Thorax Online First, published on September 27, 2007 as 10.1136/thx.2007.079608

Protection from experimental ventilator-induced acute lung injury by IL-1 receptor blockade

James A. Frank[1,2,5,6], Jean-Francois Pittet[1,3,4], Charlie Wray[6,] Michael A. Matthay[1,2,3]

Cardiovascular Research Institute[1], University of California, San Francisco, Departments of Medicine[2], Anesthesia[3], and Surgery[4], San Francisco VA Medical Center[5], Northern California Institute of Research and Education[6,] San Francisco, California USA.

Running title: IL-1 and alveolar barrier function

Word Count: 3240

KEY WORDS: Acute Lung Injury, Alveolar Epithelial Cell, Tight Junctions, Interleukin-1 Receptor Antagonist Protein, Blood-Air Barrier

Address correspondence to:

James A. Frank, M.D.
Cardiovascular Research Institute
Northern California Institute for Research and Education
Assistant Professor of Medicine
University of California, San Francisco
Director, MICU
San Francisco VA Medical Center
4150 Clement St. Box 111D
San Francisco, CA 94121 USA
1(415) 221-4810 x4137
1(415) 387-3568 (FAX)
james.frank@ucsf.edu

ABSTRACT

Background: Clinical studies have shown that injurious mechanical ventilation is associated with elevated airspace and plasma levels of interleukin-1β (IL-1β); however, the potential therapeutic value of IL-1 inhibition in acute lung injury has not been thoroughly investigated. A study was undertaken to determine if IL-1 signaling is a necessary early event in the pathogenesis of experimental ventilator-induced lung injury (VILI).
Methods: Mice deficient in IL-1 receptor type 1 (IL1R1) and rats treated with IL-1 receptor antagonist (IL-1Ra) were mechanically ventilated with high tidal volume (30 ml/kg) and the effect of IL-1 signaling blockade on lung injury severity was determined.
Results: Permeability as measured by radiolabeled albumin flux was significantly lower in IL1R1 null mice compared with wild type mice during injurious ventilation ($P<0.05$). IL-1Ra significantly decreased protein permeability and pulmonary oedema in rats during injurious ventilation. IL-1Ra also decreased airspace and plasma levels of the chemokine CXCL1 and airspace neutrophils. IL-1Ra decreased expression of NOS2 and ICAM-1 mRNA in whole lung. Bronchoalveolar lavage fluid levels of RTI40, a marker of type I cell injury, were 2.5 times lower in following IL-1Ra treatment ($P < 0.05$). In isolated type II pneumocytes, IL-1β reduced electrical resistance and increased transepithelial permeability.
Conclusions: IL-1 contributes to alveolar barrier dysfunction in VILI by promoting lung neutrophil recruitment, and by increasing epithelial injury and permeability. Because preserved alveolar barrier function is associated with better outcomes in patients with acute lung injury, these data support further testing of IL-1Ra for the treatment of acute lung injury.

INTRODUCTION

Acute lung injury, including the acute respiratory distress syndrome (ARDS), is a common cause of respiratory failure associated with 40% mortality.[1] Mechanical ventilation for patients with acute lung injury is life saving, but excessive tidal volume contributes to lung injury and is associated with higher mortality. Tidal volume reduction to 6 ml/kg predicted body weight reduces mortality,[2] but it is not certain if a truly safe strategy of mechanical ventilation exists. Furthermore, there are no validated diagnostic tools to aid in the recognition of ongoing ventilator-attributable lung injury. Although the mechanisms of the protective effects of tidal volume reduction are not completely known, previous clinical studies have demonstrated associations between airspace and plasma levels of biological markers of the inflammatory response and outcomes in mechanically ventilated acute lung injury patients (reviewed in reference [3]). For example, higher tidal volume and lower positive end-expiratory pressure (PEEP) ventilation results in persistently high plasma levels of IL-1β and is associated with non-pulmonary organ failure and mortality.[4, 5] Other studies have shown that plasma levels of IL-1-inducible mediators, such as IL-6, IL-8, IL-10 [4, 6] and nitric oxide synthase (NOS) activity [7-9] are higher in patients ventilated with larger tidal volumes. IL-1 has been identified as the major contributor to the proinflammatory activity of bronchoalveolar lavage fluid obtained from patients with ARDS.[10] In gene expression microarray studies, IL-1β has been identified as a candidate gene, potentially important in the pathogenesis of ventilator-associated lung injury.[11] Although early increases in expression levels of anti-inflammatory cytokines, soluble receptors, and receptor antagonists have been reported in ARDS patients,[12] the potential therapeutic role of exogenous IL-1 receptor antagonist (IL-1Ra), a naturally occurring inhibitor of IL-1 signaling, in patients with acute lung injury has not been thoroughly investigated.

