Evolution of the Inflammatory and Fibroproliferative Responses during Resolution and Repair after Ventilator-induced Lung Injury in the Rat


ABSTRACT

Background: The time course and mechanisms of resolution and repair, and the potential for fibrosis following ventilation-induced lung injury (VILI), are unclear. We sought to examine the pattern of inflammation, injury, repair, and fibrosis following VILI.

Methods: Sixty anesthetized rats were subject to high-stretch; low-stretch, or sham ventilation, and randomly allocated to undergo periods of recovery of 6, 24, 48, and 96 h, and 7 and 14 days. Animals were then reanesthetized, and the extent of lung injury, inflammation, and repair determined.

Results: No injury was seen following low-stretch or sham ventilation. VILI caused severe lung injury, maximal at 24 h, but largely resolved by 96 h. Arterial oxygen tension decreased from a mean (SD) of 144.8 (4.1) mmHg to 96.2 (10.3) mmHg 6 h after VILI, before gradually recovering to 131.2 (14.3) mmHg at 96 h. VILI induced an early neutrophilic infiltration. Alveolar tumor necrosis factor-α, interleukin-1β, and transforming growth factor-β1 concentrations peaked at 6 h and returned to baseline within 24 h, while interleukin-10 remained increased for 48 h. VILI generated a marked but transient fibroproliferative response, which restored normal lung architecture without evidence of fibrosis.

Conclusions: High-stretch ventilation caused severe lung injury, activating a transient inflammatory and fibroproliferative repair response, which restored normal lung architecture without evidence of fibrosis.

What We Already Know about This Topic

• High lung stretch causes severe lung injury, which is termed ventilation-induced lung injury, but the factors that promote repair are unknown

What This Article Tells Us That Is New

• High stretch ventilation causes severe lung injury, activating a transient inflammatory and fibroproliferative repair response, which restores normal lung architecture without causing fibrosis

**HIGH** tidal volume ventilation can directly cause acute lung injury/acute respiratory distress syndrome (ALI/ARDS), particularly in patients undergoing ventilation for major surgery.† 1,2 It can increase the risk of ALI/ARDS in critically ill patients that do not already have ALI/ARDS,3 and it can worsen preexisting ARDS.4 The importance of this ventilation-induced lung injury (VILI) is emphasized by the finding that ventilatory strategies that minimize lung stretch have improved patient outcome.4 While preclinical studies to date have focused on the injury phase of ALI/ARDS, many patients die after the initial “injury” phase of

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their critical illness. A greater understanding of the cellular and molecular mechanisms that mediate alveolar regeneration and repair is necessary to develop therapeutic approaches that target this phase of the disease process.

Abnormal or dysregulated repair processes are associated with increased morbidity and mortality following ALI/ARDS. The factors influencing progression to fibrosis versus resolution of the injury are incompletely understood. In particular, the effect of excessive lung stretch on lung remodeling and fibroproliferation, and the potential for mechanical stretch to lead to disordered repair and lung fibrosis, is not well characterized. Cytokines, chemokines, and growth factors released as part of the inflammatory response to lung stretch, together with inflammatory cell recruitment, may play a role in the progression from injury to fibroproliferation. Transforming growth factor-β (TGF-β) plays a critical role in fibroproliferative responses, promoting fibroblast recruitment and activation, collagen synthesis, and inhibiting collagenase production. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) have also been implicated in the remodeling process following VILI. However, the precise roles of these factors in the resolution and repair process following VILI are unknown.

We wished to characterize the inflammatory and fibroproliferative responses during resolution and repair following VILI. We hypothesized that VILI generates a sustained fibroproliferative response, and that this results in disordered repair and lung fibrosis. We established a non-lethal rodent model of repair following VILI, similar to that previously described, and characterized the inflammatory, repair, and pro-fibrotic responses, as well as the time course of injury resolution and the potential for a sustained fibrotic response following high-stretch mechanical ventilation.

Materials and Methods

All work was approved by the Animal Ethics Committee of the National University of Ireland, Galway, and conducted under license from the Department of Health, Ireland. Specific-pathogen-free adult male Sprague-Dawley rats (Harlan, Bicester, United Kingdom) weighing between 350–450 g were used in all experiments. With the exception of the collection of the physiologic data, investigators were blinded to group allocation for all analyses.

