Mucus, Phlegm, and Sputum in Cystic Fibrosis

Bruce K Rubin MEngr MD MBA FAARC

Introduction
Phlegm and the Pathogenesis of Cystic Fibrosis Airway Disease
DNA and F-Actin Polymers in Cystic Fibrosis Sputum
The Role of Mucolytics in the Treatment of Cystic Fibrosis
Classic Mucolytics
Peptide Mucolytics
Hyperosmolar Aerosols
Medications That Promote Sputum Expectoration
Decreasing the Volume of Airway Secretions: Mucoregulatory Drugs
Evaluating the Effectiveness of Interventions to Improve Sputum Clearance in Cystic Fibrosis
Summary

Although some clinicians still believe that cystic fibrosis (CF) lung disease is largely due to hypersecretion of very viscous mucus, it has never been demonstrated that there is mucus hypersecretion in CF and it is clear that there is almost no intact mucin (the principal polymeric component of normal mucus) in CF sputum. CF sputum has lower viscosity when compared to asthma or bronchitis sputa, but is highly tenacious and biochemically most closely resembles pus. Tenacity and lower viscosity lead to decreased cough clearance of infected phlegm, which is thought to induce a persistent inflammatory state in the airway, leading to bronchiectasis. There are many medications and devices either in use or under development that are meant to improve airway hygiene in CF by assisting with sputum expectoration. This paper discusses the scientific basis and potential mechanism of action for many of these interventions and briefly reviews the clinical evidence of their safety and effectiveness. *Key words: cystic fibrosis, mucus, phlegm, sputum.* [Respir Care 2009;54(6): 726–732. © 2009 Daedalus Enterprises]

Introduction

It has long been assumed that cystic fibrosis (CF) lung disease is caused by thick and viscous mucus that obstructs the airway and promotes persistent bacterial infection. So pervasive is this concept, that CF is called "mucoviscidosis" in many parts of the world. On the basis of this belief, a number of different medications meant to thin secretions have been used to try to treat CF, with mixed success. Even today it is a commonly held belief that CF

Bruce K Rubin MEngr MD MBA FAARC is affiliated with the Department of Pediatrics, Wake Forest University School of Medicine, Winston-Salem, North Carolina.

Dr Rubin has disclosed relationships with Pfizer, Ventaira, Trudell Medical International, Monaghan Medical, GlaxoSmithKline, Medihale, Pharmaxis, Syntaxin, Bayer, Hill-Rom, Boehringer Ingelheim, and RegeneRx.

Dr Rubin presented a version of this paper at the 43rd RESPIRATORY CARE Journal Conference, "Respiratory Care and Cystic Fibrosis," held September 26-28, 2008, in Scottsdale, Arizona.

Correspondence: Bruce K Rubin MEngr MD MBA FAARC, Department of Pediatrics, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem NC 27157-1081. E-mail: brubin@wfubmc.edu.

Table 1. Sputum Viscosity*

	Sputum Viscosity (poise at 1,350/s) (range)		
	Mucoid	Mucopurulent	Purulent
Chronic bronchitis	0.41 (0.08–1.25)	0.61 (0.15–1.63)	1.07 (0.46–1.38)
Bronchiectasis	ND	0.39 (0.07–0.89)	0.61 (0.21–1.63)
Cystic fibrosis	0.25 (0.04–0.46)	0.39 (0.16–0.84)	0.84 (0.39–1.60)
Asthma	0.56 (0.09–1.72)	1.46 (0.26–3.10)	ND

* Expectorated sputa from subjects with chronic bronchitis, cystic fibrosis (CF), non-CF bronchiectasis, and asthma was collected and visually categorized by the degree of "purulence." Viscosity was measured by a rotational viscometer at a single frequency. Although sputa from subjects with all diseases became more viscous with increasing purulence, CF sputum was less viscous than either asthma or bronchitis sputum with similar degrees of purulence. ND = no data available. (Data from Reference 1.)

lung disease is caused, at least in part, by thick mucus obstructing the airways.

The difficulty with this concept is that it does not stand up to scientific investigation. There have been many studies evaluating the rheologic or viscoelastic properties of expectorated sputum in CF. Most of these confirm that as secretions become more purulent, to no surprise, their viscosity increases. However, these studies also show that the viscosity of CF secretions is no greater than that of sputum from patients with bronchiectasis or chronic bronchitis, and is much less than phlegm from persons dying from asthma (Table 1). Thus, most published evidence clearly shows that the problem in CF is not secretions that are too viscous.[1-3]

It has been hypothesized that "viscous" secretions are poorly cleared by cilia and that this is what causes lung disease in CF. Mucociliary clearance is modestly compromised in the small airways of CF patients with decreased pulmonary function, but large-airway[4,5] and nasal[6] mucociliary clearance is preserved. On the other hand, persons with primary ciliary dyskinesia have congenital absence of mucociliary clearance[5] and this disease should have a more rapid progression than CF. However, clinically, primary ciliary dyskinesia is a far milder disease for most patients, strongly suggesting that failure of mucociliary clearance is not the primary cause of CF airway disease.

