Expert Opinion

General

The role of cathelicidin and defensins in pulmonary inflammatory diseases

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Antimicrobial peptides (AMPs) protect the epithelia of mucosal organs like the respiratory or the gastrointestinal tract from invading microorganisms. As an integral part of the innate immune system they display antimicrobial activity against Gram- and Gram-negative bacteria as well as against fungi and enveloped and non-enveloped viruses. Besides their microbicidal effects they have important functions in the regulation of repair and inflammation. AMPs are sometimes referred to as 'alarmins' due to their ability to recruit, modulate and activate components of the immune system. In contrast, some AMPs suppress activation of the immune system. AMPs are also involved in tissue repair, cancer biology and angiogenesis. Based on their antimicrobial and immunomodulatoy functions, AMPs are probably involved in the pathogenesis of infectious and inflammatory diseases of the lung. Inborn or acquired deficiencies contribute to susceptibility to infection and colonisation. The potential pro-inflammatory role of AMPs contributes to the disease processes in inflammatory disorders such as asthma, chronic obstructive pulmonary disease, sepsis or pulmonary fibrosis. This review summarises the knowledge about the functions of AMPs in the pulmonary innate host defence system and their role in respiratory disease.

Keywords: antimicrobial peptides, cathelicidin, defensin, innate immunity, LL-37, pulmonary disease

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1. Introduction

The epithelial barrier of the body is constantly exposed to microbial threats and, therefore, is a critical player in the defence of the body. Antimicrobial peptides (AMP) play an important role in the nonspecific defence mediated by the innate immune system. They act in the first-line of defence to defeat microbial conquests. Recent findings reveal not only antimicrobial properties but show that AMPs play a critical role in inflammation, tissue regeneration, and are involved in the pathogenesis of several diseases. The respiratory tract is shielded by a multi-component system of defence mechanisms, involving soluble and structural factors [1]. Besides parenchymal cells, such as airway and alveolar epithelium [2], soluble factors released by structural and myeloid cells play an important role. Secretions of the airways contain proteins and peptides that directly kill pathogens or modulate the inflammatory response. Well-known antimicrobial components of the airway surface fluid (ASF) are lysozyme, lactoferrin, secretory phospholipase A2 and secretory leukocyte protease inhibitor (SLPI). Other soluble factors, such as like complement, surfactant proteins, clara-cell proteins (CC10/CCSP), and proteins from the palate, nasal and epithelial clone family [3] also play an important role in host defence. An essential part of the antimicrobial activity of

human airway fluid resemble cationic polypeptides of several cellular sources including airway epithelium, serous gland cells, and myeloid cells such as neutrophils, macrophages and lymphoid natural killer cells.

2. Overview about antimicrobial peptides of the lung

2.1 Structures and families of antimicrobial peptides

AMPs can be grouped according to size, tertiary structure or predominant amino acids. The principal families of AMPs of the respiratory tract are cathelicidins and defensins.

2.1.1 *Cathelicidins*

Peptides of the cathelicidin family of antimicrobial peptides are found in many mammalian species such as bovine, mouse, rabbit and humans [4-8]. They share a highly conserved signal sequence (preregion) and a cathelin-like proregion (cathelin = cathepsin L inhibitor) but show substantial heterogeneity in the C-terminal domain that encodes the mature peptide. The microbicidal C-terminal domain can range in size from 12 to 80 or more amino acids [4] and take α-helical forms in human, rabbit and mouse [9-11] and β-sheet structures in pigs [12,13]. LL-37/hCAP-18 is the only human cathelicidin and was isolated first from bone marrow [9,14]. The peptide is expressed in myeloid cells and stored in its pro-form in specific granules but is also produced by airway epithelial cells, macrophages, lymphocytes, neutrophils and is secreted into the ASF [15,16]. It has been detected in supernatants of primary explants from respiratory epithelial cells as well as in bronchoalveolar lavage fluid from patients [15,17]. The peptide is generally cleaved by serine proteases in a cathelin N-terminal part and a C-terminal part of 38 or less amino acids. In neutrophils, LL-37/hCAP-18 is stored in specific granules in its pro-peptide form and cleaved after secretion by the serin-protease protease-3 [18]. In seminal plasma the pro-peptide is processed by the protease gastricsin to an AMP termed ALL-38 [19]. Processing of hCAP-18 by serine proteases of the kallikrein family on the skin generates several smaller peptides with increased and diverse antimicrobial activity compared with LL-37 [20,21]. The cathelin-like prosequence shows significant antimicrobial and antiprotease activity [22]. The processing of LL-37/hCAP-18 in the respiratory tract remains unclear.

