Lung disease in cystic fibrosis: is airway surface liquid composition abnormal?

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Recurrent infection and deterioration of lung function are the major causes of morbidity and mortality in cystic fibrosis. Although the genetic defect in cystic fibrosis, mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator protein (CFTR), was discovered in 1989, the mechanism by which CFTR mutations cause lung disease remains uncertain. A number of provocative and sometimes conflicting mechanisms have been proposed to link the cystic fibrosis genotype to clinical disease; some of them include defective intracellular vesicle function resulting in abnormal protein processing, loss of CFTR regulation of other membrane transporting proteins, defective airway submucosal gland secretion, abnormal airway surface liquid (ASL) composition, defective intrinsic antimicrobial function, and hyperabsorption of airway fluid. Determination of the mechanism linking genotype to disease is of critical importance in developing therapies to treat cystic fibrosis, especially because therapies indicated by some mechanisms are clearly contraindicated by others.

Recent attention has focused on possible abnormalities in the properties of the ASL, the thin layer of liquid that coats the upper and lower airways and provides a unique interface between inspired and expired air and the airway epithelium. One set of theories postulates that ASL composition is abnormal in cystic fibrosis. The “high-salt” hypothesis postulates that the normally low ASL salt concentration becomes high in cystic fibrosis, inhibiting the activity of endogenous antimicrobials such as defensins (12). The low ASL salt concentration in normal airways predicted by this hypothesis would require either a water-impermeable airway epithelium, which is not the case (3, 8), the presence of nonsalt osmolytes, or the action of a surface phenomenon capable of maintaining an osmotic imbalance. It has also been proposed that the ASL pH is abnormally low in cystic fibrosis, inhibiting bacterial clearance and antimicrobial functions. In contrast, the “low-volume” hypothesis postulates that hyperactivity of epithelial Na\(^+\) channels in cystic fibrosis results in avid salt absorption, producing a viscous, dehydrated ASL that promotes infection (9). A third set of theories implicates abnormal airway submucosal gland function in cystic fibrosis in which the glands produce a viscous or otherwise abnormal fluid promoting infection. Several recent reviews discuss these issues in more detail (1, 11, 13, 14).

The testing of these hypotheses has presented a formidable challenge because of difficulties in establishing suitable model systems and in measuring the physical parameters of the ASL. Well-differentiated airway epithelial cells grown on porous supports at an air-liquid interface have been used as a cell culture model to study ASL properties. The airway cell cultures recapitulate many native airway functions such as ion transport and ciliary beating. However, cell culture models have been highly variable from laboratory to laboratory; they cannot recapitulate the complexity in vivo airway anatomy, hormonal regulation, and cellular heterogeneity, and they are not subject to time-varying air composition (moisture, P\(\text{CO}_2\), and P\(\text{O}_2\)) and convective fluid transport as in vivo. Also, the ASL depth in airway cell culture models is \(<25 \mu\text{m}\), whereas that in intact mammalian airways is \(>50 \mu\text{m}\), raising concerns that the determinants of ASL composition and volume in cell culture models may be quite different than in the airways in vivo. Transgenic mouse models of targeted CFTR deletion or mutation are a potentially useful alternative; however, lung disease in mouse models of cystic fibrosis is quite subtle. Also, there are a number of potentially important human versus mouse species differences in airway physiology; airway submucosal glands are essentially absent from mouse airways below the larynx, and the mouse airway epithelium appears to express an alternative Cl\(^-\) channel that may substitute functionally for defective CFTR. Measurements in intact normal versus cystic fibrosis human airways are probably the most appropriate for studying ASL properties, recognizing the caveat that human airway anatomy and function are...
altered in response to recurrent infection and inflammation. It can be difficult to determine whether differences in normal versus cystic fibrosis human airways are related to the primary CFTR defect or to secondary consequences of the disease process.

ASL composition in intact airways has been determined by chemical analysis of microsamples obtained with filter paper and micropipette fluid sampling methods (2, 6, 7). A wide range of NaCl concentrations have been reported, from <50 to >180 mM. These invasive ASL sampling methods have been criticized because the sampled volumes can be substantially greater than expected fluid volume of the thin ASL film (5 μl/cm² of airway surface for 50-μm-thick ASL). The sampled ASL fluid may thus be contaminated by cellular and interstitial fluids induced by capillary suction forces and mechanical stimulation of the airway surface and submucosal glands. The study by Zahm et al. (15) in this issue of the American Journal of Physiology-Lung Cellular and Molecular Physiology utilizes cryosampling and X-ray probe methods to measure the salt content of ASL fluid in the mouse trachea.

