Pathogenesis of Haemophilus influenzae. Respiratory infection in COPD patients
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SUMMARY
In healthy individuals, bacteria do not colonize the epithelial cell layer of the lower respiratory tract. Bacteria reaching the lower airways by inhalation and aspiration are cleared from the airways by the mucociliary elevator and killed by antimicrobial polypeptides such as β-defensins produced by the airway epithelial cells. In patients with chronic bronchitis and chronic obstructive pulmonary disease (COPD), however, the epithelium of the lower respiratory tract is injured as a result of e.g. cigarette smoke and a chronic inflammatory process. Despite the presence of specific antibodies and professional phagocytes in the respiratory tract, COPD patients suffer from recurrent bacterial infections of the lower respiratory tract. The Gram-negative bacterium *Haemophilus influenzae* is one of the most frequently isolated pathogens in COPD patients. Interaction of *H. influenzae* with airway epithelial cells is important for the colonization and pathogenesis of the lower respiratory tract infection as well as for the host defense against the bacteria by inducing inflammatory mediators. *H. influenzae* colonizes *in vivo* only inflamed lower airways, such as present in COPD patients. Since the inflammatory process is characterized by the presence of phagocytes like neutrophils, which contain various antimicrobial peptides including neutrophil defensins, *H. influenzae* as well as defensins are found in the sputum of COPD patients. Therefore, the effects of defensins on the interaction of *H. influenzae* with epithelial cells were assessed in the studies described in this thesis.

First, the effect of neutrophil defensins on the adherence of *H. influenzae* to airway epithelial cells was analyzed. We showed that neutrophil defensins enhanced the adherence of all tested *H. influenzae* strains (n = 15) to both the epithelial cell line NCI-H292, derived from a human lung mucopidermoid carcinoma, and to human primary bronchial epithelial cells. This enhanced adherence was not mediated by the presence of known bacterial adhesins [chapter 2]. The defensin-enhanced adherence was dependent on the incubation time and the concentration of defensins. Since other cationic polypeptides, including poly-l-lysine and protamine, did not enhance the adherence of *H. influenzae*, the defensin-enhanced adherence can not solely be due to charge effects [chapter 2]. The defensin-enhanced adherence phenomenon was also observed using oropharyngeal epithelial cells, from which the mucus layer was not removed. This indicated that the defensin-enhanced adherence may be relevant for the *in vivo* situation. COPD patients with a deficiency of the proteinase inhibitor α1-antitrypsin (α1-AT) suffer more frequently from bacterial infections than COPD
patients without \(\alpha1\)-AT deficiency. \(\alpha1\)-AT is a neutrophil elastase inhibitor that also blocks most of the effects of defensins. We showed that the defensin-enhanced adherence was inhibited by \(\alpha1\)-AT. The difference in frequency of bacterial infection between the \(\alpha1\)-AT deficient COPD patients and COPD patients without the \(\alpha1\)-AT deficiency the might be due to such a mechanism.

The defensin-enhanced adherence was specific for selected bacterial species using the upper respiratory tract as port of entry, such as *Moraxella catarrhalis* and *Neisseria meningitidis* [chapter 3]. However, the defensin-enhanced adherence was not restricted to airway epithelial cells, since the phenomenon was also observed when studying the adhesion of *H. influenzae* to non-respiratory epithelial cells, endothelial cells (HUVEC), or fibroblast like cells (HEL) [chapter 3]. The defensin-enhanced adherence required metabolically active epithelial cells, whereas both viable and heat-inactivated bacteria showed enhanced adherence in the presence of defensins. This indicated that the bacterial component involved in the defensin-enhanced adherence is heat-stable. Lipooligosaccharide (LOS) might be such a bacterial component since the bacterial species showing the defensin-enhanced adherence are characterized by the presence of LOS in their outer membrane. Lipopolysaccharide (LPS) is not a likely candidate, since the adherence of *Escherichia coli* and *Pseudomonas aeruginosa*, both having LPS, was not affected by the presence of defensins [chapter 3]. Using LOS mutants of *H. influenzae* and *N. meningitidis*, we demonstrated that LOS was indeed involved in the defensin-enhanced adherence [chapter 4]. However, the mechanism by which defensins and LOS interact with epithelial cells to promote bacterial adherence remains to be resolved.

Interaction of *H. influenzae* with epithelial cells leads to the induction of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and interleukin-8 (IL-8). Since neutrophil defensins also induce the release of these pro-inflammatory cytokines, the effect of defensins on the *H. influenzae*-induced IL-6 and IL-8 release was assessed [chapter 5]. The release of IL-6 and IL-8 by the epithelial cell line NCI-H292 was synergistically increased more than 3-fold upon the exposure to the combination of *H. influenzae* and neutrophil defensins [chapter 5]. This synergistic effect was most evident for low bacterial loads (\(10^5\) and \(10^6\) CFU/ml) and was only partially prevented by the corticosteroid dexamethasone. The mechanism by which
H. influenzae and defensins synergistically increased the IL-6 release is not known at the moment, since the activation of transcription factors as well as the IL-6 mRNA stability were not influenced by H. influenzae and defensins. The synergistic increased IL-8 release was mainly post-transcriptionally regulated, since the stability of the IL-8 mRNA was increased.

Our findings that neutrophil defensins enhanced bacterial adherence and that defensins and H. influenzae synergized in the IL-6 and IL-8 release, leading to increased amounts of neutrophils, are in line with the vicious circle hypothesis which has been proposed for COPD patients. This hypothesis states that in COPD patients inflammation provokes colonization which will lead to bacterial infection and that this infection will lead to a further increase in inflammation. Since in COPD patients that are colonized with bacteria the inflammatory process is more pronounced than in COPD patients without bacterial colonization, the defensin-enhanced adherence and increased cytokine release might be relevant mechanisms for the continuation of the inflammatory process in these patients. Further understanding of the mechanisms by which defensins affect the interaction of H. influenzae to airway epithelial cells is needed to design strategies to break this vicious circle of inflammation and infection in COPD patients.