High- and Low-stretch Ventilation Protocols

Anesthesia was induced with intraperitoneal ketamine 80 mg/kg (Ketalar; Pfizer, Cork, Ireland) and xylazine 8 mg/kg (Xylapan; Vétoquinol, Dublin, Ireland). After confirmation of depth of anesthesia by paw clamp, intravenous access was obtained via tail vein, laryngoscopy was performed by Welch Allyn Otoscope (Welch Allyn, Buckinghamshire, United Kingdom) and the animals were intubated with a 16-gauge intravenous catheter (BD Insyte; Becton Dickinson Ltd., Oxford, United Kingdom). The animals were ventilated using a small animal ventilator (CWE SAR 830 AP; CWE Inc., Ardmore, PA). Anesthesia was maintained with repeated intravenous bolus of alphaxalone/alfadalone 10–12 mg/kg (Saffan; Schering Plough, Welwyn Garden City, United Kingdom), and muscle relaxation was achieved with cisatracurium besylate 0.5 mg/kg (GlaxoSmithKline, Dublin, Ireland).

The animals were then allocated to ventilation under conditions of high-stretch or low-stretch “protective” ventilation. The high-stretch mechanical ventilation protocol comprised of the following settings: FiO2 of 0.3, inspiratory pressure 35 cm H2O, respiratory rate of 18 min⁻¹, and positive end-expiratory pressure of 0 cm H2O. When static compliance had decreased by 50%, high-stretch ventilation was discontinued and the animals were allowed to recover, and subsequently returned to their cages. The “low-stretch” protocol comprised of the following settings: FiO2 of 0.3, respiratory rate 80/min, tidal volume 6 ml/kg, and positive end-expiratory pressure of 2 cm H2O. An additional group, which was not subjected to anesthetic or mechanical ventilation, was also included as an uninjured sham comparison.

Assessment of Injury, Inflammation, and Repair

Anesthesia and Dissection. At 6, 24, 48, and 96 h and at 7 and 14 days following ventilation or sham procedure, the animals were anesthetized with intraperitoneal ketamine 80 mg/kg and xylazine 8 mg/kg; after confirming depth of anesthesia by absence of response to paw compression, intravenous access was gained via the dorsal penile vein and anesthesia maintained with intravenous boli of alphaxalone/alfadalone. Following this a tracheotomy tube (1 mm internal diameter) was inserted and secured, and intra-arterial access (22- or 24-gauge cannulae; Becton Dickinson, Franklin Lakes, NJ) was sited in the carotid artery. Sterile technique was utilized during all manipulations. Following confirmation of the absence of a hemodynamic response to paw clamp, cisatracurium besylate 0.5 mg/kg was intravenously administered to achieve muscle relaxation, and the lungs were mechanically ventilated (Model 683; Harvard Apparatus, Holliston, MA) at a respiratory rate of 80/min, tidal volume 6 ml/kg, and positive end-expiratory pressure of 2 cm H2O for 20 min, and indices of lung damage and repair assessed.

Measurement of Physiologic Variables. Intra-arterial blood pressure, peak airway pressures, and rectal temperature were recorded continuously. Arterial blood gas analysis was performed following commencement of mechanical ventilation. Static inflation lung compliance measurements were performed by injecting incremental 1 ml of room air via the tracheotomy tube, and measuring the pressure attained 3 s after each injection, until a total volume of 5 ml was injected. At the end of the protocol, the inspired gas was altered to a FiO2 of 1.0 for 15 min, and an arterial blood sample was then taken for calculation of the alveolar-arterial oxygen gradient. Heparin (400 IU/kg, CP Pharmaceuticals, Wrexham,
United Kingdom) was then administered intravenously, and the animals were then killed by exsanguination.