The objective of this study was to test the hypothesis that IL-1 signaling is central to the initial pathogenesis of VILI. Using IL-1 receptor type 1 null mice (IL1R1KO), we tested the effect of blocking IL-1 signaling on lung injury severity in a mouse model of VILI. We then confirmed and extended the mouse studies by treating rats with an infusion of IL-1Ra, to inhibit IL-1 signaling, during mechanical ventilation and measured the effect on alveolar barrier function, lung neutrophil recruitment, lung injury severity, and IL-1-dependent mediator expression.

METHODS

Detailed methods are available in an on-line supplement.

Ventilator-induced lung injury model. Mice and rats were anesthetized and placed on a mechanical ventilator with a tidal volume of 30 ml/kg and without PEEP. An additional group of mice was ventilated with low tidal volume (6 ml/kg) and 3 cm H_2O PEEP. Animals not exposed to mechanical ventilation were used as an additional control group. Ventilation was continued for up to 3h in the mouse studies. The rat studies were continued for an additional hour (4h). For the rat studies, animals ventilated with high tidal volume received rhIL-1Ra or an equal volume of saline through a catheter in the internal jugular vein. A loading dose of 10 mg/kg in 0.5 ml was given prior to starting high tidal volume ventilation and then 10 mg/kg/hr was given as a

continuous infusion throughout the protocol. This dose was selected to provide a minimum of a 100-fold molar excess of IL-1Ra relative to IL-1 levels to ensure inhibition of IL-1 signaling.[13]

Lung endothelial and epithelial permeability to albumin and pulmonary oedema. Extravasation of intravascular ^{125}I-labeled albumin into the extravascular spaces of the lung is reported as the radioactivity of the blood-free lung expressed as a percentage of the whole-body plasma volume and normalized to non-ventilated controls. Pulmonary oedema (excess lung water) was measured using gravimetric methods.
Histological evaluation. 5 μm sections were prepared and stained with hematoxylin and eosin and scored 0-4 for oedema severity, septal thickening, and inflammatory cell infiltration in a blinded fashion.

IL-1β, CXCL1, Nitrite, and RTI40 measurements. In the mouse and rat studies, IL-1β protein levels were measured in BAL using species-specific ELISAs. BAL levels of RTI40 were measured in the rat samples using an immunoblot assay. In addition, BAL nitrite was measured in the rat samples as an index of NOS2 activity.

NOS2, and ICAM-1 gene expression. For the rat studies, mRNA expression levels of the IL-1-inducible genes NOS2 and ICAM-1 were measured in whole lung samples using semi-quantitative real time PCR. Expression levels were quantified by comparing relative expression to GAPDH mRNA expression and in reference to internal standard curves.

Primary alveolar epithelial cell isolation and permeability assay. Rat alveolar type II cells were isolated and cultured on transwells. On the 5th day, serum-free media was added to both the apical and basal compartments. The apical compartment also contained 10 μg/ml of 4 kD FITC-dextran. Recombinant rat IL-1β was then added to both sides of the monolayers. At 24h, transepithelial electrical resistance (TEER) was measured and fluorescence was measured in a sample of media from the basal compartment.
Statistics. Comparisons among groups were made by ANOVA with Student-Newman-Keuls post-hoc test for multiple comparisons. Comparisons within groups were made with paired t-tests. Parametric comparisons were appropriate for all measures based on the Kolmogorov-Smirnov goodness-of-fit test. P values less than 0.05 were considered statistically significant.

