Chapter 3
Function of Antimicrobial Peptides in Lung Innate Immunity

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Abstract The innate immune system of the lung is a complex network of different cellular and noncellular components protecting the lung from inhaled pathogens. Antimicrobial peptides (AMP) are produced by epithelial and myeloid cells as part of this system. AMPs, such as defensins and cathelicidin, are small cationic peptides with a broad microbicidal activity against respiratory bacteria, viruses, and fungi. However, their functions go beyond antimicrobial activity and include modulation of the innate and adaptive immune response to infection as well as lung repair after injury. Thus, AMPs are involved in pathophysiological processes of many lung diseases, such as acute and chronic lung infection, chronic obstructive pulmonary disease, cystic fibrosis, and lung cancer.

3.1 The Innate Immune Network of the Lung

The human lung is continuously exposed to a broad array of airborne pathogens such as bacteria, viruses, and fungi. Given its specific requirement to ensure an effective gas exchange, the lung has a unique structure that is characterized by a large contact surface combined with an extremely thin epithelial layer. Hence, the lung is a potential portal of entry for inhaled microbes. In order to avoid colonization of the lower airways with inhaled pathogens and prevent local and systemic infection, the lung is protected by a complex innate immune network consisting of various cellular and noncellular components.

The first line of defense is represented by the airway epithelium, a pseudostratified epithelium of basal cells, ciliated cells, secretory Clara cells, and goblet cells.

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J. Harder, J.-M. Schröder (eds.), *Antimicrobial Peptides: Role in Human Health and Disease*, Birkhäuser Advances in Infectious Diseases,
DOI 10.1007/978-3-319-24199-9_3

Its function within the innate immune system exceeds well-known mechanisms like barrier formation and mucociliary clearance. The epithelium of the respiratory tract is an immunologically active tissue, which exerts functions such as pathogen recognition, pathogen neutralization, and activation of further immune mechanisms. These include resident alveolar macrophages as well as inflowing neutrophils. Those professional immune cells have a high capability to neutralize microbial pathogens. In addition, the release of immunomodulatory mediators from epithelial and myeloid cells induces activation of the adaptive immune response.

Small cationic antimicrobial peptides (AMPs) from the families of defensins and cathelicidins play a key role within the innate immune network of the lung.

3.2 Expression of Antimicrobial Peptides in the Lung

AMPs are expressed by various cell types present in the lung during health and disease. The main AMPs expressed in the lung are α-defensins, β-defensins, and cathelicidins. Table 3.1 gives an overview on AMPs of the human lung.

3.2.1 Defensins

3.2.1.1 α-Defensins

α-Defensins comprise the human neutrophil peptides (HNP) 1–4 and the epithelial α-defensins HD-5 and HD-6 (Doss et al. 2010). Different to other epithelial tissues of the human body, the airway epithelium hardly produces α-defensins; only HD-5 has been detected in airway epithelial cells in small amounts (Frye et al. 2000). The main sources of α-defensins in the human lung are neutrophilic granulocytes. They

Table 3.1 Antimicrobial peptides of the human lung

Class	Peptide name	Gene name
α-Defensins	HNP-1	DEFA1
	HNP-2	DEFA1
	HNP-3	DEFA3
	HNP-4	DEFA4
	HD-5	DEFA5
β-Defensins	hBD-1	DEFB1
	hBD-2	DEFB4A
	hBD-3	DEFB103A
	hBD-4	DEFB104A
Cathelicidins	LL-37/hCAP18	CAMP

constitutively express α-defensins, which are stored in azurophilic granula and represent their main protein content. Upon infection, activation of innate immunity triggers the influx of neutrophils into the lung, where they neutralize pathogens through phagocytosis and degranulation of their azurophilic granula (Ganz et al. 1985; Selsted and Ouellette 2005).

In human alveolar macrophages, α-defensins are virtually absent; only rabbits have been found to express α-defensins in their alveolar macrophages (Ganz et al. 1985; Selsted and Ouellette 2005).