**Tissue Sampling and Assays.** Immediately postmortem, the heart-lung block was dissected from the thorax, and bronchoalveolar lavage (BAL) collection was performed as previously described. Total cell numbers per milliliter in the BAL fluid were counted, and differential cell counts were performed. The concentrations of interleukin (IL)-1, IL-8, IL-6, tumor necrosis factor-α, and IL-10 in BAL fluid were determined using a commercially available bio-plex multiplex bead-based rat cytokine assay system (Bio-Rad Life Science, Hercules, CA). The concentration of total protein in BAL fluid was determined using a Micro BCATM Protein assay kit (Pierce, Rockford, IL) as previously described. The concentration of TGF-β1 and keratinocyte growth factor (KGF) in BAL fluid was determined using quantitative sandwich enzyme-linked immunosorbent assay (R&D Systems, Abingdon, United Kingdom). This KGF assay has been validated for use in rats.

BAL fluid and homogenate MMP-2 and -9 concentrations were measured by gelatin zymography as previously described. To determine relative concentrations of MMP-1, -3, -8, -13 and TIMP-2, BAL was mixed in 1:1 ratio with βmercaptoethanol buffer as previously described and 50 μl loaded onto a 10% polyacrylamide gel. For homogenates, an aliquot containing 50 μg total protein, as determined by Bradford protein assay, was loaded onto a 10% polyacrylamide gel, and Western blotting carried out as previously described. Proteins were detected by chemiluminescence (Supersignal West pico/femto chemiluminescent substrate kit; Pierce, Rockford, IL). Primary antibodies used were polyclonal rabbit anti-rat MMP-3 antibody at 1:1,000 dilution, polyclonal rabbit anti-rat MMP-8 antibody at 1:1,000 dilution, mouse anti-rat MMP-13 at 1:400 dilution, and mouse anti-rat TIMP-2 at 1:1,000 dilution (all United Chemi-Con, Rosemont, IL). During electrophoresis, samples were placed alongside a standard aliquot of chemiluminescent marker (R&D Systems, Abingdon, United Kingdom). This KGF assay has been validated for use in rats.

**Histologic and Stereologic Analysis.** The left lung was isolated and fixed for morphometric examination as previously described. Briefly, the pulmonary circulation was first perfused with normal saline at a constant hydrostatic pressure of 25 cm H₂O until the left atrial effluent was clear of blood. The left lung was then inflated through the tracheal catheter using paraformaldehyde (4% wt/vol) in phosphate-buffered saline (300 mOsmol) at a pressure of 25 cm H₂O. Paraformaldehyde was then instilled through the pulmonary artery catheter at a pressure of 62.5 cm H₂O. After 30 min, the pulmonary artery and trachea were ligated, and the lung was stored in paraformaldehyde. The extent of histologic lung damage was determined using quantitative stereological techniques as previously described.

**Pro-collagen 1 and 3 Transcription.** Total RNA was extracted from the lungs of rats using Tri-Reagent (Sigma-Aldrich, Wicklow, Ireland) as previously described. One μg of the RNA was reverse transcribed using an Improm II Reverse Transcription System (Promega, Southampton, United Kingdom). The complementary DNA, diluted 1:20, was amplified using polymerase chain reaction primers to pro-collagen I peptide: forward 5′-TCATCGAATAACAAACCCACA-3′; reverse 5′-GCAGGGCCATGTCAT-3′; pro-collagen III peptide: forward 5′-ACACACGGTGTAATGGGACCA-3′; reverse 5′-GGCTAAGTGCACCAAAATT. Real-time polymerase chain reaction was performed using Fast SYBER Green Mastermix (Applied Biosystems, Carlsbad, CA) using the StepOne Plus Fast enabled Real time Polymerase Chain Reaction System (Applied Biosystems). After normalizing data to glyceraldehyde 3-phosphate dehydrogenase messenger RNA levels, expression relative to control rats was calculated by the comparative crossover threshold method.

**Lung Tissue Collagen Content.** The Sircol collagen assay (Biocolor Ltd., Belfast, United Kingdom) was performed following the manufacturer’s instructions. Briefly, lung homogenate was incubated in acid-pectin overnight. Sirius red reagent (50 μl) was added to each lung homogenate (50 μl) for 30 min. The collagen-dye complex was precipitated by centrifugation at 16,000 g for 5 min and dissolved in 0.5 M NaOH. Finally, the samples were introduced into a microplate reader and the absorbance determined at 540 nm.