Zahm et al. (15) sampled <10 pl of ASL from the tracheae of wild-type mice and a partial CFTR knockout mouse model. The mice were anesthetized, the tracheal mucosa was exposed by a 5-mm longitudinal incision, and within 2 min, a steel probe at liquid N₂ temperature was applied to the tracheal mucosa at a constant speed until a constant contact pressure was obtained. A small quantity of ASL at the probe surface froze immediately. The adherent fluid was transferred to an electron microscope grid and dehydrated by freeze-drying, and the elemental content (sodium, chlorine, sulfur, and calcium) was determined by X-ray spectral analysis. X-ray probe microanalysis is a well-established method to analyze electron microscopy samples that rely on the generation of X rays of unique element-specific wavelengths on irradiation of samples in an electron microscope (10). The principal conclusion of the study by Zahm et al. (15) is that the ASL salt “content” in normal and cystic fibrosis mice does not differ significantly, although absolute “concentrations” were not determined by the X-ray method used because the samples were dehydrated. The data in Table 2 in the paper by Zahm et al. permit assessment of the statistical confidence of the conclusions. The measurements of ASL sodium and chlorine content (in mmol/kg dry sample weight) had standard errors approximately equal to 20% of their mean values, with n = 8 mice/group, giving a standard deviation of ~50% of mean values. The reasons for the large sample variability were not evaluated but probably result from technical factors in mouse preparation, ASL sampling, and sample preparation for electron microscopy.

The principal conclusion of the paper, that ASL salt contents in wild-type and cystic fibrosis mice are within ~20% of each other, appears to be valid. However, several caveats in addition to the large intersample variability should be noted. It is unclear whether the incision into the trachea causes changes in ASL composition due to fluid secretion. ASL depth was not measured to evaluate secretions that may have accumulated during the time required for cryosampling. It is difficult to rule out tissue damage associated with the cryoprobe application and rapid freezing. Furthermore, it was assumed that efficiencies of elemental detection were identical in samples from normal and cystic fibrosis mice, which may not be the case if the samples differed in their content of proteins or other macromolecules. Last, as discussed above, mouse models of cystic fibrosis may not be suitable to address questions regarding ASL composition and function.

Our laboratory (5) recently developed a very different approach to measure ASL properties in situ that involves staining the ASL with fluorescent ion indicators and measuring ASL properties by fluorescence microscopy. ASL Na⁺ concentration ([Na⁺]), Cl⁻ concentration ([Cl⁻]), and pH were measured by ratio-imaging microscopy with dual-wavelength fluorescent indicators, and ASL depth was measured by rapid z-scanning confocal microscopy. ASL osmolality was measured by ratio imaging of osmotically sensitive liposomes encapsulating volume-sensitive and insensitive fluorophores (4). Measurements in airway cell culture models showed an approximately isomolar ASL, with [Na⁺] and [Cl⁻] of 100–120 mM and pH of 6.9–7.0. ASL [Na⁺] was measured in mice by ratio

![Fig. 1. Measurement of airway surface liquid (ASL) Na⁺ concentration ([Na⁺]) in mouse trachea by ratio-imaging microscopy.](http://ajplung.physiology.org/ by 10.220.33.3 on July 10, 2017)
imaging of microspheres containing at their surface a red-fluorescing $[\text{Na}^+]$-sensitive indicator and a green-fluorescing $[\text{Na}^+]$-insensitive indicator. A suspension of beads in a low boiling point perfluorocarbon was introduced onto the tracheal mucosa via a feeding needle passed through the mouth. Ratio imaging of the indicator fluorescence was done through the intact translucent tracheal wall (Fig. 1A) or through a surgically created rectangular window that was covered with Saran Wrap. The ratio of bead red-to-green fluorescence increases with $[\text{Na}^+]$ as shown in Fig. 1B. $[\text{Na}^+]$ was $114 \pm 4$ (SE) mM in wild-type mice ($n = 6$) and $105 \pm 3$ mM in CFTR-null mice. These results are in agreement with the lack of a substantial difference in ASL salt content in the study by Zahm et al. (15). We also carried out a set of measurements on freshly excised fragments of normal human bronchi that similarly showed a near-isotonic ASL.

Is the issue of abnormal ASL fluid composition in cystic fibrosis resolved by the X-ray microanalysis study reported here and the recent fluorescence data? The answer must be emphatically no, particularly because there is not yet a convincing and comprehensive mechanism to explain how CFTR mutations cause human lung disease in cystic fibrosis. It would be imprudent to dismiss the “abnormal ASL fluid composition” hypothesis until unambiguous noninvasive measurements of ASL composition are made in the upper and lower human airways. However, the recent evidence questioning this hypothesis mandates an intensified effort to identify and prove alternative mechanisms linking cystic fibrosis genotype to lung disease, such as primary abnormalities in submucosal glands and airway defense mechanisms.

REFERENCES


