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Regulation and Functional Significance of Airway Surface Liquid pH

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Summary

In gastrointestinal tissues, cumulative evidence from both *in vivo* and *in vitro* studies suggests a role for the cystic fibrosis transmembrane conductance regulator (CFTR) in apical epithelial bicarbonate conductance. Abnormal lumenal acidification is thus hypothesized to play a role in the genesis of cystic fibrosis (CF) pancreatic disease. However, consensus regarding CFTR's participation in pH regulation of airway surface liquid (ASL) and thus the contribution of ASL pH to the etiology of CF lung disease, is lacking. The absence of data reflects difficulties in both sampling ASL *in vivo* and modeling ASL biology *in vitro*. Here we evaluate the evidence in support of a lumenal acidification hypothesis in the CF lung, summarize current knowledge of pH regulation in the normal airway and illustrate how hyper-acidified airway secretions could contribute to the pathogenesis of CF lung disease.

Cystic fibrosis (CF) is a fatal hereditary disease resulting from lack of functional expression of the cystic fibrosis transmembrane conductance regulator (CFTR) in the apical membrane of epithelial cells [1, 2]. Lung disease in CF is characterized by unremitting pulmonary obstruction, infection and inflammation, accounting for 90% of the mortality and morbidity ascribed to the condition. Though it has been known for almost a decade that CFTR mediates an adenosine 3',5'-monophosphate (cyclic AMP)-regulated apical chloride conductance [3], a universally accepted paradigm linking abnormal vectorial ion transport to the complex manifestations of CF lung disease is currently lacking. Though current models of CF pathogenesis explain many aspects of its etiology [4], additional mechanisms of disease induction are also likely to be important. In this regard, accumulating evidence suggests that defective HCO_3^- transport may be pathophysiologically relevant.

A lumenal acidification hypothesis in CF has its roots in *in vivo* studies that date back more than 30 years. They demonstrated that pancreatic secretions from CF patients are acidic when compared to those of their normal counterparts [5, 6]. The more recent description of abnormally acidic seminal fluid from male patients with CFTR mutations lends further weight to this postulate [7]. Indeed, these *in vivo* studies have a resonance with the *in vitro* literature, which suggests that a CF lumenal pH defect may reflect abnormal HCO_3^- permeation through CFTR into the epithelial lumen. The relative bicarbonate:chloride conductance of CFTR *in vitro* appears to range from 0.1-0.27 [8, 9, 10]. CFTR has also been proposed to regulate lumenal bicarbonate secretion in the gut by facilitating a molecularly distinct apical anion exchanger (AE) [11]. The pathophysiological relevance of CFTR-

dependent HCO_3^- secretion is further emphasized by a recent report that a pancreatic insufficient phenotype is associated with properly processed, mutated CFTR that normally conducts chloride but exhibits fully defective HCO_3^- conductance, whereas mutants with even partial HCO_3^--transport result in a normal clinical phenotype [12].

However, the pancreas and male urogenital tract exhibit specialized HCO_3^--transport properties, and may differ in this regard from airway tissues. Although there is an evolving consensus that the defect of CFTR-dependent bicarbonate secretion plays a role in the etiology of CF pancreatic disease, consensus regarding CFTR's participation in airway surface liquid (ASL) pH (pH_{ASL}) regulation (and thus its potential role in contributing to lung disease) is lacking. Indeed, difficulties sampling ASL *in vivo* and reproducing ASL biology *in vitro* have hindered our understanding of many aspects of ASL pH regulatory physiology and thus impeded rigorous testing of the acidification hypothesis in pulmonary epithelia.

