INTRODUCTION

An efficient host defence system is required to counteract the continuous microbial challenge to which the lung is exposed. The epithelium lining the airways and alveoli is an essential element in this defence. Epithelial cells provide a passive barrier, contribute to mucociliary clearance, produce inflammatory mediators which recruit cells to the site of infection and are a main site of synthesis of antimicrobial substances referred to as antimicrobial peptides and proteins (or collectively as antimicrobial polypeptides). Therefore the epithelium plays a central role in innate immunity, a system that is rapidly activated after infection, providing efficient control of the invading pathogen. Whereas lysozyme and lactoferrin have been known for some time as key antimicrobial components of this innate immunity, in the last decade a number of new epithelial antimicrobial polypeptides have been identified. Together these endogenous antimicrobial components form an antimicrobial ‘soup’, the constituents of which act in concert to kill invading micro-organisms. Research in this area has intensified after the observation that the increased susceptibility of patients with cystic fibrosis (CF) to infection may be explained by a loss of the endogenous epithelial antimicrobial activity against Pseudomonas aeruginosa, as demonstrated in cultured epithelial cells from CF patients. This loss of activity was explained by an increased NaCl concentration in airway surface fluid (ASF) secreted by these cells which decreased the antimicrobial activity of epithelial antimicrobial polypeptides. Because of the difficulty in collecting ASF from patients and assessing its ionic composition in situ, this explanation for bacterial colonisation of the airways in CF is still a controversial one. Recent studies have provided evidence that these antimicrobial polypeptides may also be involved in inflammation, the generation of adaptive immune responses and wound repair processes.

ANTIMICROBIAL PEPTIDES AND PROTEINS

Airway surface fluid contains a variety of antimicrobial factors mainly produced by submucosal glands and surface...
epithelial cells and by macrophages and neutrophils (Fig. 1). Defensins are small, cationic antimicrobial peptides with a molecular mass of 3.5–4.5 kDa. Based on their structure (most notably the pairing of disulphide bridges) human defensins can be divided into the subfamilies of α- and β-defensins whereas in rhesus monkeys a third class of defensin has been described. These γ-defensins derived their name from their circular molecular structure. Human neutrophils store large amounts of four different α-defensins (Human Neutrophil Peptide [HNP]-1, -2, -3 and -4) in their azurophilic granules. These peptides are released into the phagolysosome where they can kill ingested micro-organisms or they are secreted into the extracellular environment. Bronchial and nasal epithelial cells express one member of the α-defensin subfamily (human α-defensin 5, HDS). In contrast, all members of the human β-defensin subfamily identified to date have been found to be expressed in epithelial cells: hBD-1, -2, -3 and -4 in their azurophilic granules. These peptides may either be constitutive, induced or decreased by a variety of stimuli. Pro-inflammatory cytokines and bacteria may increase β-defensin expression. From studies in drosophila, it is likely that pattern-recognition receptors which recognise pathogen associated molecular patterns (PAMPs) are involved in the stimulatory effect of micro-organisms on epithelial expression of antimicrobial peptides. These receptors, including Toll-like receptors (TLR), recognise microbial products such as lipopolysaccharide. Other factors involved in the regulation of β-defensin expression in epithelial cells include L-soleucine and its analogues, as demonstrated in bovine cells. It was suggested that soleucine might serve as a microbial marker for the presence of bacteria, allowing the host to mount an adequate response.

Bacteria may also decrease expression of antimicrobial peptides, as shown by the observation that Shigella spp. may decrease expression of LL-37 and hBD-1 in intestinal epithelial cells. Since, in addition to antimicrobial peptides and the complement system, phagocytes also constitute the effector mechanisms of the innate immune system, expression of these defence peptides may be modulated by pro-inflammatory mediators produced by these cells.

Figure 1 Antimicrobial polypeptides produced by the surface epithelium and submucosal glands. The airway epithelium provides protection against invading micro-organisms through its barrier function, mucociliary clearance, regulation of inflammation and immune responses and production of antimicrobial substances including polypeptides. Cells from the surface epithelium and the submucosal glands contribute to this production. Note: the schematic drawing of the submucosal gland is an oversimplification.

EXPRESSION

Antimicrobial polypeptides are produced both by surface epithelial cells and by cells of the submucosal glands. The relative contribution of these sources and the contribution of the various cell types of the surface epithelium to polypeptide synthesis are not known. In vitro epithelial cell culture studies and results from bronchial xenografts generated from surface epithelial cells, show that the surface epithelium itself may play a major role in the overall synthesis. The expression of antimicrobial polypeptides may either be constitutive, induced or decreased by a variety of stimuli. Pro-inflammatory cytokines and bacteria may increase β-defensin expression. From studies in drosophila, it is likely that pattern-recognition receptors which recognise pathogen associated molecular patterns (PAMPs) are involved in the stimulatory effect of micro-organisms on epithelial expression of antimicrobial peptides. These receptors, including Toll-like receptors (TLR), recognise microbial products such as lipopolysaccharide. Other factors involved in the regulation of β-defensin expression in epithelial cells include L-soleucine and its analogues, as demonstrated in bovine cells. It was suggested that soleucine might serve as a microbial marker for the presence of bacteria, allowing the host to mount an adequate response.