RESULTS

Transgenic Mouse Studies

BAL IL-1β protein levels are increased in mouse VILI. BAL IL-1β protein levels were significantly increased in mice ventilated with high tidal volume for 3h (36 [32-40] pg/ml, n = 7)(mean [SD]) compared with unventilated controls (7 [6-8] pg/ml, n=4, P < 0.001). After 1h of high tidal volume ventilation, BAL IL-1 β levels were not changed (6 [5-7] pg/ml, n= 4). Low tidal volume ventilation for 3h did not affect BAL IL-1β levels (6 [5-7] pg/ml, n = 4).

Absence of IL-1 signaling decreases lung albumin permeability and pulmonary oedema. IL1R1KO mice (n = 10) accumulated less extravascular radiolabeled albumin following injurious ventilation compared with wild type mice (n = 10)(P<0.05, Figure 1A). There was no

difference in baseline permeability expressed as a percentage of plasma volume between uninjured IL1R1KO mice and wild type mice (n = 10 in each group). Low tidal volume ventilation resulted in smaller, matched increases in albumin permeability in both groups ($P<0.05$ compared with unventilated controls). Similarly, there was less pulmonary oedema (excess lung water) in IL1R1KO mice following injurious ventilation (Figure 1B). Peak airway pressures in IL1R1 null mice were significantly lower compared with wild type mice following injurious ventilation while baseline respiratory mechanics were not different (not shown).

Histology and BAL neutrophil counts. Figure 1S (on-line supplement) shows representative images from each group demonstrating more severe lung injury in subjects in which IL-1 signaling was intact. Ten high-power fields from 4 individuals from each treatment group were scored 0-4 for oedema severity, alveolar septal thickening, and inflammatory cell infiltration. The 3 scores for each field were added (maximum = 12) and the mean score for each individual was determined. The average scores for each group were compared by unpaired t-test. The lung injury score for wild type mice was 3.7 [3.2-4.2](mean [SEM]) compared with 2.0 [1.8-2.2] for IL1R1 knock out mice ($P < 0.05$). Low tidal volume ventilation did not result in lung injury as measured by histology (not shown). Total BAL neutrophil counts were significantly lower in IL1R1KO mice. After injurious mechanical ventilation there were 1227 [370-2084] neutrophils/ml in BAL from wild type mice and 61 [13-108] neutrophils/ml in IL1R1KO mice (mean [SD], $P < 0.05$, n = 5 in each group).

Rat IL-1Ra Studies

BAL IL-1β protein levels are increased in rat VILI. As in the mouse studies, BAL IL-1β protein levels were also increased with injurious ventilation in rats compared with unventilated controls (Figure 2S). Similarly, IL-1β levels in lung homogenate were significantly increased with high tidal volume ventilation (Figure 3). ($P< 0.05$, n = 6 in each group). IL-1β levels were also measured in BAL from IL-1Ra-treated rats. IL-1Ra treatment appeared attenuate the increase in BAL IL-1β levels (142 [52-232], n = 5) ($P < 0.05$ compared with BAL levels in untreated, ventilated rats).

Blocking IL-1 signaling decreases lung albumin permeability and pulmonary oedema in rats. Consistent with the IL1R1KO mouse data, rats treated with IL-1Ra (n = 10) had lower lung albumin permeability in the VILI model compared with saline-treated controls (n = 11)(Figure 2A). IL-1Ra-treated rats had significantly less pulmonary oedema (Figure 2B).

Histology. Treatment with IL-1Ra significantly decreased lung injury severity (Figure 3). The lung injury score for saline treated rats was 6.9 [6.1-7.7] (mean [SEM]) compared with 3.1 [2.9-3.3] for IL-1Ra treated rats ($P < 0.05$).

IL-1Ra preserved respiratory system compliance and oxygenation in VILI. By 4h of injurious ventilation, dynamic respiratory system compliance decreased only in saline-treated rats (Table). Arterial oxygen tensions decreased only in the saline treated group (Table). There were no differences in arterial blood pressure between the two groups of rats during the ventilation protocol (not shown).

