Mechanisms and Clinical Consequences of Acute Lung Injury

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Abstract

Acute respiratory distress syndrome (ARDS) was first described in 1967, and since then there have been a large number of studies addressing its pathogenesis and therapies. Despite intense research efforts, very few therapies for ARDS have been shown to be effective other than the use of lung protection strategies. The scarcity of therapeutic choices is related to the intricate pathogenesis of the syndrome and to insensitive and aspecific criteria to diagnose this profound acute respiratory failure. The aim of this paper is to summarize advances of new ARDS definitions and provide an overview of new relevant signaling pathways that mediate acute lung injury.

Keywords: acute respiratory distress syndrome; lung injury; alveolar edema; vascular permeability

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Acute respiratory distress syndrome (ARDS) is a devastating clinical condition known to all clinicians who deal with critically ill patients. Its clinical hallmarks include severe respiratory distress, hypoxemic respiratory failure refractory to oxygen administration, standard chest X-ray showing pulmonary edema that is not the result of congestive heart failure or fluid overload, and a silent clinical history for chronic respiratory disease (1). The need for admitting these patients in the intensive care unit for mechanical ventilation with positive end-expiratory pressure (PEEP) and high $F_{I_{O_2}}$ are the therapeutic guidelines used to manage these patients.

In 1967, Ashbaugh and colleagues described 12 patients with clinical features that were reported as "remarkably similar to the infantile respiratory distress syndrome" (2). Ventilatory management with PEEP was also first reported in that article. Five patients survived. Autopsy findings in seven patients who died showed striking alveolar atelectasis, engorgement of capillaries, and hyaline membrane formation. They described these patients as having adult respiratory distress syndrome. After the original publication of Ashbaugh and colleagues, the 16th Aspen Lung Conference on acute pulmonary injury and repair expanded the clinical features, factors influencing prognosis, and principles of management of the syndromes (3).

In 1988, Murray and colleagues proposed an expanded definition of ARDS that operationally quantified the physiologic impairment through the use of a four-point scoring system (lung injury score: LIS) including the level of PEEP, $Pa_{O_2}/F_{I_{O_2}}$, the value of static lung compliance, and the degree of infiltration evident on chest X-ray. Other factors included in the assessment were the inciting clinical disorder and the presence or absence of nonpulmonary organ dysfunction.

In 1994, broad consensus was achieved when the American–European Consensus Conference Committee (AECC) recommended a new definition (4). Bernard and colleagues replaced the word "adult" with "acute" and defined ARDS as the acute onset of hypoxemia ($Pa_{O_2}/F_{I_{O_2}} \leq 200$ mm Hg) with bilateral infiltrates on frontal chest X-ray consistent with edema in the absence of left atrial hypertension. They also defined a new, broader term, acute lung injury (ALI), defined using the same criteria but with a less stringent criterion for hypoxemia ($Pa_{O_2}/F_{I_{O_2}} \leq 300$ mm Hg). Thus, ALI included ARDS, but it also included a subset with relatively mild hypoxemia (i.e., $Pa_{O_2}/F_{I_{O_2}}$, 201–300). The AECC definition has been widely adopted by clinical researchers and physicians, and two major advantages of this definition have been identified: (*1*) recognizing a less severe form of the syndrome (ALI with $Pa_{O_2}/F_{I_{O_2}} \leq 300$ mm Hg) facilitated enrollment in clinical trials, and (*2*) the definition is simple to apply in the clinical setting.

Despite this unquestionable success, a number of issues regarding the AECC definition have emerged. First, the "acute onset" demanded by the AECC but does not explicitly define acute (e.g., hours, days, or weeks), nor from when to judge the onset of the syndrome. Second, the chest X-ray criterion has been shown to have poor to moderate interobserver reliability. Third, although the definition requires a pulmonary artery wedge pressure less than or equal to 18 mm Hg (when measured), patients with ARDS frequently have elevated pulmonary artery wedge pressures often because of transmitted

airway pressures and/or vigorous fluid resuscitation. Fourth, there is evidence that ALI and ARDS, as defined using the AECC criteria, are underrecognized by clinicians, particularly in the subgroup of patients with milder hypoxemia (i.e., those with Pa_{O_2}/FI_{O_2} of 201–300). Fifth, Pa_{O_2}/FI_{O_2} is not constant across a range of FI_{O_2} in individual patients and may vary in response to ventilator settings, particularly PEEP.

