Alveolar Epithelial Ion and Fluid Transport
Recent Progress

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Studies of epithelial ion and fluid transport across the distal pulmonary epithelia have provided important new concepts regarding the resolution of pulmonary edema, specifically the removal of edema from the distal airspaces of the lung. Overall, there is convincing evidence that vectorial ion transport across the alveolar and distal airway epithelia is the primary determinant of alveolar fluid clearance (AFC). The general paradigm is that active Na and Cl transport drives net alveolar fluid clearance, as demonstrated in several different species, including the human lung. The objective of this article is to consider some areas of recent progress in the field of alveolar fluid transport under normal and pathologic conditions. More detailed reviews of this field including studies of the immature and the newborn lung are available (1–10).

In the lung, as in other epithelia, ion transporters and other membrane proteins are asymmetrically distributed on opposing cell surfaces, conferring vectorial transport properties to the polarized epithelial cells (Figure 1). There are epithelial cells in the distal airway epithelia, such as Clara cells, that are capable of vectorial ion transport. The vast majority of the surface area available for transport in the distal lung is occupied by the alveolar epithelial type I (ATI) and type II cells (ATII). Tight junctions populate these epithelial cells near their apical surfaces, thereby sustaining apical and basolateral cell polarity (11). The permeability of tight junctions is dynamic and regulated, in part, by cytoskeletal proteins and intracellular Ca concentrations and possibly by ion channels (11).

Because ATII cells can be isolated from the lung and studied *in vitro,* they have been studied extensively. ATII cells are responsible for surfactant secretion (12) as well as vectorial Na and Cl transport. Na uptake occurs on the apical surface, partly through amiloride-sensitive and amiloride-insensitive channels. Subsequently, Na is pumped actively from the basolateral surface into the lung interstitium by the Na,K-ATPase. An epithelial Na channel (ENaC) participating in Na movement across the apical cell membrane has been cloned and well characterized (13, 14). Recent evidence indicates that the CFTR is expressed in ATII cells and plays a role in cAMP-mediated fluid transport (15–18).

The role of ATI cells for AFC is less known, although several studies have established a possible contribution of ATI cells to vectorial fluid transport (19–22). ATI cells express aquaporin 5 (23), Na,K-ATPase (21), and ENaC (21, 22). The presence of Na,K-ATPase is consistent with a role for ATI cells in AFC, but is not conclusive since Na,K-ATPases are needed to maintain cell volume. However, one study using pharmacologic methods to inhibit the α_2-subunit of the Na,K-ATPase suggested a role for Na,K-ATPase in driving fluid clearance across type I cells *in vivo* (24). Another study reported a role for the α_2-Na,K-ATPase under cAMP-stimulated conditions, suggesting that type I cells may be involved, as the α_2-subunit seems to be expressed only in type I cells (25). Detailed studies of type I cells have been limited to date because of difficulty maintaining them in cell culture although recent work has demonstrated functional ion channels in freshly isolated ATI cells with electrophysiologic evidence for Na channels (ENaC), K channels, and CFTR Cl channels (26). In addition, there is evidence for ENaC expression (21, 22) and a partial amiloride inhibition of ^{22}Na-uptake in freshly isolated rat ATI cells (22). Evidence for β-adrenoceptor (βAR) expression in ATI cells has also been reported (20, 27). In addition, ATI cells may be involved in macromolecular transport due to the presence of vesicles and caveolin (28). The distal airway epithelium also actively transports Na (29–31).

ALVEOLAR FLUID CLEARANCE IN THE INTACT LUNG

A substantial number of innovative experimental methods have been developed to study fluid and protein transport from the distal airspaces of the intact mature lung, including isolated perfused lung preparations, *in situ* lung preparations, surface fluorescence methods, and intact lung preparations in living animals. More than two decades ago, studies in anesthetized, ventilated sheep demonstrated that isosmolar ion and water clearance occurred in the face of a rising airspace protein concentration over several hours (21). Evidence for active Na transport was obtained with the use of ouabain in perfused lung preparations in the rat and the sheep lung as well as in the *ex vivo* human lung (32, 33). The importance of Na uptake by apical ion channels was established in several studies in which investigators used amiloride (an inhibitor of Na uptake), including studies in sheep, rabbits, rats, guinea pigs, mice, and *ex vivo* human lungs (1–6) (Figure 2). Recently, one group of investigators adapted RNA interference (RNAi) methodology to provide more conclusive evidence for the role and importance of transepithelial Na transport in AFC in the intact lung (34). They found that 24 h after plasmid DNA (pDNA) instillation into the rat airspaces, expression of αENaC was specifically attenuated. Although the effects on basal clearance were minimal, the RNAi to αENaC inhibited AFC after βAR activation (Figure 3). In addition, amiloride did not further inhibit AFC. Thus, these results provide more evidence for a vital role for ENaC in βAR-stimulated AFC.

