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OCCASIONAL ESSAY

Resolution of Pulmonary Edema
Thirty Years of Progress

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Abstract

In the last 30 years, we have learned much about the molecular, cellular, and physiological mechanisms that regulate the resolution of pulmonary edema in both the normal and the injured lung. Although the physiological mechanisms responsible for the formation of pulmonary edema were identified by 1980, the mechanisms that explain the resolution of pulmonary edema were not well understood at that time. However, in the 1980s several investigators provided novel evidence that the primary mechanism for removal of alveolar edema fluid depended on active ion transport across the alveolar epithelium. Sodium enters through apical channels, primarily the epithelial sodium channel, and is pumped into the lung interstitium by basolaterally located Na/K-ATPase, thus creating a local osmotic gradient to reabsorb the water fraction of the edema fluid from the airspaces of the lungs. The resolution of alveolar edema across the normally tight epithelial barrier can be up-regulated by cyclic adenosine monophosphate (cAMP)-dependent mechanisms through adrenergic or dopamine receptor stimulation, and by several cAMP-independent mechanisms, including glucocorticoids, thyroid hormone, dopamine, and growth factors. Whereas resolution of alveolar edema in cardiogenic pulmonary edema can be rapid, the rate of edema resolution in most patients with acute respiratory distress syndrome (ARDS) is markedly impaired, a finding that correlates with higher mortality. Several mechanisms impair the resolution of alveolar edema in ARDS, including cell injury from unfavorable ventilator strategies or pathogens, hypoxia, cytokines, and oxidative stress. In patients with severe ARDS, alveolar epithelial cell death is a major mechanism that prevents the resolution of lung edema.

Keywords: acute respiratory distress syndrome; acute lung injury; alveolar epithelium; alveolar liquid clearance; pulmonary edema

The physiological basis for the formation of pulmonary edema was established by 1980. Cardiogenic pulmonary edema develops because of elevated vascular pressures in the lung (1), hence the term hydrostatic lung edema. Noncardiogenic pulmonary edema, clinically recognized as the acute respiratory distress syndrome (ARDS), is primarily the consequence of an increase in lung vascular and epithelial permeability (2). Several experimental studies had reported that the combination of elevated vascular pressure and an increase in lung vascular permeability would increase the accumulation of pulmonary edema (3, 4). And, in fact, elevated lung vascular pressures do coexist with an increase in lung vascular permeability in some clinical conditions that lead to ARDS, including sepsis and major trauma, as demonstrated definitively in a National Heart, Lung, and Blood Institute–supported multicenter clinical trial (5).

However, the mechanisms responsible for the resolution of pulmonary edema were not well understood until research in the 1980s identified the primary mechanism responsible for the resolution of alveolar edema—active ion transport across the alveolar epithelium. This discovery led to a more comprehensive understanding of the molecular, cellular, and physiological basis for the resolution of pulmonary edema over the last 30 years. Considerable progress has been made in understanding the absorption of perinatal lung fluid by active ion transport, though this topic is beyond the scope of this essay (6).

The first section of this essay focuses on the removal of pulmonary edema fluid in the uninjured lung, most relevant to the resolution of hydrostatic or cardiogenic pulmonary edema, with a focus on how the science in this field evolved historically. The second section considers the mechanisms that impair the resolution of lung edema in the presence of acute lung injury or ARDS. The final section briefly considers promising areas for future research.

