*Commentary*

# Is Cystic Fibrosis Lung Disease Caused by Abnormal Ion Composition or Abnormal Volume?

Mauri E. Krouse

Cystic Fibrosis Research Laboratory, Stanford University, Stanford, CA 94305-2310

The most common lethal genetic disease in the Caucasian population is cystic fibrosis (CF). In 1970, the median life expectancy was 8 yr; today, with the concerted effort of scientists and clinicians, the median survival age has risen to 30 yr. About 1 in 23 people carry a single defective copy of the CF gene in the United States population and ~40,000 people in the US have cystic fibrosis. The disease is characterized by a generalized exocrine dysfunction, and lung disease is the leading cause of death in CF patients (Davis et al., 1996). The lungs develop reoccurring (requiring hospitalization) and persistent infections often with *Pseudomonas aeruginosa* or *Burkholderia cepacia*, both of which are fairly innocuous in the normal population. The genesis of lung infection is not well understood, and has led to controversies in the CF field. The two prevalent theories of CF lung infection are termed the "high salt hypothesis" and the "low volume hypothesis," referring to the condition that leads to CF. Several recent reviews have discussed these theories (Boucher, 1999; Quinton, 1999; Wine, 1999; Travis et al., 2001; Verkman, 2001)

To understand the basis for each of the two theories, we first must understand the function of the CF gene product. The CF gene was first discovered by Riordan and colleagues (Riordan et al., 1989) in 1989. The product was named the cystic fibrosis conductance regulator (CFTR) and was found, by sequence homology, to be a member of the ATP-binding cassette (ABC) transporter family. In 1992, Bear et al. (1992) showed in lipid bilayer experiments that CFTR was a chloride channel. The gating of the channel was influenced by its level of phosphorylation (Cheng et al., 1991; Hwang et al., 1994) and ATP (Anderson et al., 1991; Hwang et al., 1994; Venglarik et al., 1994; Zeltwanger et al., 1999). It also was shown that CFTR can regulate other channels. In particular, in the lung, the activation of CFTR inhibited the functioning of the epithelial sodium channel (ENaC; Stutts et al., 1997). The dual role of CFTR as a chloride channel and an inhibitor of ENaC proved the basis for the two competing theories of CF lung pathogenesis. The high salt hypothesis and the absence of a chloride channel and the low volume hypothesis and the increased activity of the ENaC. It is this controversy that the paper by Tarran et al. (2001), attempts to address.

The high salt hypothesis was first proposed by Smith et al. (1996) in a revolutionary paper. They found in primary cultures, grown in Iowa City, from normal individuals that a modest amount of *P. aeruginosa* (30–300 colony forming units, a measure of live bacteria) added to the apical side of the cultures died within 24 h. As a control, bacteria were added to the basolateral side of the cultures and the bacteria did not die. In sharp contrast, bacteria added to the apical side of primary cultures from CF individuals did not die, but tended to grow, as did bacteria added to the basolateral side. If the apical fluid was rinsed from the cultures with distilled water the solution from normal cultures continued to kill the bacteria and the solution from the CF cells regained the ability to kill bacteria. If the fluid used to rinse the apical solution contained 150 mM NaCl then the solution from the normal cells failed to kill the bacteria, as did the solution from the CF cells. This data is summarized in Table I.

The authors hypothesized that the salt concentration was higher in the CF cultures than in normal cultures. This was borne out in direct measurements of the NaCl concentrations, with the concentrations from CF cells being significantly higher, approximately equal to plasma. The paper by Smith et al. (1996) established the existence of a salt-sensitive antimicrobial peptide in the apical solution from primary airway cultures and, by analogy, in the airway surface liquid (ASL) of the lung. The antimicrobial activity of this peptide was inhibited in CF individuals by the high salt (~150 mM NaCl) in the surface fluid. The discovery of such a compound playing a role in CF lung disease has rejuvenated the field of innate host defenses in CF.