Mucus is defined as the heterogeneous, adhesive, viscoelastic gel produced by goblet cells and submucosal glands. It is the normal, protective airway secretion whose principal polymeric components are the gel-forming mucins, predominantly MUC5AC and MUC5B.[7] Normal mucus is important for airway hydration and for entrapping and clearing bacteria and inhaled irritants. As shown in Figure 1, there is almost no intact MUC5AC or MUC5B in the CF airway.[8] Thus, biochemically and physically, CF sputum cannot be accurately described as mucus, and most closely resembles pus.

The term that we use for the purulent secretion that is a product of airway inflammation is *phlegm*, from the Greek term for inflammation. This contains breakdown products of inflammatory cells and epithelial cells, including copolymers of DNA and filamentous (F-) actin, bacteria, cell debris, and variable amounts of mucin.[9,10] When phlegm is expectorated, this substance is called sputum. Most studies of airway secretions in people with lung disease have been done using expectorated sputum because of the relative ease of obtaining specimens, compared to the difficulty in obtaining normal mucus from healthy airways.

Phlegm and the Pathogenesis of Cystic Fibrosis Airway Disease

In the previous issue of RESPIRATORY CARE, Ratjen discussed the pathogenesis of CF lung disease in detail.[11] The normal CF transmembrane ion regulator protein, CFTR (cystic fibrosis transmembrane regulator), maintains adequate airway surface liquid (ASL) volume by net sodium absorption coupled with chloride and water secretion. This periciliary fluid stands about the height of an extended cilium (roughly 7 μm in the trachea and large airways), allowing cilia to extend, and as the ciliary tips interact with the mucus layer, they engage with the mucus. There is biophysical and histological evidence that, in health, a surfactant layer separates cilia from the mucus layer (see Fig. 2), permitting efficient energy transfer without entanglement.[12,13]

The currently accepted explanation for some aspects of CF airway disease is called the low-volume hypothesis. In this hypothesis, defective chloride secretion and increased sodium absorption in the CF airway leads to an isotonic but low volume of ASL, and this impedes effective mucociliary clearance.[14] Cultures of human airway epithelium grown at an air-liquid interface show that CF epithelia have ASL volume depletion (Figure 2).[15] It is thought that this leads to mucus adhesion to the airway surface, with plaque formation and entrapment of bacteria causing chronic infection and inflammation and the development of bronchiectasis. This does not explain why there is usually less lung disease in persons who have primary ciliary dyskinesia. Ratjen reviewed the development and testing of ion-transport modifiers to improve CFTR protein channel function or allow alternative channels to reestablish the height of the periciliary fluid by increasing water secretion into the airway.[11]

It has also been hypothesized that the inhalation of hyperosmolar saline[16] or mannitol[17] draws water onto the