3.2.1.2 β-Defensins

The principal β-defensins found in the human lung are human β-defensin (hBD) 1–4 (Doss et al. 2010). Their main sources are epithelial cells and submucous glands (Kao et al. 2003; Bals et al. 1998a). However, β-defensins (hBD-1 and hBD-2) are also released from myeloid cells such as alveolar macrophages and dendritic cells (Duits et al. 2002). The expression of β-defensins shows a gene-specific behavior: hBD-1 is constitutively expressed by the epithelium, ensuring a constant basic antimicrobial activity of the airway surface liquid (McCray and Bentley 1997; Singh et al. 1998). In contrast, hBD-2, hBD-3, and hBD-4 are not released by default, but only if required; high levels can only be detected in the case of infection or inflammation (Hess et al. 2010; Harder et al. 2001; Yanagi et al. 2005; Scharf et al. 2010a, 2012; Benincasa et al. 2009). Compared to hBD-3 and hBD-4, which are predominantly expressed in other epithelial tissues, hBD-2 is mainly expressed in the respiratory tract (Bals et al. 1998a).

3.2.2 Cathelicidin

In the lung, the only human cathelicidin LL-37/hCAP-18 is expressed by different cell types, namely, myeloid cells such as alveolar macrophages (Rivas-Santiago et al. 2008), neutrophils (Rivas-Santiago et al. 2008; Cowland et al. 1995), and mast cells (Di Nardo et al. 2003), as well as airway epithelial cells (Bals et al. 1998b). It can be detected in bronchoalveolar lavage fluid (Agerberth et al. 1999). Similar to α-defensins, cathelicidin is constitutively expressed by neutrophils and stored in granula until release (Larrick et al. 1995). Degranulation is triggered by external stimulation such as TLR or cytokine receptor activation. Since degranulation can also be induced by cathelicidin itself, a feedforward loop is initiated and results in a cathelicidin burst with very high local concentrations (Vandamme et al. 2012). In granula, cathelicidin is stored as an inactive precursor protein that is processed to its active form by extracellular cleavage upon degranulation (Vandamme et al. 2012). The mechanism that activates the cathelicidin precursor protein in airway epithelial cells, which do not store it in granula, has not been identified yet (Vandamme et al. 2012).

3.3 Regulation of Antimicrobial Peptide Expression in the Lung

3.3.1 Defensins

In the lung, β-defensin expression is triggered by common respiratory pathogens including the most common causes of pneumonia. Airway epithelial cells express β-defensins upon stimulation with *Streptococcus pneumoniae* (Scharf et al. 2012; García et al. 2001), *Haemophilus influenzae* (Seiler et al. 2013), *Legionella pneumophila* (Scharf et al. 2010a), and *Pseudomonas aeruginosa* (García et al. 2001; Harder et al. 2000). β-Defensins are also induced by the most common causes of pulmonary tuberculosis, *Mycobacterium tuberculosis* and *Mycobacterium bovis* (Méndez-Samperio et al. 2007; Rivas-Santiago et al. 2005). Viral pathogens resulting in increased expression of β-defensins include *respiratory syncytial virus* (Kota et al. 2008), *rhinovirus* (Proud et al. 2004; Duits et al. 2003), and, at least in mice, *influenza virus* (Chong et al. 2008). Finally, β-defensins are induced by fungal pathogens such as *Aspergillus fumigatus* (Alekseeva et al. 2009).

The cellular components of lung innate immunity are able to recognize and specifically react to pathogens through detection of conserved microbial structures called pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors. These include transmembrane Toll-like receptors (TLRs) as well as cytosolic NOD-like and RIG-I-like receptors and others. Activation of pattern recognition receptors leads to induction of intracellular signaling pathways like the MAP kinase cascade and activation of transcription factors such as NF-κB, eventually resulting in gene expression. Apart from the constitutively expressed hBD-1, the expression of β-defensins is largely regulated by TLR signaling. The role of cytosolic pattern recognition receptors in the regulation of β-defensins remains to be discovered.

There are ten human members of the TLR family. In the lung, the whole array of TLRs is expressed by airway epithelial cells and resident innate immune cells like alveolar macrophages and neutrophils (Kovach and Standiford 2011; Kawasaki and Kawai 2014; Futosi et al. 2013; Parker and Prince 2011). Many of them have been shown to upregulate β-defensin expression upon stimulation with their specific agonists. In particular, the expression of β-defensins is induced by TLR2 (Hertz et al. 2003; Wang et al. 2003), TLR3 (Seiler et al. 2013; Proud et al. 2004), TLR4 (MacRedmond et al. 2005), TLR5 (Froy 2005), TLR6 (Froy 2005), and TLR9 (Platz et al. 2004). Since the members of this list can be activated by bacterial, mycobacterial, viral, and fungal PAMPs (Akira et al. 2006), the innate immune network of the lung can potently respond to different respiratory infections.