Alveolar Edema Fluid Clearance in the Uninjured Lung

The resolution of alveolar edema can be appreciated radiographically or

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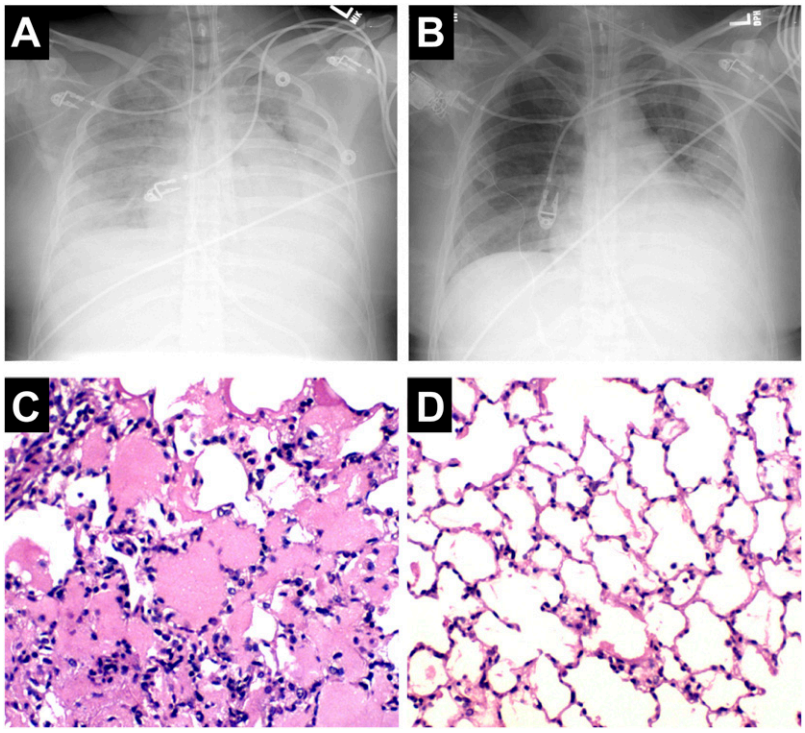

Figure 1. Resolution of pulmonary edema. (*A*) Bilateral alveolar opacities consistent with extensive alveolar edema in an intubated and ventilated patient. (*B*) Marked resolution of the bilateral pulmonary opacities in the same patient over a 24-hour period. (*C*) Standard hematoxylin and eosin–stained section, demonstrating alveolar edema with pink fluid filling most of the alveoli. (*D*) Normal air-filled alveoli, indicting reabsorption of alveolar edema fluid.

histologically, as shown in Figure 1.

The scientific challenge was to account for net reabsorption of edema fluid across the alveolar epithelium, normally a tight epithelium that restricts the passive movement of even small solutes (7). Prevailing theories had suggested that reabsorption of alveolar edema fluid might be driven by negative interstitial pressure, protein osmotic pressure differences, or by a transpulmonary airway pressure gradient, but there were no convincing data for these hypotheses. However, in the 1980s, a combination of *in vivo* and *in vitro* studies demonstrated that the primary mechanism driving the resolution of alveolar edema was vectorial transport of sodium into the interstitial space, thereby creating an osmotic gradient for absorption of water from the distal airspaces of the lung (7). Several investigators demonstrated that the excess interstitial fluid was cleared by lung lymphatics, and by bulk flow across the low-resistance visceral pleura into the pleural space (1, 8). A negative interstitial pressure gradient, even under conditions of edema, was shown to be the major force for the removal of interstitial edema fluid into lung lymphatics and the mediastinum (9).

In Vivo Studies

The development of a clinically relevant model in sheep to study the resolution of alveolar edema provided new insights into the mechanisms responsible for removal of fluid from the distal air spaces of the lung. After instillation of a known quantity of autologous serum or plasma into the distal airspaces of one lung in anesthetized and ventilated sheep, much of the liquid or water volume was removed rapidly over 4 hours while the instilled protein concentrated in the airspaces to levels above the plasma protein concentration (10). In spontaneously ventilating sheep, 75% of the liquid volume was removed over 24 hours while only 25% of the alveolar protein was cleared from the airspaces (11). In these experiments, alveolar protein concentration increased at 24 hours to 13 g/100 ml while the circulating plasma protein concentration remained at 6 g/100 ml. The alveolar protein osmotic pressure was approximately 40 cm H_2O higher than the protein osmotic pressure in the interstitial or vascular spaces of the lung (10, 11), a difference that would be expected to draw fluid into the alveolar compartment, based on the elevated alveolar protein osmotic pressure; nevertheless, net removal of alveolar fluid continued. The term alveolar fluid clearance (AFC) was used to describe this process (11). The removal of all of the instilled fluid from the lung was termed lung liquid clearance, which can be measured by standard gravimetric methods (7, 11). Normally, the rate-limiting step for the resolution of pulmonary edema is the reabsorption of edema fluid across the alveolar epithelial barrier.