Table. Arterial blood gases and respiratory physiology measurements in rats

		Baseline	2h	4h
PaO_2 (kPa)				
	NS	13.5 [12.0-15.0]	11.3 [9.3-13.3]	9.7 [8.0-11.4]*
	IL-1Ra	12.9 [12.2-13.6]	12.4 [10-14.8]	11.6 [9.5-13.7]
$PaCO_2$ (kPa)				
	NS	4.9 [4.2-5.6]	5.7 [5.2-6.2]	5.3 [3.7-6.9]
	IL-1Ra	5.4 [4.2-6.6]	5.7 [4.5-6.9]	6.1 [4.8-7.4]
pH				
	NS	7.43 [7.37-7.49]	7.39 [7.36-7.42]	7.32 [7.24-7.40]
	IL-1Ra	7.41 [7.35-7.47]	7.42 [7.34-7.48]	7.39 [7.33-7.45]
Respiratory System Compliance (ml/cm H_2O)				
	NS	0.34 [0.32-0.36]	0.28 [0.25-0.31]	0.26 [0.22-0.30]*
	IL-1Ra	0.33 [0.29-0.37]	0.31 [0.28-0.34]	0.30 [0.27-0.33]

NS = normal saline
IL-1Ra = IL-1 receptor antagonist
*P < 0.05 compared with the Baseline value in the same group
Data are mean [SD]

IL-1Ra decreases neutrophil chemokine levels and airspace neutrophils in rats. BAL levels of CXCL1 (GRO-α/KC) were lower in IL-1Ra-treated rats compared with saline-treated rats (Figure 4A). Rats treated with IL-1Ra had fewer BAL neutrophils than saline treated controls following ventilation (Figure 4B). Although total BAL cell counts were similar in each group (465 [380-546] (mean [SD]) in IL-1Ra treated rats [n = 5] and 491 [368-614] x 10^3 cells/ml in saline treated rats [n = 4]), there were significantly fewer neutrophils and more macrophages in the IL-1Ra treated group (Figure 4B). The total BAL cell count in unventilated rats (n = 4) was 561 [514-608] x 10^3 cells/ml with 99% macrophages.

IL-1Ra decreases expression of IL-1-induced biological mediators in rats. To further determine if IL-1Ra blocked IL-1 signaling, mRNA expression levels of ICAM-1 and NOS2 were measured and BAL nitrite was measured as surrogate for NOS2 activity. Following injurious ventilation, whole lung mRNA expression of ICAM-1 and NOS2 were significantly lower in rats treated with IL-1Ra compared with saline controls (Figure 3S). BAL nitrite was lower in the rats given IL-1Ra (4.8 [3.3-6.3] vs. 8.2 [6.2-10.2] μM, P < 0.05) (mean [SD], n = 5 in the IL-1Ra group and 4 in the saline group). BAL nitrite levels in unventilated rats were 3.0 [2.2-3.8] μM).

IL-1Ra reduces alveolar epithelial type I cell injury in VILI. In rats, BAL levels of the type I cell-specific protein RTI40 correlate with ultrastructural, histological, and physiological measures of acute lung injury.[14] Rats treated with IL-1Ra had lower BAL levels of RTI40 after injurious ventilation than saline controls (Figure 4S).

IL-1β decreases transepithelial electrical resistance and increases permeability to 4 kD dextran in primary rat alveolar epithelial cells. In primary rat alveolar epithelial cell monolayers, IL-1β

decreased transepithelial electrical resistance (Figure 5A) and increased permeability to 4 kD FITC-labeled dextran (Figure 5B).

DISCUSSION

Previous clinical studies have suggested that IL-1β may be a biological marker of ventilator-associated lung injury in ARDS patients.[4-6, 10] We found that airspace IL-1β protein levels were elevated early in the course of experimental ventilator-induced lung injury. Therefore, the primary objective of this study was to determine if IL-1 signaling is critical to the initial pathogenesis of VILI. Although increased airspace levels of IL-1β are not specific to ventilator-attributable lung injury, these data show that IL-1 is an important mediator of lung injury resulting from high tidal volume, low PEEP ventilation.