Apart from TLR-related β-defensin upregulation, lung infection also induces the expression of various proinflammatory cytokines derived from epithelial, innate, and adaptive immune cells. TNF-α and IL-1β are released by alveolar macrophages during bacterial infection (Hess et al. 2010) and upregulate hBD-2 in airway epithelial cells *in vitro* and *in vivo* (Hess et al. 2010; Harder et al. 2000; Albanesi et al.

2007). hBD-2 expression is also stimulated by IL-17A (Kao et al. 2004), which is derived from Th17 cells during lung infection (Ye et al. 2001), and airway epithelial cell-derived IL-17C (Kusagaya et al. 2014). Upon viral infection, interferon-gamma release is triggered, for example, by TLR3 (Akira 2009) and leads to increased hBD-3 expression (Albanesi et al. 2007).

Stimulation of TLRs and cytokine receptors leads to activation of intracellular signaling processes like mentioned above. Therefore, TLR and cytokine receptors induce β-defensin expression in an NF-κB- or MAP kinase-dependent manner (McCray and Bentley 1997; Scharf et al. 2010b). In fact, hBD-2 has been shown to be a direct target gene of the transcription factors NF-κB and the MAP kinase target AP-1 (Wehkamp et al. 2004; O'Neil et al. 1999). With a vitamin D response element within its promoter sequence, hBD-2 further is a target gene of vitamin D; however, vitamin D reactivity of the hBD-2 gene is not as strong as it is described for cathelicidin (Wang et al. 2004).

3.3.2 Cathelicidin

In contrast to β-defensins, the expression of cathelicidin is not primarily induced upon infectious or inflammatory stimulation but is regulated by vitamin D. Airway epithelial as well as myeloid cells express the vitamin D receptor (Hansdottir et al. 2008; Liu et al. 2006). Stimulation of these cells with the active form of vitamin D, 1,25-dihydroxyvitamin D3, induces the expression of cathelicidin (Wang et al. 2004; Schauber et al. 2007a; Gombart et al. 2005). The promoter of the corresponding gene contains a vitamin D response element, which makes it a direct vitamin D target gene (Wang et al. 2004). Infectious stimuli can also indirectly trigger cathelicidin expression: it has been shown that TLR activation upregulates the expression of the vitamin D receptor in human macrophages, which leads to an increased expression of cathelicidin (Liu et al. 2006). Because the effects of vitamin D rely on its conversion from its inactive proform to 1,25-dihydroxyvitamin D3 by the hydroxylase CYP27B1, cathelicidin expression is largely influenced by modulation of this reaction. TLR stimulation leads to activation of CYP27B1, resulting in increased levels of active vitamin D (Liu et al. 2006). Conversion of vitamin D to its active form is also induced by IFN-γ in alveolar macrophages (Koeffler et al. 1985). 1,25-dihydroxyvitamin D3 further amplifies the innate immune response by upregulation of TLR2 and CD14 (Schauber et al. 2007a, b).

3.4 Microbicidal Functions of Antimicrobial Peptides in the Lung

AMPs exert antimicrobial activity against many common respiratory pathogens, including bacterial, viral, and fungal species. The human β-defensins hBD-2 and

hBD-3 are bactericidal against the typic pathogen of community-acquired pneumonia, *S. pneumoniae* (Scharf et al. 2012). *In vitro* microbicidal activity against *P. aeruginosa* and *Escherichia coli*, frequent causes of nosocomial and ventilator-associated pneumonia, has been shown for hBD-2 (Harder et al. 2000). hBD-3 is further bactericidal against *Staphylococcus aureus* and *Enterococcus faecium* (Harder et al. 2001; Chen et al. 2005). hBD-4 has antimicrobial activity against *P. aeruginosa* (Yanagi et al. 2005).

The antimicrobial spectrum of cathelicidin includes a broad array of bacterial pathogens, including gram-positive species like *S. pneumoniae* and gram-negative species such as *P. aeruginosa* (Benincasa et al. 2009; Bals et al. 1998b; Saiman et al. 2001; Felgentreff et al. 2006).