In contrast, the low volume hypothesis contends that the salt concentration in both normal and CF are identical and high, approximately equal to plasma. In this theory, the lack of CFTR enhances the sodium absorption from the airway surface liquid. Because the airway surface cells form a monolayer that is leaky to water (Matsui et al., 2000), the apical fluid remains isotonic. The increased absorption of sodium is accompanied by


J. Gen. Physiol. © The Rockefeller University Press • 0022-1295/2001/08/219/3 $5.00
Volume 118 August 2001 219–221
http://www.jgp.org/cgi/content/full/118/2/219

TABLE I

*Antimicrobial Activity*

| Apical solution | In vitro | Low salt (washed with $H_2O$) | High salt (washed with 150 mM NaCl) |
|---|---|---|---|
| Normal cells | +++ | +++ | −−− |
| CF cells | −−− | +++ | −−− |

+++ means that the bacteria were killed and −−− means that the bacteria survived.

chloride that passes either through a second (non-CFTR) pathway or paracellularly. The excess absorption of salt and, thus, water in CF collapses the cilia so that the normal ciliary movement of mucus ceases (Matsui et al., 1998). When the mucus movement is stopped, the normal conveyer belt that moves bacteria out of the lung cannot function and bacteria can then colonize the lung. Because the mucus sits on a very small layer of liquid even cough clearance is reduced.

Just about all aspects of these two theories are in contention. However, three main points separate the two theories. First, is the salt concentration in the ASL from CF subjects higher than in normals (Joris et al., 1993; Knowles et al., 1997; Zabner et al., 1998)? Second, is the fluid absorption by CF tissues increased (Knowles et al., 1986; Jiang et al., 1993; Smith et al., 1994)? Third, is there is a significant alternative chloride pathway through the monolayer (Matsui et al., 1998; Zabner et al., 1998)? (Note that this alternative chloride pathway is essential for the low volume hypothesis.) Since the water permeability of the surface airway epithelium is quite large (Matthay et al., 1996; Matsui et al., 2000), the osmolarity of the solutions on each side of the epithelium must be equal or some additional force must be present to account for the different osmolarities. Zabner et al. (1998) has proposed the existence of such forces. These forces include the addition of an uncharged (unknown) osmolyte to the apical solution, capillary forces due to the surface tension on the cilia, and the ability of the mucus gel to hold water. It is these forces that the paper by Tarran and colleagues investigates (see Tarran et al., 2001 in this issue).

This paper uses well differentiated human primary airway cultures to look at each of the possible mechanisms whereby the NaCl concentration may be reduced in the apical ASL. The results are consistent with previous work (Matsui et al., 1998, 2000) and some of the figures are stunning. They find no force large enough, in isolation or in combination, that can lead to a lowering of the salt content of the ASL in normal cells. But isn't this exactly what is expected? You cannot measure the forces that lead to a lowered NaCl concentration if your cultures do not show a lowered NaCl concentration. If we expected an uncharged apical osmolyte to replace NaCl to make the apical and basolateral solutions isotonic, then we can conclude that the cultures studied in this paper do not produce or secrete such osmolytes, and in fact, no additional osmolyte accumulated in these cultures for periods of at least 2 d. If you expect the cilia to supply surface tension to hold the water level at the tips of the outstretched cilia (~7 μm) on the apical surface and if those cilia fall over as the solution level is decreased then the surface tension will not hold the water level to 7 μm, but rather to the height of the fallen cilia (3 μm). Can the surface tension of the fallen cilia lead to a lowered NaCl concentration? Matsui et al. (1998) were able to measure the chloride concentration in CF cultures, where the cilia have fallen over, and found it to be high (~120 mM). Why do the cilia not fall over in normal cultures? Tarran et al. (2001) presents a novel finding that the absorption is reduced as the periciliary liquid approaches the height of the outstretched cilia (see Fig. 6 in Tarran et al., 2001). This is accomplished by a reduction in sodium influx by an unknown mechanism. Is this control of periciliary liquid height defective in CF?

However, some questions seemed to have been resolved. This paper, along with two previous papers, (Matsui et al., 1998, 2000) present converging evidence that there is no change in the salt content in normal and CF airway cultures grown in Chapel Hill. The experiments also show that the mucus blanket acts as a donor of fluid, swelling or shrinking as the osmolarity is changed. Thus, there is no static mucus gel that can retain water. However, a comparison of the volume absorption in cultures without mucus (see Fig. 6 b in Tarran et al., 2001) and cultures with mucus (see Fig. 7 b in Tarran et al., 2001) shows that the rate of absorption is slowed at least twofold by the presence of mucus. This effect of mucus on water retention is just not large enough to lead to a significant reduction in NaCl content of the surface liquid (there appears to be at least a 10% drop in the $[Cl^-]$ in the presence of mucus; see Fig. 7 d in Tarran et al., 2001).

