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The Acute Respiratory Distress Syndrome: From Mechanism to Translation

SeungHye Han* and Rama K. Mallampalli*†‡

The acute respiratory distress syndrome (ARDS) is a form of severe hypoxemic respiratory failure that is characterized by inflammatory injury to the alveolar capillary barrier, with extravasation of protein-rich edema fluid into the airspace. Although many modalities to treat ARDS have been investigated over the past several decades, supportive therapies remain the mainstay of treatment. In this article, we briefly review the definition, epidemiology, and pathophysiology of ARDS and present emerging aspects of ARDS pathophysiology that encompass modulators of the innate immune response, damage signals, and aberrant polysis that may serve as a foundation for future therapeutic targets.


The acute respiratory distress syndrome (ARDS) is a form of hypoxemic respiratory failure that is characterized by severe impairment of gas exchange and lung mechanics, with a high case fatality rate. It is defined by acute hypoxemia (the ratio of partial pressure of arterial oxygen/the fraction of inspired oxygen $\leq 300$ mm Hg on positive end-expiratory pressure $\geq 5$ cm H$_2$O), with bilateral infiltration on chest imaging that cannot be explained fully by cardiac failure or fluid overload (1). Acute lung injury (ALI), a term that has been widely used in experimental lung injury models, is categorized as a mild form of the human disorder ARDS per the recent Berlin definition (1). The incidence of this clinical syndrome has been on the rise and now reported up to 86.2/100,000 person-years (2), which equals ~200,000 cases annually in the United States. The hospital mortality is high, at 38.5% (2), and has not significantly improved for the past several decades. The most common risk factor is severe sepsis with either a pulmonary or nonpulmonary source, explaining 79% of the cases (2). Other risk factors include aspiration, toxic inhalation, lung contusion, acute pancreatitis, trauma, transfusion, burn injury, and cardiopulmonary bypass surgery (3).

Existing treatments

Numerous treatment measures aiming to modulate inflammation or its physiological consequences have been investigated for the treatment of ARDS. However, current anti-inflammatory therapies [corticosteroids (4), neutrophil elastase inhibitors (5), GM-CSF (6), statins (7), and omega-3 fatty acid (8)] and therapies targeted at improving lung mechanics [surfactant (9), inhaled β agonists (10), and NO (11)] failed to show a mortality benefit. Only supportive therapies that minimize pressure-induced lung injury (barotrauma) during mechanical ventilation, such as lung-protective ventilation (12) with the use of neuromuscular blockers (13) or prone positioning (14), improved mortality; thus, these treatments remain the mainstay of care.

Fundamentals of pathophysiology (inflammation and immunosuppression)

The innate immune response plays a profound role in the pathophysiology of ARDS. Multiple immunologic processes involving neutrophils, macrophages, and dendritic cells participate in mediating tissue injury. Inflammatory insults, either locally from the lungs or systemically from extrapulmonary sites, affect bronchial epithelium, alveolar macrophages, and vascular endothelium, causing accumulation of protein-rich edema fluid into the alveoli and, subsequently, hypoxemia due to impaired gas exchange. Alveolar macrophages play a central role in orchestrating inflammation, as well as the resolution of ARDS (15). Once alveolar macrophages are stimulated, they recruit neutrophils and circulating macrophages to the pulmonary sites of injury. These cells partake in the elaboration of a diverse array of bioactive mediators, including proteases, reactive oxygen species, eicosanoids, phospholipids, and cytokines, that perpetuate inflammatory responses. One profound effect of these mediators is to...
damage or induce distal cell death, specifically alveolar type 2 epithelial cells. These cells serve vital functions by synthesizing and secreting pulmonary surfactant, which is an indispensable material that lines the inner lung surface to lower alveolar surface tension. Type 2 cells also actively partake in ion transport to control lung fluid. Together, these inflammatory events lead to histological changes typical of an acute exudative phase that results in significant impairment of lung mechanics and gas exchange (Fig. 1) (16). During the initial inflammatory and/or resolution phases of ARDS, alveolar macrophages also coordinate in a paracrine manner to interact with other cells, including epithelial cells (17), lymphocytes (18), and mesenchymal stem cells (19), which can result in augmentation of the inflammatory response or accentuation of tissue injury. Prolonged M1 (classically activated macrophages) or M2 (alternatively activated macrophages) phenotypes appear to be associated with nonhealing chronic ARDS (20).