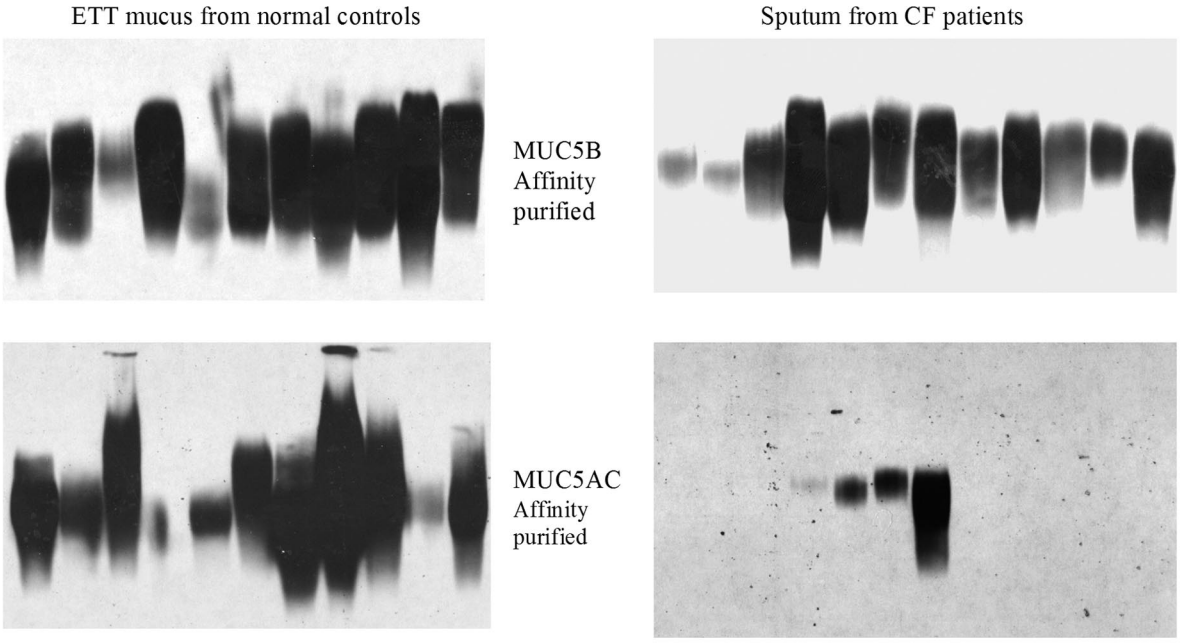

Fig. 1. The 2 principal gel-forming mucins in the human airway are MUC5AC and MUC5B. This Western gel of endotracheal tube (ETT) mucus (left) and cystic fibrosis (CF) sputum (right) was probed with antibodies to MUC5B and MUC5AC, demonstrating that there is much less intact mucin in CF sputum than in normal human mucus. (From Reference 8, with permission.)

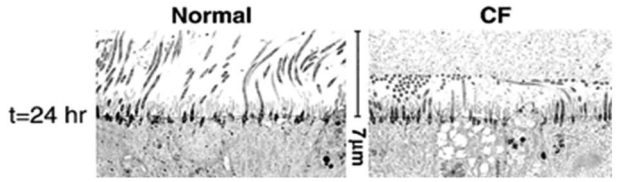

Fig. 2. On the right, cultures of airway epithelium from a human subject with cystic fibrosis (CF) were grown at an air-liquid interface to form well-differentiated mucus-secreting and ciliated epithelium. Compared with normal airway epithelial cells grown under the same conditions (left), there was a significant decrease in the height of the periciliary airway surface liquid (ASL) in CF cultures after 24 hours. This was thought to be due to CFTR (cystic fibrosis transmembrane regulator) dysfunction preventing effective ion and water secretion. (From Reference 15, with permission.)

airway surface, reestablishing ASL volume. However, if this were the principal mechanism of action of hyperosmolar aerosol in CF, the combination of a hyperosmolar aerosol with amiloride to block water and sodium absorption should further hydrate the ASL. Clinically, amiloride does not further improve mucociliary clearance in CF, and actually produces less benefit than the hyperosmolar saline alone.[18]

Another hypothesis to explain chronic airway infection in CF is that absence of intact airway mucin leaves the epithelium vulnerable to bacterial infection and may make it easier for bacteria to adhere directly to the airway epithelium and to communicate, thus enabling a change in bacteria from free-living or planktonic to the more persistent biofilm morphology.[19] This suggests that reestablishment of a protective mucus layer could be important for epithelial defense.

In response to a pulmonary exacerbation of CF the concentration of mucins increases in sputum, suggesting that mucin secretion can be augmented at times of increased infection and inflammation.[20] It is likely that mucus secretion is preserved in CF, at least in part, but there is protease degradation breakdown of this defensive layer. It has also been shown that hyperosmolar saline and mannitol are mucin secretagogues in the healthy airway, and this effect is dose-dependent.[21] These drugs may play an important role in maintaining airway health, by augmenting mucin secretion.

DNA and F-Actin Polymers in Cystic Fibrosis Sputum

CF sputum polymers are almost entirely neutrophil-derived DNA and F-actin, and these determine the rheologic properties of the sputum.[9,10] Under normal conditions such as pneumonia in an immunocompetent host, neutrophils are recruited to the site of infection, and as the infection resolves, the inflammatory cells undergo apoptosis or programmed cell death, with degradation of DNA and F-actin. However, in the CF airway, with greatly enhanced chronic inflammation, neutrophils undergo a necrotic death and DNA and F-actin copolymers form.[22] These co-polymers also act as substrates used for the formation of bacterial biofilms.[23,24]

This secondary polymer network also impedes the passage of nanoparticles, like large proteins, and is a barrier to effective gene therapy.[25,26] When directly mixed with CF sputum, dornase alfa moderately facilitated the transport of nanoparticles through CF sputum.[27]

The Role of Mucolytics in the Treatment of Cystic Fibrosis

Classic Mucolytics

By definition, mucolytics are medications that decrease the viscosity of mucus.[28,29] These have been categorized as classic mucolytics or peptide mucolytics. Classic mucolytics break down *mucin* oligomers into smaller fragments by severing disulfide bonds that connect mucin monomers across terminal cysteine residues. N-acetyl, L-cysteine (NAC) is the prototype classic mucolytic, but there are other thiol agents that have also been studied for potential mucolytic activity.[30] None have been shown to be effective as a mucolytic when inhaled as an aerosol, and there is no proven clinical benefit to their use.[31] Inhaled NAC is very irritating, with pH 2.2. Thus the primary "benefit" of inhaled NAC appears to be the induction of coughing, but at a cost of airway irritation. If intact mucin secretion is important for airway defense, inhaled NAC may be harmful to the epithelium.

