Published in final edited form as:

Expert Rev Clin Immunol. 2009 January 1; 5(1): 63–75. doi:10.1586/177666X.5.1.63.

Role of the pulmonary epithelium and inflammatory signals in acute lung injury

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Abstract

Acute lung injury (ALI) is a clinical disease marked by respiratory failure due to disruption of the epithelial and endothelial barrier, flooding of the alveolar compartment with protein-rich fluid and recruitment of neutrophils into the alveolar space. ALI affects approximately 200,000 patients annually in the USA and results in approximately 75,000 deaths. It is associated with prolonged mechanical ventilation, intensive medical care, high morbidity and mortality, and rising healthcare costs. Owing to its impact on public health, great strides have been made towards understanding the pathobiology of ALI to affect outcome. This review will focus on the role of the epithelial cell in the pathogenesis and resolution of ALI and the role of various inflammatory mediators in ALI.

Keywords

β 2-agonist; acute lung injury; acute respiratory distress syndrome; chemokine; cytokine; epithelial cell; inflammation; methylprednisolone; neutrophil; salbutamol; steroid; ventilator-induced lung injury

Acute lung injury/acute respiratory distress syndrome (ALI/ARDS) was first described in 1967 by Ashbaugh and colleagues in a group of 12 patients who shared a constellation of clinical symptoms including acute respiratory distress, hypoxemia refractory to supplemental oxygen, decreased lung compliance and diffuse pulmonary infiltrates [1]. Since this first description, the clinical criteria have evolved to include acute onset of respiratory distress, bilateral pulmonary infiltrates seen on chest radiographs, absence of left-sided heart failure and hypoxemia, with the severity of hypoxemia distinguishing ALI and ARDS (Box 1) [2]. Extrapolating from recent epidemiologic studies using these criteria, ALI affects about 200,000 patients annually in the USA and results in approximately 75,000 deaths [3]. This disease, which can be caused by common events such as pneumonia, sepsis and trauma, results in high morbidity, mortality and healthcare expenditures associated with prolonged mechanical ventilation and intensive care. Because of its impact on public health and patient outcomes, great strides have been made towards understanding the pathobiology of ALI.

When defined broadly to include all processes related to defense against microbes, other environmental insults and injury, a wide variety of cell types and proteins participate in inflammation and immunity. Leukocytes are the paradigmatic inflammatory cell type, but they are not the only cells that function in defense and immunity. The epithelial lining of all tissues forms a continuous barrier to the environment and is the first line of defense against infectious agents and toxins. Though specialized to serve distinct functions among tissues, epithelial cells respond similarly to injury and infection, and regulate leukocyte influx through the production of proinflammatory cytokines, chemokines and other factors that modulate chemokine gradients and effect leukocyte migration. This review will focus on the role of the pulmonary epithelium and inflammatory mediators in ALI. More specifically, this review addresses the

role of the alveolar epithelium in the pathogenesis of ALI, including impaired fluid transport, epithelial cell apoptosis and necrosis, cellular responses to mechanical stresses, synergistic effects of bacterial-derived products and endogenous molecules that function as alarmins. This review will also address the role of inflammatory mediators produced by the epithelium, and the role of the epithelium in wound repair and regeneration.

Anatomy of the alveolus

One of the key players involved in the pathogenesis and resolution of ALI/ARDS is the respiratory epithelium, which is involved in maintaining the integrity, function and host responses to lung injury and infection. The alveolar epithelium is comprised of two predominant cell types: the type I and II alveolar epithelial cells, or pneumocytes (Figure 1) [4]. Type I cells are very flat cells, accounting for 80% of the alveolar surface area but only 20% of the total epithelial cells, and their thin morphology allows for rapid diffusion and exchange of gases. Type II cells are cuboidal and more numerous than type I cells, making up 80% of the total alveolar epithelium but only 20% of the surface area. Both cell types have roles in host defense and immunity. In contrast to the type II cells, type I cells are susceptible to injury and cell death. The more resistant type II cells proliferate and differentiate into type I cells, important in re-epithelialization of the epithelial barrier. These cells also produce surfactant and regulate fluid balance across the epithelium [5].

Box 1. Acute lung injury and acute respiratory distress syndrome criteria

- Acute onset
- Bilateral infiltrates on chest radiographs
- Pulmonary artery occlusion pressure ≤ 18 mmHg or the absence of clinical evidence of left-atrial hypertension
- $\text{PaO}_2:\text{FiO}_2 \leq 300$ (acute lung injury) or ≤ 200 (acute respiratory distress syndrome)

From the American–European Consensus Conference Committee (1994). Data from [3].

Pathology of ALI/ARDS

Although the inciting events for ALI are diverse, the histologic changes are defining and represent a common end point. The pathologic findings in the acute phase include diffuse alveolar damage, with disruption and loss of both epithelial and endothelial cells, and exudation of protein-rich edema fluid into the alveolar spaces. Hyaline membranes form on the epithelial side of the basement membrane, and neutrophils, macrophages and red blood cells can be found in the alveolar space (Figure 1). All of these pathologic changes result in noncompliant lungs that function poorly in gas exchange. In many cases, the acute disease fails to resolve and respiratory failure continues as the disease enters a fibroproliferative phase. Histologically, this phase is marked by increased extracellular matrix deposition in the alveolar compartment with acute and chronic inflammatory cells [4].

