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REVIEW

Innate immunity in the lung: how epithelial cells fight against respiratory pathogens

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Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. R. Bals, P.S. Hiemstra. ©ERS Journals Ltd 2004.

ABSTRACT: The human lung is exposed to a large number of airborne pathogens as a result of the daily inhalation of 10,000 litres of air. The observation that respiratory infections are nevertheless rare is testimony to the presence of an efficient host defence system at the mucosal surface of the lung.

The airway epithelium is strategically positioned at the interface with the environment, and thus plays a key role in this host defence system. Recognition systems employed by airway epithelial cells to respond to microbial exposure include the action of the toll-like receptors.

The airway epithelium responds to such exposure by increasing its production of mediators such as cytokines, chemokines and antimicrobial peptides. Recent findings indicate the importance of these peptides as effector molecules of innate immunity by killing microorganisms, but also as regulators of inflammation, immunity and wound repair. Finally, the clinical relevance of the functions of the airway epithelium in innate immunity is discussed.

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The integrity of the respiratory tract critically depends on a tightly regulated host defence apparatus. The innate immune system provides initial protection against microorganisms and stimulates the adaptive immune response [1]. Cellular components of the innate immune system include phagocytes such as neutrophils or macrophages, natural killer cells, basophils, mast cells, eosinophils and others. Epithelia of the human body form interfaces between the internal milieu and the external environment. In the respiratory tract, the epithelial lining the airways is the first point of contact for inhaled substances such as environmental pollutants, cigarette smoke, airborne allergens, and microorganisms [2]. In recent years it has become clear that airway epithelial cells not only provide a passive barrier function, but also actively contribute to the innate immune system [2, 3].

The innate immune functions of airway epithelial cells are of significance for the pathogenesis of a variety of human diseases. Failure of the local host defence apparatus may result in microbial colonisation and subsequently infection of the airways and the lung parenchyma. Mechanisms of the basal immunity provided by airway epithelial cells have a critical role in these settings. Activities of the innate immune system are closely linked to inflammatory processes. All major diseases of the lung involve mechanisms of the innate or adaptive immune system. Asthma and chronic obstructive lung disease (COPD) are chronic inflammatory diseases, in which cytokines and other mediators secreted from the airway epithelium are likely to play a critical role [3]. The role of antimicrobial peptides, small endogenous antibiotics with proinflammatory, and other functions, is less clear.

The aim of this review is to highlight the role of the airway epithelium in host defence and to describe new developments in this rapidly evolving area of research. Both the recognition systems used by airway epithelial cells to 'sense the microbial world′ and the effector molecules produced in basal conditions and in response to microbial exposure are described. The focus in this latter part is on antimicrobial peptides. Whereas this review is mainly devoted to airway epithelial cells, also research on other epithelia (mainly those of the skin and the intestine) that is relevant to pulmonary research is discussed. Important input to the content of this review came from the European Respiratory Society research seminar "Host defence function of the airway epithelium" that was held in Noordwijkerhout, The Netherlands, 7–8 November, 2002.

Recognition of pathogens by airway epithelial cells

The airway epithelium senses bacterial exposure and responds accordingly by increasing its defences. This response consists of an increase in the release of *e.g.* antimicrobial

peptides into the lumen of the airways, and the release of chemokines and cytokines into the submucosa that initiate an inflammatory reaction. This inflammatory reaction includes the recruitment of phagocytes, that serve to remove microorganisms that are not cleared by the epithelium itself, and dendritic cells and lymphocytes that may aid to mount an adaptive immune response.