NAC is a precursor to glutathione and is an effective antioxidant. There are preliminary reports that high-dose oral NAC may reduce airway inflammation and improve lung health in CF, not as a mucolytic but rather as an antioxidant.[32]

Peptide Mucolytics

Peptide mucolytics break down the secondary polymer network of highly polymerized DNA and F-actin. This polymer network is characteristic of pus. More than a decade ago, dornase alfa (Pulmozyme, Genentech, South San Francisco, California) was introduced as the first peptide mucolytic aerosol for the treatment of CF lung disease. The daily use of dornase alfa significantly improves pulmonary function, and decreases the relative risk of respiratory tract infections in patients with CF lung disease, regardless of the severity of disease.[33,34] Dornase alfa is administered by jet nebulization at a dose of 2.5 mg once daily. It can be administered before or during the use of airway-clearance devices.

The purulent secondary network also contains substantial amounts of F-actin. This can be depolymerized by actin sequestering and depolymerizing peptides such as thymosin β4.[35] Thymosin β4 is the major actin sequestering peptide in prokaryotic cells. This drug is currently under development as an aerosol therapy for CF lung disease. It depolymerizes F-actin in CF sputum in vitro, decreasing sputum cohesivity and improving sputum cough clearability. Thymosin β4 also synergizes with dornase alfa so that smaller concentrations of the two mechanisms in vitro decrease sputum viscosity far more than either medication used alone. DNA/F-actin impedes sputum cough clearability in vitro, and changes in sputum DNA concentration over time inversely correlate with changes in cough transportability.[9]

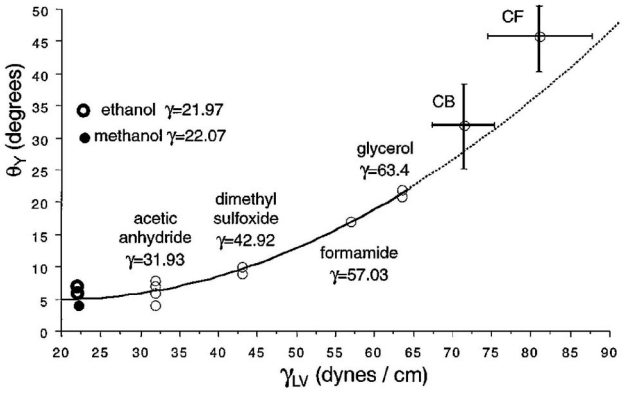

Fig. 3. Although cystic fibrosis (CF) sputum is not hyperviscous, it has a much greater interfacial tension than normal mucus, at 32 dyn/cm, or, in the absence of surfactant, than water, at 71 dyn/cm. Surfactant dysfunction makes CF sputum both hyperadhesive and tenacious, and this, in turn, impedes mucociliary and cough clearance. CB = chronic bronchitis. (From Reference 38, with permission.)

Hyperosmolar Aerosols

Hyperosmolar medications, such as hypertonic saline and mannitol, have been called mucolytic agents, but they do not meet the true definition of a mucolytic because their effect on mucus viscoelasticity is minimal.[36] Studies by Suri and colleagues suggest that hypertonic saline is not as effective as dornase alfa in improving pulmonary function in persons with CF.[37] Mannitol may be more appealing to patients, as this is administered as a dry-powder inhaler.[17]

Medications That Promote Sputum Expectoration

Mucokinetic agents improve the effectiveness of cough clearance. Although CF sputum is not particularly viscous, it is extremely tenacious when compared to sputum from patients with other lung diseases[38] (Fig. 3). There is a surfactant layer in the healthy airway that is thought to be produced by alveolar Type 2 cells or by serous cells in the submucosal glands.[12,39] Because of this, the surface tension in the normal airway surface is approximately 32 dyn/cm, far less than the surface tension of 71 dyn/cm in the absence of any surfactant.[13] This surfactant layer is thought to facilitate mucus spreading after secretion from glands and allows efficient energy transfer from cilia to mucus.[40]

Breakdown of surfactant phospholipids in the CF airway leads to the production of non-surface-active lysophospholipids that increase sputum tenacity, dramatically decreasing the cough clearability of sputum.[41] Surfactant administered as an aerosol for 2 weeks for patients with chronic obstructive pulmonary disease significantly improved pulmonary function and decreased gas trapping.[42] Similarly, phospholipid surfactant liposomes significantly improved the clearability of CF sputum in vitro.[43] Surfactant aerosol is under investigation as a mucokinetic medication for the therapy of CF.