Additional *in vivo* studies performed by several investigators in large and small animal models provided direct evidence that active sodium transport was responsible for the resolution of alveolar edema. The rate of AFC could be reduced by approximately 50% with an apical sodium channel inhibitor (amiloride), or blocked completely in the isolated perfused lung with ouabain, an inhibitor of Na/K-ATPase, or by perfusion at low temperature (12–16). Basolaterally localized Na/K-ATPase establishes a transepithelial sodium concentration gradient by extruding sodium out of polarized epithelial cells while sodium enters the apical cell surface through sodium channels. This sodium concentration gradient was thus established as the force that drives the osmotic reabsorption of water from the alveoli into the lung interstitium.

In Vitro and *In Situ* Studies

In parallel with the *in vivo* studies and perfused lung experiments, several *in vitro* studies established that cultured, polarized rat alveolar epithelial type II cells had the capacity to transport sodium from an apical to a basolateral direction. Tight monolayers of cultured type II cells formed domes of fluid, and dome formation was inhibited if sodium was not included in the culture medium or amiloride was added (17, 18). A number of different experimental preparations demonstrated a major role for Na/K-ATPase activity. As a result of Na/K-ATPase activity, the potassium electrochemical potential is larger inside the cell, and potassium efflux is regulated by channels localized to the basolateral membrane (7). In the alveolar epithelium, Na/K-ATPase is

a heterodimeric transmembrane protein composed of a catalytic α_1 subunit and a regulatory β subunit. These isoforms are needed for functional insertion of Na/K-ATPase in the cell membrane, and several mechanisms can up- or down-regulate the abundance of Na/K-ATPase on the basolateral surface of alveolar epithelial cells as well as pump activity (19). Several investigators contributed to our understanding of this process with Ussing chamber studies to measure short-circuit current and ion flux as well as with patch-clamp studies for both distal airway epithelium and alveolar epithelial cells, including novel *in situ* lung slice tissue studies (7, 20–23).

Epithelial sodium channel. The molecular basis for most of the apical uptake of sodium in the alveolar epithelium was established when the three subunits of the epithelial sodium channel (ENaC) were cloned (α, β, γ) in 1994 (24). ENaC was found to be expressed throughout the lung epithelium (as well as in the kidneys, large intestine, and other organs). Knockout of the α subunit in mice resulted in persistent lung fluid at birth and death from respiratory failure (24). Interestingly, a case report described a homozygous ENaC-α preterm infant who did not suffer any evidence of respiratory failure (25). Thus, in contrast to mice, clearance of alveolar fluid after birth in humans may not critically depend on ENaC. This report could be explained by a role for the δ ENaC subunit (26) or could be explained by up-regulation of other ENaC subunits, or a greater reliance on non-ENaC sodium channels. Several studies have shown that ENaC is regulated by transcriptional, translational, and posttranslational mechanisms, including trafficking and stability on the apical cell membrane. Also, membrane-bound extracellular serine proteases, termed channel-activating proteases, have been shown to regulate ENaC sodium conductance (27). Some investigators also established a role for other, less well-characterized channels for sodium uptake across alveolar epithelium, including nonselective cation channels, cyclic nucleotide–gated channels, and other selective cation channels (7, 21, 27). Although alveolar epithelial type II cells were initially thought to be the primary cell responsible for vectorial sodium transport, subsequent studies demonstrated that alveolar epithelial type I cells probably play an important role in driving AFC (28). Type I cells have ENaC and other sodium channels on the apical surface and Na/K-ATPase on the basolateral surface, demonstrated by elegant morphological and biophysical methods, including studies in the *in situ* lung (23, 29–31).