Some differences in basal AFC rates have been reported, where the slowest AFC rate was measured in dogs, intermediate AFC rates in sheep and goats, and the highest AFC rates were measured in guinea pigs, rats, and mice (1–6) (Figure 2). Basal AFC rates in human lungs have been difficult to estimate, but based on the estimates from resolution of alveolar edema in patients recovering from cardiogenic or hydrostatic edema, the rates of clearance seem to be similar to the fast rates reported in

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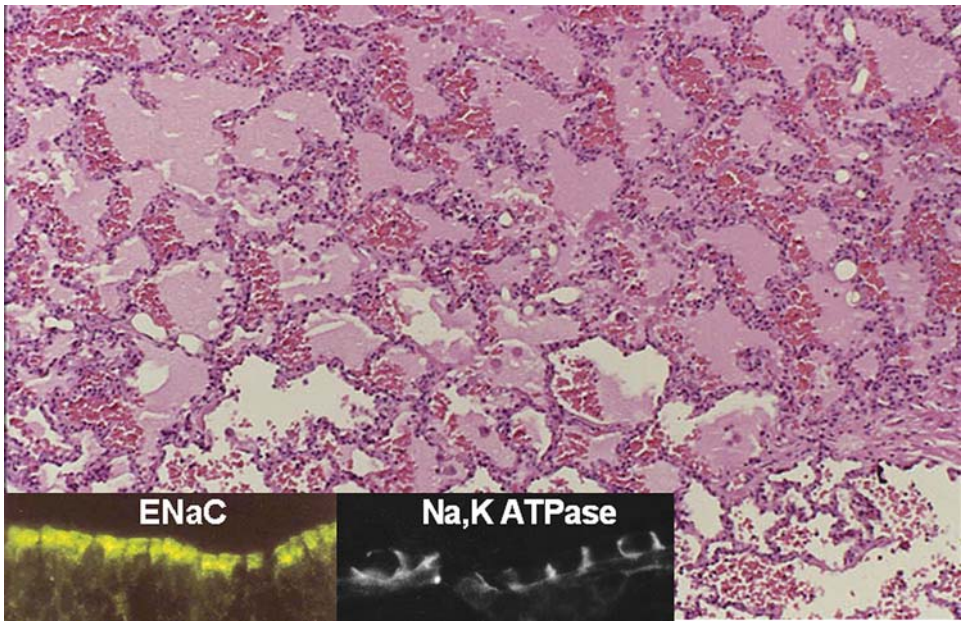

Figure 1. A section of an ARDS lung. Note the fluid-filled alveolar spaces with significant red blood cell infiltration. Insert demonstrates apical ENaC staining and basolateral Na,K-ATPase staining in lung epithelia. (Reprinted with permission from Ref. 3)

rats, mice, and guinea pigs. Unpublished observations in isolated perfused human lungs confirm these higher rates under both basal and cAMP-stimulated conditions (J. Frank and colleagues, CVRI, UCSF, unpublished data). Explanations for these species differences remain unknown, but could be related to number or activity of Na or Cl channels and/or density of Na,K-ATPases in the alveolar epithelium.

EPITHELIAL SODIUM CHANNELS IN THE LUNG

A high amiloride concentration ($\sim$ 1 mM) is required to inhibit AFC by 50–70%, possibly due to protein binding to amiloride, rapid degradation, and/or escape from the airspaces (35, 36). Molecular identification of the proteins involved in amiloride-sensitive Na influx has also been achieved in the last decade. Three homologous subunits, entitled α, β, and γENaC, make up ENaC, sharing a structure predicting two hydrophobic membrane-spanning regions, intracellular amino and carboxy termini, two transmembrane-spanning domains, and a large extracellular loop with highly conserved cysteine residues (37, 38). Important roles have been established for ENaC in distal renal tubules and collecting ducts, in colon, and in the lung (39, 40), and mRNA for all three ENaC subunits, usually with a greater expression of α than β and γ, has been identified (2, 41–43). The role of ENaC for AFC was confirmed by the generation of knockout mice with inactivated subunits of ENaC. After αENaC inactivation, neonatal mice develop respiratory distress and die within 40 h of birth (40). By contrast, β or γENaC knockout mice cleared fluid from the lungs at birth, although at a slower rate than that in wild-type mice. These mice later died from abnormal kidney electrolyte reabsorption (44, 45). Human loss-of-function ENaC mutations are present in systemic pseudohypoaldosteronism (46). These patients had no respiratory symptoms at birth, but later in life developed respiratory illnesses characterized by chest congestion and cough caused by excessive fluid volume resulting from airway Na channel dysfunction. As discussed, another approach to determine the importance of ENaC in adult animals taken recently is that of Li and Folkesson (34). Their method involved generating small interfering RNA (siRNA) against αENaC to silence its expression locally in adult rat lungs (Figure 3). When this was done, the AFC did not respond to further amiloride inhibition.

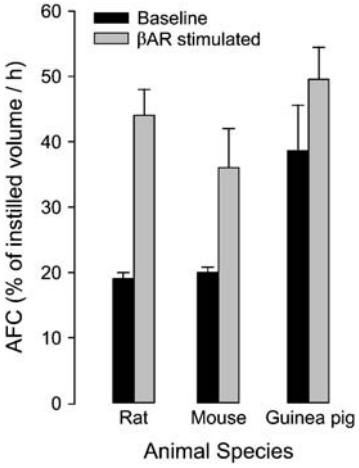

Figure 2. AFC rates in different animal species with and without βAR stimulation of AFC. All data are calculated as 1-h studies. (Data obtained from Refs. 15, 34, 61, and 132.)

