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WHAT'S NEW IN INTENSIVE CARE

ARDS: what experimental models have taught us

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Acute respiratory distress syndrome (ARDS) is characterized by severe pulmonary inflammation, increased blood–gas barrier permeability, and hypoxemia, resulting in high mortality rates. Despite extensive research, there is still no specific therapy for ARDS and management remains supportive, mostly in the form of protective mechanical ventilation. These limited therapeutic options result from the complexity of ARDS pathophysiology, which involves multiple, overlapping signaling pathways depending on etiology. ARDS models are important to elucidate the mechanisms underlying pathogenesis, progression, and resolution of this syndrome, as well as to develop therapeutic approaches [1]. This brief review will address the main features of human ARDS that may be modeled experimentally, major current models of ARDS, and what these models have taught us concerning pathophysiology and new therapeutic strategies.

An ideal model for ARDS research should reproduce the parameters found in human ARDS. However, since models that perfectly mimic human ARDS are lacking, a committee was organized to determine which features characterize ARDS in animals and identify the optimal methods to assess these features [2].

ARDS triggers are numerous, as are the animal models used to study this syndrome. The many etiologies of ARDS can be broadly classified into two categories: direct insults to the lung epithelium (pulmonary ARDS) and indirect insults to vascular endothelium (extrapulmonary ARDS), determined by an acute systemic inflammatory response [3]. Pulmonary ARDS can be induced experimentally by administration of bacteria or bacterial products such as lipopolysaccharide (LPS), hydrochloric acid or gastric particulates, high inspired fractions of oxygen, depletion of surfactant by repeated saline lavage, induction of ischemia/reperfusion by hilar clamping, or mechanical stretch secondary to injurious mechanical ventilation. Extrapulmonary ARDS can be induced using standard models of sepsis (cecal ligation and puncture [CLP], intravenous administration of bacteria or LPS, mesenteric ischemia/reperfusion), paraquat, and oleic acid. More recently, two-hit models were developed using saline lavage or LPS followed by mechanical ventilation, CLP followed by hemorrhage, or peritoneal sepsis combined with gut ischemia/reperfusion [4] (Table 1). Selection of ARDS models depends on local availability, cost, number of animals (if survival is the study endpoint, rodents are ideal), blood sampling requirements (if multiple samples are needed, large animals should be used), and measurement of inflammatory mediators, receptors, or other proteins (large animals lack specific reagents to measure these parameters).

What have animal models taught us?

Pathophysiology

ARDS pathophysiology is complex and involves multiple molecular, cellular, and physiological mechanisms, which hinders organization of these factors into a single pathogenetic pathway. Alveolar macrophages participate by orchestrating the inflammatory process, recruiting neutrophils and circulating macrophages to the site of lung damage [5]. Neutrophil extracellular traps (NETs), which are found in different ARDS models (e.g., endotoxin, influenza), are produced by neutrophils to trap bacterial, fungal, and viral pathogens [6]. These cells release cytokines, proteases, reactive oxygen species, eicosanoids, and phospholipids, thus perpetuating the inflammatory response and damaging epithelial and endothelial cells. Type II epithelial cell damage reduces surfactant production and disrupts normal fluid transport, impairing edema resolution, while endothelial cell injury increases vascular permeability, leading to edema formation.

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Table 1 Experimental models of ARDS

