The epithelium in acute lung injury/acute respiratory distress syndrome

Michael A. Gropper[a,b] and Jeanine Wiener-Kronish[a]

[a]Department of Anesthesia and Perioperative Care and [b]Critical Care Medicine, University of California San Francisco, San Francisco, California, USA

Correspondence to Michael A. Gropper, MD, PhD, Professor and Vice Chair, Department of Anesthesia and Perioperative Care, Director, Critical Care Medicine, Professor of Physiology, University of California San Francisco, 505 Parnassus Avenue, M917, San Francisco, CA 94143, USA
Tel: +1 415 353 1212; fax: +1 415 353 1990;
e-mail: gropperm@ucsf.edu

Current Opinion in Critical Care 2008, 14:11–15

Purpose of review

The mechanisms of epithelial injury in acute lung injury/acute respiratory distress syndrome have been of interest since the syndrome was first described. Cell therapies to replace injured epithelium are a futuristic dream; however, there is ongoing research to achieve this goal. We review research regarding the function of the epithelium in acute lung injury/acute respiratory distress syndrome, including potential novel therapies.

Relevant findings

Altered fluid clearance from the injured lungs in acute lung injury/acute respiratory distress syndrome patients has been consistently found and is an important prognostic finding. New research suggests that neutrophils that enter the lung late and which are enticed into the lung through a specific chemokine system may be important for causing lung injury. If this is the case, then blockers of this system could be a possible therapy. The role of fibrinolysis and coagulation abnormalities in lung injury due to infection and other perturbations is examined. These abnormal findings may be useful diagnostic tools for prognostication as well as targets for future therapies.

Summary

Epithelial damage is a hallmark of acute lung injury/acute respiratory distress syndrome. An increased understanding of the function of these cells and of the abnormalities that occur when these lung cells are injured should allow the development of novel therapies and, perhaps, lead to replacement therapies.

Keywords

acute lung injury, acute respiratory distress syndrome, barrier function, coagulopathy, fibrinolysis, lung fluid clearance, pulmonary edema

Curr Opin Crit Care 14:11–15

1070-5295

Introduction

The lung epithelium is the central player in both the pathogenesis and resolution of acute respiratory distress syndrome (ARDS). In addition to its barrier function, the alveolar epithelium regulates the water and solute content of the fluid lining the epithelium at the air–liquid interface. Regulation of this fluid layer constitutes an essential component of both homeostatic gas exchange and host defense against infection. As the epithelium is exposed to the external environment, it is a target for injury and infection, but this exposure also makes it a natural target for specific therapies using inhaled drug delivery. The review will examine the critical function of the alveolar epithelium in acute lung injury (ALI/ARDS) and novel therapies targeting these cells.

Function of alveolar epithelium

The alveolar surface comprises more than 99% of the internal surface area of the lungs. These cells must form a robust barrier against infection, but are susceptible to injury from toxic (e.g. aspiration pneumonitis) or inflammatory processes (infection). While protective, the barrier must facilitate rapid exchange of oxygen and carbon dioxide, so the diffusion distance must be minimized. The alveolar epithelium consists primarily of two cell types. Type I alveolar epithelial cells serve primarily a barrier function, but are metabolically active, participating in host defense, alveolar remodeling and antioxidant functions [1]. Type II epithelial cells are smaller and are characterized by their surfactant-containing secretory granules called lamellar bodies. In addition to surfactant production, these cells serve essential immune and progenitor functions [2].

Edema fluid clearance in acute lung injury/acute respiratory distress syndrome

There is a dynamic balance between fluid formation and clearance across the alveolar epithelium with alveolar liquid volume regulated by a balance between physical

and biochemical forces. Ionic movement is dominated by Na^+ and Cl^-, each moving through specific apical channels. Sodium moves selectively through the epithelial sodium channel (ENaC) and chloride through the channel termed the cystic fibrosis transmembrane regulator (CFTR), with adenosine playing a central role by stimulating CFTR-mediated chloride transport [3••]. Edema fluid clearance from the lung occurs when sodium undergoes vectorial transport from the alveoli into the underlying interstitium. This transport is thought to be primarily a function of alveolar type II cells. Transport occurs when sodium enters the cell through the amiloride-sensitive Na^+ channel (ENaC) and is pumped out of the cell by Na^+/K^+-ATPases located on the basolateral surface of the epithelial cells [4]. This enzymatic process of sodium transport is manipulatable; the Na^+ channel inhibitor amiloride will block fluid clearance, whereas the ATPase can be stimulated by β-agonists, increasing sodium transport and therefore trans-epithelial fluid movement [5]. Chloride is secreted to preserve electrical neutrality and likely moves through the CFTR; however, this is not well characterized. Water follows the osmotic gradient generated by Na^+ transport. The specific pathway for water movement across the epithelium is controversial: water may travel through specialized water transporting proteins called aquaporins [6]; however, in transgenic mice where aquaporins have been deleted, alveolar liquid clearance is unaffected (Fig. 1) [7,8].