Why has this controversy lasted for 5 yr? Both theories have support from many laboratories measuring the NaCl content in the ASL from cultured cells and in vivo. Is there some basic truth that we are missing? If there is, I don't know what it is. Could the explanation be simply that there is some difference between the cultures? Both cultures grown in Chapel Hill and Iowa City have cilia and mucus, so this is unlikely to be the source of the difference. But maybe the cells from Iowa City secrete an uncharged osmolyte and those from Chapel Hill do not. This would be a simple, but elegant, method for modifying the salinity of the surface fluid while maintaining

isotonicity. Is this hypothetical osmolyte also present in the ASL of normal lungs and absent in CF lungs? Alternatively, could there be some artifact that can explain all the results on one side of the controversy? Measurements of ASL in vivo have well-known possible artifacts. Filter paper and capillaries can pull fluid not only from the ASL, but possibly also from inside cells and from the basolateral side of the epithelial monolayer. Ion-sensitive microelectrodes may accidentally touch the surface of the epithelium and cause secretion, which is thought to be isotonic. So most laboratories have resorted to primary cultures. But primary cultures may not express the same features of cells in vivo. Experiments by Zabner et al. (1998; Iowa City) have shown fairly convincingly that their normal primary cultures have low NaCl levels, whereas experiments by Matsui et al. (1998; Chapel Hill) have shown that their cultures have isotonic NaCl covering their normal cells. A hallmark of the experiments in previous papers by Boucher and his colleagues (Matsui et al., 1998, 2000; Boucher, 1999) is that mucus motility in CF cultures is lost, but the movement can be restored by adding isotonic solution to the apical surface (Matsui et al., 1998). Do the CF cultures from Iowa City show rotational mucus movement? Does the ASL from Chapel Hill cultures kill bacteria when the salt content is diluted? Answers to these questions will help determine whether the two "different" culture systems are identical.

The resolution of this controversy is key to understanding the lung pathophysiology of cystic fibrosis and to the lives of individuals with cystic fibrosis. The two competing theories offer different routes to treat the lung disease. If the problem is that the surface liquid has too much salt so that antimicrobial activity is absent, then hypotonic solution to dilute the ASL will improve lung function. If the volume of the ASL is too small then hypertonic (Matsui et al., 2000) solution can be added to the ASL to draw water into the airway lumen. So a solution to this controversy must be found to advance the field and help alleviate the lung pathophysiology.

REFERENCES


Anderson, M.P., H.A. Berger, D.P. Rich, R.J. Gregory, A.E. Smith, and M.J. Welsh. 1991. Nucleoside triphosphates are required to open the CFTR chloride channel. *Cell.* 67:775–784.

Bear, C.E., C.H. Li, N. Kartner, R.J. Bridges, T.J. Jensen, M. Ramjeesingh, and J.R. Riordan. 1992. Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). *Cell.* 68:809–818.

Boucher, R.C. 1999. Molecular insights into the physiology of the 'thin film' of airway surface liquid. *J. Physiol.* 516:631–638.

Cheng, S.H., D.P. Rich, J. Marshall, R.J. Gregory, M.J. Welsh, and A.E. Smith. 1991. Phosphorylation of the R domain by cAMP-dependent protein kinase regulates the CFTR chloride channel. *Cell.* 66:1027–1036.

Davis, P.B., M. Drumm, and M.W. Konstan. 1996. Cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 154:1229–1256.

Hwang, T.C., G. Nagel, A.C. Nairn, and D.C. Gadsby. 1994. Regulation of the gating of cystic fibrosis transmembrane conductance regulator C1 channels by phosphorylation and ATP hydrolysis. *Proc. Natl. Acad. Sci. USA.* 91:4698–4702.

Jiang, C., W.E. Finkbeiner, J.H. Widdicombe, P.B. McCray, Jr., and S.S. Miller. 1993. Altered fluid transport across airway epithelium in cystic fibrosis. *Science.* 262:424–427.