Apart from bacteria, a couple of respiratory viruses are targeted by AMPs: *influenza virus*, *parainfluenza virus*, and *respiratory syncytial virus* (Klotman and Chang 2006; Tripathi et al. 2013). AMPs have also been shown to act against *Candida* spp. and *Aspergillus* spp., the most frequent species causing respiratory mycosis (Alekseeva et al. 2009; Aerts et al. 2008).

While there is much evidence for the microbicidal effect of AMPs *in vitro*, it is hard to assess their impact in the human lung. Due to their chemical structure, salt concentrations and pH in healthy or inflamed lung tissue may alter AMP function *in vivo*. In addition, physiological concentrations of AMPs may be significantly lower than the minimal inhibitory concentrations assessed by *in vitro* approaches (Bals et al. 1998b; Bowdish et al. 2005); thus, particular AMPs may not reach microbicidal concentrations *in vivo*. However, it has to be considered that in the human lung, AMPs are part of a well-orchestrated immune network with many synergistic capacities. AMPs can act in mutual synergism (García et al. 2001; Chen et al. 2005; Tripathi et al. 2013) as well as cooperative with other antimicrobial substances such as lactoferrin and lysozyme (Bals et al. 1998a). The effect of AMPs can also be enhanced by surfactant proteins (Tripathi et al. 2013) and even bacterial components (Iwase et al. 2010). Finally, AMPs have been shown to act synergistic with antibiotic agents (Xiong et al. 1999; Scott et al. 1999a, b; Hancock and Scott 2000).

3.5 Beyond Antimicrobial Activity: Modulatory Functions of Antimicrobial Peptides in the Lung

In primitive invertebrate species like the common model organism *Caenorhabditis elegans*, there is no cellular immune system. Apart from rather unspecific mechanisms such as pathogen avoidance and physical isolation, their resistance against infectious agents largely depends on the microbicidal activity of their AMPs. In the course of evolution, AMPs became part of increasingly complex immune systems in which their role changed from solely antimicrobial agents to versatile modulators of different physiologic systems.

3.5.1 Modulation of Lung Immunity

Within the innate immune network of the lung, AMPs are one of the first defense mechanisms to face invading pathogens. Apart from their antimicrobial activity, AMPs have many immunomodulatory features that are similar to those of classic cytokines. For example, AMPs act as chemoattractants for different immune cells, recruiting them to sites of inflammation. α-Defensins have been shown as chemotactic factors for monocytes, dendritic cells, and T cells (Yang et al. 2000a, 2001; Chertov et al. 1996). β-Defensins chemoattract monocytes, macrophages, and neutrophils via the chemokine receptor CCR2, immature dendritic cells and T cells via CCR6, and mast cells in a phospholipase C-dependent manner (Soruri et al. 2007; Yang et al. 1999; Röhrl et al. 2010; Niyonsaba 2002). Finally, cathelicidin is capable of recruiting neutrophils, monocytes, and T cells via FPRL1 (Yang et al. 2000b; Kurosaka et al. 2005), and mast cells via phospholipase C (Niyonsaba et al. 2002), and takes part in the regulation of dendritic cell maturation and differentiation (Davidson et al. 2004; Kandler et al. 2006).

In addition to their chemokine nature, AMPs are able to act as endogenous TLR agonists. For example, mouse β-defensin 2 activates TLR4 (Biragyn et al. 2002), and β-defensin 3 is capable of activating TLR1 and TLR2 (Funderburg et al. 2007). By that, AMPs can induce cytokine expression and activation of immune cells: β-defensins induce IL-6, IL-8, IL-10, and MCP-1 in monocytes (Boniotto et al. 2006) and IL-1β and IL-6 in dendritic cells (Biragyn et al. 2002). In myeloid and epithelial cells, IL-8 expression is upregulated by α-defensins (Khine et al. 2006). β-Defensin 2 leads to dendritic cell maturation in mice (Biragyn et al. 2002), and β-defensin 3 activates monocytes and neutrophils (Funderburg et al. 2007). Cathelicidin has been shown to indirectly enhance TLR signaling (Lai et al. 2011). Cathelicidin further induces IL-8 and MCP-1 in a MAPK-dependent manner (Scott et al. 2002; Tjabringa et al. 2003; Bowdish et al. 2004; Yu et al. 2007). Mast cell degranulation and histamine release are mediated by β-defensins and cathelicidin (Schiemann et al. 2009; Niyonsaba et al. 2001).