Model	Lung features	Advantages	Disadvantages
Pulmonary			
Intratracheal endotoxin (LPS)	Neutrophil infiltration, interstitial and alveolar edema, epithelial cell damage (apoptosis), early increase in collagen fiber	Ease of administration, reproducibility, potent activator of innate immune response	Less endothelial cell damage; purity may vary, and LPS can be contaminated with bacterial lipoproteins
Hydrochloric acid	Airway and alveolar epithelium injury, hemorrhage, necrosis and apoptosis of epithelial cells, interstitial and alveolar edema, but modest neutrophil infiltration. Late fibrosis	Useful to study physiological impact of ARDS and ventilatory strategies	Narrow range between injurious and noninjurious dose, neutrophilic response less marked in human ARDS, humans do not aspirate pure HCl
Hyperoxia	Alveolar edema, hemorrhage, neutrophilic infiltration in interstitium; proliferation of type II epithelial cells, endothelial cells, and fibroblasts	Good model of hemorrhagic damage	Neutrophilia in interstitium and alveoli less marked than in human ARDS; direct relationship to human ARDS is unclear
Bacteria (aerosolized, direct intranasal, endotracheal, or endobronchial)	Neutrophil infiltration in interstitium and alveoli, epithelial cell damage (apoptosis and necrosis), alveolar wall thickening. Late epithelial cell proliferation. Injury resolves 7 days after inoculation	Good model of pneumonia-induced ARDS	Technical difficulties due to bacteria culture, ARDS features depend on inoculum size
Surfactant depletion (saline lavage)	Few neutrophils; alveolar collapse with minimal tissue damage, unless followed by a second hit	Good model to test ventilatory strategies	Requires intubation, mechanical ventilation, and general anesthesia. Lung damage repair with the time course of ARDS
Ventilator-induced lung injury	Epithelial cell damage, interstitial edema, alveolar capillary damage	Clinically relevant, provides opportunities to change practice related to mechanical ventilation strategies	Complex model; in the absence of an additional stimulus or extremely high tidal volumes, does not induce substantial lung injury
Lung ischemia reperfusion	Increased alveolar-capillary damage with interstitial and alveolar edema, neutrophil infiltration and hemorrhage, few fibrosis	Reproduce the main features of human ARDS	Technically challenging
Extrapulmonary			
Mesenteric ischemia/reperfusion	Alveolar edema, neutrophil infiltration, endothelial cell damage	Useful to evaluate ARDS mechanisms in the presence of mesenteric ischemia–reperfusion	Technically challenging
LPS (i.v. or i.p.)	Neutrophil accumulation in capillaries and interstitium, but little inflammatory cell influx into the alveolar space; increase in pulmonary arterial pressure and intravascular thrombosis may occur. Early fibrosis that repairs late in the course of lung injury	Useful to evaluate mechanisms of sepsis-induced ARDS, easy administration, potent activator of innate immune responses	High interspecies variability in response to LPS, provides an incomplete picture of the effects of live bacteria in the lungs

Table 1 continued

Model	Lung features	Advantages	Disadvantages
Bacteria (i.v.)	If animals survive, the initial phase is followed by hemodynamic stabilization and lung damage (neutrophil infiltration, interstitial edema, intravascular congestion). Minimal epithelial cell damage	Useful to evaluate mechanisms of sepsis-induced ARDS	Alveolar epithelium is relatively resistant to i.v. bacteria; unless the inoculum is very high, there is little neutrophilic alveolitis or intra-alveolar edema formation. Experimental bacteremia is not associated with the full histopathological picture of ARDS, including epithelial damage and hyaline membrane formation
Cecal ligation and puncture	Mild lung injury with less impressive intra-alveolar inflammation and hyaline membrane, neutrophil infiltration	Best animal model of sepsis	Injury is localized mainly in the endothelium, requires major surgery, limited reproducibility. Sepsis severity depends on needle size and number of punctures
Oleic acid (i.v.)	Toxic to endothelial cells, yielding necrosis, alveolar hemorrhage, intravascular thrombosis, neutrophil infiltration, and alveolar edema. Type II epithelial cell proliferation without fibrosis	Developed as an attempt to reproduce ARDS due to lipid embolism, induces characteristics of ARDS early and rapidly, good reproducibility. Excellent model to study ventilatory strategies	Requires i.v. administration (challenging in small animals); ARDS associated with lipid injury or bone trauma is rare in humans; no evidence that the pathophysiology of oleic acid injury is similar to that underlying sepsis-associated ARDS
Paraquat (i.p.)	Diffuse alveolar damage characterized by macrophage and neutrophil infiltration, interstitial edema. Depending on dose, alveolar edema and hemorrhage can be observed	Low cost, rapid effect, ease of administration	Paraquat-induced ARDS is exceedingly rare in the clinical setting
Combination			
Saline + MV	Alveolar-capillary damage, neutrophil infiltration into airspaces and interstitium, hyaline membrane formation	Reproduces main features of human ARDS	Technically challenging
CLP + hemorrhage	Interstitial edema, intra-alveolar hemorrhage, increase in intravascular and interstitial neutrophils	Reproduces important features of human ARDS	Technically challenging
LPS + oleic acid	Accumulation of neutrophils, intra-alveolar proteinaceous debris and few hyaline membranes	Reproduces important features of human ARDS	Technically challenging
CLP + gut ischemia reperfusion	Accumulation of neutrophils in the interstitium and alveoli, proteinaceous debris in the alveolar space, thickened alveolar walls	Reproduces main features of human ARDS	Technically challenging

