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The acute respiratory distress syndrome: from mechanism to translation

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Abstract

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

1. Introduction

Definition, epidemiology, and etiology of the acute respiratory distress syndrome

The acute respiratory distress syndrome (ARDS) is a form of hypoxemic respiratory failure characterized by severe impairment in 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 (PaO_2) to the fraction of inspired oxygen (FiO_2) ≤ 300mmHg on positive end-expiratory pressure (PEEP) ≥5 cm H_2O) with bilateral infiltration on chest imaging which cannot be fully explained 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, now reported up to 86.2 per 100,000 person-years (2), which totals about 200,000 cases yearly 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

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common risk factor is severe sepsis with either a pulmonary or non-pulmonary 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 patients. However, current anti-inflammatory therapies (corticosteroids (4), neutrophil elastase inhibitor (5), granulocyte-macrophage colony stimulating factor (6), statins (7), and omega-3 fatty acid (8)) and therapies targeted at improving lung mechanics (surfactant (9), inhaled β agonists (10), and nitric oxide (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), have shown mortality improvement and 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 partake in mediating tissue injury. Inflammatory insults, either locally from the lungs or systemically from extra-pulmonary 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 in 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) that 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 non-healing 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 (BAL)

fluid and circulating plasma in ARDS subjects (21). Interestingly, systemic immunosuppression is also observed in prolonged non-resolving ARDS patients even though pulmonary inflammation is persistent simultaneously. A human observational study showed that peripheral blood samples from 23 subjects with ARDS secondary to trauma or surgery had decreased cytokine release after lipopolysaccharide exposure (22). Also, autoantibodies are rapidly produced during ARDS (23). While ARDS is characterized by lung inflammation, it is worth noting that many risk factors for ARDS can themselves induce organ-specific inflammation. For example, traumatic brain injury (24), sepsis (25), and burn injury (26) cause robust inflammation in lungs, but also systemic immunosuppression. Further studies are necessary to clarify whether ARDS contributes to this differential inflammation independent of these risk factors.

2. Emerging aspects of ARDS pathophysiology

Pattern recognition receptors: Toll-like receptors and NOD-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). Toll-like receptors (TLRs) are one of the transmembrane PRR proteins and are highly conserved molecules throughout vertebrates (27). So far, 10 functional TLRs have been identified in humans (28). They recognize non-endogenous PAMPs and trigger a rapid response to cause pro-inflammatory signaling. Recently, some TLRs are found to recognize endogenous danger (or damage)-associated molecular patterns (DAMPs) as well. Unlike TLRs, nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are cytosolic PRRs which respond to the various PAMPs and DAMPs to trigger pro-inflammatory 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, and at the same time, promotes recovery from ARDS (29). TLR4 was described as playing a pivotal role inducing ARDS in various murine models (30). TLR3 mediates hyperoxia-induced ARDS (31), and TLR2 mediates hemorrhage induced ARDS (32).

Recent studies have shown that NLRs are responsible for sterile inflammation in acute lung injury. One of them, NLRP (NLR family, pyrin domain containing) is an important component of the inflammasome, a large multi-protein 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 comprised of three components: NLRP3 (NLR family, pyrin domain containing protein-3), ASC (apoptosis-associated speck-like protein containing a CARD), and pro-caspase1. 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 into the circulation from cellular damage. 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 systematic 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 pro-inflammatory 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) both in PMN-dependent and PMN-independent fashions (42). Elevated mitochondrial DNA levels in plasma are also associated with higher mortality in patients with or without ARDS in the surgical and trauma (43), as well as the medical (44) intensive care unit. Finally, a mitochondrial-targeted inhibitor has been 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 post-translational modification process where ubiquitin is attached to a substrate protein, usually serving as the signal for their degradation via the proteasome or lysosome. Ubiquitination is carried out in three main steps performed by one or two ubiquitin-activating enzymes (E1s), several ubiquitin-conjugating enzymes (E2s), and hundreds of ubiquitin ligases (E3s). In the ubiquitination cascade, the ability of E3s 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 peripheral blood mononuclear cells by mediating the degradation of another E3 ligase subunit, Fbxl2. Fbxl2 acts as an anti-inflammatory protein, inhibiting tumor necrosis factor receptor-associated factors (TRAFs) (51). Ubiquitination has been also reported to play an important role in regulating the Na, K-ATPase and the epithelial Na^+ channel (ENaC) function during ARDS (47). Specifically, the E3 ubiquitin ligase Cblb negatively regulates TLR4 signaling to prevent hyper-activation of nuclear factor (NF)-κB 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 located 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 the expression of ENaC subunit at the apical membrane of murine alveolar epithelial cells, which may be through 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 raises opportunity for identification of unique therapeutic targets.