2.1.2 *Defensins*

The defensin family of antimicrobial peptides in vertebrates consists of two major subgroups classified by a highly conserved pattern of three disulfide bridges [23]. α-Defensins 1 – 4 were first isolated from neutrophil primary (azurophilic) granules and are, therefore, conventionally referred to as human neutrophil peptides 1 – 4 (HNP 1 – 4) [24,25]. Human α-defensin-5 (HD5) and -6 (HD6) are secreted from Paneth cells of the small intestine [26,27]. HNP 1 – 4 are present in airway secretions originating from neutrophils that invade the airway or alveolar lumen. The first human β-defensin, called human β-defensin-1 (hBD-1), was isolated from large volumes of hemofiltrate [28] and is expressed constitutively in epithelial cells of the urinary and respiratory tracts [29-31]. β-defensin-2 (hBD-2) was isolated from human psoriatic skin by affinity chromatography [32] and from airway epithelium by a database-screening approach [33]. hBD-2 is expressed by epithelia of the respiratory tract and other body surfaces [33,34] and, like hBD-1, by monocytes, macrophages and dendritic cells [35]. In airway secretions hBD-1 and -2 have been detected at μg/ml concentrations [33,34]. hBD-3 was identified in parallel by using bioscreening and computational approaches [36-38], whereas hBD-4 was identified solely by searches of genomic databases [39]. Both peptides have been identified in human milk [40] and the respiratory tract [41,42]. With the advent of detailed genomic databases, up to 28 new human and 43 new mouse β-defensin genes in 5 syntenic chromosomal regions have been identified by computer-based screening of the human and murine genomes [43]. The third class of defensins present in mammals has been solely found in rhesus monkey neutrophils and named θ-defensins or retrocyclins, according to their circular molecular structure and consist of two truncated α-defensin transcripts [44,45]. Like α- and β-defensins, they posses broad spectrum microbicidal activity against bacteria and fungi and are able to protect peripheral blood mononuclear cells against infection from several HIV strains [46,47]. In contrast to most other defensins characterised so far, their bactericidal activity is relatively unaffected by addition of sodium chloride [48]. In humans, only one pseudogene encoding a putative θ-defensin has been found [46].

2.1.3 *Regulation of antimicrobial peptides in the lung*

The expression of AMPs in the lung is regulated in a cell-type and stimulus-specific manner and can be either constitutive or inducible. The neutrophil-derived α-defensins HNP 1 – 4 and the α-defensins HD 5 – 6 from paneth cells as well as hBD-1 are constitutively expressed [24,26-28,49-51]. Increased concentrations of hBD-1, hBD-2 and LL-37 can be found in response to inflammatory stimuli in tracheal aspirates from mechanically ventilated newborn infants [52]. Expression of hBD-2, hBD-3 and hBD-4 is induced by pro-inflammatory stimuli or microorganisms [32,37-39,53,54]. hBD-2 signalling pathways involve NF-κB [55] and MAPKs [56], including Src-dependent Raf-MEK1/2-ERK [57]. Binding sites for NF-κB, AP-1 and STATs [55,58] have been identified in the promoter region of hBD-2. In epithelial cells, the transcription factor MEF acts as a transactivator of hBD-2, which functions through a mechanism independent of NF-κB [59]. Intracellular calcium and AP-1 are also involved in hBD-2 expression in airway epithelial cells [60]. *In vitro* models showed that human airway epithelial cells grown at air–liquid interface respond to bacterial lipopeptides in a toll-like receptor (TLR)-2-dependent manner with upregulation of mRNA transcript and protein levels of hBD-2 [58,61,62]. hBD-2 expression in airway epithelial cells can also be