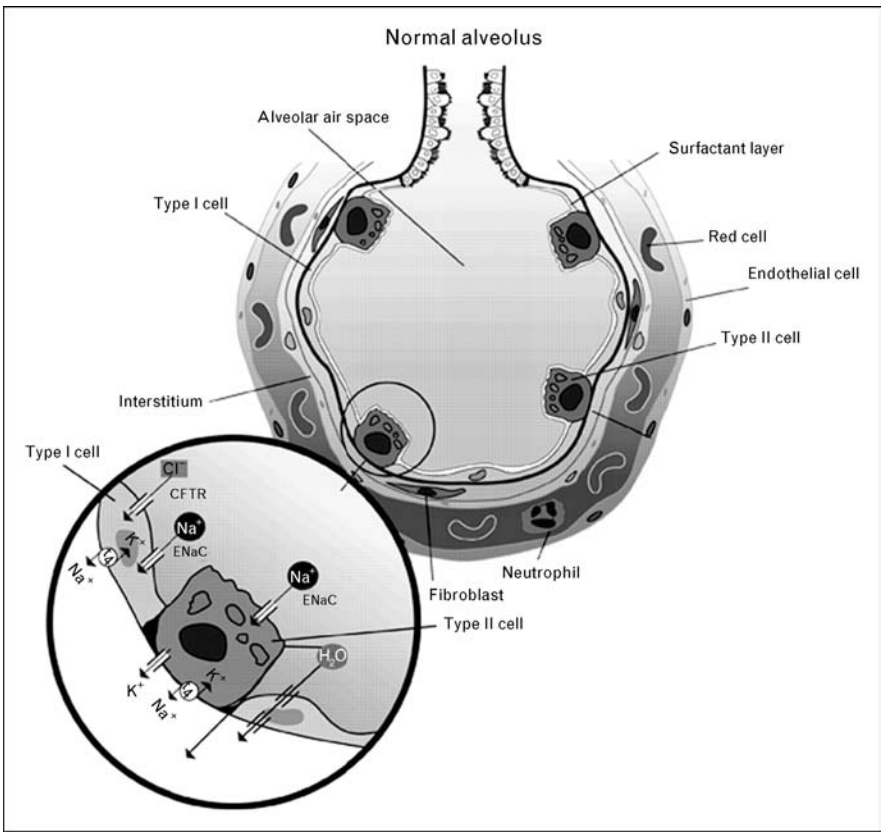
Most patients with ALI/ARDS are thought to have altered fluid clearance from the alveolar space. To assess the efficacy of edema fluid clearance from the lung, serial measurements of edema fluid protein concentration must be made. Using this technique, it has been shown that patients with impaired fluid clearance have increased mortality compared to those with normal fluid clearance [9,10]. In the most recent study, 56% of patients with lung injury had decreased alveolar liquid clearance, with only 13% having maximal liquid clearance. In the patients with hydrostatic pulmonary edema, 75% had submaximal to maximal liquid clearance. It is interesting to note that survival of patients with ALI that had maximal liquid clearance was higher compared to those patients with lower rates. These patients also had fewer days of mechanical ventilation [10].

Strategies to increase alveolar fluid clearance

If increased edema fluid clearance is associated with improved outcomes, is it possible to pharmacologically manipulate fluid clearance and therefore improve outcomes? The most commonly studied agents to improve

Figure 1 Mechanisms of alveolar fluid balance

The alveolar type II cell regulates fluid balance across the epithelium. Water follows sodium, which is transported out of the cells via Na^+/K^+-ATPases located on the basolateral membrane. Sodium enters the apical surface of the type II cell through the ENaC, and the type I cell via the ENaC and CFTR. CFTR, cystic fibrosis transmembrane regulator; ENaC, epithelial sodium channel. From [8].



fluid clearance are β-adrenergic agonists. These agents improve liquid clearance in a number of animal models of lung injury, including ventilator-induced lung injury [5] and smoke inhalation lung injury [11]. One small clinical trial has shown promise: Perkins *et al.* [12] randomized 40 patients with ALI/ARDS to either placebo or intravenous salbutamol (a selective β_2-agonist). They found decreased lung water, lower plateau pressures and a trend towards a lower lung injury score in the salbutamol group. The treatment group did have a higher incidence of supraventricular arrhythmias. Based on these promising results, the National Heart, Lung and Blood Institute ARDS Network is planning a large, multicenter, prospective trial to test the efficacy of aerosolized albuterol in mechanically ventilated patients with ALI/ARDS (clinicaltrials.gov, study # NCT00434993).

