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Synergistic and additive killing by antimicrobial factors found in human airway surface liquid

PRADEEP K. SINGH,[1] BRIAN F. TACK,[2] PAUL B. McCRAY, JR.,[3]
AND MICHAEL J. WELSH[1,4,5]
[5]*Howard Hughes Medical Institute, and Departments of* [1]*Internal Medicine,*
[2]*Microbiology,* [3]*Pediatrics, and* [4]*Physiology and Biophysics,*
University of Iowa College of Medicine, Iowa City, Iowa 52242

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Singh, Pradeep K., Brian F. Tack, Paul B. McCray, Jr., and Michael J. Welsh. Synergistic and additive killing by antimicrobial factors found in human airway surface liquid. *Am J Physiol Lung Cell Mol Physiol* 279: L799–L805, 2000.—Airway surface liquid contains multiple factors thought to provide a first line of defense against bacteria deposited in the airways. Although the antimicrobial action of individual factors has been studied, less is known about how they work in combination. We examined the combined action of six antimicrobial peptides found in airway surface liquid. The paired combinations of lysozyme-lactoferrin, lysozyme-secretory leukocyte protease inhibitor (SLPI), and lactoferrin-SLPI were synergistic. The triple combination of lysozyme, lactoferrin, and SLPI showed even greater synergy. Other combinations involving the human β-defensins, LL-37, and tobramycin (often administered to cystic fibrosis patients by inhalation) were additive. Because the airway surface liquid salt concentration may be elevated in cystic fibrosis patients, we examined the effect of salt on the synergistic combinations. As the ionic strength increased, synergistic interactions were lost. Our data suggest that the antibacterial potency of airway surface liquid may be significantly increased by synergistic and additive interactions between antimicrobial factors. These results also suggest that increased salt concentrations that may exist in cystic fibrosis could inhibit airway defenses by diminishing these synergistic interactions.

cystic fibrosis; innate immunity; lysozyme; β-defensins; lactoferrin

THE HUMAN AIRWAYS ARE PROTECTED against infection by both the adaptive and innate immune systems. Important components of innate defense are antimicrobial factors in the airway surface liquid (ASL), the thin layer of fluid that covers the luminal surface of the airway. This liquid contains multiple antimicrobial factors, including lysozyme, lactoferrin, and secretory leukocyte protease inhibitor (SLPI), that are secreted by submucosal glands and surface epithelia as well as several small antimicrobial peptides known as β-defensins and cathelicidins (3, 4, 25, 29). Lysozyme and lactoferrin are the most abundant antimicrobial factors in the airway, present in the sputum at ~1 mg/ml (5, 10). SLPI has been measured in nasal secretions at 10–80 μg/ml (6, 18). Estimates of human β-defensin (HBD)-2 concentration in ASL vary from 0.01 to 10 μg/ml (6, 25), whereas LL-37 and HBD-1 (4, 6, 25) have been detected, but their concentrations are not established.

The antimicrobial shield provided by these factors has several important features. Antimicrobial factors are continually present in ASL and are thus able to kill bacteria as soon as they are deposited. Furthermore, these factors kill bacteria without inflammation and have activity against a broad range of microorganisms. Although controversy remains, some studies suggest that the antimicrobial activity of these factors is impaired by high salt concentrations that may exist in cystic fibrosis (CF) ASL (8, 26). This disruption of innate defense may contribute to the susceptibility these patients have to bacteria that do not normally infect the lung and the persistent inflammation that eventually results in respiratory failure.

It is interesting that ASL has evolved to contain multiple antimicrobial factors. There are several potential reasons for this. One is that multiple factors might provide redundancy in maintaining the sterility of the lower respiratory tract, a requirement for survival. Multiple factors could also have evolved to increase the spectrum of antimicrobial activity. An additional possibility, which we addressed in this study, is that the antimicrobial potency of ASL is increased by the presence of multiple factors. This could occur if some factors interact additively, that is, combined activity equals the sum of individual activities; or synergistically, that is, combined activity is greater than the sum of individual activities. Increased potency produced by such interactions could make the ASL a more effective defense against bacteria and prevent the emergence of resistant organisms.

There are examples of other systems in which the activity of native antimicrobial factors is enhanced in combination. Lactoferrin and secretory IgA in human

Address for reprint requests and other correspondence: M. J. Welsh, Howard Hughes Medical Institute, Univ. of Iowa College of Medicine, 500 EMRB, Iowa City, IA 52242 (E-mail: mjwelsh@blue.weeg.uiowa.edu).

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