**Lung Tissue Myofibroblasts.** Paraffin-embedded tissue sections of 5 μm in thickness were dewaxed and rehydrated. Antigen retrieval was performed by heating in citrate buffer. After quenching endogenous peroxidase activity and blocking nonspecific binding, sections were incubated with antibodies against α-smooth muscle actin (LSAB kit; Dako, Carpinteria, CA). Antibody binding was detected using horseradish peroxidase-labeled biotin streptavidin secondary antibodies (Dako) and immunostaining visualized using 3,3-diaminobenzidine chromogen (Dako). Positive cells were identified and counted in 15 areas from each lung at 20× magnification and compared to controls. Positive-staining smooth muscle cells located in the walls of arterioles were not included in counts.
Table 1. Recovery Profile after Ventilation-induced Lung Injury

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham Ventilation</th>
<th>Low-stretch Ventilation</th>
<th>Ventilation-induced Lung Injury</th>
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<tbody>
<tr>
<td></td>
<td>6 Hours</td>
<td>24 Hours</td>
<td>48 Hours</td>
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<tr>
<td>Number of animals</td>
<td>4</td>
<td>4</td>
<td>8</td>
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<tr>
<td>Animal weight (g)</td>
<td>401 ± 14</td>
<td>394 ± 5</td>
<td>351 ± 15</td>
</tr>
<tr>
<td>Duration of ventilation (min)</td>
<td>4/4 (100%)</td>
<td>4/4 (100%)</td>
<td>8/8 (100%)</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>157.3 ± 21.3</td>
<td>148 ± 7.5</td>
<td>348.9 ± 94*</td>
</tr>
<tr>
<td>Arterial pCO2 (mmHg)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Arterial PCO2 (mmHg)</td>
<td>—</td>
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<tr>
<td>Total cell count in BAL</td>
<td>67,500 ± 3,415</td>
<td>62,878 ± 4,977</td>
<td>262,667 ± 127,549*</td>
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<tr>
<td>BAL protein concentrations</td>
<td>—</td>
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| Indices of Inflammation and Repair after VILI

No evidence of injury, inflammation, or repair was seen following low-stretch ventilation compared to sham uninjured animals (table 1; figs. 3 and 4). In contrast, high-stretch ventilation resulted in a marked inflammatory and reparative response.

Inflammatory Cells. BAL neutrophil counts increased rapidly following VILI, peaking at 24 h and returning to levels seen in sham and low-stretch ventilation animals by 96 h (fig. 3A). BAL lymphocyte counts increased more gradually following VILI, peaking at 24 h and remaining raised at 48 h before returning to levels seen in sham and low stretch ventilation animals at 7 days (fig. 3B). BAL monocyte/macrophage indices largely complete by 96 h after VILI. Arterial oxygen tension was lowest at 6 h after injury, remained low at 24 and 48 h, and progressively returned to baseline levels by 96 h (fig. 1A). The alveolar-arterial oxygen gradient followed a similar pattern (table 1). Static lung compliance decreased statistically significantly following VILI, with the maximal decrement evident at 6 h (fig. 1B). Static compliance was improved at 48 h, but did not return to normal until 14 days. BAL protein concentrations (fig. 1C), and lung wet: dry weight ratio (fig. 1D) were maximally increased at 6 h, remained abnormal at 24 h, and then decreased progressively.

VILI caused progressive derangement of histologic indices of lung injury, which was maximal at 48 h, and did not resolve until 7 days later. Quantitative stereological analysis demonstrated initial increases in acinar tissue volume fraction (fig. 2A) and decreases in acinar air-space volume fraction (fig. 2B), which are resolved by day 7. Representative samples of the lung histology at each time point following VILI are given in figures 2C, D, E, F, G, and H.

Results

Sixty animals were entered into this study. Four animals underwent low-stretch ventilation while four underwent sham ventilation, and were assessed 6 h later. The remaining 52 animals were subjected to VILI, allowed to recover, and randomized to assessment at the predefined time points. Four animals did not survive the high-stretch ventilation protocol due to the severity of the injury induced, leaving 48 animals that recovered after VILI and were subsequently assessed. There were no differences between the groups at baseline with regard to animal weight and duration of injurious ventilation required to induce injury (table 1).