Neutrophil biology/Neutrophil extracellular traps (NETs)

Neutrophil influx into the lungs in response to activated alveolar macrophages is associated with the severity of ARDS, and 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, and altered alveolar-capillary permeability in both human and animal studies (60). The neutrophil extracellular traps (NETs) are produced by neutrophils and released to the extracellular space to trap pathogens such as bacteria, fungi, viruses and protozoa (61), which is a process called 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, commonly observed in ARDS, appear to be responsible for excessive NETs in ARDS as surfactant proteins are involved in clearing NET-nucleic acid (64).

3. New potential therapeutic targets in ARDS

Targeting the ubiquitin proteasome system

Proteasome inhibitors such as bortezomib (67) and carfilzomib (68) were recently approved for the treatment of multiple myeloma by the U.S. Food and Drug Administration (FDA). Several new proteasome inhibitors are now in development as anti-cancer therapies. There is mounting evidence that inhibiting the proteasome may induce anti-inflammatory effects (69). Hypoxia-inducible factor 1 (HIF-1α), a transcription factor that controls expression of numerous genes, is targeted for ubiquitin proteasomal degradation. HIF-1α appears to be protective from ARDS as pharmacologic stabilization of HIF-1α lessens ALI severity in preclinical models (70). Inhibiting the pro-inflammatory protein, Fbxo3, effectively lessens the severity of viral pneumonia, septic shock, cytokine-driven systemic inflammation and ARDS in preclinical models, underscoring potential for targeting of the ubiquitin proteasome system in ARDS (51, 71).

Inflammasome: modification of the up- and down-stream pathways

As reviewed earlier, inflammasomes play a critical role in developing sterile inflammation during ARDS. Several agents to modify the 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 have not been tested for lung inflammation. On the other hand, a caspase-1 inhibitor decreases the release of IL-1β and IL-18 in rat endotoxemia (75), targeting a down-stream pathway to inhibit the products of inflammasome activation. Anti-IL-1 therapy is another approach to limit inflammasome activation. A monoclonal antibody against human IL-1β (Canakinumab) is licensed to treat cryopyrin-associated periodic syndromes (CAPS) (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 CAPS, respectively (77). However, the purported role of these agents as anti-inflammatory therapy for ARDS has yet to be evaluated in preclinical settings. New chemical entities directly targeting the inflammasome (NLRs) have not yet 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 (RCT) are inhaled corticosteroids, angiotensin converting enzyme (ACE) inhibitors, and peroxisome proliferator receptor (PPAR) agonists. Animal data suggests 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). On the other hand, PPARs and their respective ligands negatively control pro-inflammatory gene expression (82). Their agonists reduce inflammation and vascular leakage in animal ARDS experimental models (83). However, human RCTs are necessary to examine the effect and efficiency of all these modalities.

Cell-based therapy (stem cell)

An exciting area of investigation is assessing cell-based therapy for ARDS with 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 settings as they have practical advantages: they are easy to isolate and propagate, and do not generate ethical issues compared to embryonic stem cells. Preclinical studies show that MSC reduces 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), which seems to be mainly due to the release of keratinocyte growth factor by MSC. There was no toxicity in a recently published phase I clinical trial (87). Another phase I trial has started enrolling subjects to receive MSC (START; NCT01775774) (88).

4. 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 identification of unique druggable targets within the ubiquitin cascade, pattern recognition receptors, and protein or cell based strategies hold promise in a new frontier based on the mechanistic biology of this critical illness.