Mechanisms to recognise pathogens by the airway epithelium are therefore considered essential to mount a protective response of the innate immune system. It has been known for a long time that cells can respond to microbial products such as lipopolysaccharide and lipoteichoic acid. However, the exact mechanisms and molecules involved in this response were incompletely understood. In the last decade much has been learnt about the mechanisms that mediate this 'adaptive' arm of the innate immune system. Cells of the innate immune system, including phagocytes, dendritic cells and epithelial cells, use so-called pattern recognition molecules to bind to conserved molecular patterns that are present on microorganisms. Pattern-recognition molecules can be present in secretions and the circulation in soluble form, such as mannan-binding lectin (MBL), or they can be transmembrane molecules that mediate direct cellular responses to microbial exposure. The toll-like receptors (TLR) constitute an intensely studied family of pattern recognition receptors (10 members of the human family have been recognised to date) that are represented by various members in almost all cells of the body. Their expression has been intensely studied on dendritic cells, and it is now recognised that these, TLRs, help to shape the adaptive immune response by directing the way that dendritic cells instruct T-cells. Airway epithelial cells also express a variety of TLRs that may help them to mount an adequate response to microbial exposure. Activation of TLR on epithelial cells has now been shown to be involved in the regulation of expression of a variety of genes, including those encoding cytokines, chemokines and antimicrobial peptides.

Following the identification of toll receptors in Drosophila flies as receptors involved in the fly's response to microbial exposure [4], the search for mammalian homologs led to the discovery of TLR in mammals. A variety of bacterial, fungal and viral products have now been identified as ligands for various TLRs and other pattern recognition receptors expressed by airway epithelial cells (summarised in table 1). Among the members of the TLR family, TLR4 has been most intensively studied and its role in the response to lipopolysaccharide (LPS) has been subject of a large number of studies. Studies in LPS-hyporesponsive mice were essential in delineating the role of TLR4 in the response to LPS, and demonstrated that LPS-hyporesponsiveness in mice is associated with a mutation in the signalling domain of TLR4 [9–11]. Subsequent studies in humans revealed an association of selected polymorphisms in the human TLR4 gene with a variety of diseases, including Gram-negative bacterial infections in patients in an intensive care unit [12]. TLR4 is a central component in the response of cells to LPS. LPS is bound by LPS-binding protein (LBP), an acute phase protein that is produced not only by liver cells but also by epithelial cells in the lung [13]. LBP serves to transfer LPS to CD14, a molecule that together with the extracellular protein MD-2 is part of the TLR4 complex. This complex is involved in recognition of LPS, and this is followed by activation of a signalling complex that is associated with the intracellular domain of TLR4 and that includes the adaptor molecule MyD88 and related adaptors [5, 14] (discussed in more detail elsewhere in this section).

TLR2 recognises a wide array of microbial products from gram-positive and negative bacteria and from fungi. Like TLR4 it is present on airway epithelial cells. Whereas TLR4 appears to be the principle receptor for LPS, TLR2 also

Table 1. – Pattern recognition receptors involved in the recognition of microorganisms by airway epithelial cells

Receptor	Ligand
TLR1	Tri-acyllipopeptides
TLR2	Lipoteichoic acid, peptidoglycan, zymosan, microbial lipoproteins and lipopeptides, HSP70 (host)
TLR3	double-stranded RNA
TLR4	LPS, HSP60 and 70 (host), hyaluronic acid fragments (host)
TLR5	flagellin
TLR6	di-acyl lipopeptides
TLR7	synthetic compounds
TLR8	
TLR9	CpG DNA
TLR10	
CD14	LPS
CFTR	LPS

TLR: Toll-like receptor; HSP: heat shock protein; CpG: bacterial deoxyribonucleic acid (DNA) containing unmethylated CpG dinulceotides; LPS; lipopolysaccharide; CFTR: cystic fibrosis transmembrane conductance regulator; Refer to text and references 5–8; TLR10 expression: R. Bals, unpublished observation.

detects certain structural variants of LPS such as leptospiral LPS [15]. The function of TLR3 was revealed by studies of TLR3 knockout mice, showing that TLR3 is essential in the response to double stranded (ds) ribonucleic acid (RNA) that is produced during viral infections [16]. TLR3 has been implicated in the response of epithelial cells to *e.g.* rhinovirus, rhinovirus dsRNA and synthetic dsRNA (polyinosinic-polycytidylic acid; poly(I;C)). This response consists of an increased expression not only of chemokines [6, 17], but also of the human β-defensins (hBD)-2 and -3 [6]. TLR9 mediates the response to bacterial deoxyribonucleic acid (DNA). A recent study showed that TLR9 mediates the response of colonic epithelial cell lines to bacterial DNA, resulting in expression of interleukin (IL)-8 [18].