ENaC expression/function can be regulated by transcriptional, translational, and post-translational mechanisms (47, 48). It is, however, clear that understanding the regulation of ENaC processing, trafficking to, and stability at the cell surface is of fundamental importance. In fact, membrane-bound extracellular serine proteases, channel-activating proteases (CAPs), may be involved in regulating membrane ENaC expression and affecting AFC in the mouse (49). Those studies demonstrated that intra-alveolar aprotinin treatment abolished the terbutaline-increased Na-driven AFC in the *in situ* mouse model, while trypsin pretreatment potentiated the terbutaline-stimulated Na-driven AFC. Thus, the results indicate that endogenous membrane-bound and/or secreted CAPs can upregulate alveolar Na transport and AFC in the *in vivo* murine lung. Other studies have confirmed and provided supporting evidence for CAP participation in ENaC function. Prostasin (CAP1) has been demonstrated to activate ENaC in *Xenopus* oocyte expression studies (50–52). Other CAPs may carry out similar functions in various organs (52–54). Also, Nedd4-2 has been demonstrated to reorganize

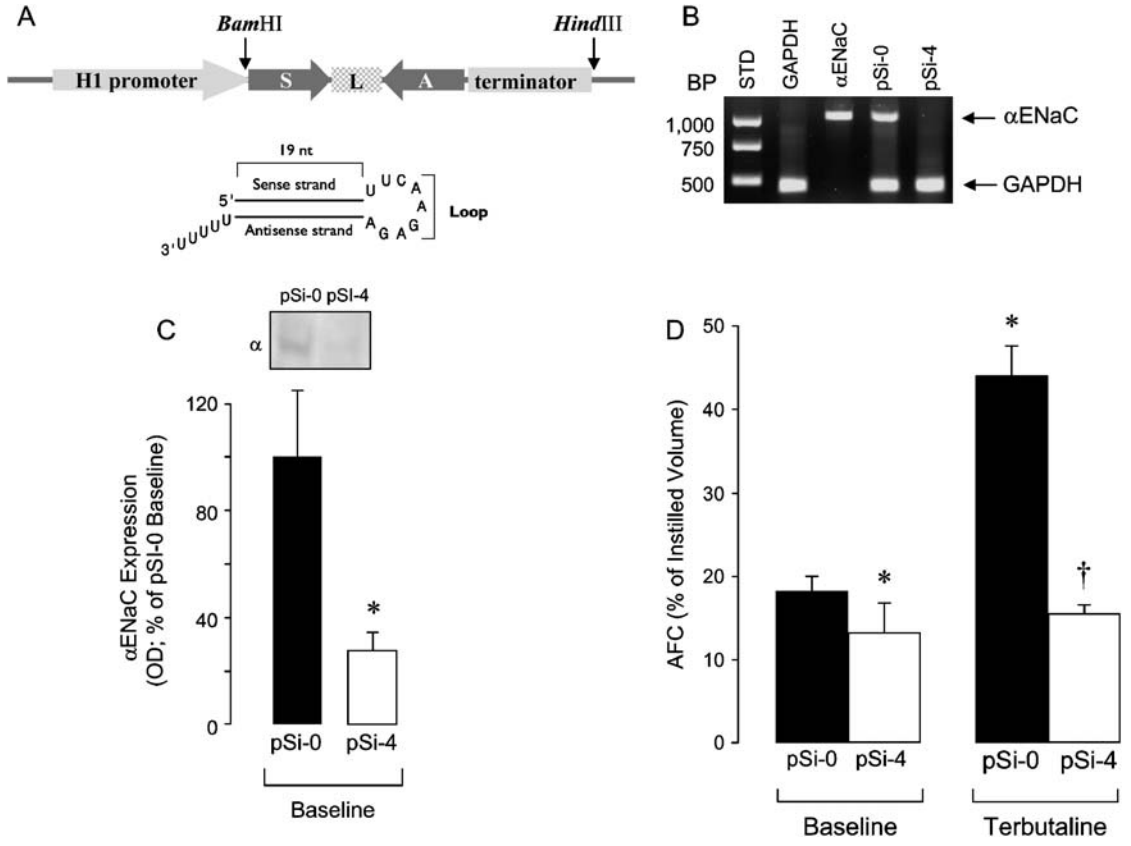

Figure 3. *A* shows a schematic representation of the H1 promoter–driven hairpin-type siRNA-expression vector. The shRNA contains a 19-nucleotide sense strand (S), a 9-nucleotide loop (L), and an antisense strand (A), followed by an RNA polymerase III terminator. *B* shows a representative RT-PCR gel of αENaC mRNA in the lung after pretreatment with the pSi-0 and pSi-4 pDNAs. It is clearly shown that pSi-4 was efficient in silencing αENaC after pDNA pretreatment, as pSi-4 dramatically reduced αENaC mRNA. *C* shows a densitometric analysis of αENaC protein in the lung by Western blot 24 h after pretreatment with pSi-0 and pSi-4 during baseline, unstimulated conditions. pSi-4 pretreatment dramatically reduced αENaC protein expression by ~80%. *D* shows AFC during baseline and after terbutaline stimulation 24 h after pretreatment with pSi-0 and pSi-4. pSi-4 pretreatment attenuated terbutaline-stimulated AFC and inhibited baseline AFC by ~30%. (Data from Ref. 34.)