Injury and Recovery Profile

No lung injury was seen following protective ventilation compared with sham uninjured animals (table 1; figs. 1 and 2). In contrast, high-stretch ventilation caused severe derangement of physiologic indices of lung function, with maximal injury seen at 6–24 h, and resolution of physiologic
Inflammatory Cytokines. BAL tumor necrosis factor-α and IL-1β concentrations peaked at 6 h following VILI and had returned to uninjured levels at 24 h (fig. 4A and B). BAL IL-6 also peaked at 6 h but remained statistically significantly increased at 24 h, before returning to uninjured levels at the later time points (fig. 4C). BAL IL-10 concentrations also peaked at 6 h and had returned to uninjured levels within 48 h (fig. 4D).

Repair Mediators. BAL TGF-β concentrations peaked at 6 h following VILI, and progressively decreased at the later time points, returning to preinjury levels by 96 h (fig. 4E). Interest-

ingly, BAL KGF increased later following VILI, and was statistically significantly increased at 14 days, but not at the earlier time points (fig. 4F).

**Fibroproliferative Response after VILI**

High-stretch ventilation caused a marked fibroproliferative response (figs. 5 and 6). In contrast, no fibroproliferative response was seen following low-stretch ventilation compared with sham uninjured animals.

**Indices of Fibrosis.** Tissue pro-collagen I peptide messenger RNA content increased dramatically after VILI, with a maximal increase at 48 and 96 h (fig. 5A). In contrast, at 7 and 14 days, pro-collagen I messenger RNA content was decreased compared with uninjured animals. Despite this, there was no change in tissue pro-collagen III messenger RNA (fig. 5B) or total lung collagen protein (fig. 5C) following VILI. Lung tissue myofibroblast content was increased at 6 to 96 h and decreased to preinjury levels at 7 and 14 days (fig. 5D).

MMP-3, a stromelysin largely derived from fibroblasts, followed a similar time course to that seen with lung myofibroblasts, with rising levels in BAL and homogenate (data not shown) at 6 h, peaking at 48 h, and reaching baseline by 96 h (fig. 6A and B). BAL MMP-13, a fibroblast collagenase, was statistically significantly increased following VILI, peaking at 24 h and subsequently falling to baseline (fig. 6B). Most of the MMP-13 identified was present as a cleaved (less than 30kDa) product (fig. 6B). BAL TIMP-1 was undetectable in BAL following sham or protective ventilation, but increased rapidly following VILI and was statistically significantly increased at 6 and 24 h before decreasing to baseline by 96 h (fig. 6D). Lung homogenates, in contrast, contained readily detectable levels of TIMP-1 at baseline. TIMP-2 was undetectable in BAL or homogenate (data not shown).

**Discussion**

A greater understanding of the mechanisms that mediate repair following lung injury is essential to the development of therapies that target this phase of ALI/ARDS. Much of the long-term morbidity following ALI/ARDS results from limitations in functional capacity partly because of ongoing impairment of respiratory function. However, most experimental studies concentrate on the early “injury” phases of ALI/ARDS. In these studies, we sought to characterize the inflammatory and fibroproliferative responses during resolution following VILI, and determine whether high-stretch is a sufficient stimulus to generate a fibroproliferative response and result in disordered repair and lung fibrosis.

**Injury and Recovery Profile**

Animals subjected to “protective” ventilation did not sustain a detectable lung injury when assessed 6 h after ventilation. In contrast, high-stretch ventilation caused a severe lung injury as evidenced by worsening of physiologic indices such as oxygenation, static lung compliance, and lung wet/dry weight ratio. Physiologic derangements were maximal at 6 h,
and then progressively resolved during the next 96 h. In contrast, histologic evidence of injury evolved more slowly and persisted for up to 7 days. These data suggest that restoration of physiologic function occurs rapidly despite histologic evidence of an ongoing response to injury.

**Cytokine, Chemokine, and Leukocyte Profile**

Cytokines, chemokines, and growth factors released in response to excessive lung stretch play a key role in the repair process. Conversely, dysregulated release of these mediators may result in fibroproliferation. Overexpression of IL1-β and tumor necrosis factor-α cause varying degrees of lung fibrosis in preclinical models. In these studies, animals subjected to low-stretch ventilation did not manifest an inflammatory response at 6 h compared with unventilated animals. This suggests that whereas low-stretch ventilation may activate innate immunity, any response is relatively short-lived following discontinuation of ventilation. In contrast, VILI caused a marked but transient response in multiple mediators, including TNF-α, IL-1β, IL-6, and IL-10, which resolved progressively with restoration of lung function. Interestingly, resolution of inflammation mirrored the time profile of recovery of physiologic, rather than histologic, indices.