It is important to note that TLRs may also mediate the response to endogenous ligands. These ligands include heat-shock proteins and extracellular matrix components, such as fragments of hyaluronic acid that are generated during inflammation. Also effector molecules of the innate immune system may serve as ligands for TLRs. The collectin surfactant protein A (SP-A) employs TLR4 to activate murine macrophages [19]. Mouse β-defensin-2 is another example of an effector molecule of innate immunity that activates cells via TLRs. This antimicrobial peptide was found to activate dendritic cells via TLR4, resulting in an increase in co-stimulatory molecules and dendritic cell maturation [20]. Whether this is the result of a classical receptor-ligand interaction remains to be determined [21].

Activation of TLRs may be involved in the regulation of a variety of genes involved in host defence, of which the cytokines and chemokines have been best characterised. It is now clear that TLR also regulate the expression of antimicrobial peptides. CD14, a part of the TLR4 receptor complex, was found to be essential in the LPS-induced induction of hBD-2 on tracheobronchial epithelial cells [7]. Subsequently, TLR2 was found to regulate the expression of hBD-2 in response to bacterial lipoprotein in A549 lung epithelial cells [22], and hBD-2 and IL-8, in response to peptidoglycan and yeast cell wall particles in human keratinocytes [23]. TLR activation may also result in an increase in the expression of TLR themselves, which are normally not present in large amounts on epithelial cells. This is illustrated by the finding that nontypeable *Haemophilus influenzae*

employs TLR2 to increase its expression on bronchial epithelial cells [24]. In addition, cytokines such as interferon (INF)-γ have been found to increase the epithelial expression of selected TLRs [25].

A specific response to microbial products appears to result, in part, from individual TLRs possibly having their own signalling pathways, resulting in specific responses. Since different cell types express distinct subsets of TLRs, the simultaneous activation of different TLRs creates a unique signal to the cells that is characteristic, both for the cell type and the micro-organism involved [5, 14]. Recent studies have partly elucidated the intracellular signalling pathways activated by TLRs following binding of TLR-ligands. The common toll-interleukin-1 receptor (TIR)-domain of TLRs plays a central role in signalling. TLRs and IL-1R will bind the adaptor molecule MyD88 through the TIR-domain that is also present in MyD88. MyD88 recruits the serine/threonine kinase IL-1R-associated kinase (IRAK) that becomes phosphorylated, allowing it to associate with TRAF6. TRAF6 will mediate signalling to downstream molecules such mitogen activated protein kinases and transcription factors such as nuclear factor κB. Recent studies have also demonstrated the existence of MyD88-independent TLR signalling pathways. This led to the discovery of four other adaptor molecules in addition to MyD88: MyD88 adaptor-like (Mal; also known as Tir domain-containing adaptor protein [TIRAP]); TIR-domain containing adaptor inducing IFN-β (TRIF; also known as TIR-containing adaptor molecule-1 [TICAM-1]), Trif-related adaptor molecule (TRAM) and sterile α- and HEAT-Armadillo motifs [26]. Whereas these adaptors, like MyD88, contain a TIR domain, they also show marked structural difference with MyD88. These may allow them to recruit different transducers resulting in specific down-stream signalling. Although the specific function of all these newly recognised adaptors remains to be fully clarified, the obvious advantage of the use of different adaptors by TLRs is to provide response specificity. Because components from different pathogens will engage different TLRs, an optimal response can be generated that results in elimination of a specific pathogen. In addition, it is tempting to speculate that these adaptor molecules may be future therapeutic targets in *e.g.* sepsis therapy aimed to block pro-inflammatory cascades initiated through TLRs while maintaining TLR-mediated protective responses [27]. However, whether blocking adaptors that mediate pro-inflammatory responses is compatible with effective clearance of microorganisms, and whether selective inhibitors of the different adaptors can be generated, is not clear.