ARDS is a systemic inflammatory disease with bidirectional involvement between the lungs and other organ systems, rather than a local pulmonary process. Inflammatory cytokines, such as IL-1β, TNF-α, IL-6, and IL-8, are elevated both in bronchoalveolar lavage fluid and circulating plasma in ARDS subjects (21). Interestingly, systemic immunosuppression is also observed in prolonged nonresolving ARDS patients, even though pulmonary inflammation is present simultaneously. An observational study in humans showed that peripheral blood samples from 23 subjects with ARDS secondary to trauma or surgery had decreased cytokine release after LPS exposure (22). Also, autoantibodies are produced rapidly during ARDS (23). Although ARDS is characterized by lung inflammation, it is worth noting that many risk factors for ARDS can induce organ-specific inflammation. For example, traumatic brain injury (24), sepsis (25), and burn injury (26) cause robust inflammation in lungs, as well as systemic immunosuppression. Further studies are necessary to clarify whether ARDS contributes to this differential inflammation independently of these risk factors.

**Emerging aspects of ARDS pathophysiology**

*Pattern recognition receptors: TLRs and nucleotide-binding oligomerization domain-like receptors.* Pattern recognition receptors (PRRs) are critical to surveillance in innate immunity, detecting components of foreign pathogens referred to as pathogen-associated molecular patterns (PAMPs). TLRs are one of the transmembrane PRR proteins and are highly conserved molecules throughout vertebrates (27). Ten functional TLRs have been identified in humans (28). They recognize nonendogenous PAMPs and trigger a rapid response to cause proinflammatory signaling. Recently, some TLRs were found to recognize endogenous danger (or damage)-associated molecular patterns (DAMPs) as well. Unlike TLRs, nucleotide-binding oligomerization domain-like receptors (NLRs) are cytosolic PRRs that respond to the various PAMPs and DAMPs to trigger proinflammatory responses.

Development and resolution of ARDS seems to be related to TLR signaling pathways. Hyaluronan, an extracellular matrix glycosaminoglycan produced after tissue injury, initiates the inflammatory response in ARDS through engagement of TLR2 and TLR4; at the same time, it promotes recovery from ARDS (29). TLR4 was described as playing a pivotal role in the induction of ARDS in various murine models (30). TLR3 mediates hyperoxia-induced ARDS (31), and TLR2 mediates hemorrhage-induced ARDS (32).

Recent studies showed that NLRs are responsible for sterile inflammation in ALI. One of them, NLRP (NLR family, pyrin domain containing) is an important component of the inflammasome, a large multiprotein complex. This complex is activated by pore-forming toxins or hypoxic cellular injury when conditions are primed with microbial ligands or endogenous cytokines (33). Specifically, the NLRP3 inflammasome is made up of three components: NLRP3, ASC, and procaspase 1. Once assembled, inflammasomes cleave pro–IL-1β and pro–IL-18, generating active IL-1β and IL-18. The NLRP3 inflammasome and its interaction with extracellular histones (34) was found to be required for the development of hypoxemia in a murine ARDS model (35). Also, inflammasome-regulated cytokines are associated with worse outcomes in ARDS subjects (36).

*Mitochondrial DAMPs.* Further compounding the inflammation from sepsis-induced ARDS is the release of mitochondrial components from cellular damage into the circulation. These mitochondrial-derived products include mitochondrial DNA, formyl peptides, and cardiolipin, which serve as DAMPs to other cells (37, 38), triggering sterile inflammation and a clinical phenotype, the systemic inflammatory response syndrome (39). Both traumatic and operative injuries (40), which are risk factors for ARDS, release mitochondrial DAMPs into the circulation and activate polymorphonuclear neutrophils (PMNs) as a proinflammatory response. Mitochondrial DAMPs are present in blood-transfusion products, suggesting a possible link with transfusion-related ARDS (41). Increased permeability of endothelial cells, which is a critical event causing hypoxemia in ARDS, is triggered by fragmented mitochondria (mitochondrial DAMP) in PMN-dependent and PMN-independent fashions (42). Elevated mitochondrial DNA levels in plasma also are associated with higher mortality in patients with or without ARDS in the surgical and trauma (43), as well as the medical (44),

**FIGURE 1.** Acute exudative phase of ARDS. Low-magnification photomicrograph showing alveolar inflammatory infiltration and filling of air sacs with protein-rich fluid (H&E, low magnification) (16).
intensive care unit. Finally, a mitochondrial-targeted inhibitor was shown to mitigate the apoptosis of mouse lung endothelial cells after irradiation (45). These mitochondrial DAMPs may also be the cause of the differential inflammation observed in trauma, sepsis, and ARDS (46), but further studies are necessary to confirm the mechanistic basis for these findings.