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system, the observation that neutrophil products such as neutrophil elastase and defensins modulate SLPI and elafin production in bronchial epithelial cells suggests another mechanism for fine-tuning the composition of the antimicrobial screen. Epithelial repair may be associated with increased expression of SLPI in the skin and hBD-2 in the cornea. Thus when the epithelial barrier function is breached, increased expression of antimicrobial peptides in repairing epithelium may protect the underlying tissue until the protective epithelial barrier is restored. Finally, the observation that glucocorticoids decrease β-defensin expression in bronchial epithelial cells indicates that anti-inflammatory drugs may alter host defense against infection.

Whereas transcriptional regulation may be an important mechanism to alter the composition of the antimicrobial soup, post-transcriptional mechanisms may also be involved. Various antimicrobial polypeptides are produced as proproteins, with a pro-region that blocks the activity of the mature antimicrobial peptide. Processing of the pro-protein by proteolytic cleavage is required to release the active peptide. The importance of this mechanism was illustrated by the observation that mice deficient in the matrix metalloproteinase matrilysin are not able to process intestinal prodefensins into the active peptides and display increased sensitivity to intestinal infections. Similar mechanisms may also operate in the lung.

**ANTIMICROBIAL ACTIVITIES**

Bacteria, fungi and enveloped viruses are susceptible to the antimicrobial activity of antimicrobial polypeptides in vitro. There is also evidence for the in vivo role of antimicrobial peptides as shown in animal models employing over-expression or knock-out models, as shown for matrilysin. These peptides kill micro-organisms by disrupting the integrity of microbial membranes by mechanisms such as pore formation, by iron chelation or by enzymatic disruption of essential microbial target structures. Little is known about the relative contribution of the various polypeptides to the overall antimicrobial activity of airway secretions. There are marked differences in the concentration of these components in the fluid. SLPI may also be a major component in ASF. Marked synergistic interactions in the antimicrobial activities of lysozyme, lactoferrin and SLPI have been described, whereas the activities of hBD-1, hBD-2 and LL-37 were additive. Synergy has also been noted between hBD-4 and lysozyme. Antimicrobial polypeptides also display differences in their activity against various target microorganisms, furthermore, the conditions (e.g. salt concentration and pH) required for optimal activity may differ. Therefore, synergistic actions, different target cell selectivity and requirements for optimal activity may explain the need for a wide variety of polypeptides in the ASF. Another explanation may be that bacteria have developed countermeasures rendering them resistant to the activity of antimicrobial peptides. Therefore, the presence of an array of polypeptides may be required to prevent colonization of the respiratory tract by micro-organisms, including those which have evolved resistance mechanisms. Bacteria do not readily develop resistance against antimicrobial polypeptides in vitro contrary to the development of resistance against conventional antibiotics. This observation, together with the fact that peptides kill micro-organisms which are resistant to conventional antibiotics has provided a rationale for the development of peptide-based antimicrobial drugs to treat infections.

The antibacterial activity of antimicrobial polypeptides is the result of an interaction of polypeptides with bacterial wall components. A major component of the outer wall of Gram-negative bacteria is lipopolysaccharide (LPS). It is well recognised that LPS displays potent proinflammatory activity by direct interaction with host cells. Whereas in most cases the outcome of this interaction of LPS with host cells will be beneficial to the host, LPS may also cause unwanted inflammatory reactions detrimental to the host. Therefore, the capacity of various antimicrobial peptides to inhibit this pro-inflammatory activity of bacterial components as LPS may help to shut off the inflammatory reaction initiated by the infectious process. This property of antimicrobial peptides also makes them attractive for the development of novel anti-inflammatory treatments.

