Defensins;where and how do they work against micro-organisms on human airways?

To the Editor:

We appreciated the fine review article by HIEMSTRA *et al.* [1] concerning defensins and pulmonary epithelium. Defensins are broad spectrum antimicrobial peptide products of neutrophils (α-defensins) [2, 3] and epithelia β-defensins) [4, 5]. Since airway infection with *Pseudomonas aeruginosa* and neutrophilic inflammation are the major cause of lung disease in cystic fibrosis (CF), bacterial killing by human β-defensins (HBD) predominantly produced by airway epithelium and in the airway surface liquid (ASL) may be of clinical/pathological importance in both healthy subjects and CF patients. Extensive evidence suggests that epithelial tissue provides the first line of defence between foreign organisms and the environment. Disruption of this barrier leads to bacterial invasion and subsequent inflammation. The first direct evidence for the expression of defensin peptides in the oral mucosa was the identification of a novel epithelial β-defensin peptide in the mammalian tongue [6]. It was shown to be upregulated in inflammation, suggesting that it participates in host defence. There is now evidence indicating that normal airway epithelial cells and tissues express two β-defensins, human β-defensins (HBD)-1 and HBD-2 [4, 5]. It has also been reported that both HBDs are detected in bronchoalveolar lavage (BAL) fluid [7, 8]. However, the antimicrobial activities ofbothHBD-1andHBD-2wereknowntobeinhibitedbyNaCl.

The key issue about the antimicrobial action of β-defensins is the composition and osmolality of ASL. WINE [9] has perceptively summarized the two current hypotheses regarding the pathogenesis of CF airway disease in reaction to the composition of ASL and defensins. In healthy subjects, ASL has been reported to be hypotonic, however elevated ASL Na^+ and Cl^- concentrations in airways in CF patients have been reported by several investigators in both *in vivo* and *in vitro* studies [10, 11]. SMITH *et al.* [12] demonstrated that normal airways reabsorb salt in greater quantities than water from the ASL, thus producing the sufficiently low NaCl concentrations needed (≤50 mM NaCl) to activate defensins, but that salt is poorly absorbed in CF airways, resulting in excessively salty ASL that disrupts the bacterial killing activities of HBDs. This is the "hypertonic (high salt) ASL in CF airways" hypothesis. However, the second hypothesis, "low, but isotonic volume of ASL in CF airways", has recently been considered to link defects in cystic fibrosis transmembrane conductance regulator (CFTR)-mediated ion transport with CF airway disease [13]. MATSUI and BOUCHER [13] have demonstrated that the airway absorbs salt/water isotonically to ad-just the volume/height of the ASI, components to maintain efficient mucus clearance. They suggested that airway epithelia are too water permeable to maintain hypotonic ASL. Several investigators have also reported that the ASL is isotonic rather than hypotonic in both healthy subjects and child and adult patients with CF [14–16]. The "low volume of ASL in CF airways" hypothesis may support one of the earliest hypotheses to explain CF lung disease, the "thick mucous" hypothesis [17, 18]. If it is true, ASL cannot produce a sufficiently low NaCl concentration to activate HBDs *in vivo*. Although the HBDs are mainly produced by airway epithelia, how and where do the HBDs work on airways? There are a number of questions still outstanding.

Firstly, do the β-defensins work in ASL in human airways? The "low salt in normal airways/high salt in CF airways" theory [10–12] strongly supports the idea that HBDs derived from the epithelium act directly against micro-organisms in the relatively hypotonic ASL of non-CF subjects, but not in CF patients. However, the "isotonic ASL in normals as well as CF airways" theory [13–16] contradicts the idea of the effective action of HBDs on the bacteria in lungs in both CF and non-CF airways.

Secondly, do the other components of ASL and the mucous inhibit or augment the action of HDBs on human airways? Since ASL is not composed of salt-water alone, (there are proteases, lysosomes, and another ions/anions)? It has been reported that ASL from healthy subjects has markedly higher K^+ concentrations than plasma [11]. In addition, α-defensins are known to increase the interleukin (IL)-8 messenger ribonucleic acid (mRNA) of epithelial cells [3].