Antimicrobial peptides and proteins produced by airway epithelial cells

Airway epithelial cells secrete a large array of molecules that are involved in inflammatory and immune processes [2, 3, 28]. This variety of substances produced by airway epithelial cells are summarised in figure 1. By secreting these mediators, the airway epithelium is capable to chemoattract and activate cells of the innate and adaptive immune system, to immobilise and kill microorganisms, to induce wound healing and angiogenesis in response to injury and to orchestrate the initiation of an adaptive immune response. Some of the secreted products have direct antimicrobial activity and likely act as endogenous antibiotics (fig. 1). These molecules include small cationic antimicrobial peptides such as the β-defensins and LL-37, but also larger antimicrobial proteins such as lysozyme, lactoferrin and secretory leukocyte proteinase inhibitor (SLPI; 29, 30). These molecules display microbicidal

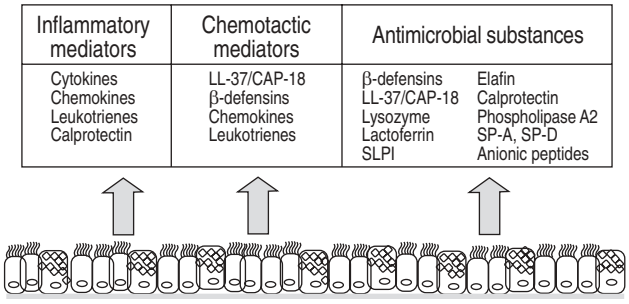

Fig. 1. – The role of the airway epithelium in host's defence against infection. Overview of secreted molecules that play a role in inflammation and host defence. Some of the depicted molecules appear to be secreted primary to the basolateral side (chemokines), whereas others are secreted to the apical side (antimicrobial peptides) of the epithelium.

activity or inhibit growth of inhaled microorganisms until they are eliminated by the mucociliary apparatus, recruited phagocytes and/or the development of an adaptive immune response.

Antimicrobial peptides are effector molecules of the innate immune system of the lung. Recently it was recognised that they have multiple additional activities besides their antimicrobial function. The term antimicrobial peptide refers to ribosomally synthesised, gene-encoded peptides, meaning that one gene in the genome codes for one peptide. Different groups of antimicrobial peptides are defined, based on structural characteristics. The defensins and cathelicidins are the principal families that are expressed in the respiratory tract. The primary translational product is a prepropeptide consisting of an N-terminal signal sequence for targeting to the endoplasmic reticulum, a pro segment, and a C-terminal cationic peptide that has antimicrobial activity after cleavage. The pro-peptide is cleaved during later stages of intracellular processing or after secretion.

Antimicrobial peptides in the human lung are mainly produced and secreted by epithelial and phagocytic cells. The expression and secretion of antimicrobial peptide genes is tightly regulated. Some peptides are produced constitutively, such as hBD-1 or mouse β-defensin 1 (mBD-1). The expression of others is increased by contact of cells with microbial products or proinflammatory mediators. It has been shown that expression of hBD-2, hBD-3, hBD-4, LL-37 and several other antimicrobial peptides is induced *in vitro* by bacterial products and inflammatory mediators [31–36]. These cell culture studies are confirmed by several patient studies, showing that the concentration of antimicrobial peptides such as β-defensins is increased in various body fluids during inflammatory or infectious diseases, such as pneumonia [37] or cystic fibrosis (38; summarised in table 2). From studies in keratinocytes it is known that the epithelial response to LPS is greatly enhanced by macrophages through the production of IL-1 [50] and similar mechanisms may operate to activate hBD-2 expression in pulmonary epithelial cells [51]. This may be an important mechanism for the amplification of the response to microbial products, since epithelial cells are markedly less sensitive to products such as LPS when compared to mononuclear phagocytes. Mechanisms involved in the epithelial regulation of human β-defensin expression by microbial products involves LPS detection by CD14 [7] and or lipoprotein recognition by toll-like receptor-2 [22]. Furthermore, growth factors involved in wound healing were found to increase expression of hBD-3, human cationic antimicrobial protein-18 (hCAP-18)/LL-37 and SLPI in human keratinocytes [52]. Regulation of LL-37 expression