Aquaporins. Although biophysicists had postulated the existence of specific water transcellular channels, the discovery in 1992 of aquaporin-1 opened a new field with identification of large numbers of aquaporins with major relevance to mammalian and plant biology (32). Aquaporin-1 was found to be distributed throughout the lung endothelium, and aquaporin-5 was expressed on the apical surface of alveolar epithelial type I cells. Type I cells were shown to be highly water permeable, primarily because of the existence of aquaporin-5 (33). However, a series of single- and double-knockout studies for aquaporin-1 and -5 in mice indicated that the absence of either or both aquaporins did not alter the rate of alveolar fluid clearance (34, 35). The osmotic clearance of water generated by the ion transport gradient across the alveolar epithelium probably occurs by paracellular pathways, although some water may cross by a transcellular route through aquaporins.

Figure 2A summarizes the pathways and principal transporters responsible for the resolution of alveolar edema in the normal lung under basal conditions.

Alveolar fluid clearance in patients with cardiogenic pulmonary edema. In ventilated patients with cardiogenic pulmonary edema the resolution of alveolar edema could be estimated by measuring sequential concentrations of total protein in undiluted edema fluid samples suctioned from a 14-gauge catheter through the endotracheal tube over 4–12 hours (36, 37). This measurement reflects net clearance of edema fluid, and thus AFC might be reduced because of ongoing alveolar edema formation. Maximal AFC in patients with hydrostatic pulmonary edema was greater than 14%/hour and submaximal clearance was 3–14%/hour (37). The submaximal clearance rate may be explained by persistently elevated lung vascular pressures in some patients, resulting in flooding, although other mechanisms are possible (38). More recent studies in the *ex vivo* perfused uninjured human lung indicate that basal AFC is approximately 20%/hour (39), a fast rate that helps to explain why cardiogenic pulmonary edema can resolve rapidly in patients once the primary cause has been successfully treated and vascular pressures in the lung microcirculation are normalized.

Regulation of alveolar fluid clearance. The rate of AFC was shown to be increased by cAMP stimulation (7, 27), an effect that can be explained in part by increased sodium conductance, recruitment of more ENaCs to the apical membrane with more open time, as well as an increase in the enzymatic activity of Na/K-ATPase (7, 27). cAMP stimulation with β-adrenergic agonists accelerated AFC in sheep, dogs, rats, and mice. The potential clinical relevance of this pathway was established by studies in an *ex vivo* human lung preparation showing that AFC could be markedly increased in human lungs by a β_2-adrenergic agonist and inhibited by amiloride or ouabain (13). In addition, experimental studies demonstrated that an increase in endogenous plasma levels of epinephrine from shock of any cause induces an acute increase in the rate of AFC that can be inhibited by β-adrenergic blockade, indicating that the up-regulation of AFC can be driven by pathological conditions that may predispose to pulmonary edema, including sepsis (27, 40).

It has been difficult to determine how chloride transfers across the alveolar epithelium. Chloride transport is required to maintain electrical neutrality to match cationic sodium clearance from the airspaces. Some *in vitro* evidence indicated that chloride may move through paracellular and transcellular pathways (41, 42), and more recent data suggested that chloride might be regulated by claudins, in particular claudin-4, by conferring relative chloride selectivity to the paracellular pathway (43). In the presence of cAMP stimulation, cystic fibrosis transmembrane conductance regulator (CFTR) opens on the apical membrane of alveolar epithelial type I and II cells, providing a permissive effect for up-regulated AFC. ΔF508 mice that lack function CFTR did not increase AFC with exogenous or endogenous β-agonist stimulation (44). Other studies indicated that CFTR plays an important role in cAMP-accelerated chloride clearance, in parallel with increased

