Review

Bench-to-bedside review: The role of the alveolar epithelium in the resolution of pulmonary edema in acute lung injury

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Abstract

Clearance of pulmonary edema fluid is accomplished by active ion transport, predominantly by the alveolar epithelium. Various ion pumps and channels on the surface of the alveolar epithelial cell generate an osmotic gradient across the epithelium, which in turn drives the movement of water out of the airspaces. Here, the mechanisms of alveolar ion and fluid clearance are reviewed. In addition, many factors that regulate the rate of edema clearance, such as catecholamines, steroids, cytokines, and growth factors, are discussed. Finally, we address the changes to the alveolar epithelium and its transport processes during acute lung injury (ALI). Since relevant clinical outcomes correlate with rates of edema clearance in ALI, therapies based on our understanding of the mechanisms and regulation of fluid transport may be developed.

Keywords active ion transport, acute lung injury, alveolar epithelium, lung fluid balance, pulmonary edema

Introduction

In the normal lung, fluid moves from the blood circulation through the capillary endothelium into the lung interstitium and then is cleared by the lymphatics on a continuous basis. Through this drainage mechanism, the alveolar surfaces are kept dry so that gas exchange can occur without a fluid barrier. When the capillary pressure is elevated, as in heart failure, or the permeability of the capillary walls is increased, as in acute lung injury (ALI), the quantity of fluid that leaves the pulmonary microcirculation is increased to a point that overwhelms the clearance capacity of the lymphatics. When this occurs, interstitial edema develops. In states of pure interstitial edema, the tight epithelial barrier protects the alveolar spaces from edema formation [1]. However, eventually alveolar edema will develop if either the amount of interstitial edema overwhelms the epithelial barrier and overflows into the airspaces (as in severe hydrostatic edema) or there is epithelial injury (as in severe ALI). Once alveolar edema develops, removal of fluid is accomplished by active

ion transport, predominantly by the alveolar epithelium. Various ion pumps and channels on the surface of the alveolar epithelial cell generate an osmotic gradient across the epithelium, which in turn drives the movement of water from the alveolar space back into the lung interstitium. Clearance of interstitial edema from the mature lung is accomplished by both lung lymphatics and the blood capillaries.

This article reviews the mechanisms of alveolar fluid clearance (AFC) based on the human, animal, and *in vitro* studies that have elucidated these mechanisms. We also discuss the changes in fluid clearance that occur in the setting of ALI and the various mechanisms that may regulate the rate of fluid clearance in both normal and pathologic states in the mature lung. In the setting of pulmonary edema, the mechanisms of fluid clearance must be upregulated in order to balance the rate of edema formation. There are some endogenous mechanisms by which this may occur, but we

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AFC = alveolar fluid clearance; ALI = acute lung injury; AQP = aquaporin; ENaC = epithelial Na$^+$ channel; HGF = hepatocyte growth factor; IL = interleukin; KGF = keratinocyte growth factor; TGF = transforming growth factor; TNF = tumor necrosis factor.

also discuss the possibility of therapeutic interventions, based on our knowledge of transport mechanisms, to further increase the rate of edema clearance in ALI. We believe that this field of research is quite clinically relevant because the rate of lung edema clearance correlates with important clinical outcomes such as duration of mechanical ventilation and survival.

The initial studies in the mature lung that proved that AFC is achieved by active ion transport in the setting of unfavorable hydrostatic and colloid gradients were done in anesthetized, ventilated sheep. When an iso-osmolar protein solution (such as autologous serum or a 5% albumin solution in Ringer's lactate) was instilled into the lungs, the protein concentration of the edema fluid increased over 4 hours, whereas the protein concentration of the lymphatic fluid decreased [2]. This suggested that there was active transport of the protein-free fraction of the airspace fluid. Subsequently, much of the research into the mechanisms of AFC and the factors that regulate it under pathologic conditions was done utilizing this basic experimental design. Assuming that the epithelial barrier is impermeable to the marker, the amount by which the marker concentration in the edema fluid increases is proportional to the amount of marker-free fluid (water) that has been reabsorbed from the alveolar spaces.