Sepsis, coagulation and lung epithelia

The interactions between epithelial cells and neutrophils, both potent sources of cytokines, play key roles in both lung injury and repair.

Neutrophils and acute lung injury

The role of neutrophils in the development and perpetuation of ALI has recently been re-explored. A rapid, early influx of mature circulating neutrophils to the injured lung is followed by a slower, sustained recruitment of neutrophils from the bone marrow [13••]. The progressive damage to the lung that characterizes ALI is attributable to this second, persistent phase of neutrophilia; indeed, the duration and severity of the neutrophilia are predictors of mortality [13••,14,15]. The recent work by Petty *et al.* [13••] documents a role for the CXCR4/stromal cell-derived factor 1 (SDF-1) chemokine in causing the persistent, sustained recruitment of neutrophils from the bone marrow to the lung in ALI caused by endotoxin. The authors also document that the source for the SDF-1 is primarily the injured lung epithelium with much smaller contributions coming from pulmonary leukocytes. This is in contrast to the first phase of cytokine release, which appears to originate from alveolar macrophages. Furthermore, the authors [13••] present data suggesting that although the SDF-1 found in the airspaces may recruit neutrophils to the injured lung, it does not appear to affect the neutrophils in the marrow. The CXCR4/SDF-1 chemokines have also been implicated in lung repair after injury [16].

Lung coagulopathy in acute lung injury/acute respiratory distress syndrome

Intra-alveolar fibrin deposition and pulmonary vascular thrombi have been noted since the beginning of pathologic descriptions of ARDS [17–19]. Research has shown that tissue factor is constitutively expressed on normal alveolar macrophages and on alveolar epithelium [20]. Fibrin deposition is not found in the lung normally because other components of the coagulation cascade, including activated protein C, antithrombin and tissue factor pathway inhibitor, prevent its formation. With lung inflammation or infection, however, tissue factor expression is induced on macrophages and on the alveolar epithelium, and lung endothelial leakage allows transudation of plasma proteins across the alveolar capillary barrier and leads to activation of Factor X to Factor Xa, which further leads to downstream products of activated coagulation [21]. Previous studies had shown that competitive blockade of Factor VIIa binding to tissue factor decreased ALI and other organ injury in baboons septic due to *Escherichia coli* [22,23]. Tissue factor–Factor VIIa inhibition also affects Factor X activation, however, so it was not clear which pathways of coagulation–inflammation crosstalk were critical for ALI. Therefore, blockade of Factor X binding to established tissue factor–Factor VIIa complexes, using a chimerized antihuman tissue factor monoclonal antibody (Sunol-cH36) that specifically blocks the Factor X-binding site on tissue factor, was utilized in a recent set of experiments. Infusion of the anti-tissue factor antibody led to improved gas exchange, decreased pulmonary hypertension, decreased interleukin-8 and -6 levels, fibrinogen depletion, and decreased thrombin–antithrombin complex formation, as well as decreased kidney injury in septic baboons [21]. These exciting data suggest that blockade of the tissue factor–Factor VIIa–Factor Xa complex may decrease lung injury as well as other organ failure, and this appears to be clinically relevant given the studies of intra-alveolar coagulation changes that occur in patients with ALI and acute lung infections.