upregulated by lipopolysaccharide (LPS) through a CD14-dependent mechanism [63], regulated by cytokines secreted by activated macrophages [64], and can be induced by neutrophil elastase [65]. The expression of the only human cathelicidin hCAP-18/LL-37 is induced by inflammatory or infectious stimuli [14,66] in neutrophils, bone marrow and testis. In human macrophages a TLR-triggered and vitamin-D mediated upregulation of cathelcidin mRNA has been reported [67,68]. The release of LL-37 at sites of inflammation triggers IL-8 release from human airway smooth muscle cells through the purinergic $P2X_7$ receptor [69]. In summary, AMPs are tightly regulated during a host defence reaction. The activity of defensins in cystic fibrosis (CF) lung disease is relatively suppressed [70]. T helper (T_H)2 cytokines are able to suppress the antibacterial host defence by inhibition of AMP induction through bacteria in airway epithelium *in vivo* and *in vitro* [71]. Much less is known about the inactivation of AMPs in chronic inflammatory diseases. Elastolytic cathepsins seem to be involved in degradation of hBD-2 and -3 [72]. Proteases secreted by common pathogenic bacteria degrade and inactivate the AMP LL-37 [73]. The antimicrobial activity of LL-37 in airway secretions is inhibited by bundle formation with F-actin and DNA [74] and by interaction with mucins [75] and bacterial polysaccharides [76].

2.2 Functions of antimicrobial peptides in the respiratory tract

AMPs have various activities and functions that are summarised in the following paragraphs. **Figure 1** illustrates these functions.

2.2.1 *Antimicrobial activity*

The antimicrobial activity of peptide antibiotics was deduced from *in vitro* tests assaying purified substances against microorganisms. AMPs have broad spectrum activity against Gram-positive and Gram-negative bacteria, as well as against fungi and enveloped and non-enveloped viruses, especially when examined under low concentrations of salt and plasma proteins [28,32,37-39,77,78]. Although the mechanism by which defensins kill microbes is not completely understood, direct interaction of micromolar concentrations with target microorganisms destabilises and disrupts their cell membranes, resulting in increased permeability and leakage of small molecules [79]. Individual antimicrobial components of the airway surface synergistically act against microorganisms.

Several groups have published results that provide proof of the host defence function of AMPs in living organisms. Indirect *in vivo* evidence for the host defence function of AMPs came from a study in a murine model with a disrupted gene for matrilysin (metalloprotease 7). Mice with missing matrilysin were more susceptible to infection with enteropathogens [80]. Studies in a human bronchial xenograft model revealed decreased antimicrobial activity of ASF after inhibition of hBD-1 transcription by antisense oligonucleotides [30]. Mice deficient in the AMP β-defensin-1 (mBD-1) revealed delayed clearance of *Haemophilus influenzae* from lung [81] or higher bacterial load in the urinary tract [82]. Mice with deleted CRAMP, the murine homologue of LL-37, showed a higher degree of infection after inoculation of bacteria on the skin [83] or the urinary tract [84]. In reverse, the overexpression of LL-37 by viral gene transfer resulted in augmentation of innate host defence in a bronchial xenograft model of CF and in murine models of pneumonia and septic shock [33,85]. The transgenic expression of a human intestinal defensin in mice protected against enteric salmonellosis [86]. A vitamin-D-dependent upregulation of LL-37/hCAP-18 expression in human macrophages protects from infection by *M. tuberculosis* via activation of TLR2/1 and vitamin D receptor [67]. Recently, it has been shown that AMPs exert broad and diverse activity against enveloped and non-enveloped viruses by either direct interaction with the virion or effects on the target cell and on innate and adaptive immunity [87]. It has been shown that bronchial and oral epithelial cells upregulate mRNA expression for hBD-2 and hBD-3 after exposure to HIV-1 and human rhinovirus [54,88,89]. Human α-defensins seem to be involved in the defence against HIV-1 [90-93]. Some microbial pathogens respond to antimicrobial threats of the host immune system rendering them less vulnerable. An increase of the phosphocholine content of the cell walls of *H. influenzae* decreases bacterial susceptibility to LL-37 [94]. When exposed to the environment found in the airways of CF patients, *Pseudomonas aeruginosa* modifies the structure of the LPS layer of the outer membrane [95]. The alterations of the endotoxin decrease the susceptibility of these bacteria to cationic AMPs.

The airway microenvironment has effects on the ability of antimicrobial peptides to defend the airways against invading pathogens. The highly cationic antimicrobial peptide LL-37 interacts with mucins of the ASF [75]. This interaction may be important in lung diseases with abnormal ASF content, such as CF [96]. Inflammatory diseases of the airways, such as chronic obstructive pulmonary disease (COPD) or chronic asthma, are associated with increased susceptibility for bacterial infections [97,98]. This might be due to insufficient mucociliary clearance [99], inactivation of host defence systems by pro-inflammatory cytokines [71], some of which are induced by the release of bacterial antigens [97,98] or by proteases released from invading bacteria that degrade and inactivate host defence functions [73].