Decreasing the Volume of Airway Secretions: Mucoregulatory Drugs

Mucoregulatory medications decrease mucin secretion or impede the formation of the DNA/F-actin secondary polymer network. Anticholinergic medications decrease the volume of mucin secreted in response to inflammatory stimuli in experimental animals[44] and in subjects with chronic bronchitis.[45] Although they can produce dry mouth as a result of blocking stimulated secretion of saliva, these drugs do not appear to increase the viscosity of airway mucus.[44,46]

The most thoroughly studied of the mucoregulatory medications are the low-dose macrolide antibiotics. Azithromycin and clarithromycin decrease interleukin 8 secretion from human airway cells in a non-linear modulation of the hyper-inflammatory response. The mechanism of action appears to be through the modulation of the extracellular regulated kinase (ERK)1/2 cascade.[47,48] Clarithromycin decreases sputum volume resulting from both inflammation and from mucus hypersecretion in patients with diffuse panbronchiolitis, sino-bronchial syndrome, and non-CF bronchiectasis.[49] Azithromycin improves pulmonary function and decreases the risk of respiratory-tract infections in persons with CF,[50] and macrolide therapy using azithromycin or clarithromycin is now considered part of the standard of care for CF.

Evaluating the Effectiveness of Interventions to Improve Sputum Clearance in Cystic Fibrosis

In 2007 RESPIRATORY CARE published proceedings from a journal conference with the theme of mucus secretion and clearance. At that conference I presented a paper discussing the appropriate design of clinical trials to evaluate mucus-clearance therapy.[51] This information was largely derived from CF research and is summarized here. Sputum color, sputum volume, and perception of sputum thickness are not useful outcomes to evaluate the effectiveness of mucoactive therapy because changes in these do not correlate with any usual measure of any clinical improvement, including pulmonary function, frequency of infection, use of antibiotics, or quality of life. Sputum rheology or viscoelasticity is useful as an in vitro method to determine a range of medication doses that may be effective in vivo. Also, changes in sputum rheology (or lack of changes) can provide information about the mechanism of action of mucoactive medications. However, viscoelasticity of sputum is too variable a measurement to determine if a medication is effective in an individual patient. Furthermore, properties measured in expectorated sputum may not be indicative of these same properties in retained airway phlegm.

Pulmonary function is also easy to measure, and when mucus clearance is improved, there can be significant increases in forced expiratory volume in the first second (FEV_1). However, FEV_1 is insensitive to all but major changes in the burden of phlegm. It is likely that measurements of gas trapping are more accurate to evaluate the effect of improved or impaired mucus clearance. It is possible that exercise testing, measured by a 6-minute walk test or by shuttle walking, is a useful way to evaluate the effectiveness of mucoactive therapy in CF, if this opens airways and reduces gas trapping.

Summary

Although CF has been thought to be due to hypersecretion of excessively viscous mucus, we know that this is not the case. In established CF lung disease, the airways are full of phlegm that is similar to pus and contains almost no intact mucin. Therefore, medications aimed at reducing mucin secretion or severing mucin polymers may be of no value and perhaps even be dangerous in CF. Medications meant to decrease the tenacity of sputum containing DNA/F-actin co-polymers are more likely to be of benefit in CF. Because the phlegm tenaciously sticks to the airway epithelium, medications that unstick secretions from the epithelium, such as surfactant, or drugs that promote water secretion into the airway should also benefit patients. The development of techniques to measure the total burden of airway phlegm will help us to better understand the pathogenesis of CF and will be valuable for evaluating therapies meant to promote sputum clearance.

REFERENCES

1. Lopez-Vidriero MT, Reid L. Chemical markers of mucous and serum glycoproteins and their relation to viscosity in mucoid and purulent sputum from various hypersecretory diseases. Am Rev Respir Dis 1978;117(3):465-477.
2. King M. Is cystic fibrosis mucus abnormal? Pediatr Res 1981;15(2):120-122.
3. Tomkiewicz RP, Albers GM, Ramirez OE, Green F, King M, Rubin BK. Rheologic properties of airway secretions in cystic fibrosis, chronic bronchitis, and fatal asthma. Biorheology 1995;32:364-365.
4. Regnis JA, Robinson M, Bailey DL, Cook P, Hooper P, Chan HK, et al. Mucociliary clearance in patients with cystic fibrosis and in normal subjects. Am J Respir Crit Care Med 1994;150(1):66-71.
5. Kollberg H, Mossberg B, Afzelius BA, Philipson K, Camner P. Cystic fibrosis compared with the immotile-cilia syndrome. A study