ENaC transport to the membrane by transferring Nedd4-2–induced ubiquitin-bound ENaCs to the lysosomal pathway for degradation (55, 56), thus reducing membrane ENaC expression. Thus, there seem to exist both positive regulation (i.e., via CAPs) and negative regulation (i.e., Nedd4-2–ubiquitin), using extracellular and intracellular serine proteases.

CATECHOLAMINE-DEPENDENT REGULATION OF ALVEOLAR FLUID CLEARANCE

In most adult mammalian species, β_2AR stimulation increases AFC. The presence of β_1ARs and β_2ARs on alveolar epithelial cells has been demonstrated *in vivo* (20, 57, 58). New evidence indicates that cAMP-stimulated Cl uptake may also be important in regulating AFC (15, 17, 18). AFC stimulation occurs rapidly after intravenous βAR agonist administration or instillation into the alveolar spaces and is prevented by βAR antagonists. The increase in AFC by β_2AR agonists can be prevented by amiloride, indicating that the stimulation is related to an increased transepithelial Na transport. This mechanism was also demonstrated after siRNA pretreatment to silence lung αENaC blocked βAR stimulation of AFC (34) (Figure 3).

Based on studies of the resolution of alveolar edema in humans, it has been difficult to quantify the effect of catecholamines on AFC (59). However, AFC studies in isolated human lungs have demonstrated that βAR agonist therapy increases AFC, and the increased AFC can be inhibited by propranolol or amiloride (33, 60). There is some new evidence that the circulating levels of catecholamines in patients with acute pulmonary edema do not seem to be high enough to stimulate AFC in the *ex vivo* human lung (61). If this is correct, then sustained catecholamine stimulation of alveolar fluid clearance in humans would require exogenous administration of a βAR agonist by aerosol or the parenteral route.

Proposed mechanisms for upregulation of Na transport proteins by cAMP include augmented open probability of ENaC (2, 62, 63), increases in Na,K-ATPase α subunit phosphorylation, and delivery of more ENaC channels to the apical membrane and more Na,K-ATPases to the basolateral cell membrane (64, 65). Although one study indicated that ENaC can be phosphorylated by PKA (66), the functional relevance of phosphorylation is not yet known and PKA does not activate an amiloride-sensitive current in *Xenopus* oocytes injected with ENaC mRNAs (67). However, delivery of PKA catalytic subunits to the lung by protein transfection increased the AFC rate in rats (68). ENaC subunits, like other ion channels and transporters, are associated with cytoskeletal proteins such as actin, ankyrin, fodrin, or spectrin, which can serve as signaling molecules. ENaC reconstitution into lipid bilayers resulted in a channel that was activated by PKA and ATP in the presence of short actin filaments (69). Thus, the increased P_o may have resulted from an increased phosphorylation of short actin filaments or other cytoskeleton proteins. Finally, terbutaline, a β_2AR agonist, may promote insertion of new channel protein from a cytoplasmic pool to the apical membrane (48, 70, 71). βAR stimulation also increases Na extrusion at the basolateral side by increasing Na,K-ATPase activity (72).

Existing evidence also indicates that agents that increase cAMP can increase Na influx through an increase in apical Cl conductance without affecting apical membrane Na conductance

(17, 18). Some cultured ATII cell studies demonstrate that cAMP-mediated apical Na uptake may depend on an initial Cl uptake (17). In cultured ATII cells under apical air interface conditions, βAR agonists acutely activated apical Cl channels with enhanced Na absorption (17).