Mechanisms of alveolar fluid transport

The alveolar epithelium consists of type I and type II cells that are connected by tight junctions, which create a polarity to the cells. The ion pumps and channels are distributed on either the apical or basolateral membrane of the cells, so that ions can be transported from the airspaces to the circulation in a directional manner. Although the alveolar type II cell has been thought to be primarily responsible for the vectorial transport of ions and fluid from the airspaces of the lungs [3], there is increasing evidence that the type I cell is also capable of active fluid transport [4]. In addition, distal airway epithelial cells, such as the Clara cell, may also participate in the vectorial transport of salt and water from the distal airspaces [5].

Most of the fluid transport from the airspaces of the lung is driven by a sodium gradient. The alveolar epithelial cells possess several types of sodium channels on their apical surface. These epithelial sodium channels have been categorized as amiloride-sensitive or amiloride-insensitive, depending on whether conduction of sodium through the channel can be inhibited by amiloride, which is known for its pharmacologic use as a potassium-sparing diuretic because of its activity on similar sodium channels in the renal tubules. Amiloride blocks 40–90% of fluid clearance in the lung by inhibiting sodium transport through certain channels (ENaC [epithelial Na^+ channel]), which are therefore known as amiloride-sensitive sodium channels. The amiloride-insensitive fraction of sodium transport is less well understood but probably depends on cyclic nucleotide-gated cation channels.

Thus, fluid clearance from the airspaces in the setting of pulmonary edema depends on sodium transport through channels that are located on the apical surfaces of alveolar epithelial cells. These channels allow for passive movement of sodium from the airspace into the epithelial cell, but this movement depends on a pre-existing sodium concentration gradient between the edema fluid and the cytoplasm. This gradient is created by the continuous extrusion of sodium from the cell to the blood, which is accomplished by Na^+/K^+-ATPase pumps that are located on the basolateral membrane of the alveolar epithelial cell. The Na^+/K^+-ATPase pumps sodium out of the cell and potassium into the cell against their respective concentration gradients. Indeed, this pump is located on the membranes of all cells in the body, and is responsible for the high sodium concentration and low potassium concentration of the extracellular space that are well known to the clinician. The function of the Na^+/K^+-ATPase is understood from experimental studies that utilized ouabain, which fully inhibits the pump. As the Na^+/K^+-ATPase pumps sodium out of the alveolar epithelial cell into the blood, a gradient is created that drives sodium movement through channels on the apical membrane into the cell. Thus, sodium is transported from the alveolar edema through the epithelial cell into the blood.

Once the sodium gradient is established water follows passively, and hence the clearance of alveolar edema is achieved. Water may be transported in part by water channels called aquaporins (AQPs) [6]. However, although osmotically driven water permeability between the airspace and capillary compartments is reduced approximately 10-fold by AQP deletion, loss of the AQP channels does not result in decreased AFC. Therefore, AQP-independent water transport, involving either alternative transcellular water channels or paracellular pathways, plays a major role in AFC [7]. Alveolar type I cells also may play a prominent role in the transport of water from the airspaces [8]. In addition to fluid transport driven by sodium ion transport, chloride transport through the cystic fibrosis transmembrane conductance regulator may play a role in fluid transport under certain conditions [9,10] (Fig. 1) (see the report by Matthay and coworkers [3] for details).

Regulation of alveolar fluid transport

There are several factors that may affect the rate of AFC, including catecholamines, hormones, and growth factors. These mechanisms are broadly categorized as catecholamine-dependent and catecholamine-independent.

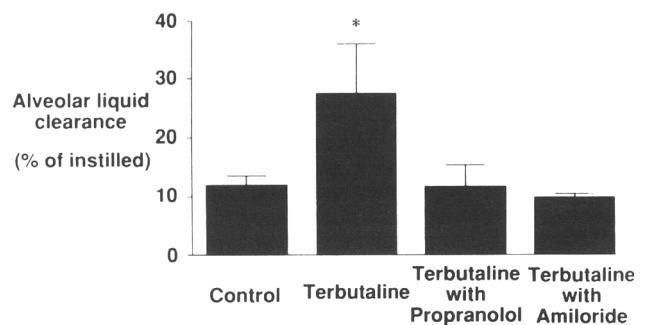### Catecholamine-dependent alveolar fluid transport

