The acute respiratory distress syndrome (ARDS) is a major cause of mortality. ARDS is characterized by its acute onset, bilateral pulmonary infiltrates, severe hypoxemia, and pulmonary edema of noncardiac origin. Pronounced morphological changes occur in the lung parenchyma and are associated with impaired lung function, which is partly reversible. Mechanical ventilation is the most important supportive therapy for patients with ARDS, but it can induce or aggravate lung injury—an entity referred to as ventilator-induced lung injury (VILI). ARDS is also characterized pathologically by an early exudative, inflammatory phase, followed in many patients by a fibrotic phase. The inflammatory phase is the focus of more studies—a PubMed search for ARDS AND inflammation yielded 561 articles, whereas a search for ARDS AND fibrosis yielded 260 articles.

In this review, the authors explore recent advances in the field of pulmonary fibrosis in the context of acute respiratory distress syndrome, concentrating on its relevance to the practice of mechanical ventilation, as commonly applied by anesthetists and intensivists. The authors focus on the thesis that mechanical ventilation—or more specifically, that VILI—may be a major contributor to lung fibrosis. The authors critically appraise possible mechanisms underlying the mechanical stress–induced lung fibrosis and highlight potential therapeutic strategies to mitigate this fibrosis.

ABSTRACT

One of the most challenging problems in critical care medicine is the management of patients with the acute respiratory distress syndrome. Increasing evidence from experimental and clinical studies suggests that mechanical ventilation, which is necessary for life support in patients with acute respiratory distress syndrome, can cause lung fibrosis, which may significantly contribute to morbidity and mortality. The role of mechanical stress as an inciting factor for lung fibrosis versus its role in lung homeostasis and the restoration of normal pulmonary parenchymal architecture is poorly understood. In this review, the authors explore recent advances in the field of pulmonary fibrosis in the context of acute respiratory distress syndrome, concentrating on its relevance to the practice of mechanical ventilation, as commonly applied by anesthetists and intensivists. The authors focus on the thesis that mechanical ventilation—or more specifically, that VILI—may be a major contributor to lung fibrosis. The authors critically appraise possible mechanisms underlying the mechanical stress–induced lung fibrosis and highlight potential therapeutic strategies to mitigate this fibrosis. (Anesthesiology 2014; 121:189-98)

T H E acute respiratory distress syndrome (ARDS) is a major cause of mortality. ARDS is characterized by its acute onset, bilateral pulmonary infiltrates, severe hypoxemia, and pulmonary edema of noncardiac origin. Pronounced morphological changes occur in the lung parenchyma and are associated with impaired lung function, which is partly reversible. Mechanical ventilation is the most important supportive therapy for patients with ARDS, but it can induce or aggravate lung injury—an entity referred to as ventilator-induced lung injury (VILI). ARDS is also characterized pathologically by an early exudative, inflammatory phase, followed in many patients by a fibrotic phase. The inflammatory phase is the focus of more studies—a PubMed search for ARDS AND inflammation yielded 561 articles, whereas a search for ARDS AND fibrosis yielded 260 articles.

In this review, we explore recent advances in the field of pulmonary fibrosis in the context of ARDS, concentrating on its relevance to the practice of mechanical ventilation, as commonly applied by anesthetists and intensivists. We focus our discussion on the thesis that mechanical ventilation—or more specifically, that VILI—may be a major contributor to lung fibrosis. We critically appraise possible mechanisms underlying the mechanical stress–induced lung fibrosis and highlight potential therapeutic strategies to mitigate this fibrosis.
Clinical Evidence of Lung Fibrosis in ARDS

Many patients with ARDS survive the acute phase, but subsequently go on to die, often with evidence of significant pulmonary fibrosis.8 Severe fibrosis was demonstrated to be a frequent complication in ARDS as early as the 1990s.9 Lung histologic studies of patients with late ARDS suggested ongoing inflammatory injury together with progressive fibrosis.10–12 Areas of exudation are found adjacent to advanced fibrosis, and epithelial and endothelial injury is pronounced in the late phase of ARDS10–12 (fig. 1).

