Defensins and cathelicidins in inflammatory lung disease: beyond antimicrobial activity

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Abstract
Innate immunity provides an effective first line of defence against infections. This is of particular importance in the lung, an organ that is exposed to a large number of pathogens that are inhaled. Antimicrobial peptides play an important role in the defence against these pathogens as effector molecules of innate immunity. These peptides are mainly produced by phagocytes and epithelial cells, and kill a wide range of micro-organisms: Gram-negative and Gram-positive bacteria, fungi and (enveloped) viruses. However, it is increasingly evident that these peptides not only act as endogenous antibiotics, but also display a range of other functions, including activities that are involved in regulating immune responses and inflammation, and wound repair. In this review, these activities are highlighted and their role in inflammatory lung disorders is discussed.

Introduction
The epithelial surface of the lungs is exposed to a large number of potentially pathogenic micro-organisms that are inhaled. Several defence mechanisms protect the lung against infection, and the production of antimicrobial peptides is one of those [1]. Epithelial cells that line the airways, submucosal glands and the alveolar epithelial cells produce a range of antimicrobial peptides that vary in structure and activity. Excellent reviews have described the structure and activities of these peptides in detail [2,3], and relevant databases are available for the interested reader (e.g. http://www.bbcm.univ.trieste.it/~tossi/amsdb.html). In humans, a large range of antimicrobial peptides and proteins have been characterized and were found to differ in cellular origin and regulation of production, activity against selected micro-organisms and other activities. This review is focused on those activities of antimicrobial peptides that are distinct from their ability to act as endogenous antibiotics, and on their expression in lung diseases. As will be discussed, these peptides have been shown to act as growth factors, pro-inflammatory mediators, chemokines and modulators of adaptive immunity. Because of this latter activity, the term ‘alarmins’ was proposed recently for a range of mediators, including defensins and cathelicidins, that share their ability to recruit and activate antigen-presenting cells [3]. This review is partly an update of two recent reviews from our research group [4,5] and is focused on the role of two antimicrobial peptide families: the defensins and the cathelicidins. These peptides are small (3.5–5 kDa) cationic peptides that are expressed in a range of species, including humans.

Defensins
Based on their structure (pairing of disulphide bridges), two human defensin families are recognized: the α- and β-defensins. Neutrophil defensins [HNP (human neutrophil peptide) 1–4] are α-defensins that are stored in the azurophilic granules of neutrophils in large amounts (5% of total neutrophil protein). Although intracellular killing of ingested micro-organisms is considered to be a main function, these peptides are also released into the extracellular environment after stimulation, and thus may affect the function of, for example, epithelial cells that line the respiratory tract [5]. Neutrophil defensins show cytotoxic activity against eukaryotic cells, including epithelial cells [6,8], but, at lower concentrations, they may also increase proliferation as demonstrated for, e.g., fibroblasts and epithelial cells [9,10]. When instilled intratracheally into the airways of mice, the peptides decrease lung function and increase inflammation [11]. This activity may in part be the result of their ability to increase production of chemokines from airway epithelial cells [12]. We explored further the mitogenic activity of defensins for epithelial cells in culture, and demonstrated that defensins increase epithelial wound closure in cultures of bronchial epithelial cells, an activity that was accompanied by its mitogenic and chemotactic activity, and by the ability to induce the production of mucins in these cells [10]. Subsequent studies revealed that areas of squamous metaplasia in central airways from smokers are characterized by increased presence of neutrophil defensins and proliferating epithelial cells [13]. These observations are in line with those from in vitro studies. In addition to these effects on structural cells of the lung, neutrophil defensins also chemoattract various leucocyte
subsets such as monocytes/macrophages, T-cells and dendritic cells [3].

**Cathelicidins**

LL-37 is the only cathelicidin that is expressed in humans, and it is produced by proteolytic processing of its precursor hCAP18 (human cationic antimicrobial protein 18) [14]. It is expressed by neutrophils that store the precursor in the specific granules, but other cell types, such as epithelial cells and mast cells, may also serve as cellular sources of hCAP18/LL-37 production. LL-37 was identified based on its broad-spectrum antimicrobial activity and ability to neutralize LPS (lipopolysaccharide). Subsequent studies revealed other activities, such as chemotaxis of neutrophils, eosinophils, T-cells and mast cells [3,15], modulation of dendritic cell function [16], induction of chemokine and cytokine synthesis in epithelial cells and monocytes [17–19], induction of angiogenesis [20] and epithelial wound healing in vitro [21].