It is well established that AFC is stimulated by β-adrenergic agonists. Both β_1- and β_2-adrenergic receptors are present on alveolar epithelial cells [11] and both are likely to mediate adrenergic stimulation of edema clearance [12–14]. Many animal and *in vitro* studies have shown that β-agonists, administered either intravenously or intratracheally, increase

Figure 1
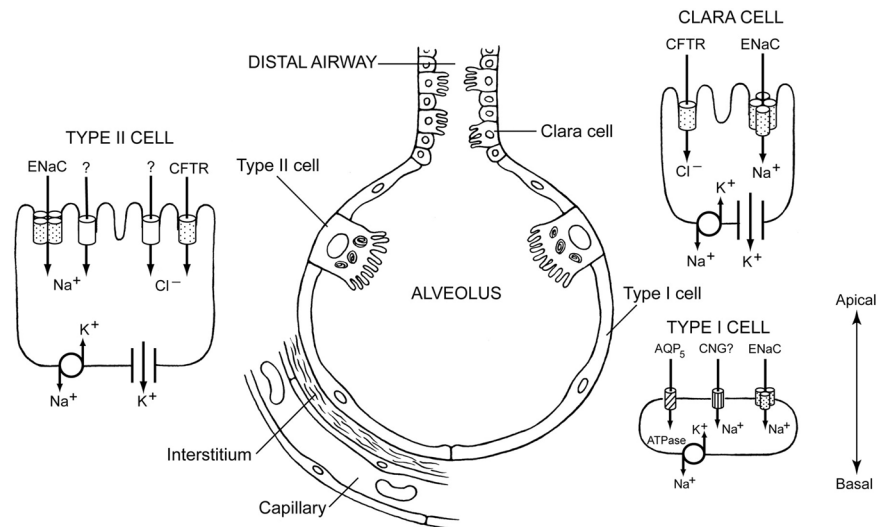
The distal airway epithelium contains alveolar type I and type II cells and Clara cells, which possess various pumps and channels that achieve clearance of edema fluid. Sodium is transported through channels on the apical membrane and extruded from the cell by the Na^+/K^+-ATPase located on the basolateral membrane. This transport generates a sodium gradient that drives the transport of water, which is accomplished in part through water channels. AQP, aquaporin; CFTR, cystic fibrosis transmembrane conductance regulator; CNG, cyclic nucleotide-gated; ENaC, epithelial Na^+ channel. From Matthay and coworkers [3], with permission from the American Physiological Society.

the rate of fluid clearance [15,16] – an effect that can be prevented by the administration of β-blockers [12,17]. As is true for many of the effects of β-agonists in the body, the stimulation of AFC is dependent on a cAMP intracellular signaling mechanism, which in turn activates various ion transporters [18]. The stimulatory effect of catecholamines on alveolar fluid transport can be prevented by administration of amiloride, suggesting that the mechanism by which catecholamines upregulate fluid transport depends on the transport of sodium through epithelial sodium channels (Fig. 2).

The underlying mechanisms by which catecholamines increase sodium transport may include increased synthesis of sodium channels [19] and recruitment of sodium channels from intracellular pools to the cell membrane [20], as well as increased open probability of the channels [21]. There is also evidence that β-agonists stimulate AFC via upregulation of the Na^+/K^+-ATPase via increased synthesis of the pump [19] and movement of pre-existing pumps from intracellular pools to the cell membrane [20]. Finally, recent evidence suggests that cAMP stimulated sodium transport may be indirectly achieved by chloride transport through the cystic fibrosis transmembrane conductance regulator channel [9,22]. Although β-agonists stimulate AFC in the setting of pulmonary edema, it is interesting that under normal conditions catecholamines do not regulate ion and fluid transport. Neither adrenalectomy nor β-blockers affect the baseline rate of edema clearance [15,17].

Figure 2

Catecholamines stimulate alveolar fluid clearance – an effect that can be inhibited by β-blockers or amiloride. This suggests that the mechanism by which catecholamines upregulate fluid transport is mediated by β-adrenergic receptors and depends on the transport of sodium through epithelial sodium channels. From Sakuma and coworkers [17], with permission from the American Thoracic Society.