Patients with severe ARDS frequently require prolonged mechanical ventilation, with a mean duration of approximately 12 days reported in this subgroup.13,14 A recent study15 showed that patients with ARDS with greater fibrotic changes required more prolonged mechanical ventilation, and this in turn was associated with an increased severity of systemic organ failure.

On the basis of the open-lung biopsies, Papazian et al.16 found evidence of pulmonary fibrosis in 53% of ventilated patients who had ARDS for 5 days or more. In a prospective cohort study of 25 consecutive patients with ARDS who were receiving mechanical ventilation, Martin et al.13 reported that the mortality rate was 57% (8 of 14 patients) in those who developed lung fibrosis with zero mortality in patients without evidence of fibrosis.

Madtes et al.17 studied 74 consecutive patients during the first 2 weeks after the onset of ARDS. Transforming growth factor-β (TGF-β) was detected in the lung lavage fluid of 90% of patients with ARDS but was not detectable in 13 normal volunteers. The mortality rate was four times higher in patients with both increased concentrations of TGF-β and procollagen type III (PCIII) in lung lavage fluids at day 7 compared with that in patients who had low TGF-β and PCIII levels. Marshall et al.18 demonstrated that the PCIII concentrations were significantly increased in nonsurvivors of ARDS as compared with survivors. The patients with ARDS who survived at least 14 days had less active fibroproliferation as reflected by lower baseline levels of PCIII in the lungs.19 These human studies suggest that, in ARDS survivors, a key event is the transition to a normal repair process, such as that evidenced by reduced collagen content in lung lavage fluids, and this is consistent with the resolution phase of ARDS.17

The development of fibrosis seems to be an important determinant of mortality attributable to mechanical ventilation regardless of the cause of ARDS.13 Taken together, the clinical data support the concept that pulmonary fibrosis represents a pathologic response in patients with ARDS.

There seems to be a “fibrosis paradox,” in that fibrosis leads to prolongation of ARDS and critical illness, and worsens outcome. Patients who die of ARDS show clear evidence of pulmonary fibrosis, even when they die in relatively early stages of ARDS. Yet, it is hard to find much trace of fibrosis in ARDS survivors. In fact, most long-term survivors of ARDS have relatively little evidence of fibrosis as measured by PCIII levels in the lung lavage although they may have mildly reduced vital capacity and diffusion capacity.20 This apparent “paradox” has two important implications. First, mechanical ventilation may be a key driver of the fibroproliferative response. Consequently, the removal of mechanical ventilation as early as possible may be a key—and previously under recognized—factor in enabling normal lung repair. Second, strategies aimed directly at attenuating the fibroproliferative response may enhance survival in patients with ARDS.

Pathophysiology of ViLi

The time course of lung structural damage in ARDS has been classically thought to occur in three phases: (1) an inflammatory exudative phase characterized by diffuse alveolar injury with necrosis of alveolar type I cells, increase in vascular permeability, and influx of inflammatory cells; (2) a proliferative phase, starting approximately 72 h after the initial insult and lasting approximately 7 days, associated with alveolar epithelial type II cell repair; mesenchymal cells, including interstitial fibroblasts and myofibroblasts, migrate, proliferate, and produce extracellular matrix (ECM) proteins such as collagen18,21–24; and (3) a fibrotic phase with up-regulation of collagen synthesis.25 The proinflammatory and profibrotic event is the transition to a normal repair process, such as that evidenced by reduced collagen content in lung lavage fluids, and this is consistent with the resolution phase of ARDS.17

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Fig. 1. Histologic findings of hematoxylin-eosin staining at open-lung biopsy in a patient with acute respiratory distress syndrome. The photomicrograph shows myxoid fibrosis, fibroblastic and inflammatory cell infiltration of the interstitium, and scattered collapsed alveoli (A) and subintimal deposition of loose myxoid collagen in an arteriole (B). Reproduced, with permission, from the American College of Chest Physicians and adapted from Meduri GU et al. Chest 1994; 105:1516–27. Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.
responses may become persistent or uncontrolled during mechanical ventilation, and can lead to pulmonary fibrosis, with subsequent decline in lung function. However, the classic time course of these events is not as distinct as portrayed above. For example, there is evidence of fibrotic change in the earliest stages of ARDS.\textsuperscript{15,18,26} Several studies have demonstrated that PC III concentrations in serum and lung lavage fluids are increased in the ARDS group compared with control patients at 24 h\textsuperscript{18} with collagen synthesis commencing within 24 h of the development of ARDS.\textsuperscript{26} In one study, 47\% of patients had computed tomography evidence of fibrosis on the first day of ARDS.\textsuperscript{15} Given the robust correlation between early collagen synthesis and mortality, mechanical ventilation may have altered collagen synthesis early in the course of ARDS.