**ROLE IN INFLAMMATION AND IMMUNITY**

**Cell recruitment**

Recruitment of leukocytes is an essential element in the defense against infections. It results in the influx of phagocytes which ingest and kill the invading microorganisms and includes cells, essential in the initiation and propagation of a specific immune response. Inflammatory cells may cause tissue injury. Chemoattractants such as chemokines play a central role in the regulation of cell recruitment. Recent data suggest that not only chemokines and other chemoattractants but also α-defensins, β-defensins and LL-37 display chemotactic activity for various cell types (Fig. 2). Interestingly these peptides were shown to employ host chemoattractant receptors to mediate this effect. Soon after the characterization of α-defensins in human neutrophils in the mid-1980s chemotactic activity of these peptides for monocytes was described. Subsequent studies showed that neutrophil α-defensins are also chemotactic for immature dendritic cells, for resting CD4 T cells, and for CD8 T cells. The receptor involved in this activity was not identified but evidence was obtained suggesting it is a chemokine receptor. The direct involvement of chemokine receptors in signalling by antimicrobial
Figure 2  Role of epithelial antimicrobial polypeptides in regulation of the influx of inflammatory and immune cells following microbial challenge. Contact of the epithelium with bacteria will result in the production of antimicrobial polypeptides and chemokines causing chemotaxis of antigen-presenting dendritic cells, monocytes, T cells and neutrophils. Once recruited, neutrophils may cause epithelial injury and further amplify cell recruitment by releasing α-defensins, LL-37 and chemokines. In addition, peptides such as neutrophil-derived α-defensins will stimulate production of chemokines such as IL-8 resulting in a positive feedback loop for neutrophil recruitment to the site of infection.

peptides was demonstrated in a study describing that β-defensins employ the chemokine receptor CCR6 to attract immature dendritic cells and memory T cells.23 Subsequent studies revealed that selected β-defensins (hBD-3 and -4) also attract monocytes.7 In contrast to the defensins, the cathelicidin LL-37 lacks chemotactic activity for dendritic cells but does attract neutrophils, monocytes and T cells. This activity is mediated by another receptor for known chemoattractants, the formyl peptide receptor-like 1 (FPRL1). Finally, other neutrophil-derived antimicrobial polypeptides display chemotactic activity for leukocytes.25 Another mechanism by which antimicrobial polypeptides may attract leukocytes to the epithelium, is local induction of chemokine production in the epithelium. Such a mechanism has been demonstrated in vitro for neutrophil α-defensins. This provides evidence for the possibility that antimicrobial peptides not only serve to protect against infection by their antimicrobial activity but may also regulate the influx of inflammatory cells. These polypeptides also appear to bridge the innate and adaptive immune systems, by attracting the cellular elements required for orchestrating an immune response.

Repair

The expression of epithelial antimicrobial polypeptides may be increased at sites of epithelial injury and repair. Whereas this may help in the protection of the exposed tissue against infection by direct antimicrobial activity and chemotactic activity, other functions are also likely. Members of the defensin and cathelicidin families and lactoferrin, may have growth-promoting activities which could play a role in the restoration of epithelial integrity. Epithelial repair is a complex biological process involving epithelial cell migration, proliferation and differentiation. Further evidence for a role of antimicrobial polypeptides in epithelial wound repair comes from a study showing that mice deficient in SLPI have a deficiency in cutaneous wound repair that is associated with increased inflammation.24 The relative contribution of the antimicrobial, proteinase inhibitory or other activities of SLPI in wound healing was not studied in detail.

Miscellaneous functions

Antimicrobial polypeptides may display activities distinct from those described above. α-defensins also regulate processes such as complement activation, fibrinolysis, mast cell degranulation and regulation of endogenous cortisol production.2 Neutrophil α-defensins stimulate the binding of selected bacteria to epithelial cells.23 This may facilitate bacterial killing as it exposes these bacteria to high concentrations of epithelial antimicrobial polypeptides or it may allow bacteria to colonise the respiratory tract. The outcome may depend on local conditions such as the salt content of the ASF. It is likely that further studies will reveal other functions for these peptides as well. A recent example of this is the observation that hBD-2 and LL-37 stimulate mast cells to release histamine and produce prostaglandin D3.26

CONCLUSIONS

During the last decade, antimicrobial peptides have been closely studied leading to the discovery of several such molecules and to the reappraisal of the function of others such as SLPI. Several new functions have now been ascribed to these components which has led to exciting new views on the link between innate and adaptive immunity. How can we use this information to understand the pathogenesis of lung disease and to optimise treatment of patients? Whereas relatively little is known about the role of these peptides in disease, it is known from a limited number of studies that their expression may be increased in inflammatory lung disease and their activity may be decreased in CF. Modulation of peptide expression in the lung may offer new treatment strategies. Understanding the role of Toll-like receptors in the regulation of innate immunity may be one of the keys towards the development of such innovative strategies. In addition, the peptides themselves or peptides based on the structure of endogenous antibiotics are being developed and evaluated in clinical trials to treat infections. Aerosol therapy using antimicrobial polypeptides designed on the basis of
structure-function studies of endogenous antibiotics may become a novel form of topical treatment of respiratory infections. As new functions of antimicrobial polypeptides are discovered, we may begin to consider other therapeutic applications for these peptides such as enhancement of wound repair and modulation of immune responses.

REFERENCES