Excessive activity of the immune system can cause self-damage of the host by immunopathological processes. Therefore, balancing of pro- and anti-inflammatory signals is crucial. Above immunostimulation, AMPs also take part in the fine-tuning of the immune response by additionally regulating anti-inflammatory mechanisms. Activation of the complement system, which can be induced in the lung (Varsano et al. 2000), is inhibited by β-defensin 2 (Bhat et al. 2007). β-Defensin 3 has been shown to inhibit ERK and, via downmodulation of TRIF and MyD88, TLR signaling, leading to a decreased production of proinflammatory cytokines in macrophages and dendritic cells (Semple et al. 2010, 2011; Pingel et al. 2008). α-Defensins have an anti-inflammatory effect on macrophages, reducing production of reactive oxygen species and inhibiting the expression of proinflammatory cytokines (Miles et al. 2009). However, compared to PAM3CSK4, another TLR1/2 agonist, β-defensin 3-derived TLR1/2 activation induces a pro- rather than an anti-inflammatory cytokine pattern in monocytes (Funderburg et al. 2011).

Excessive immunostimulation by bacterial PAMPs is considered to be a key event in the pathogenesis of septic shock. Cathelicidin inhibits proinflammatory cytokine release from neutrophils and macrophages upon stimulation with the gram-negative endotoxin LPS (Mookherjee et al. 2006; Alalwani et al. 2010; Scott et al. 2011). In particular, cathelicidin antagonizes LPS by preventing the formation of the LPS/LBP complex, which is required for TLR4-dependent LPS recognition (Scott et al. 2000; Kirikae et al. 1998). Synthetic mimics of AMPs further intercept the classical gram-positive PAMP and TLR2 agonist LTA (Scott et al. 1999a). The role of cathelicidin at the crossroads of inflammation is further underlined by its influence on bacterially stimulated neutrophils: cathelicidin increases their antimicrobial activity and phagocytotic capacity. In cathelicidin-deficient neutrophils, antimicrobial activity is impaired; instead, they react with more production of proinflammatory cytokines (Alalwani et al. 2010). Thus, AMPs may limit the adverse effects of extensive immune cell activation.

On the other hand, AMPs seem to especially promote airway epithelial innate immune mechanisms: cathelicidin leads to a decrease in epithelial permeability, thereby preventing transepithelial invasion of bacterial pathogens (Byfield et al. 2011). It further induces internalization of LPS into airway epithelial cells, which leads to activation of endosomal TLR4 and consequently LPS-induced epithelial cytokine production (Shaykhiev et al. 2010). Moreover, cathelicidin facilitates signaling via TLR3, a receptor for viral dsRNA (Lai et al. 2011). In synergy with other inflammatory mediators and TLR agonists, cathelicidin activates cytokine production in epithelial cells even in low concentrations (Filewod et al. 2009; Nijnik et al. 2012). Cathelicidin is also able to activate NF-κB and induce cytokine production in airway epithelial cells on its own (Pistolic et al. 2009).

The pro- and anti-inflammatory capacities of AMPs in different settings are still incompletely understood and even seem to be self-contradictory to some degree. Anyway, their position within the hierarchically structured immune network of the lung is strategic: at the first line of defense, they ensure an early answer to pathogen exposition, namely, through direct microbicidal activity, PAMP interception, and immunological activation of resident cells. In the next step, they trigger more potent mechanisms of the innate and adaptive immunity while simultaneously contributing to attenuate those mechanisms in order to avoid immunopathology.

In summary, AMPs are key players in the initiation and amplification of the innate immune response and contribute to activation of adaptive immunity. At the same time, they seem to protect the host from overshooting inflammation by coordination of pro- and anti-inflammatory signaling.

3.5.2 Modulation of Lung Injury and Repair

Infection and inflammation leading to epithelial injury are characteristic hallmarks of acute and chronic lung diseases. Termination and resolution of inflammation and maintenance of tissue homeostasis are active and coordinated processes which include the expression of immune cell-derived and epithelial factors such as

pro- and anti-inflammatory cytokines, the keratinocyte growth factor (KGF), and the epidermal growth factor (EGF) (Tjabringa et al. 2003; Campbell et al. 2011; Puddicombe et al. 2000; Michelson et al. 1999). AMPs qualify as mediators of wound healing and tissue repair as the expression of AMPs by inflammatory and epithelial cells is increased under infectious and inflammatory conditions and epithelial injury induced by infectious agents leads to an increased risk that pathogens invade the host (Steinstraesser et al. 2008; Lai and Gallo 2009). Indeed, there is strong evidence that AMPs are involved in epithelial repair processes, such as cell proliferation, epithelial regeneration, and reepithelialization (Lai and Gallo 2009; Heilborn et al. 2003; Tokumaru et al. 2005; Carretero et al. 2008; Beisswenger and Bals 2005; Murphy et al. 1993; Aarbiou et al. 2004).