Pathogenesis of ALI/ARDS

Numerous factors contribute to the pathobiology of ALI/ARDS, and this section will discuss the putative mechanisms of ALI/ARDS, including impairment of alveolar fluid clearance, epithelial cell apoptosis, cellular responses to mechanical stresses and synergistic effects of bacterial-derived products, as well as endogenous molecules that function as alarmins.

Alveolar fluid transport

Damage to the alveolar capillary endothelium is a well-established cause of the influx of protein-rich fluid into the alveolar compartment [4,6]. However, the epithelium is also critical in maintaining the integrity of the alveoli. Loss of the epithelial cell barrier can contribute to the influx of proteins and other macromolecules into the alveolar compartment. There is also a loss of normal epithelial cell fluid transport and reduced production of surfactant.

The resorption of pulmonary edema fluid from the alveolar space is necessary for the resolution of ALI/ARDS [7]. Epithelial cell fluid transport is dependent on Na^+ and Cl^- movement through specific channels located on the apical surface of type II cells and Na^+/K^+ -ATPases located on the basolateral surface [8,9]. To maintain equilibrium with fluid formation, water clearance is driven by sodium and chloride transport across both alveolar epithelial type I and II cells. More specifically, sodium is actively transported from the alveolar compartment into the interstitium via the apical Na^+ channel (ENaC) and the basolateral Na^+/K^+ channel, and water follows this ionic gradient. Water can travel through transcellular channels known as aquaporins. However, studies using specific aquaporin-null mice have demonstrated that these channels do not have significant roles in alveolar fluid absorption despite their importance in osmotically driven water movement across epithelial and endothelial cell barriers in the lung [10–15]. Cl^- is secreted into the alveolar space via the cystic fibrosis transmembrane regulator (CFTR) [16]; this receptor also plays a role in cAMP-mediated fluid transport [17,18].

In a study of alveolar fluid clearance rates by Ware and Matthay, the majority of patients (56%) with ALI had impaired alveolar fluid clearance, and patients with maximal alveolar fluid clearance had significantly lower mortality and a shorter duration of mechanical ventilation [19]. Alveolar fluid clearance can be enhanced using β -adrenergic agonists that stimulate Na^+ transport via the basolateral ATPases [20]. However, in Ware's study, they found no correlation between endogenous or exogenous catecholamines and alveolar fluid clearance [19]. In a small clinical trial of 40 patients, the use of salbutamol, a selective β_2 -agonist, was associated with decreased alveolar water, a more compliant lung and a trend for a lower lung injury score [21]. There was no change in oxygenation or survival; however, this study did not have enough power to detect mortality end points and larger clinical trials are underway.

Alveolar epithelial cell apoptosis

Loss of epithelial cells via necrosis or apoptosis is an important component to the pathobiology of ALI. Apoptosis, or programmed cell death, is characterized by DNA fragmentation and cellular involution, and can be triggered by external pathways that are mediated by a family of death receptors, which activate effector caspases. Alternatively, a separate internal or mitochondrial pathway can mediate apoptosis via cytochrome c and caspase activation. Lung tissue from both humans with ALI and animal models of ALI demonstrate activation of apoptotic pathways in the alveolar epithelium [22,23]. Matute-Bello and colleagues reported that bronchoalveolar fluid from patients with ALI contained soluble Fas ligand (sFasL), and the concentration of sFasL at the onset of ARDS was significantly higher in patients who died [24]. The sFasL was active and capable of inducing apoptosis of distal lung epithelial cells, which express the Fas receptor, suggesting that activation of the Fas/FasL system contributes to the severe epithelial damage that occurs in ARDS [24]. FasL can be shed by matrix metalloproteinase (MMP)7 [25], which is expressed by the lung epithelium and upregulated in lung injury [26]. Alternatively, airway epithelial cell apoptosis can also be induced by other pathways, including Toll-like receptor (TLR) signaling pathways [27] and oxidative stress pathways [28,29]. For example, stimulation of TLR3 or TLR4 signaling through the Toll/IL-1 receptor-domain-containing adaptor inducing IFN- β TRIF adaptor protein leads to cellular apoptosis [27].

Epithelial cell death may occur via necrosis in areas under high mechanical stress. Unlike apoptosis, necrosis results in the liberation of cellular contents into the environment, which can lead to further injury and inflammation. A frequent cause of necrotic cell death is disruption of the plasma membrane by toxic agents or by stress failure of the plasma membrane [30], and this latter mechanism has been identified in lungs ventilated with high tidal volumes [31].