Table 2. – Presence of antimicrobial peptides produced by airway epithelial cells and airway host defence cells in human lung disease

Component	Source	Increased levels in lung disease (references)
α-defensins	Epithelial cells	Pneumonia (39, 40)
	Inflammatory cells	Cystic fibrosis (41)
		Panbronchiololitis (42)
		ARDS (43)
		Chronic bronchitis (44)
		Idiopathic pulmonary fibrosis (45)
β-defensin (BD)	Epithelial cells	Pneumonia (37, 46)
hBD-1	Monocytes/ macrophages	Cystic fibrosis (38, 47)
hBD-2	Dendritic clls	Panbronchiolitis (48)
hBD-3		
hBD-4		
Cathelicidin	Epithelial cells,	Pneumonia (46)
LL-37/hCAP-18	Neutrophils	Sarcoidosis (49)

ARDS: acute respiratory distress syndrome.

in epithelial cells depends furthermore on the differentiation status of the cells [53, 54].

Antimicrobial peptides have a broad spectrum activity against Gram-positive and Gram-negative bacteria as well as against fungi and enveloped viruses [55]. Antimicrobial peptides show synergistic activity with other host defence molecules, such as lysosyme and lactoferrin. The antimicrobial activity is based on interactions between the peptide and surface membranes of the target organisms. Functional studies on antimicrobial activity have primarily been restricted to *in vitro* experiments using purified components. Recently, several groups published results that provided proof of the host defence function of antimicrobial peptides in living organisms. Indirect *in vivo* evidence for the host defence function of antimicrobial peptides came from a study on mice with a disrupted gene for matrilysin, also called matrix metalloprotease 7. Matrilysin-deficient mice were more susceptible to infections with enteropathogens [56]. Mice deficient in an antimicrobial peptide, mBD-1, revealed delayed clearance of *Haemophilus influenzae* from lung [57]. Mice with deleted cathelin-related antimicrobial protein-18, the murine homologue of LL-37, showed more prominent infection after cutaneous inoculation of bacteria [58]. Conversely, the overexpression of LL-37 by viral gene transfer resulted in augmentation of innate host defence in a bronchial xenograft model of cystic fibrosis and in murine animal models of pneumonia and septic shock [59, 60]. In addition, overexpression of a defensin in a transgenic murine model provided protection against enteric salmonellosis [61].

Several lines of evidence indicate the importance of antimicrobial peptides in the human lung. Using culture systems of airway epithelial cells from patients with cystic fibrosis, it was demonstrated that epithelial-cell derived antimicrobial peptides may be inactivated by the high salt concentration in the epithelial lining fluid [62, 63]. Studies in a human bronchial xenograft model revealed decreased antimicrobial activity of airway surface fluid after inhibition of hBD-1 synthesis by antisense oligonucleotides [63]. Other evidence for the importance of antimicrobial peptides comes from studies such as those showing that their levels are changed in inflammatory lung disease (table 2; 64), and the finding that nasal *Staphlococcus aureus* carriage is associated with decreased antibacterial activity of nasal fluid [65]. Finally, also the observation that hBD-1 polymorphisms are associated with COPD suggests that these peptides are important *in vivo* [66].

Antimicrobial peptides have a variety of other biological effects besides their antimicrobial activity. Based on their membrane activity, antimicrobial peptides have a concentration-dependent toxicity towards eukaryotic cells. High concentrations of α-defensins have been described in secretions of patients with cystic fibrosis [41] and chronic bronchitis [44], where these substances likely contribute to the overwhelming inflammation. This may in part be explained by the ability of α-defensins to cause lysis of lung epithelial cells and induction of IL-8 production in these cells [67]. Furthermore, whereas binding of α-defensins to proteinase inhibitors of the serpin family such as α_1-antitrypsin (α_1-AT) may restrict defensin-induced cytotoxicity, it also decreases the elastase-inhibitory activity of α_1-AT [44].