Earlier studies of cultured human airway epithelial cells (mounted in Ussing chambers), support the presence of a CFTR-dependent apical HCO_3^- conductance [13, 14]. However, since these studies were necessarily carried out under Cl^--free conditions, they cannot easily be extrapolated to more physiological circumstances. More compelling are reports suggesting that raising intracellular cAMP, in cultured normal nasal respiratory epithelium and Calu-3 cells, results in alkalinization of culture surface liquid [15, 16]. However, since similar experimental maneuvers were not performed in CFTR-deficient tissues, this alkalinization cannot confidently be ascribed to CFTR activation. Thus, though provocative, available studies in the literature fall short of demonstrating that lack of CFTR causes dysregulation of pH_{ASL} on CF pulmonary epithelium. This is a particularly important distinction, since even if airway epithelial CFTR indeed conducts HCO_3^- under physiological conditions, it presumably acts in concert with other pH_{ASL} modulatory processes, whose activity may either overwhelm CFTR or, alternatively, be altered in CF tissues, to compensate for CFTR's absence. Recent *in vivo* measurements of tracheal surface liquid failed to reveal a significant pH difference in normal and CFTR knockout mice [17]. Although these findings are provocative, and underscore the need for further clarification of CFTR's role in pH_{ASL} regulation, they must be interpreted with caution. The normal murine trachea exhibits low levels of CFTR expression [18], and the CF knockout mouse does not exhibit a pulmonary phenotype, making extrapolation of these data to the human situation problematic. Moreover, since the activity of CFTR as a regulator of ASL pH may vary under different physiological and pathological situations, even human *in vivo* measurements under basal conditions may not illuminate CFTR's full role in this process.

To date, we have lacked a sufficiently sophisticated understanding of normal pH_{ASL} regulation to resolve these important issues. We can claim a rudimentary knowledge of other pH regulatory processes on the apical membrane of airway epithelia. For example, we, and others have not detected evidence of a Na^+/H^+ exchanger in the apical membrane of pulmonary epithelia [19, 20]. However, there are suggestions that a K^+H^+ATPase, which exchanges lumenal K^+ for cytosolic protons, may acidify surface liquid. Though the presence of this ATPase in nasal airway tissue has been previously suggested [15], its molecular identification and functional role in regulation of pH_{ASL} by airway cells had not been conclusively demonstrated. However, we recently reported (in preliminary abstract form), that cultured human bronchial epithelium (in both normal and CF tissues) expresses K^+H^+ATPase at a molecular and functional level [21, 22]. In addition, the paracellular shunt could contribute to pH_{ASL} regulation. This poorly characterized pathway potentially

provides an alternative route for transepithelial ion transport, including movement of H^+ and HCO_3^- ions, though its conductivity for these species has not been characterized. Indeed, if CFTR is the sole path of bicarbonate translocation across the apical membrane of pulmonary epithelial cells, as might be suspected, the paracellular pathway could be the only mode of alkalinizing CF ASL if it becomes acidified during inflammation, infection or following aspiration of gastric contents. However, it is also possible that a 'calcium-activated chloride conductance' (CaCC) channel may also conduct bicarbonate and provide another potential route by which HCO_3^- could be transported into ASL. CaCC had been reported to compensate for the absence of CFTR in mediating HCO_3^- secretion in CF pancreatic tissue [23] and murine gallbladder [24]. CaCC has been identified in human pulmonary epithelium, though its role in pH regulation in ASL is uncertain. Its prototypical agonists are short-lived nucleotides, and its responses rapidly down-regulated, arguing against a role in eliciting sustained alterations in ASL pH. The capacity of CaCC to regulate pH_{ASL} in CF airways thus merits further attention. Anion exchangers are also known to contribute to HCO_3^- secretion in the gut. However, AEs have not, to date, been identified at a functional or molecular level in the apical membrane of airway cells. Similarly, there are no reports documenting the presence of proton translocating ATPases or $ZnCl_2$-sensitive passive proton conductance channels at the same site. Further detailed *in vitro* studies are necessary to resolve these issues. In considering such experiments, it must be borne in mind, however, that measurements of pH in 'bulk liquid' upon the culture surface may not reflect the near-membrane pH. Appreciable pH differences may be manifest across unstirred layers as a result of concentration polarization of unionized species either in liquid upon cultured epithelia or conceivably even in the thin ASL layer in vivo [25]. pH_{ASL} close to the apical membrane may also be affected by the presence therein of impermeant buffers, as is likely to be the case due to the presence of the glycocalyx, a feature of the apical surface of airway epithelia [26]. Thus, differences in pH_{ASL} at the apical membrane could, conceivably, be even greater, and studies formally addressing gradients of pH in individual compartments of ASL are necessary. Such studies are mandated because the mucous layer may also act as a diffusion barrier to acid (as it does in the stomach) and thus modulate pH_{ASL} [27]. Figure 1 models what we feel to be the most likely scenario for movement of H^+ and HCO_3^- across the apical membrane of normal and CF airway epithelium.