Epithelial cell responses to mechanical stretch

The pathologic changes characteristic of ALI/ARDS result in stiff, noncompliant lungs and contribute to an inability to maintain adequate gas exchange, which can be overcome with mechanical ventilation. Within the past decade, there have been significant advances in our understanding of the cellular responses to mechanical forces required to ventilate and distend the injured lung [32]. Overdistention of the alveolar wall can lead to disruption of the epithelial and endothelial cells, basement membrane, epithelial cell death and activation of proinflammatory pathways, which gives rise to the clinical entity known as ventilator-induced lung injury (VILI).

In Webb and Tierny's classic 1974 study, it was demonstrated that very high inspiratory pressures could induce lung injury in rodents, characterized by edema, alveolar flooding and diffuse alveolar damage at the most extreme pressures [33]. Replication of these studies in other animal models has demonstrated similar findings of nonhydrostatic pulmonary edema and ultrastructural evidence of endothelial and epithelial disruption and destruction with denuded basement membranes [34–38]. Importantly, reduction in tidal volumes has been shown to reduce epithelial and endothelial cell injury. Using a rat model of lung injury, Frank *et al.* examined the effect of three different tidal volumes (3, 6 and 12 ml/kg) on lung injury in rats. Their investigation showed that alveolar epithelial injury was a critical part of the pathology because there were elevated plasma levels of rat type I cell 40 kD protein, a marker of type I epithelial cell injury and impaired alveolar fluid clearance, effects that were reduced by lower tidal volume ventilation [39].

Radiographically, ALI/ARDS is a heterogeneous process, with areas of normal lung adjacent to fluid-filled alveoli [4]. Thus, normal alveolar units may be subject to much higher local distending pressures when ventilated with a given tidal volume. Importantly, VILI is not only induced by alveolar overdistention but also by repeated opening and closing of alveolar units. This concept is supported by studies that demonstrate that cyclic stretch is more injurious than tonic stretch [40], and animal studies that demonstrate a protective effect of positive end-expiratory pressure (PEEP) [33].

In addition to the early cellular disruption induced by stretch, recruitment of neutrophils is another important component in the pathogenesis of VILI. Belperio and colleagues demonstrated that mice lacking the major neutrophil chemokine receptor, CXCR2, are protected from VILI [41]. Other groups demonstrated that neutrophil depletion reduces IL-8 expression in the mechanically-ventilated lung [42], and inhibition of neutrophil elastase ameliorates VILI [43].

Sepsis or bacterial infection is an important risk factor for the development of ARDS, and it is also a common complication occurring during the chronic management of someone afflicted with ARDS. Numerous experimental models of VILI have demonstrated additive or synergistic effects of bacterial products with mechanical ventilation on the development or exacerbation of lung injury [44–48]. Mechanical ventilation using moderate tidal volumes in mice enhanced lipopolysaccharide (LPS)-induced expression of TNF- α , IL-8, growth-related protein- α and monocyte chemoattractant protein (MCP)-1 [44]. A similar finding in rabbits has also been observed by Bregeon and colleagues [46]. In rabbits treated with mechanical ventilation and intravenous endotoxin, they observed increased neutrophilic lung inflammation, hypoxemia,

altered lung mechanics and increased concentrations of the chemokines IL-8 and growth-related protein- α [46]. Enhanced lung injury is also seen with mechanical ventilation and *Staphylococcus aureus* pneumonia. The combination of ventilation and bacterial infection also augmented the injury of nonpulmonary organs, suggesting that mechanical ventilation exacerbates systemic inflammatory responses to bacteria and contributes to the pathogenesis of the multiple organ dysfunction syndrome [45].

Activation of the innate immune system and mechanotransduction has synergistic effects on lung injury, and animal studies suggest that they work in a reciprocal manner. For example, mechanical ventilation can enhance innate immune responses by upregulating CD14 [49], a LPS-binding coreceptor important in TLR pathways. The TLRs comprise a family of receptors that recognize bacterial products and other molecules to quickly activate the immune response. More recently, TLRs have been shown to interact with endogenous molecules released during injury, known as 'alarmins', thus providing evidence of common inflammatory pathways shared by injury and infection [50]. These molecules include high-mobility group box-1 (HMGB1), matrix fragments and oxidized phospholipids [51], and provide alternative pathways by which nonbacterial products could further augment lung injury [52].

Translation of these early experimental observations has resulted in one of the only therapy modifications that has significantly changed ALI/ARDS mortality rates in the past decade. In the NIH-sponsored ARDSnet trial of two different ventilatory strategies in ARDS, patients receiving lower tidal volumes (6ml/kg) had improved mortality from 41 to 30% compared with conventional tidal volumes (10–12 ml/kg) [53]. This ARDSnet trial has resulted in a paradigm shift towards a lung-protective ventilatory strategy as standard of care. Additional studies from this trial demonstrated that, in patients with ALI, baseline plasma levels of IL-6, -8 and -10 were associated with increased mortality and a decrease in ventilator-free and organ failure-free days. Patients receiving lower tidal volumes had reduced plasma levels of IL-6 and -8, indicating that ventilatory management can modify circulating inflammatory cytokines [54]. Another study of a lung-protective strategy demonstrated reduced lung inflammation and reduced concentrations of inflammatory cytokines in plasma and bronchoalveolar lavage (BAL) fluid associated with a lung-protective strategy [55]. Stuber and colleagues examined the kinetics of cytokine expression associated with tidal volume changes. As they increased tidal volumes from 6 to 12 ml/kg in patients with ALI, they found an associated increase in both pro- and anti-inflammatory cytokine levels in the plasma (IL-8, TNF- α , IL-1Ra, IL-10 and IL-6). Furthermore, these levels returned to baseline after resuming lung-protective ventilation [56].

