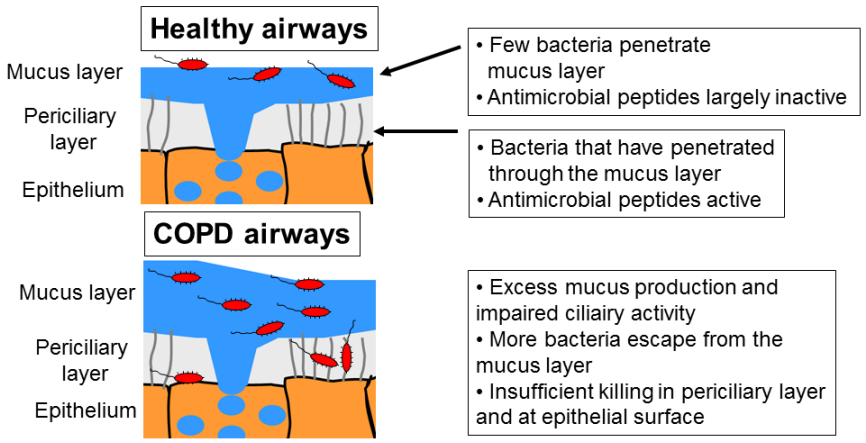

Figure 2. Mucus, antimicrobial peptides, and microbial pathogens. In COPD, mucus hypersecretion and decreased mucociliary clearance may allow bacteria to escape from the mucus layer in numbers that are too large for efficient killing in the periciliary layer and epithelial surface

prevents this process. Mucus itself does display antimicrobial activity because of its barrier and biochemical properties, but microorganisms have developed escape mechanisms such as flagella-mediated motility and enzymatic degradation of mucus (71). Mucins are large, heavily glycosylated glycoproteins that are essential components of mucus. Mucins have been shown to restrict the antimicrobial activity of LL-37 (72, 73), and therefore, these peptides may not contribute optimally to antimicrobial activity of mucus. In healthy airways, this is not

a problem, since mucus is removed. Furthermore, the bacteria that escape from the mucus layer are likely to be killed by antimicrobial peptides present in the periciliary layer (Figure. 2). However, because of excess mucus production and impaired ciliary activity, in COPD more bacteria may penetrate the mucus layer and reach the epithelial surfaces. However, also other factors in the inflamed airways of COPD patients may have a negative impact on the antimicrobial activity of AMPs. It has been shown that a wide range of microbial and host proteases are able to degrade and inactivate AMPs (74-76). In addition, products such as F-actin and DNA (77) that are released by dead cells, glycosaminoglycans (78, 79), and bacterial polysaccharides (80) may inhibit antimicrobial activity of AMPs. Finally, bacteria have evolved mechanisms to escape the antimicrobial activity of peptides. One such mechanism is the development of biofilms that provide protection against the action of the host immune system. Interestingly, bacteria in biofilms may not be fully protected against the action of antimicrobial peptides, since, e.g., LL-37 (81) and lactoferrin (82) have been shown to prevent biofilm formation and to act on bacteria present in biofilms.

CONCLUDING REMARKS

Recent studies point to a clear role of respiratory infection and AMPs in COPD. Microbiome analysis using unbiased molecular biological methods is still in its infancy but is pointing toward an altered microbiome also in stable COPD. Local excessive release of AMPs by, e.g., neutrophils may contribute to inflammation and possibly autoimmunity, whereas a local deficiency as a result of epithelial smoke exposure or inactivation of AMPs may contribute to respiratory infections. Biofilm formation impairs host defense against infection, but some AMPs may contribute to the fight against biofilm formation. Whether enhancement of local AMP production by, e.g., vitamin D treatment or administration of novel drugs based on the structure of endogenous AMPs holds a future in COPD treatment requires additional studies.

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