Joris, L., I. Dab, and P.M. Quinton. 1993. Elemental composition of human airway surface fluid in healthy and diseased airways. *Am. Rev. Respir. Dis.* 148:1633–1637.

Knowles, M.R., M.J. Stutts, J.R. Yankaskas, J.T. Gatzy, and R.C. Boucher, Jr. 1986. Abnormal respiratory epithelial ion transport in cystic fibrosis. *Clin. Chest Med.* 7:285–297.

Knowles, M.R., J.M. Robinson, R.E. Wood, C.A. Pue, W.M. Mentz, G.C. Wager, J.T. Gatzy, and R.C. Boucher. 1997. Ion composition of airway surface liquid of patients with cystic fibrosis as compared with normal and disease-control subjects [published erratum appears in *J. Clin. Invest.* 101:285]. *J. Clin. Invest.* 100:2588–2595.

Matsui, H., B.R. Grubb, R. Tarran, S.H. Randell, J.T. Gatzy, C.W. Davis, and R.C. Boucher. 1998. Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. *Cell.* 95:1005–1015.

Matsui, H., C.W. Davis, R. Tarran, and R.C. Boucher. 2000. Osmotic water permeabilities of cultured, well-differentiated normal and cystic fibrosis airway epithelia. *J. Clin. Invest.* 105:1419–1427.

Matthay, M.A., H.G. Folkesson, and A.S. Verkman. 1996. Salt and water transport across alveolar and distal airway epithelia in the adult lung. *Am. J. Physiol.* 270:L487–L503.

Quinton, P.M. 1999. Physiological basis of cystic fibrosis: a historical perspective. *Physiol. Rev.* 79:S3–S22.

Riordan, J.R., J.M. Rommens, B. Kerem, N. Alon, R. Rozmahel, Z. Grzelczak, J. Zielenski, S. Lok, N. Plavsic, J.L. Chou, et al. 1989. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science.* 245:1066–1073.

Smith, J.J., P.H. Karp, and M.J. Welsh. 1994. Defective fluid transport by cystic fibrosis airway epithelia. *J. Clin. Invest.* 93:1307–1311.

Smith, J.J., S.M. Travis, E.P. Greenberg, and M.J. Welsh. 1996. Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. *Cell.* 85:229–236.

Stutts, M.J., B.C. Rossier, and R.C. Boucher. 1997. Cystic fibrosis transmembrane conductance regulator inverts protein kinase A-mediated regulation of epithelial sodium channel single channel kinetics. *J. Biol. Chem.* 272:14037–14040.

Tarran, R., B.R. Grubb, J.T. Gatzy, C.W. Davis, and R.C. Boucher. 2001. The relative roles of surface forces and ion transport in the modulation of airway surface liquid volume and composition. 118:223–236.

Travis, S.M., P.K. Singh, and M.J. Welsh. 2001. Antimicrobial peptides and proteins in the innate defense of the airway surface. *Curr. Opin. Immunol.* 13:89–95.

Venglarik, C.J., B.D. Schultz, R.A. Frizzell, and R.J. Bridges. 1994. ATP alters current fluctuations of cystic fibrosis transmembrane conductance regulator: evidence for a three-state activation mechanism. *J. Gen. Physiol.* 104:123–146.

Verkman, A.S. 2001. Lung disease in cystic fibrosis: is airway surface liquid composition abnormal? *Am. J. Physiol.* 281:L306–L308.

Wine, J.J. 1999. The genesis of cystic fibrosis lung disease. *J. Clin. Invest.* 103:309–312.

Zabner, J., J.J. Smith, P.H. Karp, J.H. Widdicombe, and M.J. Welsh. 1998. Loss of CFTR chloride channels alters salt absorption by cystic fibrosis airway epithelia in vitro. *Mol. Cell.* 2:397–403.

Zeltwanger, S., F. Wang, G.T. Wang, K.D. Gillis, and T.C. Hwang. 1999. Gating of cystic fibrosis transmembrane conductance regulator chloride channels by adenosine triphosphate hydrolysis. Quantitative analysis of a cyclic gating scheme. *J. Gen. Physiol.* 113:541–554.