The function of AMPs in the repair of epithelial injury has been particularly studied in wound healing of the skin. Cathelicidin, for instance, induces reepithelialization after skin injury via transactivation of the EGF receptor (Heilborn et al. 2003; Tokumaru et al. 2005; Carretero et al. 2008). Cathelicidin also plays an important role in angiogenesis and thereby contributes to wound repair (Koczulla et al. 2003). As cathelicidins and defensins induce proliferation of diverse airway epithelial cell lines and primary airway epithelial cells *in vitro*, it is suggested that AMPs also mediate wound healing in the lung via the activation of pro-proliferative signaling cascades (Beisswenger and Bals 2005; Murphy et al. 1993). Cathelicidin activates airway epithelial cell via metalloproteinase-mediated cleavage of membrane-anchored EGF receptor ligands as well as MAP kinase-dependent signaling pathways (Tjabringa et al. 2003) and stimulates airway epithelial wound closure in an EGF receptor-, G protein-coupled receptor-, and MAP kinase-dependent manner (Shaykhiev et al. 2005). A function of cathelicidins in tissue repair is further supported by a mouse study which associates deficiency of the mouse cathelicidin CRAMP with increased lung injury during infection with gram-negative bacteria (Kovach et al. 2012).

Like cathelicidin, α-defensins from human neutrophils induce proliferation of airway epithelial cell lines via activation of MAP kinase signaling cascades (Aarbiou et al. 2002) and enhance wound closure in an EGF-dependent manner (Aarbiou et al. 2004). α-Defensins also increase proliferation of lung fibroblasts and collagen synthesis (Han et al. 2009).

However, *in vitro* studies also suggest that cathelicidins and defensins induce the release of inflammatory mediators by airway epithelial cells and that AMPs are cytotoxic at higher concentrations (Tjabringa et al. 2003; Shaykhiev et al. 2005; Sakamoto et al. 2005). Therefore, the proinflammatory, cytotoxic, and pro-proliferative functions of AMPs may also have adverse consequences by perpetuating inflammation and potentially contribute to epithelial injury in lung diseases (Shaykhiev et al. 2005; Sakamoto et al. 2005). Moreover, concentrations of AMPs have been found to be increased in severe lung diseases. Plasma concentrations of α-defensins, for instance, are increased in pulmonary fibrosis (Mukae et al. 2002) and β-defensins are increased in bronchoalveolar lavage fluids of patients with diffuse panbronchiolitis and in bronchiolitis obliterans syndrome after lung transplantation (Ross et al. 2004; Hiratsuka et al. 2003). Cathelicidin associates with bronchial inflammation in cystic fibrosis lung disease (Chen et al. 2004).

In summary, AMPs may contribute to tissue regeneration and epithelial regeneration in the lung during infection and inflammation by inducing proliferation of structural cells, but they also potentially contribute to epithelial injury and fibrotic remodeling during airway inflammation when present at high concentrations.

3.6 Clinical Perspectives

3.6.1 Acute Respiratory Tract Infections

Respiratory tract infections are very common. Especially pneumonia is a major cause of global morbidity and mortality in children as well as in adults (Klugman and Garau 2009). Due to their microbicidal properties ("endogenous antibiotics"), the role of AMPs in acute respiratory tract infection has drawn much attention. AMP levels are increased in patients with pneumonia. High levels of β-defensins and cathelicidin can be detected in plasma or sputum of patient with acute pneumonia (Hiratsuka et al. 1998; Herr et al. 2009; Schaller-Bals et al. 2002; Ishimoto et al. 2006).