Ubiquitin biology in lung injury. Ubiquitin is a small regulatory molecule found universally in most tissues in eukaryotic organisms. Ubiquitination is a posttranslational modification process whereby ubiquitin is attached to a substrate protein, usually serving as the signal for its degradation via the proteasome or lysosome. Ubiquitination is carried out in three main steps performed by one or two ubiquitin-activating enzymes, several ubiquitin-conjugating enzymes, and hundreds of ubiquitin ligases (E3 ligases). In the ubiquitination cascade, the ability of E3 ligases to target a specific substrate for its degradation provides an elegant mechanism for protein disposal in cells but also opens up attractive opportunities for relatively selective therapeutic intervention.

ARDS is characterized by activation of the ubiquitin proteasome system (47), increased expression of ubiquitin within alveolar (type II) epithelia (48), and release of ubiquitin proteasome components into lung fluid (49). Ubiquitin components (E3 ligases) are also activated by endotoxin (50). An endotoxin-responsive ubiquitin E3 ligase component, termed Fbxo3, is elevated in subjects with sepsis. It profoundly stimulates cytokine release when expressed in human PBMCs by mediating the degradation of another E3 ligase subunit, Fbxl2. Fbxl2 acts as an anti-inflammatory protein, inhibiting TNF receptor-associated factors (51). Ubiquitination also was reported to play an important role in regulating the Na, K-ATPase and epithelial Na+ channel (ENaC) functions during ARDS (47). Specifically, the E3 ubiquitin ligase Cblb negatively regulates TLR4 signaling to prevent hyperactivation of NF-kB in a sepsis-induced ARDS murine model (52). The disruption of the alveolar–capillary barrier is one of the key pathophysiologic events causing lung edema and hypoxemia in ARDS. The Na, K-ATPase is localized in the basolateral surface of alveolar type 2 epithelial cells (53), contributing to lung liquid clearance. During hypoxia, the Na, K-ATPase is internalized and degraded by endocytosis via ubiquitination, resulting in alveolar epithelial barrier dysfunction and, consequently, decreased alveolar fluid clearance (54, 55). ENaC is responsible for salt and fluid absorption in lung epithelia, and its cellular abundance is regulated by the E3 ligase Nedd4-2 (56). Interestingly, hypoxia inhibits expression of the ENaC subunit at the apical membrane of murine alveolar epithelial cells, which may occur via Nedd4-2–mediated ubiquitination (57). Furthermore, Nedd4-2–knockout mice develop sterile lung inflammation with some similarities to an ARDS phenotype (58). The emerging role of these protein-degradation factors in ARDS presents the opportunity for identification of unique therapeutic targets.

Neutrophil biology/neutrophil extracellular traps. Neutrophil influx into the lungs in response to activated alveolar macrophages is associated with the severity of ARDS, and it may directly influence the development of this disorder (59). Several chemokines, including IL-8 (CXCL8), seem to play a central role in regulating neutrophil recruitment and consequent tissue damage, as well as altered alveolar–capillary permeability in both human and animal studies (60). Neutrophil extracellular traps (NETs) are produced by neutrophils and are released to the extracellular space to trap pathogens, such as bacteria, fungi, viruses, and protozoa (61), a process known as NETosis. In a murine ARDS model, NETs are formed in lung tissue, directly inducing the cell death of lung epithelia and endothelia (62). NETs are found in a variety of ARDS models, including both infection-related injury [influenza (63), bacterial endotoxin (64), and fungi (65)] and sterile lung injury [transfusion-related ARDS (66)]. The lower levels of surfactant proteins A and D that are commonly observed in ARDS appear to be responsible for excessive NETs in ARDS, because surfactant proteins are involved in clearing NET nucleic acid (64).

Potential new therapeutic targets in ARDS

Targeting the ubiquitin proteasome system. Recently, proteasome inhibitors, such as bortezomib (67) and carfilzomib (68), were approved for the treatment of multiple myeloma by the U.S. Food and Drug Administration. Several new proteasome inhibitors are now in development as anticancer therapies. There is mounting evidence that inhibiting the proteasome may induce anti-inflammatory effects (69). Hypoxia-inducible factor (HIF)-1α, a transcription factor that controls the expression of numerous genes, is targeted for ubiquitin proteosomal degradation. HIF-1α loses its ability to induce the expression of many genes when inhibiting its degradation with proteasome inhibitors, underscoring the potential for targeting of the ubiquitin proteasome system in ARDS (51, 71).