To define the role of Cl transport across the lung distal pulmonary epithelium, our research group used *in vivo* lung studies. In those experiments, the potential role for CFTR under basal and cAMP-stimulated conditions was tested in mice in which CFTR was not functional due to failure in CFTR trafficking to the cell membrane (ΔF508 mice) (15). The results supported the hypothesis that CFTR is essential for cAMP-mediated upregulation of AFC because AFC could not be increased in the ΔF508 mice with either βAR-agonists or forskolin, unlike in the wild-type control mice (15). Other investigators used additional *in vivo* strategies that demonstrated the link between CFTR expression and the presence of intact β_1 and β_2AR in the distal lung epithelium (73). Further studies using pharmacologic CFTR inhibition in mouse and human lungs with glibenclamide or a novel CFTR inhibitor, $CFTR_{inh}$-172, also supported the conclusion that cAMP-mediated transport requires CFTR (15, 16) (Figure 4A). Very recent studies have demonstrated the CFTR is present in rat alveolar type II cells (74), human alveolar type II cells (16), and in rat and human ATI cells (26). Pharmacologic inhibition of CFTR prevents cAMP-stimulated fluid clearance across human ATII cell monolayers (16). Although absence of CFTR in upper airways results in enhanced Na absorption (75), the data in these studies provide evidence that absence of CFTR prevents cAMP-upregulated AFC, a finding that is similar to work on the importance of CFTR in mediating cAMP-stimulated Na absorption in human sweat ducts (76). *In vivo* mice studies demonstrated that the lack of CFTR can result in a greater accumulation of pulmonary edema fluid during hydrostatic stress, thus demonstrating a potential physiologic importance of CFTR in upregulating AFC (15). Finally, there are some data supporting the participation of the Na,K,2Cl-cotransport in AFC in adult guinea pigs (77) (Figure 4B). The new data on a role for CFTR for cAMP-upregulated AFC raise further interest in determining how CFTR and ENaC interact and contribute to net AFC. The relative conductances for Cl and Na are difficult to measure in the *in vivo* lung epithelium. Under open circuit conditions, net transfer of Cl and Na across the distal lung epithelium must be equal—that is, there cannot be significant net charge accumulation. It is possible that with cAMP stimulation, Cl and Na conductances increase in parallel. This hypothesis would be in accord with data on isolated ATII cells where CFTR protein was immunolocalized and forskolin-stimulated short-circuit currents were inhibited by glibenclamide (78).

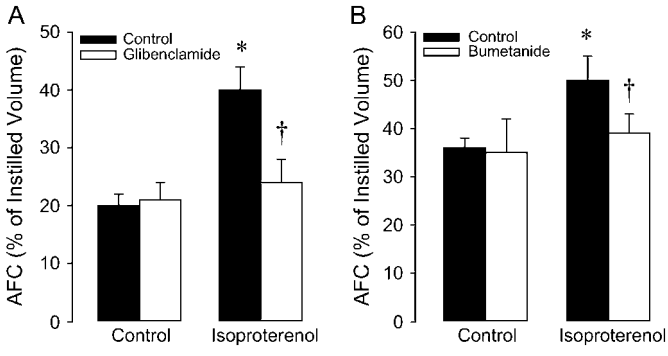

Figure 4. (*A*) Effect of glibenclamide inhibition of CFTR on AFC in mouse lungs with and without βAR stimulation with isoproterenol. AFC extrapolated as 1-h experiments. Glibenclamide only attenuated isoproterenol-stimulated AFC, thus providing evidence of Cl and CFTR participation in βAR stimulated AFC. (*B*) Effect of bumetanide inhibition of the Na,K,2Cl-cotransporter on AFC in guinea pig lungs with and without βAR stimulation with isoproterenol. AFC measured as 1-h experiments. Bumetanide only attenuated isoproterenol-stimulated AFC, thus providing evidence of Cl and the Na,K,2Cl-cotransporter participation in βAR-stimulated AFC. (Data from Refs. 15 and 77.)

ATII cell β_2AR overexpression in transgenic mice may enhance AFC and edema resolution (79, 80). Evidence is also present that β_2AR overexpression was associated with increased ENaC (α subunit) and Na,K-ATPase expression in apical and basolateral cell membrane fractions isolated from peripheral lungs (80, 81). These data provide evidence for a potential development of gene therapy to enhance pulmonary edema resolution.

Some studies suggest that prolonged exposure to exogenous catecholamines may impair the ability of the alveolar epithelium to remove alveolar edema fluid and that this impairment was associated with a reduction in epithelial βAR number and post-receptor intracellular signaling (82). In a recent study, Maron and colleagues (83) demonstrated that isoproterenol-induced βAR downregulation over 48 h of infusion recovered spontaneously at 96 h of infusion, even in the continued presence of the βAR agonist. Thus, it is important to bear in mind that although an impairment may be evident at clinically used doses, the stimulatory effect from βAR agonists may recover spontaneously and thus enable infused βAR agonists as well as instilled βAR agonists to regain their abilities to stimulate AFC above baseline levels.

Several catecholamine-independent mechanisms have been identified that upregulate AFC, including hormonal factors, such as glucocorticoid and thyroid hormones (39, 84–88). Growth factors work by either a transcriptional and/or direct membrane effect, and/or by enhancing the number of ATII cells (89–93). Dopamine has in several investigations been demonstrated as a factor that can stimulate AFC (94–96). There is also evidence that a proinflammatory cytokine, tumor necrosis factor-α, and also leukotriene D4, can upregulate AFC by novel mechanisms (97–99). Finally, as discussed previously, serine proteases can regulate the activity of ENaC and potentially increase AFC (100).