The type I IL-1 receptor (IL1R1) is required for IL-1 signaling, while the type 2 receptor serves a decoy function.[15] Mice deficient in IL1R1 developed significantly less lung injury compared with wild type mice. Alveolar barrier permeability to albumin was significantly lower in IL1R1 null mice and IL1R1 null mice had less pulmonary oedema following injurious ventilation (Figure 1). Histological evaluation also demonstrated less severe lung injury in IL1R1 null mice (Figure 1S). Previous experimental studies have shown that IL-1Ra may limit cytokine release and lung neutrophil recruitment in other models,[16-19] raising the possibility that exogenous IL-1Ra may limit the severity of alveolar barrier disruption in ventilator-induced acute lung injury. Therefore, to extend the mouse studies, we tested the effect of pharmacologic doses of IL-1Ra delivered as a continuous infusion beginning with the initiation of mechanical ventilation on lung injury severity in a rat model of VILI. Although *in vivo* studies in transgenic mice are valuable, there are some advantages to rat studies, including improved ability to monitor circulatory physiology and arterial blood gases and more reliable administration of medications as a continuous infusion.We extended the duration of the ventilation protocol an additional hour in the rat studies to examine the effect of IL-1Ra over a longer time period. Rats treated with IL-1Ra developed less pulmonary oedema and had lower lung permeability to albumin than saline-treated rats. In addition, arterial oxygenation was preserved and respiratory system compliance did not decrease in the IL-1Ra treated rats consistent with less severe lung injury (Table).

IL-1 induces expression of IL-8, ICAM-1, and NOS2 via activation of NFKB [20-22] to promote neutrophil recruitment from the circulation during injury. Previous studies have shown that VILI is dependent on the recruitment of neutrophils into the lung.[23-27] We measured expression levels of CXCL1 (GRO-α, an IL-8 orthologue), ICAM-1, and NOS2 expression and activity to provide additional evidence that IL-1Ra blocked IL-1 signaling, and to determine the mechanisms for the IL-1Ra-mediated protection from VILI. We found that protein levels of the neutrophil chemokine CXCL1 were lower in the airspaces of ventilated rats treated with IL-1Ra. Lung ICAM-1 and NOS2 mRNA expression levels were also lower in rats treated with IL-1Ra and airspace nitrite levels, a marker of NOS2 activity, were lower with IL-1Ra treatment. Interestingly, one recent study found that mice deficient in NOS2 were protected from lung injury in a VILI model.[28] Others have reported that NOS2 activity results in increased neutrophil sequestration in the lung in experimental sepsis.[29] Taken together these data show

that IL-1 is a proximal mediator of inflammation and neutrophil recruitment in the initial pathogenesis of VILI.

In the present study, IL-1Ra treatment was associated with significantly less alveolar epithelial cell injury in the rat model. Rats treated with IL-1Ra had lower BAL levels of RTI40, a type I alveolar epithelial cell-specific membrane protein. Previous studies from our group and others have shown that RTI40 levels correlate well with histological, ultrastructural, and physiological measures of acute lung injury.[14] Therefore, inhibition of IL-1 signaling resulted in decreased lung neutrophil recruitment, decreased lung protein permeability, and decreased alveolar epithelial cell injury in experimental VILI.

It is not certain if the increased IL-1β protein levels measured in the present study were the result of increased IL-1 secretion from alveolar epithelial cells, or from other cell types. However, alveolar macrophages may be one important source of IL-1β protein in VILI. Alveolar macrophages are known to be a major source of IL-1 in the lungs of ARDS patients.[30] Previously, we found that alveolar macrophages are activated within minutes of initiating high tidal volume, zero PEEP ventilation, and macrophage depletion results in decreased neutrophil recruitment and less severe lung injury in the rat VILI model.[31] In the present study, there were fewer BAL macrophages in the saline-treated rats compared with the IL-1Ra-treated rats (Figure 4), consistent with increased activation-associated macrophage adhesion to the alveolar epithelium in the saline group. Therefore, inhibition of IL-1 signaling may limit autocrine or paracrine activation of alveolar macrophages in this model.

To further explore the mechanisms for the IL-1-mediated loss of alveolar barrier function, we studied the effect of Il-1 on epithelial barrier properties in vitro. Primary rat alveolar epithelial cells were cultured on membrane supports and transepithelial electrical resistance (TEER) and permeability to 4 kD FITC-dextran were measured in the presence or absence of IL-1β. Under serum-free conditions, IL-1β induced a dose dependent decrease in TEER with a concomitant increase in permeability to labeled dextran. These data suggest that IL-1 may also act through neutrophil-independent mechanisms to disrupt alveolar barrier function in VILI, including directly increasing alveolar epithelial permeability. The molecular mechanisms of the observed increase in epithelial permeability *in vitro* remain to be investigated; however, previous studies have reported that IL-1 increases oxidative stress in alveolar epithelial cells [32] and activates platelet activating factor – a potent mediator of pulmonary edema.[33] Interestingly, IL-1β has also been shown to decrease expression of the alpha subunit of the epithelial sodium channel (ENaC), resulting in decreased epithelial sodium transport.[34] In VILI, this would be expected to decrease oedema fluid clearance from the airspaces resulting in more severe pulmonary oedema.