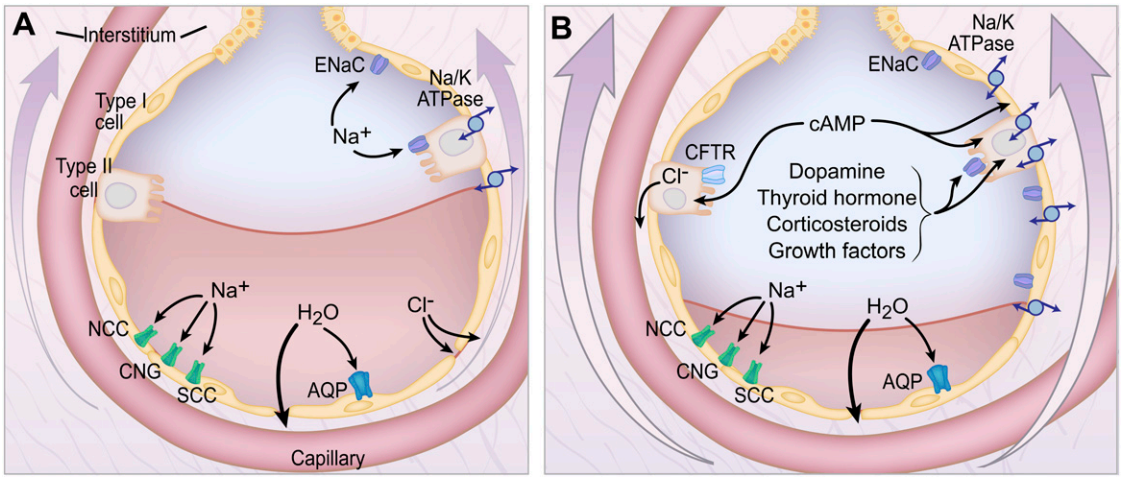

Figure 2. (*A*) Normal alveolar fluid clearance pathways. The interstitial, capillary, and alveolar compartments are shown with edema fluid in the alveolus. Type I and II cells are indicated. Apical channels on type I and II cells for absorption of sodium (Na^+) are shown, including the epithelial sodium channel (ENaC), as well as other apical sodium channels, including nonselective cation channels (NCC), cyclic nucleotide–gated channels (CNG), and other selective cation channels (SCC). Chloride (Cl^-) is shown crossing the alveolar epithelium either by a transcellular or a paracellular route, the latter possibly facilitated by claudins. Water (H_2O) is shown crossing through an aquaporin (AQP) channel or by an intercellular route. Note that the basolaterally located sodium/potassium ATPase pump (Na/K-ATPase) is shown on both type I and II cells. The large *purple arrows* in the interstitium indicate that after alveolar edema fluid is absorbed into the interstitium, the fluid moves to the lung lymphatics for clearance, which are present in the extraalveolar interstitium. (*B*) Up-regulated clearance pathways. Shown here are mechanisms by which the rate of alveolar fluid clearance can be increased by exogenous or endogenous factors. The best described mechanism involves cyclic adenosine monophosphate (cAMP), which is an intracellular messenger, stimulated by activation of β receptors on alveolar type I and II cells, which stimulates adenyl cyclase, which increases intracellular cAMP. cAMP can be stimulated by endogenous elevations of epinephrine or exogenous dobutamine or epinephrine. Other mechanisms for increased clearance include dopamine, thyroid hormone, corticosteroids, and growth factors, in particular keratinocyte growth factor. Note in *B* that the quantity of alveolar edema fluid has declined in the presence of accelerated alveolar fluid clearance. CFTR = cystic fibrosis transmembrane conductance regulator.

transport of sodium through ENaC and other apical sodium channels (27, 42).

Several investigators reported that β-adrenergic receptor–independent factors, including dopamine, corticosteroids, thyroid hormone, reactive oxygen species, and keratinocyte growth factor, and even leukotriene D4, tumor necrosis factor-α, membrane-bound extracellular serine proteases, and nucleotides, can increase AFC by transcriptional and translational pathways (7, 22, 27, 45). Also, purinergic agonists have been shown to regulate ENaC as well as a large number of intracellular signaling pathways (22).

Figure 2B summarizes several of the pathways that increase the rate of AFC.

The net effect of all of these pathways in the setting of pulmonary edema in patients has not been clearly defined. For example, there is evidence that cAMP agonist stimulation with an inhaled β_2-adrenergic agonist (salmeterol) can prevent high-altitude pulmonary edema (46) and that inhaled β_2-agonist therapy can reduce lung water and improve oxygen saturation in patients with probable postoperative hydrostatic pulmonary edema (47). However, as is discussed in the next section, cAMP stimulation does not enhance the resolution of alveolar edema in patients with lung injury from ARDS.