Alveolar concentrations of TGF-β, which plays a critical role in the pathogenesis of lung fibrosis, increased in the early phases following VILI. However, the elevation of TGF-β was transient, and mirrored closely the time profile.
seen with pro-inflammatory cytokines. In contrast, alveolar concentrations of KGF, an epithelial-specific growth factor produced by mesenchyme, which may be an important endogenous stimulus for alveolar epithelial proliferation and repair, was increased later in the repair process, becoming significantly increased only at day 14 after VILI. KGF may therefore have a role in suppressing fibroproliferation after stretch injury.

VILI resulted in rapid alveolar neutrophilic infiltration, which peaked at 24 h, returning to baseline levels by 96 h. Neutrophils phagocytose debris, and produce lipid and protein mediators important in orchestrating tissue repair. Alveolar lymphocytes, which are important in mediating resolution of lung injury, accumulated more gradually and remained increased at 96 h, a pattern consistent with previ-
ous studies. Alveolar monocytes/macrophages, which induce neutrophil apoptosis and are pivotal in the progression to lung repair, peaked at 48 h, and remained increased up to 7 days. The pattern of monocyte/macrophage infiltration mirrored the resolution of histologic evidence of injury, suggesting a role in regulating the repair process.

Role of MMPs in Repair after VILI

A favorable balance of MMPs to their TIMPs is believed necessary to facilitate cell detachment from basement membrane and migration during wound healing. Conversely, an imbalance between collagen-catabolizing MMPs and their specific inhibitors, TIMPs, can result in excessive collagen production and/or breakdown. MMPs have been implicated in the pathogenesis of ARDS, and may contribute to loss of the alveolo-capillary barrier and intercellular junctions. Interestingly, some MMPs, such as MMP-9, appear to exhibit a protective profile in ALI.

In our studies, alveolar concentrations of MMPs and their TIMPs increased rapidly following VILI, but had decreased to preinjury levels by 96 h. Alveolar concentrations of the collagenase MMP-8 and the gelatinolytic enzyme MMP-9, which are produced by neutrophils, exhibited similar time profiles to those seen with neutrophil infiltration. MMP-3, a stromelysin largely derived from fibroblasts, and the fibroblast collagenase MMP-13, followed a similar time course. MMP-8 and MMP-13 are the major collagenolytic species in rats. Despite a relatively small increase in MMP-13 compared with MMP-8, most of the MMP-13 present was in the active small (less than 30kDa) form, indicating the potential for rapid collagen degradation. MMP-9 is produced by epithelium, neutrophils, and macrophages, and it can damage basement membrane contributing to alveolar edema; however, it is also necessary for epithelial repair. MMP-3 is produced predominantly by stromal cells in the lung, particularly when activated by inflammatory cytokines. It cleaves and activates collagenases that degrade type I collagen, and

Fig. 5. Fibroproliferative response following ventilation-induced lung injury (VILI). (A) Scatter plot representing lung tissue procollagen I messenger RNA (mRNA) content with sham and low-stretch ventilation, and at each time point following VILI. (B) Scatter plot representing lung tissue procollagen III mRNA content with sham and low-stretch ventilation, and at each time point following VILI. (C) Scatter plot representing lung tissue collagen content with sham and low-stretch ventilation, and at each time point following VILI. (D) Scatter plot representing lung tissue myofibroblast counts with sham and low-stretch ventilation, and at each time point following VILI. For sham and protective ventilated group, n = 4; for other VILI groups, n = 8. * Significantly different from sham and low-stretch ventilated animals (P < 0.05, ANOVA). Sham = animals that received sham ventilation; vent = animals that received low-stretch ventilation.