Release of chemokines, cytokines & other inflammatory modulators

Inflammation is propagated from the initial site of tissue injury or infection by the diffusion of small molecules that act locally and more distantly. Exogenous mediators, including bacterial products such as endotoxin, are potent activators of host immune responses. Among the endogenous inflammatory mediators are cytokines and chemokines that induce the migration of leukocytes to sites of injury or infection and activate the cells to mount an immune response [57]. Although alveolar macrophages are a major source of chemokines in the air spaces [58], the pulmonary epithelium also produces these proinflammatory cytokines and chemokines [59,60]. *In vitro*, type II epithelial cells can produce greater chemoattractant signals than alveolar macrophages in a manner dependent on TNF- α and IL-1 β [60]. Furthermore, the epithelium also expresses other proteins, such as heparin sulphate proteoglycans, which help bind and restrain chemokines to aid in leukocyte retention and migration [61].

Chemokines are named and grouped by their chemical structure, more specifically, their cysteine-residue positioning (Table 1). The CXC subfamily has the first two cysteines

separated by one nonconserved amino acid residue. This group is further subdivided based on the presence or absence of a Glu-Leu-Arg (ELR) motif preceding the first cysteine residue. In general, ELR-positive chemokines regulate neutrophil migration via an interaction with the CXCR2 chemokine receptor, and the ELR-negative chemokines modulate mononuclear leukocyte migration [62].

Acute lung injury is characterized by neutrophilic influx into the lung and alveolar spaces, and these leukocytes are key players in the pathogenesis of ALI and VILI [41]. Although critical for innate immunity and pathogen clearance, they also cause tissue destruction by releasing proteases such as neutrophil elastase, MMP8, MMP9, MMP25, cathepsin G and protease 3, and reactive oxygen intermediates (ROIs), such as hydrogen peroxide (H₂O₂), hypohalous acids, chloramines and hydroxyl radicals [63]. Their tissue recruitment is mediated by the ELR and CXC chemokines, growth-related oncogene (GRO), epithelial neutrophil-activating protein-78, granulocyte chemotactic protein-2 and IL-8 (or the murine homologs, GRO/macrophage inflammatory protein-2/KC and granulocyte chemotactic protein-2/LIX), and both alveolar macrophages and alveolar type II cells can highly express many of these chemokines in response to proinflammatory cytokines or bacterial products [64].

Several animal studies have shown that inhibition of the CXC ligand–CXCR receptor axis reduces neutrophil recruitment to the lung and ALI (Table 2). For example, cytokine-induced neutrophil chemoattractant (CINC), the homolog of human GRO- α , is increased in inflammatory models in rats, and pretreatment of rats with a neutralizing antibody to CINC protects rats against pancreatitis-associated lung injury [65]. Passive immunization of rabbits with neutralizing antibodies to IL-8 prevented neutrophil recruitment and tissue injury in a rabbit model of acid aspiration lung injury [66] and ischemia–reperfusion injury [67,68]. Furthermore, neutralizing epithelial neutrophil-activating protein-78 with antibodies attenuated lung injury in a rat model of hepatic ischemia/reperfusion injury [69].

Petty *et al.* identified stromal cell-derived factor (SDF)-1 as the major chemokine important in sustained neutrophilic responses in the endotoxin-treated murine lung [70]. They also demonstrated the progressive upregulation of its receptor CXCR4 on neutrophils. This chemokine is also important for the migration of bone marrow-derived stem cells *in vitro*, although recruitment of this specific population of cells in ALI is unknown [71].

Extending these observations in animals to human disease, Steinberg and colleagues demonstrated that, in patients with ARDS following sepsis, there was a trend for higher neutrophil levels in the alveolar compartment in those who died compared with those who survived [72]. Aggarwal and colleagues investigated the levels of GM-CSF, G-CSF and IL-8 in the lungs of 31 patients with ARDS and determined their relationship to the level of neutrophils in the alveolar compartment and the clinical outcome. They found that G-CSF and IL-8, but not GM-CSF, correlated with BAL neutrophil levels, and only G-CSF correlated with survival, with higher levels associated with decreased survival [73].

Modification of chemokine gradients in ALI

The function of chemokines is regulated by the level of biosynthesis, the expression of cognate receptors, proteolytic processing and compartmentalization. This latter mechanism includes both the immobilization of chemokines to cell surfaces or ECM proteins and the generation of chemotactic concentration gradients that provide directional cues for leukocyte migration. MMPs are proteinases that can indirectly control influx of inflammatory cells by cleaving proteins in the pericellular environment that bind chemokines [74–76].