Unresolved issues. Although much has been learned, there are several unanswered questions. Is there an important contribution of distal airways to AFC that has been underestimated (48)? Under some pathological conditions, could secretion of Na^+ or Cl^- contribute to alveolar edema (49)? Also, we do not adequately understand how the alveolar liquid subphase is maintained in the normal lung, although one study measured a fluid pH of 6.9, suggesting that it is actively regulated (50).

Resolution of Lung Edema in the Injured Lung (ARDS)

Clinical studies established that the capacity to remove pulmonary edema is impaired in most patients with ARDS (51). Impaired AFC correlated with higher mortality (51), and furthermore the severity of shock in sepsis-induced ARDS has been reported to be associated with lower rates of AFC (52). The mechanisms responsible for impaired AFC have been studied in a variety of clinically relevant experimental models of ARDS (19, 27).

Moderate hypoxemia reduced AFC by 50% (53) by decreasing apical sodium uptake by transcriptional effects and by impaired trafficking of ENaC. Hypoxia inhibited the function of Na/K-ATPase on alveolar epithelial cells, in part by triggering endocytosis through reactive oxygen species and phosphorylation of the α_1 subunit (19, 54). Restoration of normoxia rapidly reversed the depressant effects of hypoxia in rats (53). Thus, the simple administration of supplemental oxygen to patients with pulmonary edema may in itself enhance the resolution of alveolar edema. There is also new evidence that hypercapnia can impair alveolar fluid clearance (55, 56).

Experimental work indicated that high tidal volume and elevated airway pressures injure the alveolar epithelium, markedly reducing AFC in the injured lung, in part by inducing cell death, inflammation, and disrupting cell junctions. The success of lung-protective ventilator strategies in

reducing mortality in ARDS is likely explained in part by the preservation or restoration of more normal AFC in the injured lung, a finding that was demonstrated in a clinically relevant rat model of acid-induced lung injury (57).

Several cytokines present in the airspaces of patients with ARDS, including IL-1β, IL-8, and transforming growth factor-β, were shown to decrease vectorial fluid transport across alveolar epithelium by reducing the expression and function of Na/K-ATPase and ENaC (58–62). Viral infection with influenza was found to impair the function of ENaC. Some investigators reported that bacterial and viral products directly injured alveolar epithelial cells, either through their effects on neutrophils, platelets, and monocytes, or by direct toxic injury to epithelial cells, potentially through oxidant, proteolytic, and other pathways (63–65). There is also evidence that alveolar epithelial cell injury and dysfunction may in part result from mitochondrial injury, resulting in low intracellular ATP levels that can impair surfactant secretion and perhaps vectorial ion and fluid transport (66).

If lung vascular hydrostatic pressures are elevated in the setting of persistent endothelial or epithelial injury, then ongoing alveolar flooding could impair net removal of edema fluid from the alveoli. This concept may help explain why a conservative fluid strategy resulted in more ventilator-free days in patients with ARDS in a large randomized clinical trial (5), although there is experimental and clinical evidence that lower vascular pressures may also reduce proinflammatory pathways (67, 68).

The failure of either inhaled or intravenous β_2-adrenergic agonists to improve survival in phase 3 clinical trials (69, 70) could be explained by several of the mechanisms, including the loss of an intact alveolar epithelial barrier, because apoptosis and necrosis play an important role in lung injury in ARDS (71, 72). There is also evidence that IL-8 can impair the stimulatory effect of β_2 agonists on AFC (62). Inhaled β_2 agonist also did not increase alveolar fluid clearance or oxygenation in a randomized trial in brain-dead subjects who were potential lung transplant donors if their lung function had improved (73).

Figure 3 summarizes these pathways to explain impaired AFC in ARDS. It is likely that several of these pathways simultaneously reduce the resolution of alveolar edema in most patients with ARDS.