New aspects of ARDS pathophysiology

Both the development and resolution of ARDS seem to be related to toll-like receptor (TLR) signaling pathways. TLRs are transmembrane proteins that recognize pathogen-associated molecular patterns (PAMPs) (bacterial cell wall components) and damage-associated molecular pattern (DAMPs) (intracellular proteins, namely heat shock proteins and extracellular matrix fragments) [5]. Stimulation of TLRs by PAMPs or DAMPs leads to activation of transcription factors (e.g., AP-1, NF-κB) and production of mediators. Nucleotide-binding oligomerization domain-like receptors (NLRs) are cytosolic receptors that respond to different PAMPs and DAMPs and are responsible for the sterile inflammation response. ARDS is also characterized by activation of the ubiquitin–proteasome system, increasing expression of ubiquitin within type II epithelial cells. Despite substantial progress, further experimental studies are required to elucidate the aforementioned mechanisms and help design future ARDS therapies.

Potential new therapeutic targets in ARDS

Different therapies have been tested in animals before clinical studies. The challenge is to extrapolate the data obtained from animal studies to human patients.

Several anti-inflammatory agents have failed to show any mortality benefit in ARDS. Inhaled corticosteroids, angiotensin-converting enzyme inhibitors, peroxisome-proliferator receptor agonists, tyrosine kinase inhibitors [7], proteasomes, and inflammasomes have been studied in experimental ARDS. Various new approaches aim to repair the endothelium. FG-4497 has been shown to support the integrity of adherens junctions, thus preventing loss of endothelial barrier function [8]. Mesenchymal stromal cells (MSCs) are also effective in experimental ARDS, as they secrete paracrine factors that regulate alveolar-capillary permeability and reduce inflammation, fibrosis, and infection in experimental ARDS [9]. Gene therapy aiming to increase expression of the ion channels and pumps required for alveolar fluid clearance is another possible future therapy [10]. Use of low tidal volumes (V_T) improves survival in ARDS, but V_T itself does not seem to play an important role in ventilator-associated lung injury (VALI), unlike driving pressure, for which there is no safe limit. As VALI is an important cause of poor clinical outcomes in ARDS patients, strategies that reduce its incidence and severity are being sought. Variable ventilation, management of spontaneous breathing, and different recruitment maneuver techniques have been evaluated in experimental ARDS [11–13]. Additionally, evaluation of transpulmonary pressure will provide a new approach to mechanical ventilation settings [14].

Summary

Improving the course and outcome of patients with ARDS presents a considerable challenge. Animal studies attempting to mimic human ARDS have been useful and will continue to provide valuable insight into both the mechanisms underlying pathogenesis, progression, and resolution of this syndrome and ways in which its course can be modulated therapeutically. Unbiased methodologies, including metabolomics, proteomics, gene expression analysis, and genome-wide association studies, have the potential to identify new mediators and pathways that are mechanistically important. Although no single model perfectly resembles human ARDS, the best model is that which best addresses researchers' experimental issues and can simulate all adjuvant therapies used in the ICU. The most exciting current treatment strategy is to reduce ARDS incidence with preemptive application of protective mechanical ventilation to patients at high risk of developing ARDS.

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Compliance with ethical standards

Conflicts of interest

The authors declare no conflicts of interest.

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