Inflammasomes: modification of the upstream and downstream pathways. As reviewed earlier, inflammasomes play a critical role in the development of sterile inflammation during ARDS. Several agents to modify NLRP3 inflammasome signaling have been studied. Antioxidants (72) and glyburide (73) block upstream signaling of the NLRP3 inflammasome in vitro. P2X7R antagonists (74) also inhibit upstream pathways before inflammasomes are assembled, but they have not been tested for lung inflammation. In contrast, a caspase-1 inhibitor decreases the release of IL-1β and IL-18 in rat endotoxemia (75), targeting a downstream pathway to inhibit the products of inflammasome activation. Anti-IL-1 therapy is another approach to limit inflammasome activation. A mAb against human IL-1β (canakinumab) is licensed to treat cryopyrin-associated periodic syndrome (76), a rare genetic disease caused by autosomal-dominant mutations of the NLRP3 gene. Recombinant IL-1Ra (anakinra) and IL-1 Trap (rilonacept) are approved to treat rheumatoid arthritis and cryopyrin-associated periodic syndromes, respectively (77). However, the purported role of these agents as anti-inflammatory therapy for ARDS has not been evaluated in preclinical settings. New chemical entities directly targeting the inflammasome (NLRs) have not been identified.

Modulating inflammation. Numerous anti-inflammatory agents have failed to show any mortality benefit in ARDS subjects. Currently untested alternatives for the treatment and prevention of ARDS in human randomized controlled trials are inhaled corticosteroids, angiotensin-converting enzyme inhibitors,
and peroxisome proliferator receptor agonists. Animal data suggest that nebulized corticosteroids improve dynamic lung compliance and oxygenation and decrease lung inflammation in sepsis-induced ARDS models (78). Angiotensin II induces NF-κB gene expression (79); hence, blocking the renin–angiotensin axis may be beneficial to ARDS patients based on animal data (80) and an observational human study (81). In contrast, peroxisome proliferator receptors and their respective ligands negatively control proinflammatory gene expression (82). Their agonists reduce inflammation and vascular leakage in animal ARDS experimental models (83). However, human randomized controlled trials are necessary to examine the effect and efficiency of all of these modalities.

**Cell-based therapy (stem cell).** An exciting area of investigation is assessing cell-based therapy for ARDS using stem cells, because these cells have the potential to differentiate into alveolar epithelial or lung endothelial cells and directly replenish the alveolar–capillary barrier during cellular injury (84). Mesenchymal stem cells (MSC) are rapidly advancing to the clinical setting because they have practical advantages: they are easy to isolate and propagate and do not generate the ethical issues associated with embryonic stem cells. Preclinical studies showed that MSCs reduced lung inflammation and mortality in a murine ARDS model (85). This beneficial effect of MSC therapy was reproduced in ex vivo–perfused human lungs (86); this seems to be primarily due to the release of keratinocyte growth factor by MSCs. There was no toxicity in a recently published phase I clinical trial (87). Another phase I trial started enrolling subjects to receive MSCs (START; NCT01775774) (88).

**Conclusions**

In summary, the highly complex signaling and cellular networks that mediate tissue injury in ARDS present significant challenges in devising novel therapies for this disorder (Fig. 2). However, this pathobiologic model provides a mechanistic platform offering unique opportunities to identify new targets for intervention. The pathobiology of ARDS involves cellular, biochemical, and organelle-based mediators, with activation of components within the innate immune response that incites significant pulmonary inflammation. The identifica-

![Figure 2](http://www.jimmunol.org/)

**Figure 2.** Pathophysiology of ARDS. Initial inflammatory insults, including mitochondrial DAMPs, activate alveolar macrophages via TLR and NLR signaling pathways. Activated alveolar macrophages release proinflammatory cytokines and recruit circulating macrophages and neutrophils to injured sites. Excessive neutrophils and persistently activated macrophages cause extensive damage to lung epithelia and endothelia, resulting in an impaired alveolar–capillary barrier. Disruption of this barrier allows protein-rich fluid to enter the alveoli, causing fluid accumulation in alveolar spaces (pulmonary edema) that interferes with gas exchange. Ubiquitination ( Ub ) plays an important role in modulating the abundance of key proteins in ARDS, resulting in secretion of cytokines, lower levels of surfactant proteins, and decreased function of ion channels ( Na , K-ATPase and ENaC). ARDS is associated with surfactant depletion, leading to increased NETosis, a process that alters lung cell viability. Mitochondrial DAMPs can directly increase microvascular permeability independently of leukocytes. MΦ, macrophage.
tion of unique druggable targets within the ubiquitin cascade related to PRRs or stem cells holds promise in advancing a new frontier for treating this critical illness based on the mechanistic biology.

Disclosures

R.K.M. has an equity interest and serves as Chairman of the Scientific Advisory Board for E3 Therapeutics.

References


Corrections


The reference listed as Ref. 81 is incorrect. The correct reference is listed below.


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