For these reasons, and to reflect new information and experience acquired, the European Society of Intensive Care Medicine—with the endorsements of the American Thoracic Society and the Society of Critical Care Medicine—convened an international expert panel to revise and adjust the AECC definition of ARDS (5). The essential aspects of the new definition (the "Berlin definition") are as follows. First, acute was defined as 1 week or less. Second, the term acute lung injury was abandoned. Third, measurement of the Pa_{O_2}/FI_{O_2} ratio was changed to require a specific minimum amount of PEEP. Fourth, three categories of ARDS were proposed (mild, moderate, and severe) based on the Pa_{O_2}/FI_{O_2} ratio. Fifth, chest radiograph criteria were clarified to improve interobserver reliability. Sixth, the wedge pressure criterion was removed, and additional clarity was added to improve the ability to exclude cardiac causes of bilateral infiltrates. The "conceptual model" of ARDS (i.e., how clinicians recognize patients with the syndrome) was formalized as the following: ARDS is a type of acute diffuse lung injury associated with a predisposing risk factor, characterized by inflammation leading to increased pulmonary vascular permeability and alveolar collapse. Hypoxemia and bilateral radiographic infiltrates, increased pulmonary right-to-left venous admixture, increased physiological dead space, and decreased respiratory system compliance are the hallmarks of the syndrome. Diffuse alveolar damage (i.e., lung edema, inflammation, hyaline membrane, and alveolar hemorrhage) is the characteristic morphological finding. Of interest, this definition was empirically evaluated for predictive validity for mortality, using data derived from multi- and single-center clinical trials that included 3,670 patients (5). Mortality rate is 27% for mild ARDS, 32% for moderate ARDS, and 45% for severe ARDS. Effective definition will give the opportunity to select more homogenous patient population that will be enrolled in reproducible and consistent clinical studies. However, syndrome definitions still evolve over time, and it is hoped they will be enriched by the research on molecular mechanisms of lung injury, for example through identification of novel biomarkers. Exposure to several risk factors (i.e., pneumonia, sepsis, shock) causes acute hypoxemic respiratory failure that requires invasive mechanical ventilation when alveolar gas exchange is severely impaired. Pathological hallmarks of this clinical phenotype are injury to the epithelial/endothelial cells of the alveolar barrier, surfactant dysfunction, activation of innate immune response, and coagulation. In fact, depending on which risk factor is responsible for respiratory failure, endothelial and/or epithelial monolayers are first damaged, impairing their barrier function. Moreover, the effect of noxious stimuli is amplified by activation of the innate immune system that contributes, for example, to the clearance of invading pathogens but also to amplify lung damage. Although better understanding of the physiological aspects of acute lung injury led to clinical trials that showed efficacy of lung-protective ventilation and of limitation of fluid overload to improve outcome, therapeutic interventions to attenuate overwhelming inflammatory response failed to demonstrate any clinical benefit. This demonstrates the urgent need to understand which are the most relevant signaling pathways (Table 1) that mediate acute lung injury. Particular attention should be paid to understand the role of endothelial hyperpermeability, epithelial barrier disruption, and leukocytes in promoting damage or repair of the lungs.