2.2.2 *Antimicrobial peptides and inflammation*

AMPs have a variety of other biological effects beside their antimicrobial activity. Based on their membrane activity, AMPs act in a concentration-dependent manner, not only towards microorganisms but also towards eukaryotic cells. In patients with inflammatory diseases such as CF and chronic bronchitis, high concentrations of α-defensins in airway secretions have been reported [100,101]. Besides the

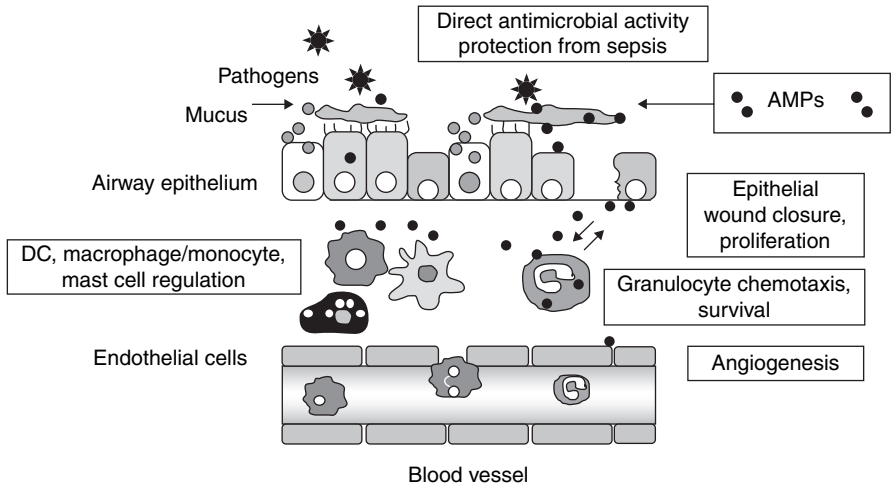

Figure 1. Overview about the functions AMPs in pulmonary biology. AMPs are produced from several cell types including airway epithelial cells and host defence cells (macrophages, neutrophils and lymphocytes). They are endogenous antibiotics with direct antimicrobial activity. In addition, they have activities as mediators and modulate diverse processes ranging from angiogenesis and wound healing to inflammation and immunity.
AMP: Antimicrobial peptide; DC: Dendritic cell.

direct cytotoxic effect caused by high concentrations of α-defensins [102,103], the inflammatory reaction is exacerbated by induction of IL-8 expression through α-defensins [104,105] in bronchial epithelial cells and subsequent recruitment of neutrophils [106]. Furthermore, inflammation may be driven by binding of α-defensins to protease inhibitors of the serpin family, such as α1-antitrypsin, thereby inactivating their antiprotease activity [107]. When instilled into the respiratory tract of animals, neutrophil defensins mediate an acute inflammatory response [108]. Cathelicidins, defensins and other AMPs, such as eosinophil-derived neurotoxin (EDN) or high mobility group box protein 1 (HMGB1), are often referred to as 'alarmins' because of their immediate release after pathogen challenge and their ability to recruit and activate components of the immune system [109]. On the other hand, in severe inflammatory situations like septic shock, cathelicidins are able to bind and inactivate LPS [110] and protect from septic shock in animal models [111].

2.2.2.1 *Antimicrobial peptides, acquired and innate immunity*

There are many ways in which defensins and cathelicidins impinge on the acquired immune system. The development of an acquired immune response can be boosted by α-defensins by enhancing systemic IgG but not IgA, antibody responses through costimulatory $CD4^+$ T_H1- and T_H2-type helper cytokines and foster B and T cell interactions thereby linking innate and adaptive immunity [112]. Many AMPs are chemoattractants for leukocytes and other immune cells. HNP 1 – 3, hBD-1, hBD-2, hBD-3, mBD-2 and mBD-3 have been found to be chemotactic for immature dendritic cells [113]. Human α-defensins also chemoattract lymphocytes [114,115], and HNP 1 – 3 are chemotactic for monocytes via a G-protein-coupled receptor [114,115]. Murine β-defensin-2 linked to a fusion protein has a chemotactic effect on CCR6 expressing immature dendritic cells [116]. hBD-1 and hBD-2 were found to bind to the chemokine receptor CCR6 [117], which is expressed on immature dendritic and memory T cells ($CD4^+$/$CD45RO^+$). hBD-3 and hBD-4 chemoattract monocytes by mechanisms that have not yet been clarified [38,39]. hBD-2 directly and specifically induces mast cell migration through a pertussis toxin-sensitive and phospholipase C-dependent pathway [118].