One such example of this mechanism is MMP7-dependent shedding of syndecan-1 in ALI. Syndecan-1 is a transmembrane heparan sulfate proteoglycan (HSPG) present on the

basolateral surface of the epithelium. By way of its heparin sulfate chains, syndecan-1 is capable of binding numerous extracellular ligands and can direct these ligands for recycling or degradation, enhance ligand–receptor interactions or release the entire complex via shedding [61]. The CXC chemokine KC (the mouse functional orthologue of IL-8/CXCL8) is one such ligand that binds to and is spatially restrained by the heparin sulfate chains of syndecan-1 at the basolateral surface of epithelial cells. In response to lung injury, both KC and MMP7 are induced, and MMP7 cleaves syndecan-1 within its juxtamembrane motif, thereby releasing the KC–syndecan-1 ectodomain complex to generate a chemokine gradient. Mice deficient in either MMP7 (*Mmp7*^{−/−}) or syndecan-1 (*scd1*^{−/−}) are unable to create an effective KC gradient and, thus, neutrophils fail to efflux into the alveolar space, thereby protecting the lung from ALI [74].

Mmp9^{−/−} mice are also protected from lung injury and lethality of pulmonary infection with *Francisella tularensis* via modification of neutrophil chemokine gradients [77]. These mice have diminished neutrophil accumulation in the lung and reduced lung injury due to a significant decrease in Pro-Gly-Pro (PGP), a chemotactic fragment generated from MMP9-mediated hydrolysis of collagen that has homology to KC and binds and activates the KC receptor CXCR2 [78]. In addition, MMP9 deficiency protects against mortality in an LPS model of peritoneal sepsis, presumably by mitigating systemic inflammatory responses that can have deleterious effects on the host [79].

Another modifier of chemokine gradients includes the Duffy blood group antigen. This receptor binds selective CXC and CC chemokines and is expressed on erythrocytes and endothelial cells. However, it does not transmit a signal via G proteins, as occurs with other seven-transmembrane receptors. The receptor serves as a chemokine reservoir, regulating tissue and plasma chemokine levels, thus affecting neutrophil migration to various tissues, including the lung [80–83]. Mice deficient in this receptor demonstrate impaired neutrophil migration to the lung, intestines and peritoneum, and altered chemokine levels in the serum and tissue [82,83]. In endothelial cells expressing the Duffy antigen, neutrophil migration toward GRO- α /CXCL1 and IL-8 (IL-8/CXCL8) was enhanced, suggesting that the Duffy antigen participates in the movement of neutrophil chemokines across the endothelium [81].

Release of other proinflammatory mediators

Other inflammatory mediators have been shown to be upregulated in edema fluid from patients with ARDS, including substance P [84], a neuropeptide that binds to its receptor NK1. In addition to its known effects in nociception, it also has proinflammatory roles [85], and absence or inhibition of this receptor is protective against ALI [86–88]. Hydrogen sulfide (H₂S) has also recently been shown to be an important mediator of inflammatory responses and may induce the production or release of substance P [89,90]. Although H₂S may be proinflammatory, in certain models of ALI, H₂S production is decreased, and administration of the H₂S donor sodium hydrosulfide decreased proinflammatory cytokines IL-6 and IL-8, and the resultant ALI [91]. Thus, further studies are needed to better define the interplay of H₂S and substance P in mediating ALI. Although animal studies suggest modification of these pathways may be a novel target to mitigate lung injury, additional studies are warranted.

Other inflammatory mediators, such as IL-1 β , have been found to be upregulated in animal models of ALI as well as in BAL fluid from patients with ARDS [92,93]. IL-1 β expression augments neutrophil recruitment and also impairs fluid clearance via down-regulation of the epithelial sodium channel (ENaC) [94]. Early blockage of IL-1 β is protective in a murine model of VILI, suggesting that IL-1 β may be a potential target in ALI [92]. However, IL-1 β has some roles in epithelial wound repair [95,96], and further evaluation of its role in both the pathogenesis and resolution of ALI is needed.

Epithelial cell repair

By maintaining a tight barrier against the external environment, secreting antibiotic peptides and producing chemokines and homing receptors, the epithelium is the first line of defense. Injury disrupts the barrier function of the epithelium, creating a portal for microbes and toxins. Fortunately, injured epithelial cells respond rapidly to close wounds, a process that involves cell proliferation, spreading and migration. Repair involves an orderly progression of events to re-establish the integrity of the injured tissue. The initial injury sets off a programmed series of interdependent yet separate responses, such as re-epithelialization and epithelial proliferation, inflammation, angiogenesis, fibroplasia, matrix accumulation and eventually resolution.