The important role of AMPs in respiratory tract infection is especially underlined by studies in animal models. Cathelicidin treatment has been shown to reduce pro-inflammatory cytokine release during MRSA pneumonia and improves the histopathological outcome (Hou et al. 2013). In cathelicidin-deficient mice, the immune response to gram-negative pneumonia with *K. pneumoniae* is delayed, eventually resulting in more inflammation and increased lung injury (Kovach et al. 2012). During gram-negative pneumonia with *P. aeruginosa*, overexpression of β-defensin 2 in rats reduces bacterial load in the lung as well as inflammation and lung injury and increases survival rate (Shu et al. 2006; Hu et al. 2010). In β-defensin 1-deficient mice, clearance of *H. influenzae* in the lung is impaired (Moser et al. 2002).

The effect of AMPs is also modulated by host-microbe interactions. The *S. aureus* virulence factor staphylokinase acts synergistically with cathelicidin to promote fibrinolysis during mouse pneumonia, which leads to a more invasive infection (Braff et al. 2007). Staphylokinase is also able to inactivate α-defensins (Jin et al. 2004).

While there are natural limitations in evaluation of the *in vivo* impact of AMPs in human pneumonia, the high AMP levels found in patients with pneumonia together with the studies mentioned above suggest a crucial role of AMPs in the response to lung infection.

3.6.2 Chronic Lung Diseases

Cigarette smoke is the major risk factor for the development of the chronic obstructive pulmonary disease (COPD). COPD is characterized by chronic inflammation of the lung leading to tissue destruction, emphysema, and loss of pulmonary function (Sethi and Murphy 2008; Sethi 2010). Stable COPD patients are frequently colonized with bacterial pathogens (e.g., *H. influenzae*); recurrent infections of the

respiratory tract and chronic bronchitis are common in COPD (Sethi and Murphy 2008; Sethi 2010; Moghaddam et al. 2011; Garmendia et al. 2012). Microbial infections of the lung evoke harmful inflammatory responses that contribute to clinical deterioration of COPD patients (Sethi 2010). Even in healthy individuals, smoking suppresses pulmonary host defense making the lung more susceptible for microbial colonization and infection (Herr et al. 2009; Sethi 2010; Mehta et al. 2008). *Ex vivo* and *in vitro* studies showed that the expression of AMPs is inhibited by cigarette smoke and that COPD is associated with skewed AMP expression in the respiratory tract. Analyses of human samples showed that current or former smoking is associated with significantly reduced levels of the β-defensin hBD-2 in pharyngeal washing fluids and sputum from patients with acute pneumonia (Herr et al. 2009). *In vitro* studies demonstrated that exposure to cigarette smoke and reactive oxygen species inhibit the expression of hBD-2 in respiratory epithelial cells in response to inflammatory mediators or bacteria (Herr et al. 2009). The effects of cigarette smoke on β-defensin expression are likely due to the suppression of cellular signaling cascades by cigarette smoke, such as NF-κB and AP-1 signaling (Pace et al. 2012; Kulkarni et al. 2010; Laan et al. 2004). In currently smoking COPD patients, hBD-2 expression is decreased in central but not in distal airways, inversely correlating with cigarette smoke exposure (Pace et al. 2012). This has been underlined by the finding that hBD-2 mRNA expression in peripheral lung samples has been found to be increased in COPD patients and was associated with higher IL-8 expression and poor lung function (Liao et al. 2012). Cathelicidin has also been shown to be increased in small airways of COPD patients, where it may contribute to airway remodeling (Sun et al. 2014). In addition, a proteomic study showed that the α-defensins are increased in bronchoalveolar lavage fluids of COPD patients (Merkel et al. 2005). In summary, the expression pattern of AMP seems to be deranged in COPD with low antimicrobial activity in the central airways. Altered AMP expression may lead to airway colonization with pathogens in smokers and patient with COPD, providing reservoirs for recurrent infection. Enhanced susceptibility to recurrent respiratory tract infections subsequently leads to chronic inflammation, aberrant expression of AMPs, and disease progression (Herr et al. 2009; Sethi 2010).