Bronchoalveolar lavage (BAL) studies in patients with severe pneumonia documented similar changes in local fibrin turnover as had been described with ARDS patients [24•]. Similar, but less severe, changes have been found in patients with less severe lung infections that did not require mechanical ventilatory support [25]. The protein C system is suppressed in patients with ventilator-associated pneumonia (VAP) and plasminogen activator inhibitor-1 (PAI-1) concentrations are increased in the plasma and BAL of ALI/ARDS patients, and in the BAL of patients with pneumonia [25,26,27••,28]. Retrospective measurements of plasma levels of protein C and PAI-1 in plasma samples were analyzed from 779 patients in a trial of a protective ventilatory strategy for ARDS/ALI patients and were compared to 99 samples from patients with acute cardiogenic pulmonary edema. Baseline protein C levels were low and baseline PAI-1 levels were elevated in the samples from the patients with ARDS/ALI. In fact, decreased levels of plasma protein C and increased levels of PAI-1 were independent risk factors for mortality and adverse clinical

outcomes in these patients [29•]. Notably, other studies document that the PAI-1 and other evidence of depressed fibrinolysis precedes the clinical occurrence of VAP by days, suggesting that these coagulation changes are sensitive markers of airspace perturbations and that these markers stay localized in the lungs unless patients have lost their epithelial barriers, as is seen with the development of ARDS [24•,25]. Finally, a series of experiments from one group of investigators suggests that lung epithelial cells are active participants in producing members of the coagulation cascade [30,31]. The group showed that cultured A549 cells, small airway epithelial and primary human alveolar epithelial type II cells were found to express thrombomodulin, endothelial protein C receptor and tissue factor after cytokine stimulation. Also, all three of these cultured cell lines activated protein C. Shedding of endothelial protein C receptor and thrombomodulin from the alveolar epithelium was inhibited by metalloproteinase inhibitors, suggesting that shedding is mediated by a metalloproteinase [30].

Conclusion

The alveolar epithelium plays a key role in the formation and clearance of ALI/ARDS. Trans-epithelial ion transport functions to remove excess liquid from the alveolar space and this ability to remove excess water from the alveoli has been correlated with outcomes in patients with ALI/ARDS. New clinical trials will target this pathway to determine if outcomes can be improved by increasing the rate of liquid clearance through β-adrenergic stimulation. There is a delicate balance that exists between the alveolar epithelium, the coagulation pathway and lung leukocytes. The development of an alveolar procoagulant state can result in inflammation, edema and fibrosis. Novel anticoagulant therapies such as activated protein C may provide the ability to improve lung function without concomitant immunosuppression.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 105).

1 Qiao R, Zhou B, Liebler JM, *et al.* Identification of three genes of known function expressed by alveolar epithelial type I cells. Am J Respir Cell Mol Biol 2003; 29:98–105.

2 Wright JR. Immunoregulatory functions of surfactant proteins. Nat Rev Immunol 2005; 5:58–68.

3 •• Factor P, Mutlu GM, Chen L, *et al.* Adenosine regulation of alveolar fluid clearance. Proc Natl Acad Sci U S A 2007; 104:4083–4088.
This is a key study that identified the importance of adenosine in regulating the CFTR channel.

4 Matthay MA, Folkesson HG, Verkman AS. Salt and water transport across alveolar and distal airway epithelia in the adult lung. Am J Physiol 1996; 270:L487–L503.

5 Folkesson HG, Matthay MA. Alveolar epithelial ion and fluid transport: recent progress. Am J Respir Cell Mol Biol 2006; 35:10–19.

6 Borok Z, Verkman AS. Lung edema clearance: 20 years of progress: invited review: role of aquaporin water channels in fluid transport in lung and airways. J Appl Physiol 2002; 93:2199–2206.

7 Verkman AS. Role of aquaporins in lung liquid physiology. Respir Physiol Neurobiol 2007; Epub ahead of print.

8 Berthiaume Y, Matthay MA. Alveolar edema fluid clearance and acute lung injury. Respir Physiol Neurobiol 2007; Epub ahead of print.

9 Matthay MA, Wiener-Kronish JP. Intact epithelial barrier function is critical for the resolution of alveolar edema in humans. Am Rev Respir Dis 1990; 142: 1250–1257.

10 Ware LB, Matthay MA. Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. Am J Respir Crit Care Med 2001; 163:1376–1383.

11 Palmieri TL, Enkhbaatar P, Bayliss R, *et al.* Continuous nebulized albuterol attenuates acute lung injury in an ovine model of combined burn and smoke inhalation. Crit Care Med 2006; 34:1719–1724.

12 Perkins GD, McAuley DF, Thickett DR, Gao F. The beta-agonist lung injury trial (BALTI): a randomized placebo-controlled clinical trial. Am J Respir Crit Care Med 2006; 173:281–287.

13 •• Petty JM, Sueblinvong V, Lenox CC, *et al.* Pulmonary stromal-derived factor-1 expression and effect on neutrophil recruitment during acute lung injury. J Immunol 2007; 178:8148–8157.
Interesting paper in animals that suggests a mechanism for the later neutrophil invasion of injured lungs and how these neutrophils may be essential in causing further lung injury.