Apart from α- and β-defensins, the cathelicidin LL-37 is able to modulate the adaptive immune response. LL-37 was found to bind to formyl peptide receptor-like 1 (FPRL1), a promiscuous receptor expressed on a variety of cells including neutrophils, monocytes and lymphocytes [119]. It has recently been shown that interaction of LL-37 with FPRL1 and $P2X_7$ receptors on neutrophils suppresses neutrophil apoptosis in bacterial infections [120]. By activation of this G-protein-coupled receptor, LL-37 attracts neutrophils, monocytes and CD4 T cells, and activates mast cells [121]. In human primary monocytes but not in B or T lymphocytes, LL-37 activates the extracellular signal-regulated kinase (ERK) and p38 kinase via a G-protein-coupled receptor-independent mechanism not yet determined [122]. LL-37 also leads to IL-1β processing and release form monocytes [123]. This effect appears to be mediated by the $P2X_7$ receptor. LL-37 also influences dendritic cell differentiation and dendritic cell-induced T cell polarisation [124]. Dendritic cells generated from blood monocytes under the influence of LL-37 have significantly upregulated endocytic capacity, modified expression of phagocytic receptors, enhanced costimulatory molecule expression and secretion of T_H-1-inducing cytokines [124]. LL-37 is able to block dendritic cell TLR4 activation and

allergic contact sensitisation by direct interaction with the cell membrane [125,126].

The biological impact of the receptor-mediated activities is completely unclear. Although recent observations implicate immunomodulatory properties of AMPs, it remains unclear whether AMPs act as principal ligands for the mentioned receptors or whether they modulate the activities of other ligands.

2.2.2.2 *Wound healing and proliferation*

Besides their multiple roles in host defence and immunity, AMPs are also involved in wound healing and proliferation. Human neutrophil defensins induce proliferation of airway epithelial cell lines via an EGF receptor-independent, MAPK signalling pathway [127]. Furthermore, neutrophil defensins enhance lung epithelial wound closure and mucin gene expression *in vitro* [128]. LL-37 activates human airway epithelial cells by activation of the MAPK/extracellular signal-regulated kinase (ERK) and increased release of IL-8 [129]. *In vitro* data suggest that LL-37 transactivates the EGF receptor involving metalloproteinase-mediated cleavage of membrane-anchored EGF receptor ligands [129]. LL-37 also plays a role in wound closure and re-epithelialisation of human skin and airway epithelium [130,131] and stimulates angiogenesis in endothelial cells by activation of FPRL1, resulting in increased proliferation and formation of vessel-like structures [132]. Application of LL-37 results in neovascularisation in the chorioallantoic membrane assay and in a rabbit model of hindlimb ischaemia [132]. Although completely different in structure PR-39, a porcine member of the cathelicidin family of antimicrobial peptides, stimulates angiogenesis by binding to the α-7 subunit of the 26S proteasome and modulates the ubiquitin-proteasome pathway without affecting overall proteasome activity [133]. Further, PR-39 is chemoattractive for neutrophils in a calcium-dependent and pertussis toxin inhibitable reaction and contributes to wound healing by stimulating the expression of syndecans, cell surface heparane sulfate proteoglycans [134].

In summary, vertebrate AMPs have a variety of additional functions beside their microbicidal function. The impact of these non-microbicidal functions on the pathogenesis of diseases is speculative. The non-microbicidal functions offer interesting opportunities to investigate the roles of AMPs in inflammatory diseases; however, they might also cause side effects when these peptides are used as therapeutics.