MECHANISMS THAT CAN IMPAIR ALVEOLAR FLUID CLEARANCE

While several factors are capable of upregulating AFC, atrial natriuretic peptide (ANP) can downregulate AFC. ANP plays a pivotal role in volume and electrolyte homeostasis through potent biological effects, including natriuresis, diuresis, and vasorelaxation. Besides being a target organ for ANP from atrial origin, the lung is a site of bioactive ANP synthesis and release (101). The lung has the highest tissue concentration of ANP-binding sites (102) and expresses both guanylate cyclase (GC) receptor subtypes with a functional predominance of GC-A over GC-B receptors, but does not possess clearance receptors (103, 104). The functional role of ANP on AFC is still unclear. ANP increased alveolar epithelial permeability and decreased active Na transport, thus decreasing AFC (105). In sheep with left atrial hypertension there was an increase in plasma ANP levels that may have inhibited normal AFC upregulation in the presence of a rise in endogenous catecholamines (104). ANP decreased amiloride-sensitive ^{22}Na influx (103), but did not change basal Na,K-ATPase activity (103, 104). Thus, on balance, it appears that ANP can impair AFC directly by reducing Na transport.

Hypoxia may occur under a variety of pathologic conditions associated with acute and chronic respiratory disease. Therefore, it is important to understand the effect of hypoxia on AFC. Both *in vitro* and *in vivo* studies clearly show that decreased O_2 tension reduces the capacity of alveolar epithelial cells to actively transport Na. In ATII cells, hypoxia (0% and 3% O_2) inhibits dome formation (106), decreases amiloride-sensitive ^{22}Na influx and Na,K-ATPase activity, and decreases the amiloride-sensitive short-circuit current (106, 107). The mechanisms whereby hypoxia decreases Na transport protein activity depend on the severity and length of hypoxic exposure. For long exposure times ($>$ 12 h), the decrease in amiloride-sensitive Na channel and Na,K-ATPase activity was associated with a parallel decline in mRNA levels of the three subunits, α, β, and γ of ENaC and two subunits α_1 and β_1-Na,K-ATPase, and the rate of αENaC protein synthesis. For short exposure times (3 h exposure), the decrease in ^{22}Na influx and Na,K-ATPase activity preceded detectable changes in mRNA levels, findings that suggest other mechanisms may be involved in regulation, including decreased efficiency in the translation of ENaC mRNA or in apical membrane trafficking of ENaC subunits, abnormal degradation or internalization of the channel protein, or hypoxia-induced modification of intracellular signals (106). Also, one group of investigators demonstrate that hypoxia decreases Na,K-ATPase activity in alveolar epithelial cells by triggering its endocytosis through mitochondrial reactive oxygen species and phosphorylation of the Na,K-ATPase α_1-subunit (108).

The effect of hypoxia under *in vivo* conditions has been studied primarily in rats, where hypoxia was found to decrease AFC by inhibiting the amiloride-sensitive component (109). In contrast to *in vitro* studies, hypoxia increased αENaC and β_1-Na,K-ATPase mRNA transcripts with little no change in protein expression, suggesting that a post-translational mechanism such as a direct change of Na transporter protein activity or protein internalization was involved (109). This latter hypothesis was supported by the normalization of AFC by a cAMP agonist (terbutaline) (109), which is known to increase trafficking of Na transporter proteins from the cytoplasm to the membrane (65, 110).

HYDROSTATIC STRESS AND ALVEOLAR FLUID CLEARANCE

Studies of AFC have been done in intubated, ventilated patients by measuring the concentration of total protein in sequential samples of undiluted pulmonary edema fluid aspirated from the distal airspaces with a standard suction catheter passed through the endotracheal tube into a wedged position in the distal airways (59, 111–113). This method for measuring AFC in patients was adapted from the method for aspirating fluid from the lung distal airspaces in experimental studies in small and large animals (1, 3). The clinical procedure has been validated in patients by demonstrating that there is a relationship between AFC, the improvement in oxygenation, and the chest radiograph (59, 111).

In patients with severe hydrostatic pulmonary edema, predominantly from acute or chronic left ventricular dysfunction, there was net AFC in the majority of the patients during the first 4 h after endotracheal intubation and onset of positive pressure ventilation (111) (Figure 5). AFC in these patients varied between maximal ($>$ 14%/h) in 38% and submaximal (3–14%/h) in 37%; thus, 75% of the patients had intact AFC. There was no correlation between AFC and endogenous epinephrine plasma levels, although twice as many of the patients with intact AFC received aerosolized βAR therapy as those with impaired AFC. The lack of AFC in 25% of the patients was not simply related to elevated pulmonary vascular pressures. Experimental studies may provide some insight into mechanisms that may downregulate AFC in the presence of elevated pulmonary vascular hydrostatic pressures. Because hydrostatic pulmonary edema is associated with an uninjured epithelial barrier, the studies of hydrostatic pulmonary edema provide an important comparison group to the patients with pulmonary edema from acute lung injury, in whom some degree of morphologic or functional epithelial injury probably occurs. Also, there is experimental evidence that accelerated AFC can occur during the resolution of hydrostatic pulmonary edema in patients (114).