**Biophysical Insults**

The forces generated during mechanical ventilation can impact this time course by causing VII\textsuperscript{25} and can impact clinical outcomes. For example, a ventilation strategy aimed at decreasing lung stretch significantly decreased mortality.\textsuperscript{28} Conversely, ventilation with high transpulmonary pressures can lead to injury due to “barotrauma,” that is, air leaks caused by overdistension. Alveolar overdistension can also lead to “volutrauma,” characterized by increased alveolar-capillary leak and pulmonary edema.\textsuperscript{29,30} Moreover, repeated opening and collapsing of alveolar space can also contribute to injury \textit{via} a mechanism called “atelectrauma.”\textsuperscript{31} There is emerging evidence demonstrating that barotrauma, volutrauma, or atelectrauma may influence the course of lung remodeling.\textsuperscript{32,33}

At the cellular level, mechanical stretch of alveolar epithelial cells can result in loss of tight junction structure and cell–cell attachment associated with a decrease in the intensity of the peripheral occludin band and actin perturbations.\textsuperscript{34} Disruption of the epithelial layer and failed repair mechanisms after mechanical stretch can result in epithelial–mesenchymal transition (EMT),\textsuperscript{35} a mechanism discussed in greater detail later in the article. A recent study using a three-dimensional cell culture system observed F-actin clumps during mechanical stretch, suggesting that actin cytoskeleton remodeling plays an important role in fibrosis formation\textsuperscript{40} (fig. 2).

Biomechanical interactions between cells and the ECM proteins can lead to the reorganization and remodeling of the ECM. Collagen is the most important stress-bearing constituent of the parenchymal tissue and plays a critical role in the ECM. Collagen is the most important stress-bearing constituents of the parenchymal tissue and plays a critical role in fibrosis formation\textsuperscript{36} (fig. 2).

**Fig. 2.** Mechanical stretch impairs alveolar epithelial integrity. The alveolar epithelial tight junction is consists of several constituents of connected proteins. Occludin is a transmembrane protein known to be associated with F-actin, either directly or indirectly modulating the tight junction structure. Mechanical stretch of alveolar epithelial cells can result in loss of tight junction structure and cell–cell attachment associated with decrease in the expression or increase in degradation of occludin and actin perturbations. The actin cytoskeleton remodeling plays an important role in fibrosis formation in the lung. ATI = alveolar type I; ATII = alveolar type II.

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**Biochemical Insults**

It has become clear that mechanical ventilation causes injury not only by structural disruption of the lung but also by induction of inflammatory responses associated with mediator release that can worsen lung injury and potentially cause systemic organ dysfunction.\textsuperscript{46} This is termed “biotrauma.” The physical forces generated during mechanical ventilation can induce the release of cytokines, chemokines, and growth factors\textsuperscript{47} in lungs with preexisting injury or in previously healthy lungs (fig. 3).

The type-1 and type-2 helper T-cell (Th1 and Th2) cytokines and chemokines released in response to lung stretch or strain, together with inflammatory cell recruitment, may play a role in the progression from injury to fibroproliferation. The Th1 response, with increased expression of interferon-γ, interleukin (IL)-2, IL-12, and IL-18, may play a role helping tissue repair, whereas the Th2 cytokines, including IL-4, IL-5, IL-10, and IL-13, tend to promote fibroproliferation.\textsuperscript{48} Certain chemokine receptors expressed on lung epithelial cells, such as CXCR3\textsuperscript{49–51} for Th1 responses, and CCR4,\textsuperscript{52} CCR8,\textsuperscript{53} and CXCR4\textsuperscript{54–56} for Th2 responses, can modulate
the transition from lung restoration and repair to progressive lung fibrosis.