Fig. 6. Matrix metalloproteinase (MMP) response following ventilation-induced lung injury (VILI). (A) Scatter plot representing BAL (BAL) matrix metalloproteinase-3 (MMP) concentrations with sham and low-stretch ventilation, and at each time point following VILI. (B) Representative western blot of BAL MMP-3 and MMP-13 with sham and low-stretch ventilation, and at each time point following VILI. (C) Scatter plot representing BAL MMP-13 concentrations with sham and low-stretch ventilation, and at each time point following VILI. (D) Scatter plot representing BAL tissue inhibitor of metalloproteinase-1 concentrations with sham and low-stretch ventilation, and at each time point following VILI. For sham and protective ventilated group, n = 4; for other VILI groups, n = 8. * Significantly different from sham and low-stretch ventilated animals (P < 0.05, ANOVA). AU = arbitrary units; BAL = bronchoalveolar lavage; sham = animals that received sham ventilation; TIMP = tissue inhibitor of metalloproteinase; vent = animals that received low-stretch ventilation.
Evidence for Fibroproliferation During Repair after VILI

Fibroproliferation is an early response to lung injury. The factors influencing progression to fibroproliferation, in particular the role of mechanical stretch versus resolution, are poorly understood. A recent in vitro study demonstrated how cyclic mechanical stretch can induce epithelial-to-mesenchymal transition in alveolar type II epithelial cells, providing a putative link between lung stretch and fibrosis.

Our findings demonstrate that stretch-induced lung injury causes a pronounced early pro-fibrotic stimulus. Tissue pro-collagen I messenger RNA increased dramatically, with a maximal increase seen at 48 to 96 h. In contrast, at 7 and 14 days following VILI, pro-collagen I messenger RNA was decreased compared with that seen in uninjured animals. This suggests that transcription of collagen I is initially stimulated but later suppressed following VILI. Lung tissue myofibroblasts, which are considered the key effector cell in lung fibrogenesis, followed a similar pattern, increasing early following injury before decreasing to preinjury levels in the later stages.

Despite these changes, total lung collagen content was not increased at the later time points, suggesting that active resorption of collagen may have occurred during the later phases of the resolution process. Taken together, these findings strongly suggest that sufficient collagenases (such as MMP-3, MMP-8, and MMP-13) are produced in a timely fashion to limit collagen deposition in the lung.

Limitations

A number of limitations need to be considered. First, the model chosen was an isolated high-stretch model. Though high tidal volume ventilation can directly cause ALI/ARDS, VILI is generally seen in the context of other disease processes. However, we wished to focus on whether high lung stretch alone can generate an ongoing fibroprolific response. Second, high airway pressures, beyond that seen clinically, were used to cause a severe stretch-induced injury in these studies. However, ventilation with a peak inspiratory pressure as high as 45 cm H₂O is commonly used in preclinical studies to induce VILI. This practice is supported by evidence that regional lung areas may be subject to gross overdistension in ALI/ARDS patients. Third, we did not provide a low-stretch ventilation control group for each time point. However, our finding that “protective” ventilation did not result in detectable injury, inflammatory or fibroproliferative response at 6 h suggests that any response to low-stretch ventilation is transient. Fourth, the inclusion of groups with additional injury types and with differing degrees of stretch-induced injury, over longer durations, would have provided useful additional comparison groups. However, this would have required a large number of additional groups and are best examined in future studies. Lastly, the observational design of these studies precludes assessment of a cause-and-effect relationship between the mediator profile and the time course of injury and repair following VILI.

Clinical Implications

Our data suggest that repair following VILI demonstrates a pronounced early pro-inflammatory and pro-fibrotic phenotype. However, this is balanced by later events, such as MMP-3, MMP-8 and MMP-13 secretion that results in collagen reabsorption, and does not lead to an increase in lung fibrosis in the setting of uncomplicated VILI. High lung stretch alone, particularly when not sustained, may not constitute a sufficient stimulus to produce lung fibrosis. Nevertheless, there is clear potential for additional stimuli, such as infection or additional episodes of stretch induced injury, to disrupt this finely balanced process. Finally, we here establish a relevant preclinical model of the repair and resolution phase of VILI that can be used to test the efficacy of strategies targeted at this phase of the disease process.

Conclusions

These studies establish a rodent model of repair following VILI and characterize the time course of injury and repair following VILI. High lung stretch causes severe injury, resulting in a pronounced early pro-inflammatory and pro-fibrotic phenotype, but this response is balanced by later events that result in restoration of normal lung architecture and function.

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References