Migration and proliferation of type II alveolar epithelium is required for regeneration of the alveolar architecture, and several mediators are critical for proper, rapid re-epithelialization of the alveolus [97]. In addition to its role in facilitating neutrophil efflux, MMP7 is essential for re-epithelialization. In the absence of this proteinase, alveolar and airway repair is markedly impaired [26,74,98]. Cytokines that promote epithelial cell migration include EGF, TGF- α and KGF/FGF-7, among others [16]. EGF and TGF- α share a common receptor, EGF receptor (EGFR). TGF- α is elevated in pulmonary edema fluid from patients with ALI/ARDS and has been shown to induce alveolar epithelial repair *in vitro* [99]. The role of IL-1 β in epithelial repair also appears to be mediated by EGF and TGF- α , as blockade of this pathway abrogates epithelial cell migration *in vitro* [96]. KGF has been shown to induce alveolar type II proliferation *in vitro* and *in vivo*, and it reduces disease severity and mortality of murine models of ALI [100–107]. It also appears to function through a common pathway via EGFR [97].

Expert commentary

The pulmonary epithelium is critical to maintaining the integrity and function of the respiratory unit. Disruption and injury to the alveolar epithelium leads to impaired barrier function, with release of protein-rich fluid into the alveolar space, reduced fluid clearance, epithelial necrosis or apoptosis, release of inflammatory signals and influx of leukocytes. Although our attempts to understand the complex pathobiology of the mediators of ALI have led to improvements in clinical outcomes, particularly in terms of low tidal volume ventilation [53], ALI/ARDS still remains a devastating disease with a significant impact on public health. Therapy should be aimed at reducing lung injury by initiating lung-protective ventilation and strategies to reduce nosocomial infections that could potentially augment systemic inflammatory pathways and lung injury. Other ventilatory strategies, such as augmented PEEP or different ventilatory modes, have not changed outcomes significantly [108]. Efforts should be made to maintain conservative fluid management to reduced pulmonary edema and improve gas exchange [109,110]. Currently, larger clinical trials have demonstrated no role for steroids in ARDS [111]. The use of other disease-modifying therapy, including antioxidants to decompose ROS and detoxify toxic oxidation products that participate in lung injury, and β_2 -agonists to restore epithelial fluid transport, remain under study. Further investigation of the critical function of the epithelium in regulating these and other reparative processes is necessary to effect clinical outcomes.

As we continue to advance our understanding of the molecular pathways that are involved in the initiation, progression and resolution of ALI, we will continue to develop and study individual and combination drugs that target these specific pathways. The growing field of genomics may allow us to identify individuals who are at risk for developing ALI, and understanding these individual risk factors may further elucidate the complex pathobiology of ALI and allow new molecular targets to be discovered.

Five-year view

Attempts to understand the complex pathobiology of the mediators of ALI have led to improvements in clinical outcomes, particularly in terms of low tidal volume ventilation [53]. Ventilation with low tidal volumes has dramatically improved the survival in ARDS, in part by reducing stretch-induced epithelial and endothelial cell damage and the release of inflammatory mediators. Although lung-protective ventilation works in part by reducing lung inflammation, inhibition of inflammatory pathways with anti-inflammatory agents, such as steroids, has failed to demonstrate reliable improvement in outcomes [112–115]. In a small trial of steroids in late-stage ARDS, there was improvement in mortality [116]. Re-examination of this issue with a larger patient population revealed that patients receiving methylprednisolone had improved oxygenation and lung compliance, and were able to be liberated from mechanical ventilation sooner, but had a higher rate of recurrent respiratory failure and no difference in mortality [111]. One critique of this study design is that the steroids were rapidly tapered, possibly resulting in an exacerbation of lung injury. Importantly, increased infectious complications were not seen, but other complications of steroids were increased, including neuromuscular weakness. It is possible that steroids improved the lung physiology, but that these modest improvements were offset by side effects.

Instead of using a shotgun approach to inhibit ongoing inflammatory pathways, targeting specific chemokines or cytokines may prove beneficial. Indeed, small-molecule antagonists to chemokine receptors, such as CXCR2, have been developed [64]. As new therapeutic approaches are considered and developed, attention needs to be paid to potential outcomes that arise due to altering one process that impacts on others. For example, any strategy designed to inhibit neutrophil influx or activation must be balanced against a loss of the antimicrobial benefits of these granulocytes. Furthermore, the redundancy of chemokine pathways may necessitate the use of multiple antagonists.

Additional therapeutic advances under investigation include the role of antioxidants by dietary modification using omega-3 fatty acids, γ -linolenic acid and antioxidant supplementation. In response to various inflammatory stimuli, lung endothelial and epithelial cells, alveolar macrophages and other inflammatory cells produce ROS that damage the lungs and initiate proinflammatory cascades. Furthermore, the addition of oxygen can enhance the production of these toxic species. Natural antioxidants, such as glutathione, tocopherol (vitamin E) and flavonoids, and antioxidant enzymes reduce oxidized cellular components, decompose ROS and detoxify toxic oxidation products. Modification of this balance via antioxidants has been explored in animal models of lung injury, with some studies demonstrating attenuation of ALI [28,29,117–119], and recent clinical trials have demonstrated improvement in lung neutrophilia, oxygenation and decreased length of stay in the intensive care unit in those who received eicosapentaenoic acid and γ -linolenic acid compared with controls [120,121]. Currently, there are larger clinical trials in progress evaluating the effect of dietary modification with antioxidant supplementation on ALI and inflammatory biomarkers.