The first major study evaluating the effect of acute hydrostatic pulmonary edema on AFC was done in anesthetized, ventilated sheep in which left atrial pressure was elevated acutely to 18–25 cm H_2O (104). The rise in left atrial pressure created lung interstitial edema, with the expected increase in protein-poor lung lymph flow. Alveolar flooding was simulated by instillation of large volumes of isosmolar 5% albumin Ringer's lactate solution into the distal airspaces of both lungs. Remarkably, AFC remained normal over 4 h (104). This finding provided direct evidence that an intact distal lung epithelium could actively remove fluid even though there was interstitial edema and a moderately elevated left atrial pressure. In isolated perfused rat lungs, 15 cm H_2O pulmonary venous pressure reduced AFC by 50%, while smaller elevations had no effect (115). The lower level at which vascular pressure impaired AFC in this rat model compared with the sheep studies may be explained by the lack of functioning lung lymphatics in the isolated perfused lung studies. Thus, as in sheep, there was no evidence of sustained epithelial barrier injury, correlating well with clinical studies of resolution of hydrostatic pulmonary edema (111).

In a sheep study, βAR agonist aerosolization failed to increase AFC over 4 h in the presence of left atrial pressure elevation to 18–24 cm H_2O (114). When left atrial pressure was normalized (6 cm H_2O), the aerosolized βAR agonist markedly increased AFC (104). Studies in rats also demonstrated that a β_2AR-agonist could enhance lung edema resolution and improve

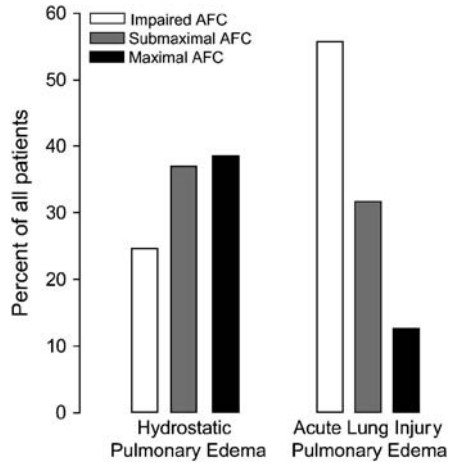

Figure 5. Percentage of patients with three categories of AFC: impaired (*open bars*; $<$ 3%/h), submaximal (*shaded bars*; 3–14%/h), and maximal (*solid bars*; $>$ 14%/h) during hydrostatic pulmonary edema and during acute lung injury–induced pulmonary edema. AFC was measured during the first 4 h after intubation and mechanical ventilation. There was a greater number of patients with submaximal-maximal AFC after hydrostatic pulmonary edema than after acute lung injury–induced pulmonary edema. In acute lung injury–induced pulmonary edema the majority of patients studied displayed an impaired AFC. (Data from Refs. 111 and 113.)

oxygenation in the resolution phase of hydrostatic pulmonary edema (114).

ACUTE LUNG INJURY AND ALVEOLAR FLUID CLEARANCE

The majority of patients with increased permeability edema and acute lung injury demonstrate impaired AFC (Figure 5), a finding associated with a prolonged respiratory failure and a high mortality. In contrast, a minority of patients can remove alveolar edema fluid rapidly, and these patients have a higher survival rate (59, 112, 113). These results indicate that a functional, intact distal lung epithelium is associated with a better prognosis in patients with acute lung injury, thus supporting the hypothesis that the degree of injury to the distal lung epithelium is an important determinant of patient outcome with increased permeability pulmonary edema from acute lung injury.

The effect of acute endotoxemia on enhancing lung vascular permeability has been well described in sheep (116). However, subsequent studies in sheep demonstrated that the alveolar–epithelial barrier was resistant to the injurious effects of endotoxin, whether administered intravenously or in the airspaces. In fact, AFC remained normal even when high doses of endotoxin were administered and lung vascular permeability was increased as measured by an increase in protein-rich lung lymph flow (117).

In sharp contrast to intra-alveolar endotoxin, live bacteria disrupt alveolar epithelial barrier integrity and decrease AFC (117). *Pseudomonas aeruginosa* products (e.g., exoenzyme S and phospholipase C) are important in determining the extent of injury (118). Subsequent studies indicated that bacterial pneumonia may progress to septic shock when the infecting gram-negative organism generates proinflammatory cytokines in the lung airspaces that are released into the circulation as bacterial-mediated injury results in sufficient injury to the distal lung epithelial barrier (119). In another recent 4-h study of severe *E. coli* pneumonia, the acute increase in plasma catecholamines attenuated the increase in lung edema by both increasing alveolar fluid transport and decreasing lung endothelial permeability (120). Although this finding of the protective effect of acute increase in endogenous catecholamines has been demonstrated in other animal models of septic and hypovolemic shock, as well as neurogenic pulmonary edema (1), it seems unlikely that the effect would be sustained in most critically ill patients because use of analgesics and sedatives reduced endogenous catecholamine levels and the actual levels measured in ventilated patients with acute pulmonary edema are below the levels expected to increase the rate of AFC in the human lung (61). Since sepsis produces a procoagulant environment in the lung and hyaline membranes are a feature of acute lung injury, it is important to note that thrombin impairs AFC (121).

There are several inflammatory factors that may upregulate or downregulate alveolar fluid clearance. One recent commentary discussed the evidence for an increase in alveolar fluid clearance stimulated by either tumor necrosis factor-α or leukotriene D4 (122). There is also evidence that interleukin-1β and transforming growth factor-β can depress alveolar fluid clearance (123, 124).