Catecholamine-independent alveolar fluid transport

Glucocorticoids also upregulate AFC through several mechanisms. They have been shown to increase synthesis of ENaC and the Na^+/K^+-ATPase [23]. In addition, glucocorticoids enhance sodium transport by altering channel activity at the post-transcriptional level [24]. Aldosterone also increases fluid clearance by upregulating synthesis of the Na^+/K^+-ATPase subunits [25] and increasing the expression

of some apical sodium channels [26]. Other hormones, such as thyroid hormone, may also stimulate increased AFC in certain settings [27,28]. Finally, there is some evidence that insulin may increase alveolar sodium transport [29], and this may be achieved by an increased open probability of apical sodium channels [30].

Several growth factors can upregulate AFC. Epidermal growth factor increases active sodium transport and fluid clearance via an increase in Na^+/K^+-ATPase activity [31,32]. Transforming growth factor (TGF)-α increases AFC in a dose-dependent manner that is partially independent of cAMP and may be mediated by an intracellular tyrosine kinase [33]. Finally, keratinocyte growth factor (KGF) stimulates proliferation of alveolar epithelial cells and therefore increases fluid transport [34,35]. There is also some evidence that KGF directly increases expression of Na^+/K^+-ATPase [36]. Hepatocyte growth factor (HGF) is also known to be a potent mitogen for type II alveolar cells and probably has similar effects on AFC [37].

There is also evidence that certain factors may have a negative effect on AFC, including atrial natriuretic peptide [38], halogenated anesthetics [39], and hypoxia [40]. Nitric oxide has also been shown to inhibit AFC by downregulation of both ENaC and the Na^+/K^+-ATPase via a cGMP dependent mechanism [41].

In summary, clearance of pulmonary edema from the airspaces depends on active ion transport, which leads to an osmotic gradient that drives the movement of fluid from the alveolar space back into the interstitium and eventually to the blood circulation. There are several factors that influence the rate at which the transporters that drive this process function.

## Alveolar fluid transport in the presence of acute lung injury

In ALI, an inflammatory process damages the lung endothelium, resulting in high permeability of the lung capillaries to fluid, which leads to clinical pulmonary edema. In contrast to the endothelium, the alveolar epithelium is often spared in ALI, and therefore active ion and fluid clearance can be preserved [42]. Therefore, investigations are ongoing into whether the mechanisms known to stimulate or inhibit AFC in the normal lung are effective in ALI and might be endogenously or exogenously upregulated in ALI. Presumably, if we could understand what factors can effectively regulate AFC in ALI, either endogenously or exogenously, then effective therapies could be designed to increase AFC in ALI.

Remarkably, despite the increased permeability of the alveolar barrier in ALI, there is abundant evidence that the rate of AFC in ALI can be preserved or perhaps even increased. In one rat model of severe septic shock, there was a 100% increase in the rate of AFC [43], and a similar increase of 76% has been shown in an ischemia/reperfusion

model of ALI [44]. The increase in AFC in ALI may be due to increased synthesis and/or activity of Na^+/K^+-ATPase [45,46]. There is also evidence that synthesis and open probability of ENaC increase during ALI [21]. Finally, hyperplasia of the alveolar epithelium may contribute to increased AFC in ALI [47]. Many of the factors discussed above that are known to stimulate AFC in healthy lungs have been shown to be active in ALI. The upregulation of AFC in the setting of ALI may be thought of as an adaptive mechanism and may be triggered by endogenous secretion of catecholamines, glucocorticoids, and cytokines (see below).