Studies also suggest that skewed expression of AMPs and inhibition of the bactericidal activity of AMPs play a role in cystic fibrosis (CF). AMPs are detectable in sputum or bronchoalveolar lavage fluid of CF patients with α-defensins and cathelicidin being present at high levels (Felgentreff et al. 2006; Chen et al. 2004; Soong et al. 1997). Moreover, enhanced levels of cathelicidin correlate with severity of CF lung disease (Chen et al. 2004). β-Defensin levels do not seem to correlate with the severity of inflammation, but decreased levels of hBD-2 are associated with severity of CF (Chen et al. 2004; Dauletbaev et al. 2002; Bals et al. 2001). *In vitro*, the bactericidal activity of cationic AMPs is salt sensitive. Thus, it is suggested that increased salt concentrations in airway fluids of CF patients contribute to chronic lung infection by inhibiting bactericidal functions of AMPs (Bals et al. 1998a). In line with this hypothesis, bactericidal activity of hBD-1 has been shown to be decreased in airways of CF patients and CF airway fluids fail to kill *S. aureus* and *P. aeruginosa* (Goldman et al. 1997; Bals et al. 1999). However, in a human bronchial xenograft

model, salt-independent dysfunction of antimicrobial activity was corrected by adenovirus-mediated gene transfer of CFTR (Bals et al. 2001). Thus, the role of altered salt concentrations in airway fluids of CF patients is controversial and yet unknown mechanisms may contribute to the reduced bactericidal activity in the fluids lining the mucosal surfaces of CF patients. Excessive mucus production, for instance, also potentially contributes to impaired bactericidal activities of AMPs in CF patients as mucus interferes with AMPs (Felgentreff et al. 2006).

3.6.3 Lung Cancer

For several tumor types such as ovarian cancer and malignant melanoma, adverse effects of AMPs on tumor development and progression have been shown. In lung cancer, overexpression of different AMPs was found. Cathelicidin is overexpressed in up to 25 % of NSCLC samples (Von Haussen et al. 2008). It is also released by myeloid cells in the tumor microenvironment (Li et al. 2014). Cathelicidin has been shown to transactivate EGFR in airway epithelial cells (Tjabringa et al. 2003). EGFR signaling is one of the critical pathways in lung carcinogenesis (Mitsudomi and Yatabe 2010). Through interaction with the EGFR pathway, cathelicidin promotes tumor growth *in vitro* and *in vivo* (Von Haussen et al. 2008), which is further induced by cigarette smoke (Li et al. 2014).

As for β-defensins, hBD-1 and hBD-2 are overexpressed in about 50–60 % of NSCLC tissue samples (Shestakova et al. 2008). In serum of patients with lung cancer, concentrations of hBD-1 and hBD-2 are significantly higher as compared to healthy controls and even patients with pneumonia. Therefore, hBD-1 and hBD-2 have even been proposed as lung tumor markers (Arimura et al. 2004).

AMPs take part in the orchestration of important cellular functions that contribute to oncogenesis and tumor promotion, as they modulate proliferation, cell migration, and angiogenesis. Thus, given their central role in chronic lung disease, AMPs might link chronic airway inflammation to the development of lung cancer.

In contrast to cathelicidin and β-defensins, the α-defensin HNP-1 has been shown to act as an inhibitor of tumor growth. HNP-1 is cytotoxic to tumor cells (Van Wetering et al. 1997; Okrent et al. 1990) and induces apoptosis in lung cancer and other cells (Xu et al. 2008; Lichtenstein 1991). This even led to successful gene therapy approaches with direct intratumoral administration of plasmid DNA encoding HNP-1 in a murine xenograft tumor model (Xu et al. 2008).

3.7 Conclusions

In the human lung, AMPs are abundantly expressed by different cell types, including epithelial and immune cells. The expression of defensins is largely triggered upon stimulation with PAMPs and inflammatory mediators, whereas cathelicidin

expression mainly underlies a vitamin D-dependent mechanism. At least *in vitro*, AMPs are capable of neutralizing a broad array of bacterial, viral, and fungal species, including the most common respiratory pathogens. While their microbicidal impact *in vivo* is still not completely clear, it is now recognized that the abilities of AMPs within a complex immune network by far exceed antimicrobial activity. AMPs take part in the initiation, amplification, and modulation of lung innate immunity. Remarkably, AMPs seem to be involved in balancing of pro- and anti-inflammatory signaling, thus protecting the host from excessive immunostimulation. Thus, AMPs seem to have an important role in acute lung infection. However, in the context of chronic inflammation and infection-related lung diseases, AMPs can be dysregulated and even act adversely: AMP dysfunction may contribute to the pathogenesis of COPD, asthma, and cystic fibrosis. Furthermore, while AMPs contribute to lung repair after injury, they may also have harmful effects regarding fibrogenesis and lung tumor growth.

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