There is also data that influenza virus infection can specifically alter epithelial ion transport by inhibiting the amiloride-sensitive Na current across the mouse tracheal epithelium (125). The inhibitory effect of influenza virus was caused by binding the viral hemagglutinin to a cell surface receptor, which then activated phospholipase C (PLC) and protein kinase C (PKC). It is well known that PKC can reduce ENaC activity so that influenza infections in the lung may thus inhibit ENaC functions (126). In addition, one recent study investigated influenza virus strain A/PR/8/34 on ENaC in rat ATII cells on amiloride-sensitive fluid clearance in rat lungs *in vivo*. The influenza virus rapidly reduced the net volume transport across monolayers and reduced the open probability of single ENaC channels in apical cell-attached patches. Intratracheal administration of the influenza virus produced a rapid inhibition of amiloride-sensitive (i.e., ENaC-dependent) lung fluid transport. These results showed that influenza virus rapidly inhibits ENaC in ATII cells and that this rapid inhibition of ENaC and formation of edema when the virus first attaches to the alveolar epithelium might facilitate subsequent influenza infection and may exacerbate influenza-mediated alveolar flooding that could lead to acute respiratory failure and death (127). In addition, another recent study demonstrated that mycoplasma lung infection decreases AFC and functional ENaC channels via the production of reactive oxygen–nitrogen intermediates (128). Given the importance of Na channels for AFC, these results provide new evidence that may explain in part the accumulation of alveolar edema fluid in patients with viral pneumonia and acute lung injury.

FUTURE DIRECTIONS

Important advances have been made in the understanding of the reabsorption of fluid and solutes by the distal epithelia with characterization of Na transport and water pathways under both physiologic and pathologic conditions. ATI cells, ATII cells, and distal airway epithelial cells, such as Clara cells, are implicated in Na and fluid transport, but their relative contribution in both physiologic and pathologic conditions are not well defined. Innovative approaches, such as studies of lung slices, are needed, along with other improved models to assess the differential contribution of ATI and ATII cells to AFC.

Another important area of research is the characterization of the Na transporters involved in AFC and their regulation. Amiloride-sensitive Na transport is one of the major pathways for Na entry across distal epithelial cells, but several questions remain unsolved. For example, are the molecular and biophysical characteristics of these channels *in vitro* representative of their *in vivo* characteristics, and how are these channels regulated during physiologic and pathologic conditions? The mechanisms regulating ENaC and Na,K-ATPase trafficking between cytoplasm and the membrane need to be evaluated in distal lung epithelia. Increased insertion of transport proteins may be one important mechanism for increasing Na and AFC under pathologic conditions and may potentially contribute to regulating edema fluid clearance. In addition to amiloride-sensitive Na transport, a characterization of ion transporters involved in amiloride-insensitive Na transport needs to be done. Also, pathways for Cl reabsorption under basal and stimulated conditions need to be further determined, with particular attention to the role of CFTR under cAMP-stimulated conditions. Well-designed RNAi experiments may be useful to determine the role and regulation of the various Na and Cl transporters and the regulatory proteins suggested to be involved in AFC.

Recent advances have been made with transgenic mouse models to define the role of Na and water channels for AFC. Knockout of the three subunits of ENaC has clearly established the preponderant role of α compared with β and γENaC in alveolar transepithelial Na absorption. siRNA studies of αENaC knockdown during baseline and terbutaline-stimulated conditions in adult rats have further conferred an important role for αENaC in AFC, but at the same time suggested that a large fraction of baseline AFC is non–ENaC-dependent. Similarly, knockout mice for several aquaporin water channels have revealed that these channels are not essential for lung water

transport. However, genomic disruption of genes expressed during development or in multiple tissue types complicates the phenotypic analysis. A solution to this problem may be provided by conditional knockouts and/or further siRNA studies. Those systems permit control of timing for cell-specific expression of specific proteins, thereby circumventing both embryonic lethality and complex adaptive responses occurring when physiologic observations follow gene knockout events by days or weeks. In those systems, gene expression is regulated temporally and spatially using cell specific promoters, such as SP-C for ATII cells, in combination with a regulatory on-off system or administration of specific siRNAs designed to silence their targets by RNA interference. These approaches may provide major opportunities to advance our understanding of the roles of Na, Cl, and AFC during physiologic and pathologic conditions, such as during reabsorption of edema from the distal airspaces of the lung.

One recent placebo-controlled small clinical trial reported that β_2AR agonist therapy reduces extravascular lung water in patients with acute lung injury (129). These results, coupled with several encouraging preclinical experimental studies (130) indicate that large controlled clinical trials are needed to test the role of intravenous and aerosolized β_2AR agonists in patients with acute lung injury. β_2AR agonists have the capacity to reduce lung edema in acute lung injury by improving lung vascular permeability as well as by enhancing the removal of alveolar edema fluid (131). However, their efficacy can only be established by well-designed placebo-controlled clinical trials.

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