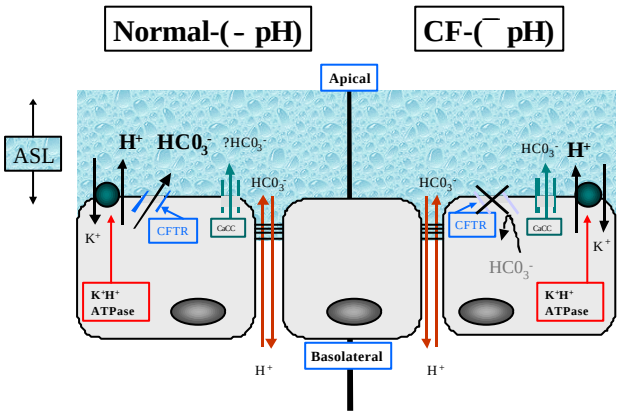

Figure 1. Schematic representation of hypothesized pH_{ASL} regulatory mechanisms in normal and CF bronchial epithelium.

Regulation of pH_{ASL} on normal cultured bronchial epithelium may reflect the acidifying effects of a K^+H^+ATPase opposed by bicarbonate/H^+ entry/exit into ASL via the paracellular pathway and a cAMP activated CFTR-dependent mechanism, in addition, potentially, to calcium-activated chloride conductance channels. In CF, however, only apically directed paracellular bicarbonate (+/-CaCC) movement opposes K^+H^+ATPase function and ASL (which is reduced in volume compared to normals) becomes correspondingly more acidic.

If absence of functional CFTR-dependent epithelial HCO_3^- transport renders ASL abnormally acidic, how might altered airway surface liquid pH impinge on host defense processes? Despite its shallow depth (about 5-40 μM), ASL is a critical determinant of airway epithelial defense. The efficient killing and

removal of microorganisms in ASL result from the concerted action of a many independent biological processes. Since so many of these processes are pH dependent, the importance of tightly regulated proton concentrations in ASL is obvious. It was correctly noted recently that this is a neglected area of epithelial biological and, in particular, CF research [28].

Hyper-acidified ASL is predicted to negatively impact upon the rheological properties of mucus [29]. The polymeric gel (and thus viscoelastic) properties of the mucus layer of ASL are determined by hydration of mucin molecules and their interactions [30]. pH not only determines the degree to which the hydrophobic regions of the protein component of the mucin molecule are exposed, influencing non-covalent mucin-mucin interactions [31], but $[H^+]$ is a determinant of net charge of sulphated and sialated carbohydrate sidechains of the molecule (which have a net neutral pKa) and, consequently, its hydration state [32]. Reducing pH will diminish the electrostatic repulsive forces between mucins and increase ASL viscosity. In addition to promoting the gel transition of mucins already integrated into the mucus layer, more acidic ASL may also adversely affect the initial hydration of freshly-exocytosed mucins and, in this regard, the near membrane pH is likely to be critical. The effects of low ASL pH in CF may be even more undesirable, since the prediction is that low pH will also promote interactions between mobile gel forming mucins and membrane surface tethered mucins, likely attenuating cough-clearance of mucous plaques [33]. This situation may be compounded by low pH_{ASL} in CF, since it has recently been suggested that acidic lumenal pH increases ENaC activity [34], which may exacerbate hyperabsorption of apical liquid, already a putative hall mark of CF epithelia, and diminish the solvent volume for mucins therein.