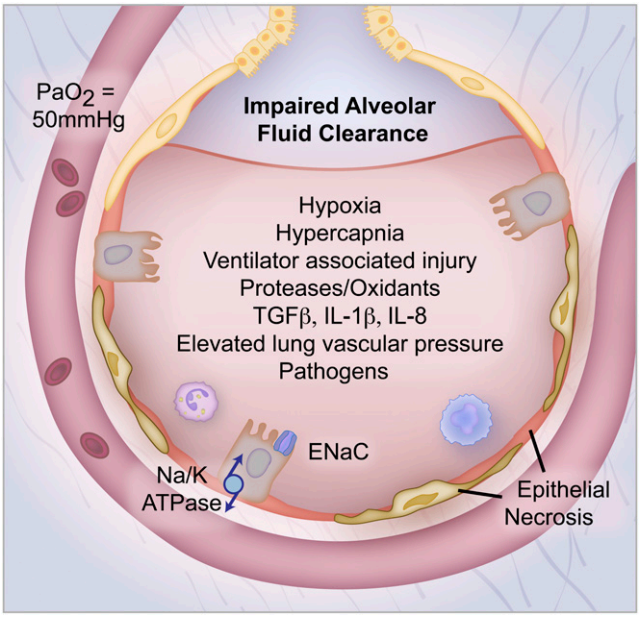

Figure 3. Impaired alveolar fluid clearance. Shown are some of the clinically relevant mechanisms that decrease the rate of alveolar fluid clearance in patients with acute respiratory disease syndrome. Note that type I and type II alveolar epithelial cell necrosis is shown. This is an important mechanism because of the loss of epithelial barrier function and the ability to generate net alveolar epithelial sodium and fluid clearance. The other factors and mechanisms shown are discussed in text.

Future Research

The challenge is to accelerate the resolution of alveolar edema in patients with ARDS, which also includes enhancing the clearance of pathogens, cell debris, fibrin, and excess matrix (74). A critical barrier to restoring normal AFC is the lack of functional alveolar epithelium, probably in large part from death of type I and type II cells, perhaps explaining why aerosolized and intravenous β-adrenergic agonists did not improve outcomes in patients with ARDS (69, 70). Thus, we need to understand more completely the mechanisms of lung epithelial cell death and obtain more insights into how the injured alveolar epithelium is regenerated. Some progress has been made in mouse models indicating the likely contribution of several progenitor cell populations to alveolar repair (75–78). However, we do not know how to accelerate this process of reestablishing an alveolar epithelium that will function normally for three essential properties of normal alveolar epithelium: (*1*) a tight barrier that guards against alveolar flooding, (*2*) surfactant secretion, and (*3*) vectorial ion and fluid transport to facilitate the resolution of alveolar edema. It is likely that repair of the nearby injured microvascular endothelium in ARDS is also required for normal AFC to be established across the alveolar epithelium.

Current ARDS trials are testing therapies that have the potential to enhance the resolution of alveolar edema. Cell-based therapy with mesenchymal stem (stromal) cells is being tested in phase 1 and 2 trials in adults with ARDS. Mesenchymal stem cells are attractive because they have multiple beneficial properties, including inhibition of apoptosis, reduction of inflammation, and enhancement of AFC in the presence of endotoxin and bacterial–induced injury (79–81), and the capacity to deliver mitochondria to injured alveolar epithelium that can restore depleted ATP levels to a normal level (66). Although inhaled nitric oxide did not reduce mortality in ARDS, there is interest in testing safe levels of inhaled carbon monoxide because of promising data in preclinical models (82). Antiplatelet therapies are attractive, in part because of their capacity to reduce lung injury and also to increase pro-resolving lipid mediators (83). Improved methods to decrease ventilator-associated lung injury with prone positioning (84) or new methods

to identify optimal levels of positive end-expiratory pressure may enhance AFC (85) and the resolution of alveolar edema by reducing epithelial injury and allowing more rapid restoration of a normal alveolar epithelium. ■

Author disclosures are available with the text of this occasional essay at www.atsjournals.org.

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