Finally, if serine protease inhibitors are to be studied in CF clinical trials, it will be necessary to have appropriate markers of the lung serine protease burden. Although bronchoalveolar lavage is a useful tool to study the protease-antiprotease balance in the lung, the relative invasiveness of such a procedure makes it impractical for large scale clinical trials. Sputum markers such as elastase activity, IL-8 and protein degradation products may be helpful, but will likely give highly variable results since sputum is not a homogeneous sample. It is therefore probable that serum and plasma markers of the lung protease burden will be needed to follow patients in clinical trials. One such marker is the elastase-α1AT complex measured by ELISA, however additional indicators of the lung serine protease burden in CF may be needed in the near future.

References
procedures in bacteria (8). The method of choice involves production of these peptides by fusion protein technology wherein a four part fusion protein is encoded by a plasmid in E. coli or S. aureus. This fusion protein contains (from N- to C-terminus) an affinity binding region (for ease of purification), an anionic stabilizing fragment (to neutralize the cationic peptide portion and prevent its bactericidal action and cleavage by bacterial proteases), a methionine (to permit release of the cationic peptide by cyanogen bromide) and a cationic antimicrobial peptide. The latter three regions are encoded by synthetic DNA and their sequence can be changed rather simply permitting a large range of cationic peptides to be made by this technology. After purification, a peptide produced by recombinant DNA technology was indistinguishable from one made by protein chemical means, except that it cost only 5% as much to produce.

Using this technology, we have produced a variety of different peptides including a human defensin, alpha helical hybrids of moth cecropin and bee venom melittin (CEME and a variant MBI.28), indolicidin, bactenacin and apidecin. The alpha helical class was studied in detail. These peptides were shown to access the self promoted uptake pathway in P. aeruginosa (and Enterobacter cloacae) (9). They demonstrated very rapid killing even at the MIC (cf. all other known classes of antibiotics), were equally effective against parental strains and antibiotic resistant mutants and engendered no resistance themselves in vitro experiments. They also breached the outer membrane permeability barrier of Pseudomonas aeruginosa and enhanced the uptake of lysozyme and certain antibiotics. In addition, they bound tightly to endotoxin and neutralized it. We propose that cationic peptides offer potential as an alternative to classical antimicrobial therapy against Pseudomonal lung infections in patients with cystic fibrosis.

References

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S14.4 Effect of rhDNase Administration on Sputum Bacterial Density
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rhDNase decreases sputum viscosity in vitro and aerosol administration to patients with Cystic Fibrosis improves pulmonary function. Assuming that less viscous sputum permits facile clearance of respiratory secretions by the mucociliary elevator and cough, we questioned whether sputum bacterial density would decrease with aerosol administration of rhDNase. We enrolled 74 patients from 5 CF Centers, quantitatively culturing their sputum prior to and after 29 and 169 days of administration of placebo (24 patients) 2.5mg, or rhDNase once (22 patients) or twice (28 patients) daily.

All initial sputum cultures grew organisms commonly isolated from CF patients. Comparing the mean enrollment density of P. aeruginosa to that observed on day 29 (in 54 patients) and day 169 (in 45 patients) we found no significant difference among the three treatment groups (all p values >0.20). This lack of difference remained when those patients who received parenteral, aerosol, or oral anti-pseudomonal antibiotics in the two weeks prior to each culturing were excluded. Similarly, when Staphylococcus aureus densities were compared there were no significant differences among treatment groups before or after adjustment for prior antibiotic administration. Patients yielding H. influenzae, C. albicans, and B. cepacia were distributed equally among the treatment groups at all sampling times, but were too few to permit statistical analysis. We conclude that aerosol administration of rhDNase (at 2.5mg once or twice daily for 29 or 169 days) does not produce a significant change in sputum density of S. aureus and P. aeruginosa.