Thirdly, although the natural bacterial killing activity of HBD is upregulated by inflammatory stimuli including bacteria, fungi, and lipopolysaccharides (LPS), it is not easy to maintain airway cells in culture without antibiotics, *e.g.* penicillin and tobramycin. The antimicrobial activity of HBDs may be not potent enough to protect airway cells in a culture dish against the contaminated bacteria.

Finally, the problem is that ASL exists as a very thin layer of fluid, making the collection of a sufficient volume for reliable analysis difficult [18, 19]. After many false starts, investigators are now coming closer to understanding the fundamental pathogenesis of CF [9]. The further knowledge about the difference and/or similarity, in the regulation of depth and composition of ASL between CF patients and healthy subjects may be the key to the real understanding of the function of defensins as well as CF airway disease pathogenesis [18, 19].

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From the authors:

We would like to thank S. Teramoto and Y. Ouchi for their comments in response to our review on neutrophil serine proteinases and defensins in the *European Respiratory Journal* [1]. Our review was focused on the effects of neutrophil serine proteinases and defensins on pulmonary epithelium, rather than addressing the recently developed new insights into the role of defensins in increased susceptibility to bacterial infection in cystic fibrosis (CF). Indeed, studies by SMITH *et al.* [2] and GOLDMAN *et al.* [3] have indicated that the activity of antimicrobial peptides produced by epithelial cells is possibly decreased in the airway surface liquid (ASL) of patients with CF as a result of an increased salt concentration in this fluid [4]. These studies are in line with the "high salt hypothesis"[1]. Although they have received much attention and led to new research initiatives in the field, it has to be noted that the results were obtained using cultured epithelial cells (including the elegant bronchial xenograft model). Therefore, their relevance to the *in vivo* situation remains to be shown. Studies in which the Na^+ and Cl^- concentrations of ASL sampled from the airways of patients with CF is measured may provide important information. However, such studies have sometimes provided conflicting results, which may in part be related to the fragility of the bronchial mucosa especially in inflamed areas. Improved sampling techniques for the collection of ASL from the airways may provide more consistent data on this matter. Much of the attention on the inactivation of antimicrobial peptides in ASL in CF is focused on β-defensins. In addition, the activity of other cationic antimicrobial peptides and proteins, including that of secretory leukocyte proteinase inhibitor (SLPI); [5], is decreased under conditions of increased ionic strength.

The questions raised by S. Teramoto and Y. Ouchi in their letter are interesting. Indeed, based on the "low volume hypothesis", it would seem unlikely that β-defensins are active inASL under normal conditions due to the isotonic nature of this fluid. Little is known about the effect of various components present in ASL on the activity of antimicrobial peptides. Therefore studies aimed at exploring the activity of antimicrobial peptides in their "natural environment" are needed. What is the impact of this new information on airway epithelial cell culture? While it is true that it appears to be difficult to maintain airway epithelial cells in culture without antibiotics, this does not necessarily imply that the antimicrobial peptides secreted by the cells in culture are not active. This observation is probably explained by the fact that these cells are cultured in isotonic culture medium. Because of the salt content of the medium, it does not seem likely that antimicrobial peptides display optimal activity against micro-organisms in this medium. This also partially explains the results of a recent study [6], in which neutrophil defensins were found to increase the adherence of *Haemophilus influenzae* to cultured bronchial epithelial cells, in the absence of antimicrobial activity against the bacteria. The composition of the culture medium used in this study formed a reasonable explanation for the lack of antibacterial activity of the neutrophil defensins in these experiments. These results suggest that in conditions of high salt, as predicted in CF, neutrophil defensins do not kill but rather enhance the adherence of selected micro-organisms.

In summary, the "high salt hypothesis" for the inactivation of antimicrobial peptides is an appealing addition to the various explanations that have been proposed to explain the increased susceptibility to bacterial infection in CF. It has led to rapid progress in research into endogenous antimicrobial peptides, and ultimately may aid in the development of new therapies.

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