The processes regulating acute inflammation and the innate immune response in the lung in response to injury are complex, involving a variety of cell types and responses to several inputs such as growth factors, cytokines, chemokines, toll-like receptors and other pathways.[35-40] It is not yet clear to what extent TNF-α and other mediators of lung injury function in parallel or in series with IL-β in this model, but blockade of IL-1 signaling significantly reduced lung neutrophil recruitment and protected the alveolar epithelium during injurious ventilation.

This study has potential implications for clinical ventilator-associated lung injury. There are currently no effective pharmacologic therapies for acute lung injury. A previous phase III trail of IL-1Ra in patients with sepsis tested the effect of IL-1Ra (100 mg loading dose and up to 2 mg/h for 3 days) on 28-day mortality.[41] There was no overall mortality benefit for patients receiving IL-1Ra in the entire group; however, in the subgroup of 223 patients with ARDS and sepsis, there was a significant reduction in mortality in the those who received IL-1Ra (34% in patients receiving the 2 mg/h dose compared with 49% in the placebo group, P=0.04). For ARDS patients with an expected mortality of greater than or equal to 24% (n=150), the apparent benefit of IL-1Ra treatment on mortality was even greater (34% compared with 60%, P=0.01).[41] These data were not confirmed in a subsequent study;[42] however, in the second study of sepsis patients, the subgroup of ARDS patients was not further stratified by disease severity or predicted mortality. It is possible that the lack of a clear beneficial effect of IL-1Ra in ARDS patients may have resulted from heterogeneity within this subgroup with respect to disease severity, as well as variability in the timing of onset of ARDS and mechanical ventilation and the initiation of IL-1Ra. Of course, neither study was designed to examine the effect of IL-1Ra on lung injury outcomes. In the case of clinical ventilator-associated lung injury, it is possible that a treatment strategy could be started before the injurious stimulus, that is just before mechanical ventilation is started. The data from the present study are consistent with the hypothesis that early activation of the inflammatory response, including increased IL-1 signaling, is a major mechanism of alveolar barrier dysfunction in ventilator-attributable injury. Because alveolar epithelial injury and the loss of alveolar barrier function are associated with mortality in acute lung injury patients,[43, 44] this study supports further testing of the therapeutic value of IL-1Ra in patients with acute lung injury.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Leland Dobbs for the generous gift of the rat RTI40 antibody and Dr. Jorge Gutierrez for measuring the BAL RTI40 levels.

COMPETING INTERESTS

The authors have no competing interests to declare.

FUNDING

This work supported by National Institutes of Health, National Heart, Lung and Blood Institute grants HL69900 (JAF) and HL51854 (MAM).