of mucociliary clearance, ciliary ultrastructure, clinical picture and ventilatory function. Scand J Respir Dis 1978;59(6):297-306.
6. McShane D, Davies JC, Wodehouse T, Bush A, Geddes D, Alton EW. Normal nasal mucociliary clearance in CF children: evidence against a CFTR-related defect. Eur Respir J 2004;24(1):95-10.
7. Voynow JA, Rubin BK. Mucins, mucus, and sputum. Chest 2009; 135(2):505-512.
8. Henke M, Renner A, Huber RM, Seeds MC, Rubin BK. MUC5AC and MUC5B mucins are decreased in cystic fibrosis airway secretions. Am J Respir Cell Mol Biol 2004;31(1):86-91.
9. Kater A, Henke MO, Rubin BK. The role of DNA and actin polymers on the polymer structure and rheology of cystic fibrosis sputum and depolymerization by gelsolin or thymosin beta 4. Ann N Y Acad Sci 2007;1112:140-153.
10. Rubin BK. Mucus structure and properties in cystic fibrosis. Paediatr Respir Rev 2007;8(1):4-7.
11. Ratjen FA. Cystic fibrosis: pathogenesis and future treatment strategies. Respir Care 2009;54(5):595-602; discussion 603-605.
12. Morgenroth K, Bolz J. Morphological features of the interaction between mucus and surfactant on the bronchial mucosa. Respiration 1985;47(3):225-231.
13. Im Hof V, Gehr P, Gerber V, Lee MM, Schürch S. In vivo determination of surface tension in the horse trachea and in vitro model studies. Respir Physiol 1997;109(1):81-93.
14. Boucher RC. Evidence for airway surface dehydration as the initiating event in CF airway disease. J Intern Med 2007;261(1):5-16.
15. Matsui H, Grubb BR, Tarran R, Randell SH, Gatzy JT, Davis CW, Boucher RC. Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. Cell 1998;95(7):1005-1015.
16. Elkins M, Robinson M, Rose B, Harbour C, Moriarty C, Marks G, et al; National Hypertonic Saline in Cystic Fibrosis (NHSCF) Study Group. A controlled trial of long-term inhaled hypertonic saline in patients with cystic fibrosis N Engl J Med 2006;354(3):229-240.
17. Jaques A, Daviskas E, Turton JA, McKay K, Cooper P, Stirling RG, et al. Inhaled mannitol improves lung function in cystic fibrosis. Chest 2008;133(6):1388-1389.
18. Donaldson SH, Bennett WD, Zeman KL, Knowles MR, Tarran R, Boucher RC. Mucus clearance and lung function in cystic fibrosis with hypertonic saline. N Engl J Med 2006;354(3):241-250.
19. Rubin BK. Mucus and sputum in chronic lung disease: biophysical properties and airway clearance. *In*: Respiratory drug delivery 8. River Grove, Illinois: Davis Healthcare Publishing; 2008:19-28.
20. Henke MO, John G, Germann M, Lindemann H, Rubin BK. MUC5AC and MUC5B mucins increase in cystic fibrosis airway secretions during pulmonary exacerbation. Am J Respir Crit Care Med 2007; 175(8):816-821.
21. Kishioka C, Okamoto K, Kim J-S. Rubin BK. Hyperosmolar solutions stimulate mucus secretion in the ferret trachea. Chest 2003; 124(1):306-313.
22. Lethem MI, James SL, Marriott C, Burke JF. The origin of DNA associated with mucus glycoproteins in cystic fibrosis sputum. Eur Respir J 1990;3(1):19-23.
23. Moreau-Marquis S, Stanton BA, O'Toole GA. *Pseudomonas aeruginosa* biofilm formation in the cystic fibrosis airway. Pulm Pharmacol Ther 2008;21(4):595-9.
24. Walker TS, Tomlin KL, Worthen GS, Poch KR, Lieber JG, Saavedra MT, et al. Enhanced *Pseudomonas aeruginosa* biofilm development mediated by human neutrophils. Infect Immun 2005;73(6):3693-3701.
25. Dawson M, Wirtz D, Hanes J. Enhanced viscoelasticity of human cystic fibrotic sputum correlates with increasing microheterogeneity in particle transport. J Biol Chem 2003;278(50):50393-50401.
26. Broughton-Head VJ, Smith JR, Shur J, Shute JK. Actin limits enhancement of nanoparticle diffusion through cystic fibrosis sputum by mucolytics. Pulm Pharmacol Ther 2007;20(6):708-717.
27. Sanders NN, De Smedt SC, Van Rompaey E, Simoens P, De Baets F, Demeester J. Cystic fibrosis sputum: a barrier to the transport of nanospheres. Am J Respir Crit Care Med 2000;162(5):1905-1911.
28. Rubin BK. Mucolytics, expectorants, and mucokinetic medications. Respir Care 2007;52(7):859-865.
29. Rogers DF, Rubin BK. Mucolytics for COPD. *In*: Stockley RA, Rennard SI, Rabe K, Celli B, editors. Chronic obstructive pulmonary disease: a practical guide to management. Oxford: Blackwell Publishing; 2006.
30. Henke MO, Ratjen F. Mucolytics in cystic fibrosis. Paediatr Respir Rev 2007;8(1):24-29.
31. Grandjean EM, Berthet P, Ruffmann R, Leuenberger P. Efficacy of oral long-term N-acetylcysteine in chronic bronchopulmonary disease: a meta-analysis of published double-blind, placebo-controlled clinical trials. Clin Ther 2000;22(2):209-221.
32. Tirouvanziam R, Conrad CK, Bottiglieri T, Herzenberg LA, Moss RB, Herzenberg LA. High-dose oral N-acetylcysteine, a glutathione prodrug, modulates inflammation in cystic fibrosis. Proc Natl Acad Sci USA 2006;103(12):4628-4633.
33. Shah PI, Bush A, Canny GJ, Colin AA, Fuchs HJ, Geddes DM, et al. Recombinant human DNase I in cystic fibrosis patients with severe pulmonary disease: a short-term, double-blind study followed by six months open-label treatment. Eur Respir J 1995;8(6):954-958.
34. Quan JM, Tiddens HA, Sy JP, McKenzie SG, Montgomery MD, Robinson PJ, et al; Pulmozyme Early Intervention Trial Study Group. A two-year randomized, placebo-controlled trial of dornase alfa in young patients with cystic fibrosis with mild lung function abnormalities. J Pediatr 2001;139(6):813-820.
35. Rubin BK, Kater AP, Goldstein AL. Thymosin ß4 sequesters actin in cystic fibrosis sputum and decreases sputum cohesivity in vitro. Chest 2006;130(5):1433-1440.
36. Daviskas, E Anderson SD, Gomes K, Briffa P, Cochrane B, Chan H-Kim, et al. Inhaled mannitol for the treatment of mucociliary dysfunction in patients with bronchiectasis: effect on lung function, health status and sputum. Respirology 2005;10(1):46-56.
37. Suri R, Metcalfe C, Lees B, Grieve R, Flather M, Normand C, et al. Comparison of hypertonic saline and alternate-day or daily recombinant human deoxyribonuclease in children with cystic fibrosis: a randomised trial. Lancet 2001;358(9290):1316-1321.
38. Albers GM, Tomkiewicz RP, May MK, Ramirez OE, Rubin BK. Ring distraction technique for measuring surface tension of sputum: relationship to sputum clearability. J Appl Physiol 1996;81(6):2690-2695.
39. Rubin BK. Surface properties of respiratory secretions: Relationship to mucus transport. *In*: Baum G, editor. Cilia, mucus, and mucociliary interactions. New York: Marcel Dekker; 1998:317-324.
40. Allegra L, Bossi R, Braga P. Influence of surfactant on mucociliary transport. Eur J Respir Dis 1985;142:71-76.
41. Girod S, Galabert C, Lecuire A, Zahm JM, Puchelle E. Phospholipid composition and surface-active properties of tracheobronchial secretions from patients with cystic fibrosis and chronic obstructive pulmonary diseases. Pediatr Pulmonol 1992;13(1):22-27.
42. Anzueto A, Jubran A, Ohar JA, Piquette CA, Rennard SI, Colice G, et al. Effects of aerosolized surfactant in patients with stable chronic bronchitis: a prospective randomized controlled trial. JAMA 1997; 278(17):1426-1431.
43. Girod de Bentzmann S, Pierrot D, Fuchey C, Zahm JM, Morançais JL, Puchelle E. Distearoyl phosphatidylglycerol liposomes improve surface and transport properties of CF mucus. Eur Respir J 1993; 6(8):1156-1161.
44. Kishioka C, Okamoto K, Kim JS, Rubin BK. Regulation of secretion from mucous and serous cells in the excised ferret trachea. Respir Physiol 2001;126(2):163-171.