Mechanisms of Lung Injury

Vascular Hyperpermeability—Role of the Endothelium

Integrity of the epithelial/endothelial barrier, which prevents alveolar edema formation and leukocyte extravasation, involves epithelial (E-cadherin)- and vascular endothelial cadherin (VE-cadherin)-mediated adherence junction bonds (6, 7). VE-cadherin is a type I transmembrane protein with a calcium-dependent adhesive function in vascular cell–cell contacts. The extracellular portion of the protein forms dimers through homophilic interactions, whereas the cytoplasmic tail of VE-cadherin is associated with the cell cytoskeleton via proteins of the catenin family. The amount and adhesive function of VE-cadherin mediates paracellular routes of edema formation and leukocyte diapedesis through a well-coordinated balance between activity of tyrosine kinases and phosphatases. In particular, proinflammatory and permeability-inducing factors such as vascular endothelial growth factor, tumor necrosis factor (TNF), or histamine induce Src-mediated phosphorylation of VE-cadherin at Tyr685 (p-Y685), which leads to vascular permeability. Additionally, leukocytes activate phosphatase SHP-2, which leads to the dephosphorylation of the phosphorylated Tyr731 residue (p-Y731) of VE-cadherin. Dephosphorylated Tyr731 associates with the AP-2 complex and elicits endocytosis of VE-cadherin, thus promoting leukocyte extravasation (8, 9). In addition, the endothelial-specific vascular endothelial protein tyrosine phosphatase mediates an alternative pathway that has no effect on the Tyr 731 residue. In an animal model of vascular hyperpermeability, LPS or vascular endothelial growth factor administration has been associated with dissociation of vascular endothelial protein tyrosine phosphatase from VE-cadherin and increased neutrophil infiltration into the lungs (10). Moreover, lipid mediators may affect VE-cadherin–mediated vascular permeability. In particular, the sphingolipid metabolite sphingosine-1-phosphate (S1P) binds the G-protein–coupled receptor S1Pr1 and induces VE cadherin localization at the membrane of endothelial cells (11). In an animal model of systemic vascular leak, S1P knockout mice showed higher lung edema as demonstrated by higher lung wet/dry ratio and increased peribronchial fluid on histology. This effect was a consequence of exaggerated formation of interendothelial cell gaps. Of interest, transfusion of blood from wild-type animals or administration of S1P receptor agonist rescued the permeability phenotype. These results highlight that S1P maintains a barrier-protective tone, and S1P receptor agonists may represent pharmacological candidates to enhance vascular integrity. However, given the role

Table 1. Signaling pathways involved in epithelial–endothelial barrier stabilization

Signaling Pathway	Mediator	Biological Effect	Reference
Epithelial cells			
Phospholipase D1 PI3K1α NOX4	TGF-β	Internalization of β subunit of ENaC	14
GRK2/PI3K	IL-8	Desensitization and deregulation of β_2AR	15
PKC and PLC	Viral hemagglutinin	Decrease activity of ENaC	16
Transmembrane protein channel M2	Influenza virus	Endocytosis and proteasome-mediated destruction of ENaC	16
Caspase 8–mediated apoptosis	Fas and TGF-β	Cell death and denudation of alveolar epithelial barrier	16, 21
PI3K/Akt–ERK1,2	Mechanical stretch	Epithelial cells apoptosis	22, 23
Endothelial cells			
Src-mediated phosphorylation VE-cadherin	VEGF, TNF, IL-1	Vascular hyperpermeability	8, 9
Robo4 /p120/ VE-cadherin	Slit2N	Blockade of internalization of VE-cadherin at levels of intercellular junction after challenge with LPS or H5N1 infection	27
G-protein–coupled receptor S1Pr1/VE cadherin	Sphingosine1-phosphate (S1P)	Higher localization of VE-cadherin at membrane surface	11
CD73-mediated adenosine release/A2BAR/cAMP	IFN-β	Enhanced activity of amiloride-sensitive fluid transport and elevation of pulmonary cAMP levels promoting alveolar fluid clearance	28

Definition of abbreviations: GRK2 = G-protein–coupled receptor kinase 2; NOX4 = nicotinamide adenine dinucleotide phosphate reduced oxidase 4; PI3K1α = phosphatidylinositol-3-kinase-1 alpha; PKC, protein kinase C; PLC, phospholipase C; TGF = transforming growth factor; TNF = tumor necrosis factor; VE-cadherin = vascular endothelial cadherin; VEGF = vascular endothelial growth factor.

of S1P in regulating migration and activity of lymphocytes, caution is warranted before introducing any immune-modulatory agent as putative therapeutic agent.