In a model of endotoxin-induced lung injury in mice, investigators demonstrated that a subset of CD4+ lymphocytes named T regulatory cells, expressing the surface marker CD25 (IL-2 receptor α) and the transcription factor Forkhead box protein 3, played an important role in resolving lung injury. This finding suggests that the influx of inflammatory cells such as T regulatory cells during the exudative phase may play a critical role in the crosstalk between innate and adaptive immune systems that normally would modulate the transition from injury to repair in resolving lung injury. ATI = alveolar type I; ATII = alveolar type II.

Cellular Mechanisms of Mechanical Ventilation–associated Lung Fibrosis

The pathophysiology of mechanical ventilation–associated lung fibrosis may involve a number of distinct cellular mechanisms including activation of stretch-sensitive ion channels in pulmonary epithelial and endothelial cells, disruption of cell plasma membranes, and direct conformational changes in membrane-associated molecules and their cell–cell or cell–ECM interactions. We will focus the following discussion on possible cell sources and activation of a few signaling pathways in the context of mechanical ventilation–associated lung fibrosis (fig. 4).

Cell Sources

Epithelial–Mesenchymal Transition. Epithelial–mesenchymal transition is a biological process whereby epithelial cells undergo transition to a mesenchymal phenotype, that is, fibroblasts and myofibroblasts. EMT plays an important role in the process of epithelial repair after injury. EMT can be characterized by loss of lung epithelial markers including collagenase production seem to be the key events contributing to the lung-remodeling process after VILI, leading to the development of pulmonary fibrosis.
surfactant proteins (surfactant protein B, C, and D, junctional and cell–cell adhesion proteins (i.e., E-cadherin and claudin), and cytoskeletal organization proteins (i.e., cytokeratin-8) associated with gain of mesenchymal markers including α-smooth muscle actin, N-cadherin, vimentin, and fibronectin.65,68–70 The failure of lung epithelial repair stretch induced EMT in vitro in patients with ARDS may potentially lead to activation of EMT-signaling pathways. In a recent study, the application of cyclic mechanical stretch induced EMT in vitro in primary murine alveolar type II epithelial cells.55 Using an in vivo mouse model of acid aspiration–induced ARDS followed by a ventilatory strategy causing overdistension, we demonstrated impaired lung mechanics associated with increased lung hydroxyproline content and higher expression of TGF-β, β-catenin, and mesenchymal markers including α-smooth muscle actin and vimentin at both gene and protein levels.59 In contrast, the expression of epithelial markers including cytokeratin-8, E-cadherin, and prosurfactant protein B was decreased.99 The specific EMT pattern in response to mechanical stretch was reproduced in an in vitro system using cultured human lung epithelial cells.59 These results suggest that mechanical stretch alone can induce EMT and hence may play an important role in mediating the VILI-associated lung fibrosis.59 Fibroproliferation. Resident fibroblasts can proliferate and participate in the lung repair process. Pulmonary fibroblasts can be activated in response to Th2 cytokines and growth factors, thereby increasing fibroproliferation.71 Mechanical ventilation using high tidal volumes (30 ml/kg) has been demonstrated to induce pulmonary fibroproliferation in a mouse model of ARDS.72 Similar results were obtained in rats ventilated with more clinically relevant (7 ml/kg) tidal volumes.73 In fetal human lung fibroblasts, exposure to cyclic stretch for 48 h resulted in an increased expression of the cell cycle–regulated gene calcyclin. Calcyclin gene expression was also up-regulated in isolated-perfused rat lungs exposed to high stretch for 4 h.74 Whether the calcyclin gene is a biomarker of the fibroblast cell cycle or plays a direct role in the mechanotransduction signaling pathways leading to fibroproliferation remains to be elucidated.

In a prospective observational cohort clinical study, Ichikado et al.15 performed high-resolution computed tomography in 85 patients on the day of diagnosis of ARDS. They demonstrated that lung fibroproliferation as assessed by computed tomography was a prognostic indicator for ventilator dependency, increased mortality, and increased susceptibility to multiple organ dysfunction.