Besides this rather nonspecific toxicity caused by high concentrations, antimicrobial peptides bind to cellular receptors at low concentrations and activate intracellular signalling pathways. α-defensins are able to stimulate a variety of cells by mechanisms not yet identified. They attract human CD4/CD45RA+ or CD8+ T cells [68, 69], immature dendritic cells [69], and monocytes [70]. They also induce the release of IFN-γ, IL-6, and IL-10 from T-cells [71]. hBD-1 and hBD-2 were found to bind to a chemokine receptor known as CCR-6 [72]. This receptor is found on immature dendritic and memory T cells (CD4+/CD45RO+) and consequently these findings are interpreted as a link between innate and adaptive immune mechanisms mediated by defensins. hBD-3 and hBD-4 chemoattract monocytes by mechanisms that have not yet been clarified [36, 73]. Cathelicidins may display similar activities as defensins, since LL-37 binds to formyl peptide receptor like 1 [74] and attracts neutrophils, monocytes, and CD4 T cells and activates mast cells [75]. It is interesting to note that whereas antimicrobial peptides may act as chemokines, the reverse has also been observed since several intact or truncated forms of chemokines display antimicrobial activity [76]. In addition to these roles of defensins and LL-37 in inflammation and immunity, defensins and LL-37 have also been implicated in wound repair processes. Neutrophil α-defensins were found to cause proliferation of airway epithelial cells and mediate epithelial wound repair [77], whereas β-defensins may promote differentiation of keratinocytes [78]. In line with this, LL-37 is involved in re-epithelialisation of cutaneous wounds [79], and induces angiogenesis *in vitro* and *in vivo* [80].

Despite the progress that has been made in the understanding of the basic biology of antimicrobial peptides, their role in human disease is still incompletely understood. Based on their direct host defence and various receptor-mediated functions, antimicrobial peptides could have a central role in infectious and inflammatory diseases. Table 2 summarises disease conditions that are characterized by altered concentrations of antimicrobial peptides.

Antimicrobial peptides have emerged as effector substances of the innate immune system involving not only activities as endogenous antibiotics but also as mediators of inflammation. Further analysis of the biologically relevant functions of antimicrobial peptides will reveal the role that these molecules have in diseases of the lung. Here, antimicrobial peptides might contribute to the development of diseases not only as endogenous host defence substance but also as pro- or anti-inflammatory mediators. Development of antimicrobial peptides as drugs involves optimized strategies for candidate identification, for modification of pharmacodynamic and pharmacokinetic profiles and for production. Studying the biology of antimicrobial peptides should allow the development of novel therapeutics for infectious or inflammatory diseases.

Conclusions

It is evident that there is a renewed and increased interest in innate immunity that has intensified studies on the role of the airway epithelium that forms the primary interface between the environment and the host. The epithelium contributes to host defence in a number of ways, including ciliary beat activity and mucus production, but also production of chemokines, cytokines, antimicrobial peptides, proteinase inhibitors and surfactant proteins. The recognition systems used by epithelial cells to sense microbial exposure do have a certain degree of specificity, and serve to mount a quick and efficient response to deal with a certain pathogen. Studies to elucidate the role of these mechanisms in human lung disease are ongoing, and include genetic studies to identify the association between polymorphisms in genes of innate immunity and lung disease. Such studies have identified MBL, hBD-1 and TLR4 as potential modifier genes in human disease such as cystic fibrosis and COPD.

Current studies are exploring the possibility to modulate the innate immune response of the epithelium to increase local defence or to employ the present knowledge of antimicrobial peptides to develop a new class of antibiotics. These are all promising developments in the application of our increased knowledge of the basic elements of the innate immune system to the development of new therapies for infectious and inflammatory lung disease. This is especially important in view of the emergence of microorganisms resistant to conventional antibiotics, which forms a threat to human health and constitutes an economic burden for health care.

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