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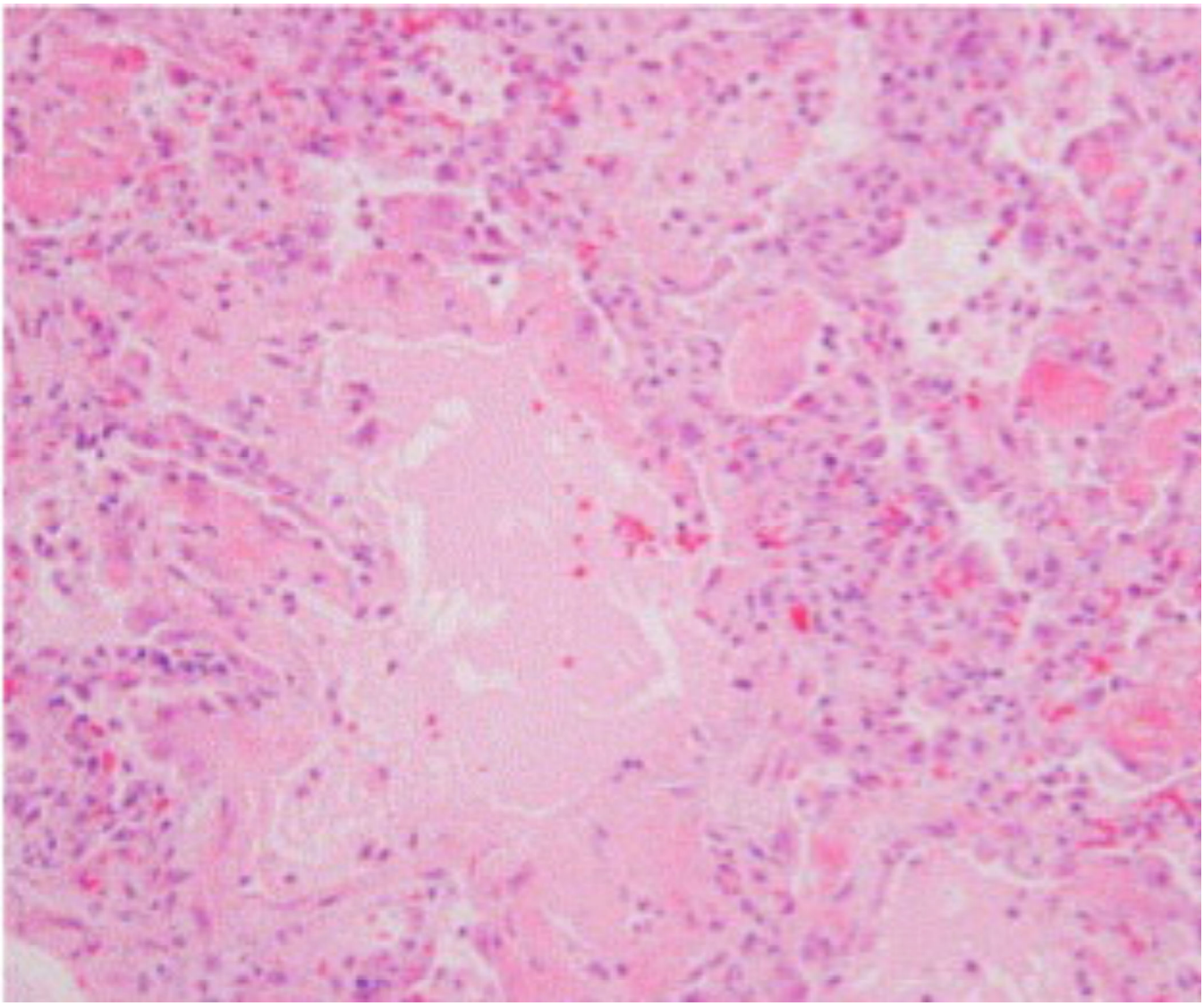

Figure 1.
The acute exudative phase of ARDS. Shown is a low-magnification hematoxylin and eosin stain micrograph showing alveolar inflammatory infiltration and filling of air sacs with protein-rich fluid (16).