3. Role of antimicrobial peptides in pulmonary disease

AMPs may have a role in a variety of infectious and inflammatory diseases of the lung. Based on their functions, several pathogenic models are relevant:

- Inborn or acquired deficiencies of AMPs result in loss of function and subsequent increased susceptibility to infections. Only very limited data on states of decreased activity of AMPs are available. In morbus Kostmann [135], a severe congenital neutropenia, periodontal disease has been linked with the deficiency of AMPs in neutrophils [136]. A deficiency in the expression of AMPs may account for the susceptibility of patients with atopic dermatitis to skin infection with *Staphylococcus aureus* [137]. Chronic inflammation seems to be related with depressed expression of AMPs [71,138-140]. Cigarette smoke has adverse effects on the expression of AMPs. The concentration of hBD-2 in pharyngeal washing fluid and sputum from smokers or former smokers is significantly diminished as compared to people that never smoked (own unpublished results). Cigarette smoke also decreased the expression of hBD-2 in differentiated primary human airway epithelia cells following bacterial challenge (own unpublished results). Extracts from tobacco smoke also downregulates the LPS-induced expression of pro-inflammatory cytokines *in vitro* and thereby contributes to higher susceptibility of smokers to infection of the airways [141].
- Several pulmonary diseases are linked to inflammation that is associated with overexpression of AMP genes. Based on the receptor-mediated functions of AMPs, increased concentrations may have a pro-inflammatory effect. Several examples are given below.
- Polymorphisms of genes of AMPs may predispose to the development of pulmonary diseases. Several polymorphisms have been found in genes of β-defensins [142-145]. Polymorphisms of hBD-1 are associated with oral *Candida* carriage [146]. Variable numbers of copies of defensin genes contribute to the genetic complexity of these peptides [147].

3.1 Pneumonia and tuberculosis

Infections of the respiratory tract are one of the most common disease groups. High numbers of hospital-acquired pneumonias and increasing numbers of infections with multiple resistant bacteria are prominent problems. Several studies have found increased concentrations of defensins during infectious pulmonary diseases such as neonatal and adult pneumonia [52,71,148]. Elevated levels of α-defensins were found in patients with empyema [149]. Tuberculosis is an infectious disease that in most cases involves the lung. The number of multi-resistant *Mycobacterium tuberculosis* strains is increasing. Levels of defensins are increased in plasma or bronchioalveolar lavage fluid in pulmonary tuberculosis [150] and infections with *Mycobacterium avium-intracellulare* [151]. In a mouse model of mycobacterial infection, there was an initial rapid expression of murine β-defensin-3 and -4 by respiratory epithelial cells that decreased again during the later progressive phase of infection [152]. Recent results provide a direct connection between exposure to sunlight and susceptibility to microbial infections in humans. Sunlight, especially ultraviolet B, triggers the conversion of 7-dehydrocholesterol to vitamin D_3, which is converted

to 1,25-dihydroxy-D_3 (1,25-D_3) (e.g., in keratinocytes) [153]. TLR2/1 heterodimers on the surface of circulating macrophages are activated by *M. tuberculosis* in the lung and induce the expression of the vitamin D receptor (VDR). VDR together with 1,25-D_3 directly induces the expression of hCAP-18/LL-37 in circulating macrophages and protects from *M. tuberculosis* [67,68,154].

3.2 Cystic fibrosis and diffuse panbronchiolitis

Cystic fibrosis and diffuse panbronchiolitis are characterised by chronic infection associated with overwhelming neutrophilic inflammation. CF is caused by a genetic defect of the CF transmembrane conductance regulator (CFTR). CF represents a model disease for defects of the innate host defence and research on this disease attracted significant attention to the field of AMPs in the late 1990s. Whether AMPs have a direct role in the initial processes that link the defective CFTR with impaired host defence is unclear. The biogenesis or secretion of functional antimicrobial substances may be altered by intracellular defects in airway epithelial cells, as suggested by a salt-independent decrease of antimicrobial activity of CF airway secretions [155]. In contrast, it appears that AMPs contribute to the overwhelming inflammatory activity. In CF airways, α-defensins have been found at increased levels [100]. Several reports have found increased β-defensin protein concentration in CF airways [34,155]. In contrast, the expression of β-defensins in CF airway epithelial cells seems to be suppressed in the setting of chronic inflammation [70,156].

Diffuse panbronchiolitis is a chronic inflammatory lung disease of unknown origin that is phenotypically related to CF [157]. Neutrophil-derived defensins are elevated in the airways in diffuse panbronchiolitis and may be a marker of neutrophil activity in this disease [158]. Increased concentrations of β-defensins have been found in plasma and bronchoalveolar lavage fluid (BALF) of patients with this disease [159].