45. Tamaoki J, Chiyotani A, Tagaya E, Sakai N, Konno K. Effect of long term treatment with oxitropium bromide on airway secretion in chronic bronchitis and diffuse panbronchiolitis. Thorax 1994;49(6):545-548.
46. Saito Y, Azuma A, Morimoto T, Fujita K, Abe S, Motegi T, et al. Tiotropium ameliorates symptoms in patients with chronic airway mucus hypersecretion which is resistant to macrolide therapy. Intern Med 2008;47(7):585-591.
47. Shinkai M, Foster G, Rubin BK. Macrolide antibiotics modulate ERK phosphorylation and IL-8 and GM-CSF production by human bronchial epithelial cells. Am J Physiol Lung Cell Molec Physiol 2006;290(1):L75-L85.
48. Shinkai M, Henke MO, Rubin BK. Macrolide antibiotics as immunomodulatory medications: proposed mechanisms of action. Pharmacol Ther 2008 117(3):393-405.
49. Tamaoki J, Takeyama K, Tagaya E, Konno K. Effect of clarithromycin on sputum production and its rheological properties in chronic respiratory tract infections. Antimicrob Agents Chemother 1995; 39(8):1688-1690.
50. Saiman L, Marshall BC, Mayer-Hamblett N, Burns JL, Quittner AL, Cibene DA, et al. Azithromycin in patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa*: a randomized controlled trial. JAMA 2003;290(13):1749-1756.
51. Rubin BK. Designing clinical trials to evaluate mucus clearance therapy. Respir Care 2007;52(10):1348-1358; discussion 1358-1361.