Circulating Fibrocytes. Fibrocytes are a distinct subpopulation of bone marrow–derived fibroblast-like cells which can be found in tissue95 or as circulating cells in peripheral blood.76 They are defined as cells that dually express leukocyte (CD45) and mesenchymal (collagen I) markers.77,78 Fibrocytes can express α-smooth muscle actin, indicative of myofibroblast differentiation.79 Studies have shown that circulating fibrocytes can travel to the lung and serve as progenitors for interstitial fibroblasts.78,80,81 In a clinical setting, Quesnel et al.82 reported that fibrocytes were detected in bronchoalveolar lavage fluid of 90 of 92 patients (98%) with ARDS treated with mechanical ventilation. They suggested that a fibrocyte percentage greater than 6% in lung lavage fluid was an independent predictor for mortality in mechanically ventilated patients with ARDS.52 Larger studies are needed to confirm these findings.

Immune Cells. Neutrophil infiltration into the alveolar space is a hallmark of ARDS.83 Although immune cells—other than fibrocytes—are not directly transformed into fibroblasts, the persistence of neutrophils in the lung likely plays a role in modulating the fibrotic process.84,85 Activated neutrophils release matrix proteins and chemotactic factors that not only induce further neutrophil lung infiltration86–89 but also facilitate epithelial cell remodeling.90 A correlation between the numbers of alveolar macrophages and pulmonary fibrosis was reported in a recent study.91 The investigators demonstrated that ventilated patients with ARDS who had greater numbers of fibroblasts in lung lavage fluid had a higher percentage of alveolar macrophages although whether the alveolar macrophages contributed to the fibrotic development was not addressed in this clinical study.91 It is noteworthy that activated macrophages are categorized into M1 and M2 phenotypes depending on the patterns of their inflammatory responses. Stimulation of normal human alveolar macrophages with the Th2 cytokines IL-4 and/or IL-10 resulted in an alveolar macrophage phenotype shift to M2 that has been implicated in the pathogenesis of pulmonary fibrosis.92 Taken together, the literature suggests that alveolar macrophages may not simply be bystanders but may play an important role contributing to the development of pulmonary remodeling in ARDS.

Signaling Pathways Involved in Mechanical Ventilation–associated Lung Fibrosis

Extracellular matrix plays an important role in the biomechanical behavior of the lung parenchyma, and mechanical ventilation has been shown to activate ECM elements such as collagen, elastin, fibronectin, laminin, lumican, and proteoglycan.41,93–97 The expression of different components of the pulmonary ECM varies during the course of lung fibrosis. PCIII fibers are predominant early in the course of lung injury, whereas PCI is more prevalent in the late phase.94 The ECM interacts with TGF-β and mesenchymal tissue growth factors modulating the lung-remodeling processes.98–101 Mechanical stretch induced accumulation of the short fragment hyaluronan (sHA) of ECM glycosaminoglycan associated with an increase in IL-8 production in pulmonary fibroblasts and lung epithelial cells.102 The increased inflammatory responses, lung injury, neutrophil infiltration, cyto-kine production, and lung edema observed in wild-type mice ventilated with high tidal volume were attenuated in hyaluronan synthase-3 gene knockout mice.102,103 Moreover, a recent study demonstrated that cyclic mechanical stretch of primary
Murine alveolar type II epithelial cells for 4 h resulted in an increased expression of the sHA in the absence of fibroblasts. Moreover, mechanical stretch–induced sHA production has been shown to up-regulate the Wnt-inducible signaling protein 1. In turn, the Wnt/β-catenin signaling pathway is known to mediate EMT, which has been reported to be activated by mechanical ventilation at high tidal volumes in animals without preexisting lung injury. These data suggest that sHA produced and released during lung injury induces EMT in alveolar type II epithelial cells, and sHA may be a novel therapeutic target in ventilator-associated lung fibrosis.

It is noteworthy that, in the kidney, mechanical stretch and subsequent renal tubular epithelial cell distension induced up-regulation of reactive oxygen species that in turn activated the cytoplasmic proline-rich tyrosine kinase-2 (Pyk2). Although this pathway has not been examined in the mechanically ventilated lung or in pulmonary epithelial cells subjected to in vitro mechanical stretch, human lung epithelial cells do express Pyk2. In mice deficient in the Pyk2 gene, the renal expression of TGF-β1 and connective tissue growth factor induced by mechanical stretch was significantly reduced. Thus, Pyk2 may be important in initiating stretch-induced fibrosis in the lung although this hypothesis has not been directly examined.