3.3 Asthma and chronic obstructive pulmonary disease

Asthma and COPD are obstructive pulmonary diseases that are characterised by airflow limitation and a chronic inflammatory process of the airways. These diseases have outstanding medical and economic importance. Inflammation in asthma is characterised by T_H2 orchestrated inflammation; whereas in COPD, chronic smoke exposure (or other rare causes) results in neutrophil influx and activation of proteases. Based on their function as inflammatory mediators, AMPs likely are to be involved in the pathogenesis of these diseases. Allergic airway inflammation suppresses the expression of AMPs [71]. A polymorphism of the hBD-1 gene is found at higher frequency in COPD patients [143], although more recent linkage studies have found only weak linkage between polymorphisms in defensin genes and susceptibility to COPD [160].

3.4 Acute respiratory distress syndrome

Acute respiratory distress syndrome (ARDS) is a catastrophic inflammatory pulmonary disease that can be caused by a variety of conditions such as trauma, hypoxia, infection or intoxication. Neutrophil defensins are elevated in plasma and in BALF from patients with ARDS [158]. Cathelicidins can protect from and neutralise effects found after administration with high concentrations of LPS in models of septic shock [161,162], which is a major component of the bacterial cell wall. The anti-LPS activity of cathelicidin could potentially be used to protect from septic shock.

3.5 Pulmonary fibrosis, sarcoidosis and pulmonary alveolar proteinosis

Pulmonary fibrosis is a descriptive term for a group of lung diseases characterised by various amounts of inflammation, destruction of lung parenchyma and replacement by fibrous materials accompanied by loss of pulmonary function. Plasma concentrations of α-defensins are raised in patients with pulmonary fibrosis [163]. Sarcoidosis is an inflammatory disease that mainly involves the pulmonary system. LL-37 is found to be upregulated in lungs [17] and α-defensin is significantly upregulated in BALF and serum of patients with this disease [164]. In pulmonary alveolar proteinosis, a disease characterised by excessive accumulation of surfactant lipoprotein in pulmonary alveoli, with associated disturbance of pulmonary gas exchange [165], elevated levels of α-defensins and high levels of β-defensins 1 and 2 have been found in BALF compared with controls or patients with pneumonia and sarcoidosis [166].

4. Conclusion

AMPs have emerged as multifunctional effector substances of the pulmonary innate immune system. On one side, convincing evidence has accumulated that these molecules are indeed endogenous antibiotics. On the other side, AMPs have additional functions as mediators of inflammation in the host defence scenario. A significant number of *in vitro* studies investigated these non-antimicrobial functions. Whether these activities are relevant *in vivo* is speculative. Therefore, at this time the role of AMPs in inflammatory lung disease is also speculative. Nevertheless, studies on the receptor-mediated functions of AMPs shed light on traditional therapies like phototherapy curing *M. tuberculosis* infections. Based on the presented data, AMPs qualify as potential innovative drugs. The broad spectrum of antimicrobial activity and the low incidence of bacterial resistance make them attractive candidates for prototypic novel antibiotics. The potential pro-inflammatory of AMPs could also be detrimental in this setting. Synthetic modifications could be used that eliminate the inflammatory activity and conserve the antimicrobial activity. If their role as mediators in inflammatory lung disease can be proven, appropriate strategies could be developed to influence human disease.

5. Expert opinion

There are several areas of recent interest in the field of AMPs:

- What is an antimicrobial peptide? The field started by isolating peptides with antimicrobial properties. These peptides are gene-encoded and this is the classical definition of a vertebrate AMP. During the years it became clear that many more peptides and especially cleavage products of larger proteins have antimicrobial properties. Antimicrobial function of proteins/peptides is becoming a complex field of many components involved. In parallel to other '-omics' approached this area will develop into a 'defenceomics' area. An integrative approach that includes the analysis of the host and the microbe is necessary.
- Are AMPs antimicrobials? What is the 'main' function of an AMP? Recent results indicate that individual AMPs might have various functions that depend on the place of activity, the concentration and many other factors. This is not a surprise as many other actors such as cytokines are multifunctional and act within a network. *In vivo* studies (animal experiments and clinical studies) will have to show the real role of AMPs in immunity.
- Can AMPs be used as drugs? Yes and no. As demonstrated in the past, the structures of AMPs can serve as models for the development of antibiotic drugs. Most candidates failed during development in preclinical studies or clinical trials. The reasons for these failures are complex and include factors such as weak *in vivo* efficacy, toxicity, high production costs or the need for topic instead of systemic treatment. Some of the classic AMPs might be more useful in other indications, such as angiogenesis, cancer, wound healing or sepsis.

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