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Alveolar fluid transport: a changing paradigm

Lucky Jain and Douglas C. Eaton

Department of Pediatrics and Physiology and The Center for Cell and Molecular Signaling, Emory University School of Medicine, Atlanta, Georgia

UNLIKE OTHER EPITHELIAL TISSUES that transport large amounts of solutes and water, the alveolar epithelium is faced with a unique physiological challenge. To facilitate optimal gas exchange and to trap airborne particulate matter, it must maintain a thin layer of fluid on the luminal surface of its extensive alveolar network. However, the thickness of the fluid layer must be tightly regulated, since even slight increases in the fluid depth could adversely affect gas exchange, whereas dehydration of the surface would impair ciliary clearance. The alveolar flooding associated with acute respiratory distress syndrome or the airway dehydration in cystic fibrosis represents two pathological extremes of a failure to maintain proper airway fluid balance. Both of these conditions account for a significant burden of morbidity and mortality worldwide (16, 20). Extensive research done in the last two decades has shown that precise regulation of alveolar surface fluid layer is achieved through vectorial transport of solutes between the alveolar surface and interstitial spaces with active Na^+ transport across the alveolar epithelium generating the osmotic force for movement of water (12, 17). The broadly accepted paradigm for Na^+ transport in the alveoli involves a two-step process: movement of Na^+ through cation channels in the apical cell membrane constitutes the first step, and extrusion of the excess Na^+ thus acquired via basolateral Na^+-K^+-ATPase is the second step (19). However, despite extensive research using many different approaches that support this paradigm, gaps still exist in our understanding of how this fine balance is achieved in vivo (19). In particular, there are several unresolved questions that, when resolved, could significantly affect our view of alveolar fluid clearance. First, type 1 cells constitute ~95% of the alveolar surface area, but their role in ion transport is not completely clear. If there are significant differences between the transport characteristics of type I and type II cells, the current paradigm describing alveolar fluid clearance would need to be modified. Second, although cation permeability pathways have been described fairly well, the precise pathways for movement of anions are unclear. The character of these channels is important since anion channels could form either co-ion pathways for the concomitant movement of Na^+ and Cl^- or a secretory pathway to increase alveolar fluid. Finally, alveolar fluid clearance appears to consist of a basal and stimulated component of reabsorption. The relative contribution of different ion channels and different cell types to these two components of clearance is far from clear. These questions are all underscored by the differences between the robust increases in transport that can be produced by several different treatments in vitro and the often weak clearance response of the in vivo lung epithelium to the same treatments under conditions of pathologically abnormal fluid accumulation (1, 21).

As mentioned above, there has been long-standing controversy about the transport pathways involved in basal lung Na^+ and water movement and the precise role that epithelial Na^+ channels (ENaC) play in the absence of agents known to enhance ENaC activity (18). Notwithstanding the significant differences that may exist across several animal models used in different studies, one camp maintains that ENaC contributes to basal lung fluid clearance, whereas the other camp suggests that ENaC only plays a role under extraordinary circumstances (at birth, in pulmonary edema, or after stimulation by β-agonists, etc.). The report from Li and Folkesson, the current article in focus (Ref. 14a, see p. L649 in this issue), attempts to close the gap in our understanding of regulation of alveolar fluid clearance in the basal state and after β-agonist stimulation. Using an elegant in vivo design, they present data to show that ENaC channels are responsible for most, if not all, of the β-agonist-induced increase in alveolar fluid clearance but contribute significantly less to baseline fluid clearance. The authors have employed a novel approach using plasmid delivery of sequences that encode short hairpin RNA (that acts via RNA interference) into the air spaces of the lungs to decrease ENaC expression in intact epithelia. This strategy was successful since α-ENaC was undetectable in type II cells isolated from small interfering RNA (siRNA)-pretreated lungs, and the dose-response curve shows that lesser degrees of inhibition occurred at lower doses of siRNA. This approach had high subunit specificity, with no changes in β-ENaC message or protein expression in target epithelia and no changes in α_1-Na^+-K^+-ATPase protein expression. The treatment was also confined to the lung epithelium with no changes in renal expression of ENaC subunits. However, the presence of the target ENaC subunit was not evaluated in type I alveolar cells, and therefore, it is not clear if the strategy worked equally well in these cells. This is important since there is a growing body of evidence to show that type I cells express ENaC and Na^+-K^+-ATPase and may have a significant role to play in alveolar salt and fluid transport (3, 14, 22).