In addition to modifying proinflammatory pathways, other therapeutic considerations include modification of the resolution of lung injury via fluid absorption in the lung [122] and restoration of the epithelial lining [97]. Assessing these and other variables or interventions may one day be made easier via genetic profiling to help identify patients with genetic predispositions to ALI. A pharmacogenomics approach to understanding what genes or combination of genes places one at risk for developing the disease will be an important step in discovering new pathways that could be exploited to modify ALI disease.

Key issues

- Acute lung injury/acute respiratory distress syndrome (ALI/ARDS) clinical criteria include:
 - Acute onset of respiratory distress
 - Bilateral pulmonary infiltrates seen on chest radiographs
 - Absence of left-sided heart failure
 - Hypoxemia, with the severity of hypoxemia distinguishing ALI and ARDS
- The pathologic findings in the acute phase include:
 - Diffuse alveolar damage
 - Disruption and loss of both epithelial and endothelial cells
 - Exudation of protein-rich edema fluid
 - Presence of hyaline membranes on the epithelial side of the basement membrane
 - Neutrophilic influx
- In ALI, there is disruption of epithelial cell function and increased alveolar epithelial cell apoptosis.
- The majority of patients with ALI have impaired alveolar fluid clearance, which correlates with survival, and fluid clearance can be modified pharmacologically with β_2 -agonists with uncertain clinical benefit.
- Mechanical ventilation with high tidal volumes can augment ALI, and ventilation with a lung-protective strategy reduces mortality and is associated with changes in inflammatory biomarkers in the plasma and bronchoalveolar lavage.
- In animal models, inhibition of neutrophil chemokines or disruption of these chemokine gradients in the lung attenuates ALI.
- Molecules associated with enhanced wound closure, such as epithelial cell growth factors, reduce ALI in animal models.
- Reactive oxygen species are enhanced in ALI and propagate inflammatory cascades and lung injury. Antioxidants can attenuate ALI in animal models, and clinical trials evaluating the benefit of antioxidants in modifying ALI are underway.
- The future of ALI research and therapy will require further elucidation of the molecular pathways involved in the initiation, progression and resolution of ALI, and the development of combination therapies targeted at multiple pathways. In addition, the use of biomarkers and genomics to identify at-risk populations may enable initiation and modification of therapies early in the disease process.

Acknowledgments

Financial & competing interests disclosure

This work was supported in part by the NIH grant HL084385. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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- of considerable interest

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Figure 1. Anatomy of the normal alveolus versus the injured alveolus

In the normal, uninjured alveolus, the majority of the surface area is comprised of type I epithelial cells. The cuboidal type II cells are involved in surfactant production, fluid transport and repopulation of the alveolar epithelium following injury. In the injured alveolus, there is apoptosis or necrosis of the epithelium, denudation of the basement membrane, influx of inflammatory cells, activation of macrophages and neutrophils, with release of proteases, oxidants, cytokines and other inflammatory mediators, deposition of fibrin and influx of protein-rich fluid into the alveolar space.

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Table 1

Chemokine receptor families.

Systemic name	Human ligand	Mouse ligand	Chemokine receptor
<i>C chemokine family</i>			
XCL1	Lymphotactin/SCM-1 α /ATAC	Lymphotactin	XCR1
XCL2	SCM-1 β	Unknown	XCR1
<i>CC chemokine family</i>			
CCL1	I-309	TCA-3/P500	CCR8
CCL2	MCP-1/MCAF/TDCF	JE	CCR2
CCL3	MIP-1 α /LD78 α /PAT464/GOS19/HSIS α	MIP-1 α	CCR1, CCR5
CCL4	MIP1- β /ACT-2, HC21, MAD-5, LAG-1	MIP-1 β	CCR5
CCL5	RANTES	RANTES	CCR1, CCR3, CCR5
CCL6	Unknown	C10/MRP-1	Unknown
CCL7	MCP-3	MARC	CCR1, CCR2, CCR3
CCL8	MCP-2	MCP-2	CCR3, CCR5
CCL9	Unknown	Unknown	Unknown
CCL10	Unknown	Unknown	Unknown
CCL11	Eotaxin	Eotaxin	CCR3
CCL12	Unknown	MCP-5	CCR2
CCL13	MCP-4	Unknown	CCR2, CCR3
CCL14	HCC-1/NCC-2	Unknown	CCR1, CCR5
CCL15	HCC-2/LKN-1/MIP1- δ /NCC-3	Unknown	CCR1, CCR3
CCL16	HCC-4/LEC/LCC-1/NCC-4	Unknown	CCR1, CCR2
CCL17	TARC	TARC/ABCD-2	CCR4
CCL18	DC-CK1/PARC/AMAC-1	Unknown	Unknown
CCL19	MIP-3 β /ELC/exodus-3	MIP-3 β /ELC/exodus-3	CCR7
CCL20	MIP-3 α /LARC/exodus-1	MIP-3 α /LARC/exodus-1	CCR6
CCL21	6CKINE/SLC/EXODUS-2	6CKINE/SLC/exodus-2/TCA-4	CCR7
CCL22	MDC/STCP-1	ABCD-1	CCR4
CCL23	MPIF-1/CK β 8/CK β 8-1	Unknown	CCR1
CCL24	Eotaxin-2/MPIF-2/CK β 6	MPIF-2	CCR3
CCL25	TECK	TECK	CCR9
CCL26	Eotaxin-3	Unknown	CCR3
CCL27	CTACK/ILC	ALP/CTACK/ILC/ESKINE	CCR10
CCL28	MEC	CCL28	CCR3, CCR10
<i>CXC chemokine family</i>			
CXCL1	GRO α /MGSA	GRO/MIP-2/KC	CXCR2
<i>CXC chemokine family</i>			
CXCL2	GRO β /MGSA- β	GRO/MIP-2/KC	CXCR2
CXCL3	GRO γ /MGSA- γ	GRO/MIP-2/KC	CXCR2
CXCL4	PF4	PF4	Unknown
CXCL5	ENA-78	GCP-2/LIX	CXCR2
CXCL6	GCP-2	GCP-2/LIX	CXCR1, CXCR2
CXCL7	NAP-2	Unknown	CXCR2
CXCL8	IL-8	Unknown	CXCR1, CXCR2
CXCL9	MIG	MIG	CXCR3
CXCL10	IP-10	IP-10	CXCR3
CXCL11	I-TAC	I-TAC	CXCR3
CXCL12	SDF-1 α / β	SDF-1/PBSF	CXCR4
CXCL13	BCA-1	BLC	CXCR5
CXCL14	BRAK/bolekine	BRAK	Unknown
CXCL15	Unknown	Lungkine/WECH	Unknown
CXCL16	Unknown	Unknown	CXCR6
<i>CX3C chemokine family</i>			
CX3CL1	Fractalkin/CMKBRL1/V28	Neuroactin/ABCD-3	CX3CR1