Discussion

Geller: How should a respiratory therapist explain the goals to the CF patient? What are we doing to their mucus with these drugs? We have to put it in words they can understand.

Rubin: Easy. The phlegm in your lungs is not good for you. It's blocking your airways and it makes you feel bad. It includes bacteria, inflammatory products, all of which contribute to damage your lungs. We know that the more of this stuff we get out, the better you'll be able to breathe, and the less will be down there to damage your airways. Your body is always producing more, so it's important that we do these airway clearance maneuvers and take these medications to always keep them clear. You've got to stay on top of it, because the sputum is going to keep having to be coughed up, and as long as you keep on top of it and keep them clear, there'll be less there and it will be better for your lung health.

Rosenblatt: So if a patient tells me they are coughing up more sputum—and I get worried if they tell me that—or if they tell me they feel like they are more congested and can't get the sputum out, that means nothing?

Rubin: No, sorry; what I meant is that these are not good outcomes for evaluating a therapy. The therapy can cause them to cough up a lot more and they'll feel better, it'll cause them to reduce the volume so they can swallow more. But if a patient is coming in on their baseline therapy and saying, "I'm coughing up more, it's greener, I'm feeling sicker, it's harder to cough it out," that usually goes along with all the other signs of what we call a pulmonary exacerbation: decreased pulmonary function, often weight loss, feeling puny. Those are very good signs associated with an exacerbation in somebody at their baseline. I wouldn't use those, though, for an outcome to see if they're getting any *improvement* with a new therapy.

Rosenblatt: If they come in with those findings that indicate they're having an exacerbation, but then they go back to their baseline, we can't say that they are getting any better?

Rubin: No, I would. But if you have somebody coming in and you're initiating dornase therapy (Pulmozyme) or you're teaching them to do active cycle breathing, for example, and you say, "I want to see how this is going to work for you and what we're going to do is measure how much sputum you can cough up each morning, or look at the color of the sputum," that really doesn't give you any indication if the therapy is working. Studies that have used those, where they've quantified the volume of secretion, or looked at the color or texture, haven't correlated at all with how the patient feels, respiratory-tract exacerbations, use of antibiotics, or pulmonary function. So, although those are useful in a patient when they're feeling bad, and are decent clinical symptoms that go along with an exacerbation, they aren't useful outcome measurements for determining if an intervention is of benefit.

Rosenblatt: So they are good clinical markers at the bedside to determine how well a patient is doing, but they should not be used as markers in an objective study.

Rubin: Sure. If you put somebody on a vest, which we know is an effective method of airway clearance, and they say, "I'm coughing up a lot more," the next questions should be, "Well, how do you feel? What can you do? What is your energy?" Some of these new things that we're bringing on—hyperosmolar solutions are an example—they're mucus secretagogues; they've been shown to induce secretion of mucus, of true mucus with mucins. If somebody is expectorating a lot more, you don't know if it's a good thing, if it's a bad thing, or if it's neutral. So it's not the volume or color of what you're coughing up as a response to therapy, but what you can cough up and how you feel do tend togo hand in hand on your baseline. Is that a little bit more clear? It's not useful as an outcome for therapeutic interventions, but it appears to be useful as a clinical sign of deterioration or a recovery.

Rosenblatt: I'm a little viscous, so it's OK.