Alveolar Edema Accumulation and Clearance—the Role of Alveolar Epithelial Cells and Leukocytes Infiltration

Unlike the endothelium that limits vascular hyperpermeability and leukocyte infiltration, epithelial cell monolayer stabilizes the alveolar barrier, producing surfactant and ensuring alveolar fluid clearance (AFC), which is an active reabsorption process of sodium, chloride, and water from the air compartment to the basolateral surface to keep the lung dry. In particular, AFC is impaired in patients with ARDS and correlates with higher mortality. Several factors have been shown to be responsible for the lower rate of AFC. Hypoxia and hypercapnia from impaired alveolar gas exchange may decrease the density of Na/K ATPase on the basolateral membrane. This mechanism seems to be related to the production of higher levels of reactive oxygen species that enhance phosphorylation of α1 subunit and subsequent endocytosis. In addition, mechanical ventilation with high inspiratory pressures seems to impair cAMP-dependent alveolar fluid clearance by impairing nitric oxide production or by disrupting the cell junction and causing epithelial cell death (12). In this regard, signaling pathways involving adenosine and its receptors on epithelial cells increases the intracellular levels of cAMP and Na-K pump activity, and it may be useful in attenuating lung edema and inflammation (13). Recently, two key inflammatory mediators in the acute phase of ARDS, IL-8 and transforming growth factor (TGF)-β, have been shown to impair AFC (14). TGF-β activates phospholipase D1, phosphatidylinositol-4-phosphate 5-kinase 1 (PI3K1α), and nicotinamide adenine dinucleotide phosphate reduced oxidase 4 (NOX4) to oxidize and internalize the β subunit of ENaC, which is the subunit responsible for stability of ENaC on cell membrane surface. Remarkably, this mechanism of impaired AFC by TGF-β has been reproduced in an *in vivo* mouse model of bleomycin-induced lung injury and in an *in vitro* model of epithelial cells that were challenged with bronchoalveolar lung fluid from patients with ARDS containing TGF-β (14). On the other hand, IL-8 has been shown to inhibit β_2-adrenergic receptor (β_2AR)-mediated AFC. In particular, alveolar epithelial type II cells challenged with IL-8 showed desensitization and deregulation of β_2AR-mediated chloride vectorial transport through activation of the G-protein–coupled receptor kinase 2 (GRK2)/PI3K signaling pathway (15). Recent evidence showed that impairment of AFC is a mechanism of lung injury in influenza-associated severe ARDS. Lung epithelial cells are the first barriers encountered by the viruses avian origin subtype H5N1 and H7N9 and swine origin H1N1, which have different tropisms for type I and II pneumocytes, based on the expression of different sialosaccharides on the cell surface. In fact, viral hemagglutinin may decrease activity of ENaC on the apical surface through activation of protein kinase C and phospholipase C. In addition, epithelial cell–induced expression of the virus transmembrane protein channel M2 was associated with higher levels of reactive oxygen species, with enhanced endocytosis and proteasome-mediated destruction of ENaC (16). Alternative pathways have been demonstrated to be involved in lung injury from noninfectious causes, such as acid aspiration. Oxidized phospholipids generated by oxidative stress from acid challenge induced lung injury via IL-6

production, independently from the canonical TLR4-MyD88 pathway, suggesting that this high-preserved signaling pathway has a role for infectious and noninfectious risk factors of ARDS (17). New emerging pathways may mediate the degree of lung injury. One emerging pathway is the renin angiotensin system via effects on pulmonary vascular tone/permeability, epithelial cell survival, and fibroblast activation. Several experimental models of acid aspiration and sepsis-associated ARDS have shown that opposing to ACE, AGII, and AT1 receptor, the angiotensin-converting enzyme 2 (ACE2) mitigates lung injury as demonstrated by reduction in edema and neutrophil accumulation. In addition, ACE2 has been identified as receptor for severe acute respiratory syndrome coronavirus (18). *In vivo*, ACE2 knockout mice showed lower titers of severe acute respiratory syndrome coronavirus and reduced signs of lung damage (18). Lung injury mediated by neutrophil infiltration is characterized by disassembly of tight junctions and release of soluble mediators such as elastase, metalloproteinase, defensins, and oxidants that in turn lead to epithelial cell death. In fact, massive migration of activated neutrophils into the lungs causes formation of large epithelial wounds due to separation of adjacent epithelia at tight junctions. On the other hand, neutrophil transmigration activates β-catenin signaling in epithelial cells that leads to activation of target genes involved in cell proliferation, thus contributing to epithelial repair after injury (19). Interestingly, inhibition of β-catenin has been shown to delay reepithelization of the denuded epithelium. β-Catenin signaling is activated in lung epithelial cells via elastase-mediated cleavage of E-cadherin during neutrophil transmigration (19). In addition, neutrophil sequestration into the lungs is enhanced by high tidal volume mechanical ventilation because of increased permeability and the chemotactic gradient of chemokines KC/CXCL1 and MIP-2/CXCL2/3 (20). The magnitude of neutrophil infiltration into the lungs parallels the expression of cell surface expression of chemokine receptor CXCR2, not only on leukocytes but also on epithelial cells, endothelial cells, and fibroblasts. In particular, treatment with specific antibodies that block interaction between CXCR2 and KC-MIP 2 was effective in reducing neutrophil recruitment and tissue damage in a mouse model of ventilator-induced lung injury (VILI) (20).