However, there is also evidence that AFC can be decreased in ALI. For example, one model of hyperoxic lung injury demonstrated a 44% decrease in AFC [48]. In fact, the majority of patients with ALI have impaired AFC [49]. Na^+/K^+-ATPase activity appears to be decreased in experimental ALI in certain circumstances [50]. Decreased synthesis of ENaC in lung injury may also contribute [47]. Recent work has begun to identify several potential mechanisms that impair AFC in states of lung injury, including hypoxia, reactive oxygen and nitrogen species, ventilator-associated lung injury, and atelectasis. For example, hypoxia downregulates the synthesis and activity of both ENaC and the Na^+/K^+-ATPase [40,51,52]. Interestingly, this effect is completely reversed after reoxygenation [53]. In addition, reactive oxygen and nitrogen species associated with the inflammatory processes of ALI may damage the sodium transport machinery in the epithelial cells, leading to decreased edema clearance [54,55]. In ALI, ventilator-associated lung injury also contributes to the decrease in AFC, probably via decreased Na^+/K^+-ATPase activity [56,57]. There is also evidence that lung collapse might decrease AFC in the setting of ALI, via reactive oxygen and nitrogen species. This effect is reversed by lung inflation [58]. There is some recent evidence that endotoxin leads to decreased expression of the proteins that comprise the tight junctions between epithelial cells and the formation of alveolar edema [59]. The loss of epithelial tight junctions might lead to decreased AFC due to the loss of polarity of epithelial cells. In addition, the loss of epithelial tight junctions may lead to increased edema formation via increased paracellular permeability. More work is needed to elucidate better the effect of endotoxin on epithelial tight junctions, because other studies have demonstrated a lack of effect of endotoxin on the integrity of the epithelial barrier [42].

The conflicting findings of preserved versus impaired AFC in ALI may be partly explained by the theory that, in mild ALI, injury to the endothelium occurs with sparing of the epithelium and its transport functions, whereas severe ALI results in a damaged epithelium and decreased AFC (Fig. 3) [42,60]. In lung transplant patients with reperfusion injury, a greater degree of histologic injury correlated with decreased rates of fluid clearance [61]. Some studies have also

Figure 3
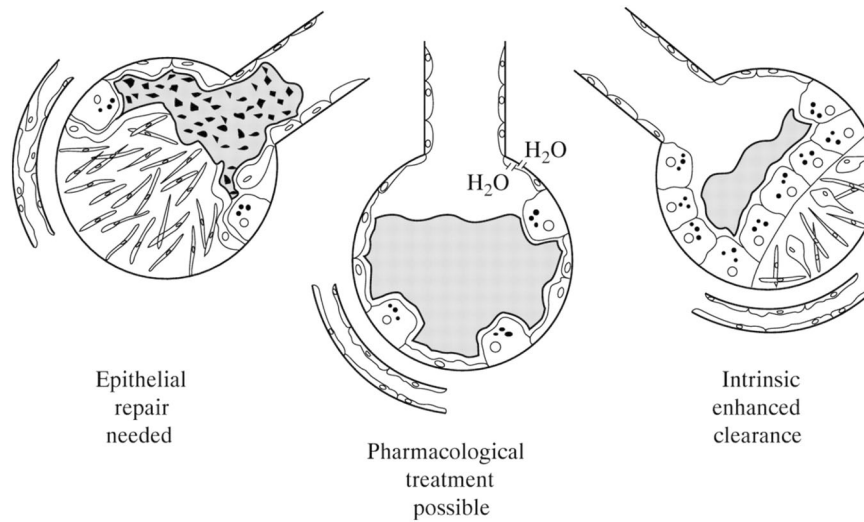
In severe acute lung injury (ALI) the alveolar epithelium is damaged to such an extent that epithelial repair is needed before fluid clearance can be achieved. In contrast, in mild ALI the epithelium and its transport functions are spared, and so pharmacologic stimulation of fluid clearance is possible. If epithelial cell proliferation occurs after injury, either endogenously or due to the administration of mitogens such as keratinocyte growth factor, then fluid clearance may be enhanced. From Berthiaume and coworkers [93], with permission from *Thorax*.

suggested that AFC is reduced immediately after injury, but then is stimulated to levels above baseline during the recovery phase of ALI [62].

Regulation of alveolar fluid transport in acute lung injury

β-Agonists

In a model of septic shock in rats, endogenous plasma adrenaline (epinephrine) levels are 100 times higher than normal, and this increase is associated with a 100% increase in AFC – an effect that is prevented with β-blockers. Because amiloride reverses this effect, endogenous stimulation of AFC by catecholamines in ALI is mediated by an increase in sodium transport [43]. This finding has been confirmed in a rat model of hemorrhagic shock [63].