**Neutrophil defensins, LL-37 and cellular receptors**

A range of mainly in vitro, but also some in vivo, studies have demonstrated that neutrophil defensins and LL-37 may alter the activity of host cells. Previous studies have identified cellular receptors that are involved in activation of host cells by these peptides. These receptors include chemokine receptors [FPRL1 (formyl peptide receptor-like 1)], EGFR (epidermal growth factor receptor) and purinergic receptors. LL-37 was found to use formyl peptide receptors, most notably FPRL1, to chemotact neutrophils and eosinophils [22]. We demonstrated that LL-37 activates airway epithelial cells not via formyl peptide receptors, but via transactivation of the EGFR. This transactivation results from metalloprotease-mediated shedding of membrane-bound ligands for the EGFR [19]. A similar mechanism was found to occur in keratinocytes [23], and heparin-binding epidermal growth factor receptor was identified as the principal EGFR ligand that was involved in this transactivation in these cells. Other studies revealed the involvement of purinergic receptors in the ability of defensins and LL-37 to activate cells. Purinergic receptors bind extracellular nucleotides such as ATP and allow the cells to respond to exposure to these nucleotides. These nucleotides signal through two types of purinergic receptors: P2Y G-protein-coupled receptors and P2X ion-channel-forming receptors. Recently, the antimicrobial peptide LL-37 has been shown to activate monocytes through the P2X7 receptors [24], whereas neutrophil defensins were found to activate a lung epithelial cell line [7]. Where these studies clearly implicate an interaction between cellular receptors and antimicrobial peptides, alternative mechanisms have been suggested. A recent study by Braff et al. [25] showed that both L- and D-forms of LL-37 induce an increase in IL-8 (interleukin 8) mRNA in keratinocytes, indicating that structure-dependent interactions between LL-37 and surface receptors may not be required for keratinocyte activation. However, LL-37 may use cellular receptors such as G-protein-coupled receptors and EGFR in further downstream signalling, as was also demonstrated by these authors [25].

**Antimicrobial peptides and inflammatory lung disease: what drives production?**

A large number of studies have been directed at identifying processes that drive the production of antimicrobial peptides. The presence of neutrophil-derived antimicrobial peptides in the lung is regulated by processes such as neutrophil migration and degranulation, and processing of peptides. Epithelial cells are the main source of β-defensins, the anti-microbial proteinase inhibitors SLPI (secretory leucocyte protease inhibitor) and elafin, and also produce hCAP18/LL-37. Microbial products, pro-inflammatory cytokines and growth factors are the main regulators of the production of these peptides. Obviously, the presence of these factors in the lung partly explains the higher levels of these peptides found in the lungs of subjects with inflammatory lung disease [4,26]. In diseases such as cystic fibrosis [27] and α1-antitrypsin deficiency [28], the levels of antimicrobial peptides are increased secondary to a genetic defect. It is unknown to what extent genetic polymorphisms in the genes encoding antimicrobial peptides determine levels or activities of these peptides, but two independent studies have shown associations of a polymorphism in the gene encoding hBD-1 (human β-defensin-1) with COPD (chronic obstructive pulmonary disease) [29,30]. However, the functional impact of these specific polymorphisms has not yet been demonstrated.

Previous studies showed that cytokines expressed in the epithelium in patients with atopic disorders may downregulate expression of these peptides in skin [31]. These studies were prompted by the observation of an increased risk of skin infection in atopic dermatitis, when compared with patients with psoriasis, and showed that the expression of hBD-2 and LL-37 is lower in lesional skin in atopic dermatitis when compared with lesional skin in psoriasis [31]. These studies were recently extended by de Jongh et al. [32] in a microarray analysis of purified epidermal cells from lesional skin, followed by immunohistochemical evaluation of lesional skin. This revealed higher expression of a range of antimicrobial peptides and proteins in psoriasis when compared with that of atopic dermatitis. Decreased expression of hBD-2 and LL-37 in atopic skin disease may in part be explained by the presence of IL-13 and IL-10, cytokines expressed by TH2 and regulatory T-cell subsets [33]. Another level of regulation of peptides is brought about by alternative proteolytic processing of released peptides, as demonstrated for LL-37 in skin by proteases that are present in human sweat [34]. This study demonstrated that some processing variants of LL-37 have lost their ability to increase chemokine release from keratinocytes, but retain antimicrobial activity. This is an interesting feature of these peptides from a therapeutic perspective, as it raises the possibility to use modified antimicrobial peptides that lack
pro-inflammatory activity, but retain antimicrobial activity, for the treatment of infectious disorders. We have recently produced a series of variants of LL-37, and developed a lead component that may meet part of these requirements [35].

Concluding remarks

Antimicrobial peptides display a range of activities that may optimize their activity in host defence, but may also contribute to inflammatory disorders. An increased knowledge of the activities of these peptides, the relative importance of these activities and the mode of action contributes to our understanding of inflammatory lung disease. In addition, it will aid the development of new therapeutic strategies to treat infectious and inflammatory lung disease.

References

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