Alveolar Epithelial Cell Death

Although neutrophil apoptosis may limit alveolar damage induced by leukocyte infiltration, epithelial and endothelial cell death severely impairs the barrier function of the alveolar wall. Bacterial toxins produced by *Staphylococcus*, *Escherichia coli*, and *Pseudomonas* directly induce cell necrosis, which causes overwhelming inflammation and further damage to the surrounding tissue. Furthermore, mechanical ventilation, depending on the degree of mechanical stress applied on the cell membrane surface, may cause cell death by apoptosis or necrosis. In fact, in an animal model of ARDS, Imai and colleagues showed that mechanical ventilation with low distending pressures at end inspiration caused high levels of pulmonary apoptosis, whereas high mechanical stretch was associated with decreased apoptosis and increased necrosis (21). In fact, mechanical stretch may modulate intracellular stretch-activated signaling cascades that are involved in cell survival (22). In an isolated model of lung injury, mechanical stretch phosphorylates key proteins such as Akt and ERK1-2 that are essential in regulating cell survival and death (23). In light of these findings, modulation of apoptosis might be a promising strategy to mitigate injury in the lung and in distal organs, depending on the phase of the disease. Pulmonary edema–associated influenza infection derives also from direct cytotoxic effects of viruses on epithelial cells that die though both apoptosis and necrosis. In this regard, caspase 8–dependent mechanisms of cell death induced by Fas and TGF-β have been described. In addition, viral infection of human bronchial epithelial cells induces the release of chemokine CXCL8 and macrophage inhibitor factor, which is 0massively released after cell death by necrosis (24).

Clinical Future Directions

Despite deeper understanding of ARDS pathophysiology, available therapies to limit morbidity and mortality of the syndrome are scarce. Limitation of end-inspiratory stretch of alveolar units and negative fluid balance are currently the two therapeutic approaches that have been proven to be effective in ARDS (1, 25, 26). In addition, prone position and use of neuromuscular blocking agents may be effective in severe ARDS. Ongoing randomized multicenter clinical trials will test the hypothesis that further lowering of tidal volume to 4 ml/kg and plateau pressures to 25 cm H_2O may reduce the risk of VILI and improve survival. Toward this end, partial or total extracorporeal support techniques will be applied to patients with moderate and severe ARDS. The rationale of these nonconventional supportive therapies is to improve gas exchange—clearance of carbon dioxide and oxygen supply—and minimize the distending pressure applied to the lungs. Future research will test the hypothesis that resting the lung with extracorporeal support will improve lung repair. In addition, based on the most recent advances in mechanisms of lung injury as mentioned before, prevalence and severity of high-permeability lung edema seems to be one of main clinical feature of ARDS. Therefore, there is urgent need for new specific therapies aimed to restore the sealing of the alveolar–endothelial barrier and modulate the innate immune response to limit injury and promote resolution by cell-based therapies. Candidate pathways involved in stabilizing intercellular junctions seem to be promising: (*1*) The Slit Robo 4 interaction inhibits VE-cadherin tyrosine phosphorylation and prevents the dissociation of p120catenin from VE-cadherin avoiding microvascular leak (27). In fact, in *in vitro* studies, the N-terminal proteolytic fragment of Slit2 has been shown to reduce LPS-, TNF-, and IL-1–induced permeability; this effect has been abrogated in the presence of small interfering RNA directed against the receptor Robo4. The mechanism of this protection appears to be by the higher expression of VE-cadherin induced by Robo4-dependent Slit2N signaling; in particular, this signaling pathway promoted association between VE-cadherin and p120-catenin and blocked internalization of VE-cadherin at levels of intercellular junction. This protective effect has been confirmed also *in vivo* in a mouse model of LPS-associated lung injury. Intratracheal administration of Slit2 reduced protein exudates and inflammatory cell accumulation in bronchoalveolar lung