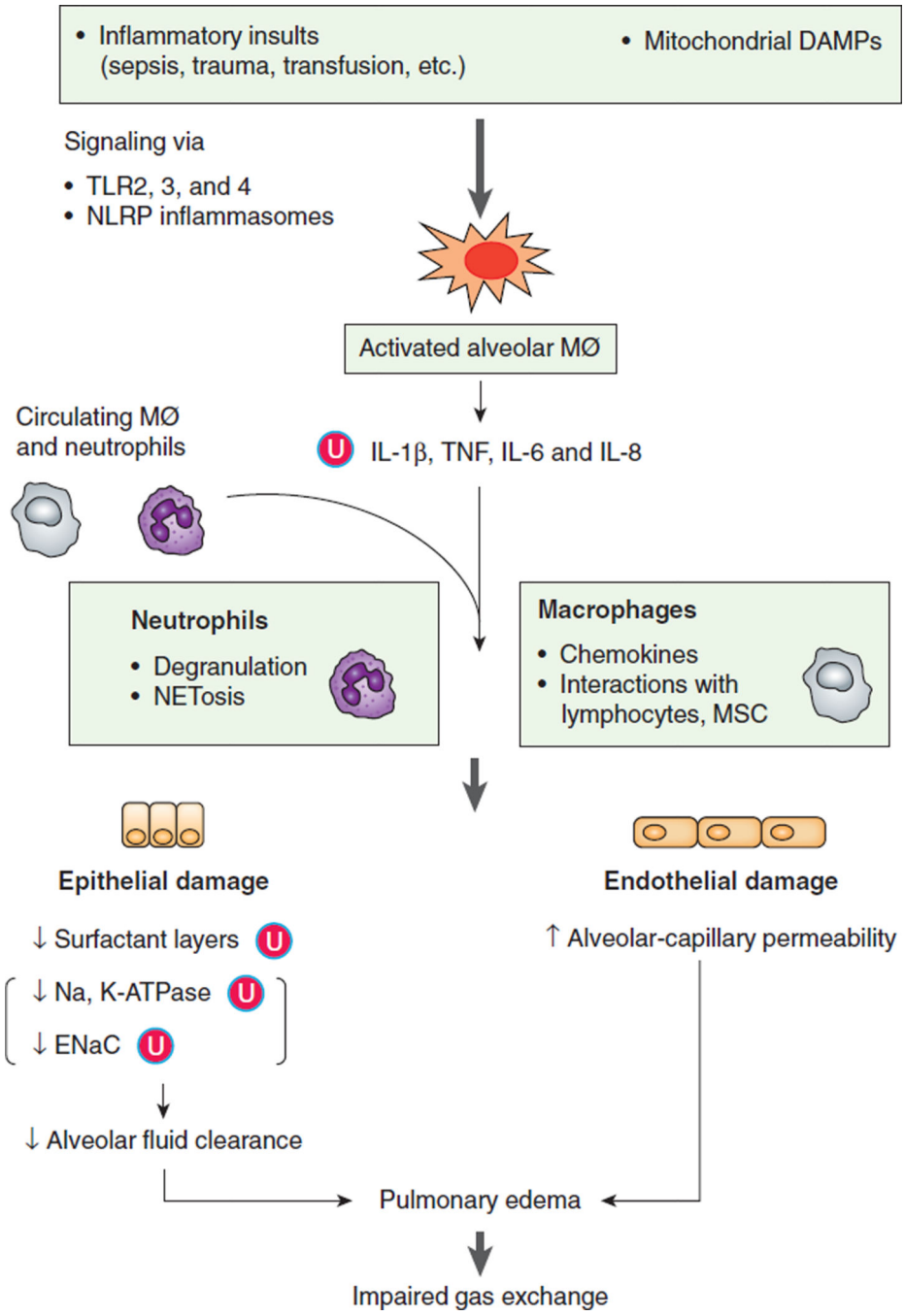

Figure 2. Pathophysiology of the acute respiratory distress syndrome

Initial inflammatory insults including mitochondrial DAMPs activate alveolar macrophages via TLRs and NLRs 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) interfering with gas exchange.

Ubiquitination (Ⓤ) 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 independent to leukocytes.

Mitochondrial DAMPs, mitochondrial-derived products released by cellular damage, which serve as danger-associated molecular patterns; TLRs, Toll-like receptors; NLRP, nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family, pyrin domain containing; Alveolar MØ, alveolar macrophage; NETs, neutrophil extracellular traps; MSC, mesenchymal stem cell; ENaC, epithelial Na+ channel; Ⓤ, ubiquitination.