**Therapeutic Strategies**

There are no pharmacological therapies that have been proven to be effective in large-scale trials for ventilator-induced lung fibrosis. Meduri et al. reported that all patients with unresolved ARDS had a progressive increase in PCI and PCIII in plasma and in bronchoalveolar lavage fluids, and that administration of methylprednisolone decreased these molecules and improved patient outcome. In the ARDS Network study, patients with low baseline lung lavage levels of PCIII had a 60-day mortality of 35% in the methylprednisolone-treated arm versus 9% in the placebo arm. In contrast, there was a trend to lower mortality in the methylprednisolone group (4%) compared with that in the placebo group (19%) in patients with high baseline lavage levels of PCIII. This study suggests that the corticosteroids may be beneficial in the subset of patients most at risk of developing pulmonary fibrosis.

The mechanisms of action of methylprednisolone may be related to its antiinflammatory and/or antifibrotic properties. Corticosteroids have numerous actions that mitigate inflammation, such as inhibition of the adhesion, migration of leukocytes across the capillary wall, and blockade of nuclear factor-κB nuclear translocation. A recent review article suggested several therapeutic targets to limit ARDS-associated lung fibrosis by pharmacological interventions, including tyrosine kinase inhibitors, Src kinase inhibitors, histone deacetylase inhibitors, monoclonal antibodies, and blocking peptides that directly bind to growth factors or block receptor ligation binding to inhibit synthesis of matrix proteins. The potential for these approaches to attenuate mechanical stretch–induced fibrosis is unknown.

Because both ARDS and VILI are associated with inflammatory injury to the alveolar epithelial cells, mesenchymal stromal cells (MSCs) are interesting candidates to facilitate lung regeneration and repair (fig. 5). The demonstration that human MSCs exert benefit in a variety of in vitro and in vivo preclinical lung injury models is particularly exciting. MSCs are reported to secrete multiple paracrine factors that can protect epithelial cell membranes from damage, decrease inflammation, and inhibit bacterial growth.

Mesenchymal stromal cells have been demonstrated to enhance repair and restoration of physiologic function after VILI. The effects seem to be paracrine mediated and dependent on keratinocyte growth factor produced by the stromal cells. Both intratracheal and intravenous MSC delivery seem to enhance lung repair after VILI. A recent study in a mouse model of endotoxin-induced lung injury demonstrated that bone marrow–derived MSCs could transfer their mitochondria into lung epithelial cells resulting in increased alveolar adenosine triphosphate concentrations.

The investigators speculated that the mitochondrial transfer might have enhanced cellular bioenergetics and improved lung function. However, one has to be careful in potential application to humans because MSCs derived from mouse bone marrow and human umbilical cord blood have been shown to produce soluble factors that mediate lung fibroblast growth. At present, although MSCs demonstrate...
considerable promise, additional studies are needed to address significant deficits in our knowledge regarding mechanism(s) of action and the efficacy and the safety of MSCs in the treatment of patients with ARDS.

Conclusions
Recent evidence demonstrates that mechanical ventilation, particularly where significant overstretch occurs, may drive the pathogenesis of fibrosis in patients with ARDS. The application of mechanical ventilation in animal models of acute lung injury or the application of mechanical stress in vitro in lung epithelial cells can induce the development of lung fibrosis through fibroproliferation and EMT. Future studies are required to improve our understanding of these mechanisms so that we can develop novel approaches—pharmacologic or other—to prevent or treat the pulmonary fibrosis associated with mechanical ventilation in patients with ARDS.

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Correspondence
Address correspondence to Dr. Zhang: St. Michael’s Hospital, University of Toronto, Room 619 LKSKI, 30 Bond Street, Toronto, Ontario M5B 1W8, Canada. zhangh@smh.ca. This article may be accessed for personal use at no charge through the Journal Web site, www.anesthesiology.org.

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