fluid. This effect was lost in Robo4 knockout mice and was independent of leukocyte migration because of the confined expression of the receptor on endothelial cells. These findings were expanded also in a mouse model of polymicrobial sepsis, in which Slit2N significantly reduced vascular permeability in the kidney and spleen and improved mortality from 80 to 33%. Finally, Slit2N significantly mitigated vascular endothelial hyperpermeability in the lung and improved mortality 3 days after H5N1 infection in mice. This effect was independent from the lung viral titers. In conclusion, the Slit-induced signaling pathway, leaving unchanged the immune activation after several infections, might be considered as a key modulator of vascular integrity reducing capillary leak, multiorgan failure, and death. (*2*) Recently, safety and efficacy of IFN-β-1a administration has been tested in phase 1 to 2 trials of patients with ARDS. Thirty-seven patients treated with the optimal tolerated dose of IFN-β-1a (10 μg for 6 d) had lower mortality compared with control subjects (8 vs. 32%), and they did not experience adverse effects. The mechanism of protection seemed to be related to a higher release of antiinflammatory molecule induced by IFN-β (28). These results will be confirmed in a double-blind multicenter randomized controlled trial. IFN-β1-a belongs to the wide family of interferons, and it has been demonstrated to induce higher expression of the enzyme CD73 on several cells, including those in blood and lymphatic lung vessels. This enzyme critically regulates the rate of conversion of adenosine monophosphate to adenosine, a potent antiinflammatory molecule. In fact, adenosine and its receptor adenosine 2β (A2BAR) enhance stability of alveolar epithelial–endothelial barrier. In a mouse model of VILI, adenosine has been shown to enhance amiloride-sensitive fluid transport and elevation of pulmonary cAMP levels promoting alveolar fluid clearance (13). In addition, in a mouse model of LPS-associated lung injury, genetic deletion or pharmacological inhibition of the A2BAR revealed higher degrees of lung inflammation and pulmonary edema. Interestingly, pretreatment with A2BAR agonist significantly attenuated this proinflammatory lung phenotype (29). (*3*) Cell-based therapy with mesenchymal stem cells has recently emerged as future pharmacologic therapy for patients with ARDS. Several mechanisms have been advocated to explain their putative role in lung protection. First, stem cells secrete paracrine-soluble factors, such as keratinocyte growth factor (30), angiopoietin-1 (31), IL-1 receptor antagonist (32), IL-10 (33), prostaglandin E_2 (33), and antimicrobial peptide LL-37 (34), or interact directly with injured cells (35), thus promoting clearance of alveolar edema, resolution of inflammation, and tissue repair. Interestingly, bone marrow–derived stromal cells have been shown to enhance recovery from lung injury, increasing ATP concentration in alveoli. These cells were able to form connexin 43 (Cx43)-containing gap junctional channels with the alveolar epithelia releasing mitochondria-containing microvesicles (31). Second, multipotent stem cells engraft and differentiate in lung resident cells, regenerating the alveolar epithelial–endothelial barrier, although this mechanism seems to be not predominant. The potential therapeutic role of mesenchymal stem cells in lung injury has been covered in a comprehensive review and is not discussed at length here.

In conclusion, the severity of acute lung injury derives from the cumulative effects of several risk factors. Vascular hyperpermeability, impairment of edema clearance, cell death, and dysregulated accumulation of leukocytes into the alveolar space are all associated with lung injury. The degree of alveolar barrier dysfunction precipitates the severity of hypoxia that requires the institution of mechanical ventilation and eventually of extracorporeal support when conventional treatment fails. Novel signaling pathways that promote the sealing of alveolar epithelial–endothelial barrier seem to be "druggable" targets that represent putative specific therapies for patients with ARDS. ■

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

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