The report by Li and Folkesson (14a) is important and is being highlighted for several reasons. First, their data clearly demonstrate that gene silencing with specific siRNA can provide a viable alternative to traditional gene knockout technology (15). Generating appropriate knockouts requires more time and resources as well as knowledge of a genomic sequence. As the dose-response curve in the authors' work demonstrates, this approach can be useful in achieving partial gene silencing to study lethal genes. This is particularly important since knockouts for α-ENaC survive for only a few hours and die from a failure to clear their lungs of fluid, whereas β- or γ-ENaC knockouts survive only a few days and die of electrolyte imbalances (11). A high degree of organ specificity was achieved with this approach, and this is highly desirable for

Address for reprint requests and other correspondence: L. Jain, Emory Univ. School of Medicine, 2015 Uppergate Dr., Atlanta, GA 30322 (e-mail: ljain@emory.edu).

functional proteins like ENaC that are expressed in multiple organs.

Second, this work sheds new light on the net contribution of ENaC to basal alveolar fluid clearance. Pretreatment with α-ENaC siRNA-generating plasmid DNA under unstimulated conditions blocked the baseline lung fluid absorption by only 30%. This treatment also attenuated amiloride sensitivity of lung fluid absorption. The investigators did not explore the pathways responsible for the remaining 70% of alveolar fluid clearance, and there is no consensus about the ion transport mechanisms responsible for this large fraction of baseline fluid transport, although some investigators have attributed this to cyclic nucleotide-gated channels (17).

Third, this work shows that ENaC is required for the β-agonist-induced increase in alveolar fluid clearance. Pretreatment with α-ENaC siRNA-generating plasmid DNA blocked most of the terbutaline-associated increase in fluid transport. There is considerable evidence to show that, in animal models challenged with excess alveolar fluid, increasing the activity of ENaC and Na^+-K^+-ATPase can enhance edema resolution, raising the hopes for therapeutic interventions (4, 5, 21). Candidates for therapeutic intervention include β-adrenergic agents, steroids, growth factors like keratinocyte growth factor and EGF, and gene technologies to upregulate salt transporters (5). However, in spite of promising animal research data, few reliable strategies are available to the clinician to treat lung edema. Although this disconnect is perplexing, a few possible explanations have been proposed. Lung edema often occurs as a secondary event following a wide variety of lung insults, resulting in a heterogeneous group of patients. These patients have different levels of cytokine and hormonal responses, epithelial barrier injury and leakiness, and compensatory responses to alveolar flooding. The underlying state of the lung before the onset of lung injury and edema accumulation will also determine the ability of the lung to clear edema rapidly and may contribute to the heterogeneity in response (2, 8, 9).

Overall, it should come as no surprise that the regulation of apical and basolateral Na^+ transport in the lung epithelium is likely to be coordinated (24). Many agents such as steroids, β-agonists, and dopamine that increase the number and/or activity of apical Na^+ channels (10) also have a similar effect on Na^+-K^+-ATPase (23). In this regard, lung epithelium differs from other epithelia responsible for Na^+ transport (like the colon and kidney) in that its primary goal is not net absorption of salt, but maintenance of a precisely regulated fluid layer through a combination of fluid absorption and secretion. Having control over the apical entry as well as the basolateral extrusion step would be useful under different physiological and pathological circumstances. In the final analysis, the ability of the lung epithelium to respond to sudden changes in fluid flux would be contingent upon coordinated changes in the activity of Na^+ channels and the pump (6) and the ability of the epithelium to move appropriate amounts of chloride (7, 13). Understanding which of these components is lacking in an individual patient may hold the key to our success in treating lung edema.

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