ACT: Activation-induced T-cell derived and chemokine related; BCA: B cell-attracting chemokine; BLC: B-lymphocyte chemoattractant; BRAK: Breast and kidney-expressed chemokine; CTACK: Cutaneous T-cell-attracting chemokine; DC-CK: Dendritic cell-derived CC chemokine; ELC: EBL-1-ligand chemokine; ENA: Epithelial-derived neutrophil attractant; GCP: Granulocyte chemoattractant protein; GRO: Growth-related oncogene; HCC: Hemofiltrate CC chemokine; ILC: IL-11 receptor α locus chemokine; IP-10: IFN-inducible protein; I-TAC: IFN-inducible T-cell α chemoattractant; JE: Murine equivalent to MCP; LAG: Lymphocyte activation gene; LARC: Liver and activation-regulated chemokines; LCC: Liver-specific CC chemokine; LEC: Liver-expressed chemokine; LIX: LPS-induced CXC chemokine; LKN: Leukotactin; MAD: MAX dimerization protein; MARC: Macrophage/monocyte activation-regulated chemokine; MAX: MCAF: Monocyte chemotactic and activating factor; MCP: Monocyte chemotactic protein; MDC: Macrophage-derived chemokine; MEC: Mucosae-associated epithelial chemokine; MGSA: Melanoma growth stimulatory activity; MIG: Monokine induced by interferon- γ ; MIP: Macrophage inflammatory protein; MPIF: Myeloid progenitor inhibitory factor; MRP: Macrophage inflammatory protein-related protein; NAP: Neutrophil-activating peptide; NCC: New chemokine CC; PARC: Pulmonary and activation-regulated chemokine; PBSF: Pre-B-cell growth-stimulating factor; PF: Platelet factor; RANTES: Regulated upon activation, normal T-cell expressed and secreted; SCM: Single cysteine motif; SDF: Stromal cell-derived factor; SLC: Secondary lymphoid tissue chemokine; STCP: Stimulated T-cell chemotactic protein; TARC: Thymus and activation-regulated chemokine; TCA: Thymus-derived chemotactic agent; TDCF: Tumor-derived chemotactic factor; TECK: Thymus-expressed chemokine; WECH: Weird chemokine.

Table 2

Inhibition of the CXC–CXCR chemokine axis in animal models of acute lung injury.

Chemokine	Animal model	Method	Effect of ALI	Ref.
CINC (homolog of human GRO- α) IL-8	Pancreatitis-induced ALI in rat	Neutralizing ab	Protective	[65]
	Acid aspiration-induced ALI in rabbit	Neutralizing ab	Protective	[66]
	Ischemia-reperfusion injury			[67]
	Endotoxin-induced ALI			[69,123]
ENA-78 CXCR2	Hepatic ischemia–reperfusion in rat	Neutralizing ab	Protective	[69]
	Ventilator-induced lung injury in mouse	CXCR2 knockout	Protective	[41]
	Acid and LPS-induced ALI in mouse	CXCR2 inhibitor		[124]

ab: Antibody; ALI: Acute lung injury; CINC: Cytokine-induced neutrophil chemoattractant; CXCR: CXC chemokine receptor; ENA: Epithelial cell-derived neutrophil-activating peptide; GRO- α : Growth-related protein- α .