However, in an endotoxin model of ALI, the increased rates of AFC did not seem to be mediated by β-agonists [64]. Furthermore, in patients with ALI, rates of AFC do not appear to correlate with endogenous catecholamine levels [49]. In fact, the increased levels of catecholamines observed in most patients with ALI are probably not high enough to stimulate AFC. In some models of inflammation, reactive nitrogen species may impair the stimulation of AFC by catecholamines [55].

In addition to the effect of endogenous catecholamines on AFC in ALI via increased plasma catecholamines, exogenous β-agonists have similarly been shown to increase AFC in experimental models of ALI by upregulating active sodium transport [65,66]. Even in some models of ALI in which AFC

is decreased by the lung injury, β-agonists are still able to stimulate increased rates of edema clearance [48]. In patients with a predisposition to high altitude pulmonary edema, the pathogenesis of which may involve both hydrostatic and increased permeability, edema formation is reduced by prophylactic β-agonist inhalers [67].

Again, the conflicting data regarding the ability of fluid transport to be stimulated by β-agonists in the injured lung may be reconciled by the hypothesis that severe insults may result in such extensive injury to the alveolar epithelium that the ability to upregulate the machinery for ion and fluid transport in response to β-agonists is destroyed [68]. Nonetheless, at least in mild-to-moderate ALI the evidence suggests that the therapeutic use of β-agonists in ALI is promising. In fact, it has been shown that conventional nebulized administration of β-agonists to ventilated patients can achieve the concentrations in edema fluid that have been shown experimentally to stimulate AFC [69]. These data suggest that the therapeutic use of β-agonists to stimulate AFC in patients with ALI may be accomplished without the toxicities associated with systemic administration of β-agonists.

Glucocorticoids

The increased rates of AFC seen in many models and clinical studies of ALI may be in part due to increased levels of endogenous glucocorticoids. Although, as discussed above, glucocorticoids stimulate AFC in many animal models, clinical studies have demonstrated that pharmacologic gluco-corticoids do not prevent the development of ARDS in

patients with septic shock [70]. Furthermore, it has been feared that clinical use of glucocorticoids in ALI, especially when due to sepsis, may result in decreased immune function and poor outcomes. However, glucocorticoids have recently been shown to confer a survival benefit in early septic shock, perhaps because of the frequency of relative adrenal insufficiency in sepsis [71]. Because it has been established that glucocorticoids can safely be given in severe septic shock, clinical studies of the rates of AFC in ALI patients treated with glucocorticoids should be considered.

Cytokines

IL-8 has been shown to mediate injury to both the endothelium and epithelium in models of acid-induced ALI, leading to high permeability edema formation and decreased AFC. In various animal models of ALI, pretreatment with anti-IL-8 antibodies successfully restored the rate of AFC to normal [72,73], probably because IL-8 attenuates injury to the epithelium. Surprisingly, tumor necrosis factor (TNF)-α, which is well known for its proinflammatory properties, has a stimulatory effect on AFC in ALI. In one rat model of pneumonia, the rate of AFC was increased by 43–48% over baseline, and this increase was reversible not with β-blockers, but with anti-TNF-α antibody [74]. The same stimulatory effect of TNF-α on AFC has been demonstrated in an ischemia/reperfusion model of ALI [44]. TNF-α might have a direct stimulatory effect on ENaC [75]. Finally, leukotrienes increase AFC by recruitment of Na^+/K^+-ATPase from the intracellular compartment to the basolateral cell membrane [76].

There is some evidence that, if eventually found to be therapeutic for ALI, cytokines might be administered through an aerosolized route [77], which could theoretically achieve the desired benefit for AFC without the systemic toxicity associated with cytokines.

Growth factors

KGF has been shown to prevent lung injury and decrease mortality in rat models of ALI, suggesting a possible therapeutic use. In rat models of bleomycin-induced and radiation-induced lung injury, pretreatment with KGF decreased both histologic evidence of lung injury and mortality [78,79]. In an experimental model of pseudomonas pneumonia, pretreatment with KGF was shown to increase AFC, as well as decrease translocation of bacteria [80]. However, KGF is not effective in restoring the injured epithelium if it is administered after the injury [81]. In a study of patients with ALI, very high concentrations of HGF were found in the edema fluid; interestingly, HGF levels were inversely correlated with survival [82]. A similar study revealed high levels of TGF-α in the edema fluid of patients with ALI [83]. If TGF-α has the same stimulatory effect on AFC in patients as has been demonstrated experimentally, then this could be another possible therapeutic approach to ALI. The use of growth factors in the treatment of ALI may be critical, because no amount of stimulation of the transport

machinery will be effective in clearing edema fluid in severe lung injury unless the integrity of the epithelium is restored. Importantly, macromolecules the size of cytokines and growth factors may actually be deliverable to patients with ALI via an inhaled route [84].