Inability to normally regulate ASL pH may compromise the function of airway immune cells and thereby promote lung damage in CF. An early and persistent inflammatory cell influx into the airway lumen of CF patients is characteristic of the condition [35]. Perplexingly, there is a failure to kill resident bacteria therein. Instead, immune cell-derived proteases and oxidants contribute to progressive pulmonary parenchymal destruction typical of CF [36, 37, 38]. An acidic extracellular pH has been shown to suppress intracellular oxidant generation, a key component of the polymorphonuclear leucocytes (PMNs) bacteriacidal armamentarium, while increasing release of H_2O_2 into the extracellular compartment, in a manner likely to accelerate host damage [39]. Moreover, extracellular acidification increases the release of neutrophil azurophil granule contents [40] that include myeloperoxidase, which generates long-lived toxic oxidant species, and neutrophil elastase, a broad spectrum and potent protease with the propensity to permanently degrade pulmonary connective tissues. Extracellular acidification is not only chemotactic for neutrophils but also inhibits their apoptotic involution and thereby potentially prolongs the life span of ineffective and thus potentially harmful cells on the airway surface.

Finally abnormally low pH of CF ASL may facilitate bacterial survival in the airway lumen. Phagocytic cells are less efficient at ingesting and killing bacteria at lower extracellular pH [41, 42]. In addition, *pseudomonas aeruginosa*, the organism most typically associated with CF, carries a net negative surface charge. In this context, lowering the pH may eliminate electrostatic repulsive charges between organisms and facilitates "tighter" biofilm formation, potentially hindering ease of access of immune cells to the organism [43]. In addition, electrorepulsive forces between bacteria and negatively charged mucins and glycocalyceal proteins may be similarly reduced at low pH, altering bacterial interaction with mucins and the cell surface. Clearance of bacteria may also be sub-optimal at low pH since ciliary beat frequency in bronchial

epithelium is reduced when external pH falls [44]. These processes are modeled in Figure 2. Therefore, ASL pH appears likely to play an important role in the modulation of key biological processes implicated in normal lung host defense. Since, in addition, it may be abnormal in CF patients, future investigations, utilizing both *in vitro* and *in vivo* strategies, are required to accurately define its mode of regulation.

Key words Bicarbonates; Cystic Fibrosis Transmembrane Conductance Regulator; Epithelium; H(+)-K(+)-Exchanging ATPase; Lung

Abbreviations AE: anion exchanger; AMP: adenosine 3',5'-monophosphate; ASL: airway surface liquid; CaCC: calcium activated chloride conductance; CF: cystic fibrosis; CFTR: cystic fibrosis transmembrane conductance regulator; PMNs: polymorphonuclear leucocytes

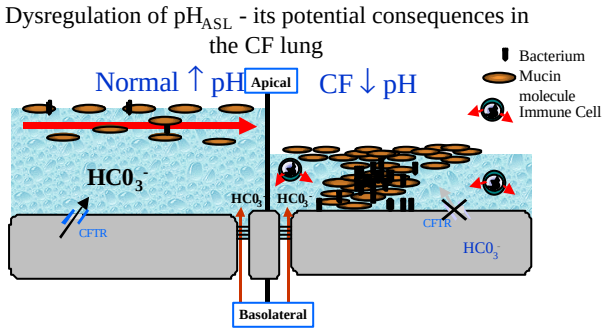

Figure 2. Predicted adverse effects of reduced ASL pH on CF airway epithelium.
On normal airway relatively higher pH results in a less viscous surface layer, facilitating transport of retained bacteria in the mucous layer in a cephalad directions. Putatively, in CF, lower pH results in an increasingly viscous "low-volume" gel, adherence of soluble and tethered mucins, resulting in mucous plaques, persistence of dysfunctional immune cells and bacteria.

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