14 Steinberg KP, Milberg JA, Martin TR, *et al.* Evolution of bronchoalveolar cell populations in the adult respiratory distress syndrome. Am J Respir Crit Care Med 1994; 150:113–122.

15 Aggarwal A, Baker CS, Evans TW, Haslam PL. G-CSF and IL-8 but not GM-CSF correlate with severity of pulmonary neutrophilia in acute respiratory distress syndrome. Eur Respir J 2000; 15:895–901.

16 Gomperts BN, Belperio JA, Rao PN, *et al.* Circulating progenitor epithelial cells traffic via CXCR4/CXCL12 in response to airway injury. J Immunol 2006; 176:1916–1927.

17 Bachofen M, Weibel ER. Structural alterations of lung parenchyma in the adult respiratory distress syndrome. Clin Chest Med 1982; 3:35–56.

18 Carvalho AC, Quinn DA, DeMarinis SM, *et al.* Platelet function in acute respiratory failure. Am J Hematol 1987; 25:377–388.

19 Tomashefski JF Jr, Davies P, Boggis C, *et al.* The pulmonary vascular lesions of the adult respiratory distress syndrome. Am J Pathol 1983; 112:112–126.

20 Chapman HA Jr, Reilly JJ Jr, Kobzik L. Role of plasminogen activator in degradation of extracellular matrix protein by live human alveolar macrophages. Am Rev Respir Dis 1988; 137:412–419.

21 Welty-Wolf KE, Carraway MS, Ortel TL, *et al.* Blockade of tissue factor-factor X binding attenuates sepsis-induced respiratory and renal failure. Am J Physiol Lung Cell Mol Physiol 2006; 290:L21–L31.

22 Welty-Wolf KE, Carraway MS, Miller DL, *et al.* Coagulation blockade prevents sepsis-induced respiratory and renal failure in baboons. Am J Respir Crit Care Med 2001; 164:1988–1996.

23 Chapman HA, Stahl M, Allen CL, *et al.* Regulation of the procoagulant activity within the bronchoalveolar compartment of normal human lung. Am Rev Respir Dis 1988; 137:1417–1425.

24 • Schultz MJ, Levi M. Pulmonary coagulopathy: a potential therapeutic target in different forms of lung injury. Thorax 2007; 62:563–564.
Review of the coagulation abnormalities found in injured lungs, the importance of these abnormalities and potential treatments.

25 Gunther A, Mosavi P, Heinemann S, *et al.* Alveolar fibrin formation caused by enhanced procoagulant and depressed fibrinolytic capacities in severe pneumonia. Comparison with the acute respiratory distress syndrome. Am J Respir Crit Care Med 2000; 161:454–462.

26 Schultz MJ, Millo J, Levi M, *et al.* Local activation of coagulation and inhibition of fibrinolysis in the lung during ventilator associated pneumonia. Thorax 2004; 59:130–135.

27 •• El Solh AA, Choi G, Schultz MJ, *et al.* Clinical and hemostatic responses to treatment in ventilator-associated pneumonia: role of bacterial pathogens. Crit Care Med 2007; 35:490–496.
Important paper documenting coagulation abnormalities in BAL fluids and how different bacteria are associated with different degrees of abnormalities.

28 Choi G, Schultz MJ, van Till JW, *et al.* Disturbed alveolar fibrin turnover during pneumonia is restricted to the site of infection. Eur Respir J 2004; 24:786–789.

29 Ware LB, Matthay MA, Parsons PE, *et al.* Pathogenetic and prognostic • significance of altered coagulation and fibrinolysis in acute lung injury/acute respiratory distress syndrome. Crit Care Med 2007; 35:1821–1828.
This paper reports on studies performed on 779 blood samples from clinical trials on lung injury in the US. PAI-1 levels were elevated and protein C levels were low in these baseline blood samples, and were associated with mortality and adverse clinical outcomes.

30 Wang L, Bastarache JA, Wickersham N, *et al.* Novel role of the human alveolar epithelium in regulating intra-alveolar coagulation. Am J Respir Cell Mol Biol 2007; 36:497–503.

31 Bastarache JA, Wang L, Geiser T, *et al.* The alveolar epithelium can initiate the extrinsic coagulation cascade through expression of tissue factor. Thorax 2007; 62:608–616.