Ventilatory strategy

Low tidal volume mechanical ventilation is well known to improve survival [85] but has also been shown to improve the rate of AFC by protecting the epithelium and endothelium [86]. Low tidal volume ventilation decreases the leakage of surfactant proteins from the alveolar space into the blood, providing further evidence that epithelial injury is mitigated [87]. There is some evidence that a high tidal volume ventilation strategy decreases AFC by downregulating Na^+/K^+-ATPase – an effect that is avoided with low tidal volume ventilation [56].

Gene therapy

In rats with ALI due to hyperoxia, gene therapy with the cDNA for the subunits of the Na^+/K^+-ATPase yielded a greater than 300% increase in the rates of AFC and improved survival [88]. Gene therapy with β-receptor DNA has also been shown to increase β-stimulated sodium and fluid transport *in vitro* [89]. There is evidence that selective delivery of the gene therapy to the alveolar space by an intranasal route can effectively achieve gene transfer and increase enzymatic activity *in vivo* [90].

Stem cell repair

Finally, although little research into the possibility has yet been conducted, we propose that stem cell transplantation may be a productive area of future research in the therapeutic possibilities for ALI. The pathologic insult in severe ALI involves destruction of the alveolar epithelium to such an extent that stimulation of the fluid transport machinery is futile. Because epithelial mitogens such as KGF and HGF are known to improve fluid clearance in ALI, perhaps regeneration of the epithelium through stem cell transplantation would be a possible treatment in the future (Fig. 3). In a mouse model of radiation pneumonitis, stem cells from a bone marrow transplant engrafted in the lung and adapted the functional role of alveolar type I and type II pneumocytes [91]. It is also possible that progenitor cells that persist within the adult lung might be stimulated to differentiate into specialized epithelial cells and repair an injured lung.

Conclusion

Ideally, our understanding of the mechanisms of AFC in normal states and ALI will lead to better treatment modalities for ALI, a diagnosis that carries significant morbidity and mortality. More specifically, it has been postulated that some of the factors that are known to stimulate AFC should be implemented therapeutically in ALI to accelerate resolution of edema and therefore improve mortality. We know that clinical improvement of patients with pulmonary edema, as estimated

Figure 4
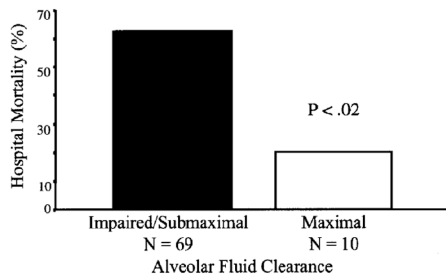
In patients with acute lung injury, the rate of edema clearance correlates with important clinical outcomes such as survival. From Ware and Matthay [49], with permission from the American Thoracic Society.

by the alveolar–arterial oxygen gradient, radiographic findings, duration of mechanical ventilation, and survival, correlates with the rate of active ion and fluid transport from the lungs (Fig. 4) [49,92]. Therefore, therapeutic endeavors to increase the rates of edema clearance in ALI may have a significant impact clinically.

More research is needed, both on the basic science and clinical levels, before these interventions could be implemented in patients. Challenges to converting scientific evidence to clinical practice will include toxicities of treatment, both anticipated and unanticipated, and the route of delivery of treatment. In addition, most of the research discussed here has been done by administering the modulator of AFC before the lung injury, raising the question of whether such treatments would be effective in patients who have already sustained lung injury. Nevertheless, investigations into these and other issues are ongoing, and we hope that if the field continues to progress at the current rate then stimulation of edema clearance will become a major therapeutic goal in ALI in the near future.

Competing interests

The author(s) declare that they have no competing interests.

Acknowledgments

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