REFERENCES

1. **Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, Stern EJ, and Hudson LD.** Incidence and outcomes of acute lung injury. *N Engl J Med* 2005;**353**: 1685-1693.
2. **ARDSNetwork.** Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The Acute Respiratory Distress Syndrome Network [see comments]. *N Engl J Med* 2000;**342**: 1301-1308.
3. **Frank J, Parsons P, and Matthay M.** Pathogenetic significance of biological markers of ventilator-attributable lung injury in clinical and experimental studies. *Chest* 2006;**130**: 1906-1914.
4. **Ranieri VM, Suter PM, Tortorella C, De Tullio R, Dayer JM, Brienza A, Bruno F, and Slutsky AS.** Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome: a randomized controlled trial. *Jama* 1999;**282**: 54-61.
5. **Ranieri VM, Giunta F, Suter PM, and Slutsky AS.** Mechanical ventilation as a mediator of multisystem organ failure in acute respiratory distress syndrome. *Jama* 2000;**284**: 43-44.
6. **Parsons PE, Eisner MD, Thompson BT, Matthay MA, Ancukiewicz M, Bernard GR, and Wheeler AP.** Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. *Crit Care Med* 2005;**33**: 1-6.
7. **Gessner C, Hammerschmidt S, Kuhn H, Lange T, Engelmann L, Schauer J, and Wirtz H.** Exhaled breath condensate nitrite and its relation to tidal volume in acute lung injury. *Chest* 2003;**124**: 1046-1052.
8. **Broccard AF, Feihl F, Vannay C, Markert M, Hotchkiss J, and Schaller MD.** Effects of L-NAME and inhaled nitric oxide on ventilator-induced lung injury in isolated, perfused rabbit lungs. *Crit Care Med* 2004;**32**: 1872-1878.
9. **Frank JA, Pittet JF, Lee H, Godzich M, and Matthay MA.** High tidal volume ventilation induces NOS2 and impairs cAMP- dependent air space fluid clearance. *Am J Physiol Lung Cell Mol Physiol* 2003;**284**: L791-798.
10. **Pugin J, Ricou B, Steinberg KP, Suter PM, and Martin TR.** Proinflammatory activity in bronchoalveolar lavage fluids from patients with ARDS, a prominent role for interleukin-1. *Am J Respir Crit Care Med* 1996;**153**: 1850-1856.
11. **Ma SF, Grigoryev DN, Taylor AD, Nonas S, Sammani S, Ye SQ, and Garcia JG.** Bioinformatic identification of novel early stress response genes in rodent models of lung injury. *Am J Physiol Lung Cell Mol Physiol* 2005;**289**: L468-477.
12. **Park WY, Goodman RB, Steinberg KP, Ruzinski JT, Radella F, 2nd, Park DR, Pugin J, Skerrett SJ, Hudson LD, and Martin TR.** Cytokine balance in the lungs of patients with acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2001;**164**: 1896-1903.
13. **Ohlsson K, Bjork P, Bergenfeldt M, Hageman R, and Thompson RC.** Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. *Nature* 1990;**348**: 550-552.
14. **Frank JA, Gutierrez JA, Jones KD, Allen L, Dobbs L, and Matthay MA.** Low tidal volume reduces epithelial and endothelial injury in acid-injured rat lungs. *Am J Respir Crit Care Med* 2002;**165**: 242-249.
15. **Dinarello CA.** Biologic basis for interleukin-1 in disease. *Blood* 1996;**87**: 2095-2147.

16. **Abraham E and Allbee J.** Effects of therapy with interleukin-1 receptor antagonist on pulmonary cytokine expression following hemorrhage and resuscitation. *Lymphokine Cytokine Res* 1994;**13**: 343-347.
17. **Shanley TP, Peters JL, Jones ML, Chensue SW, Kunkel SL, and Ward PA.** Regulatory effects of endogenous interleukin-1 receptor antagonist protein in immunoglobulin G immune complex-induced lung injury. *J Clin Invest* 1996;**97**: 963-970.
18. **Laffon M, Lu LN, Modelska K, Matthay MA, and Pittet JF.** alpha-adrenergic blockade restores normal fluid transport capacity of alveolar epithelium after hemorrhagic shock. *Am J Physiol* 1999;**277**: L760-768.
19. **Leff JA, Bodman ME, Cho OJ, Rohrbach S, Reiss OK, Vannice JL, and Repine JE.** Post-insult treatment with interleukin-1 receptor antagonist decreases oxidative lung injury in rats given intratracheal interleukin-1. *Am J Respir Crit Care Med* 1994;**150**: 109-112.
20. **Guo RF and Ward PA.** Mediators and regulation of neutrophil accumulation in inflammatory responses in lung: insights from the IgG immune complex model. *Free Radic Biol Med* 2002;**33**: 303-310.
21. **Warner RL, Paine R, 3rd, Christensen PJ, Marletta MA, Richards MK, Wilcoxen SE, and Ward PA.** Lung sources and cytokine requirements for in vivo expression of inducible nitric oxide synthase. *Am J Respir Cell Mol Biol* 1995;**12**: 649-661.
22. **Geller DA, Nussler AK, Di Silvio M, Lowenstein CJ, Shapiro RA, Wang SC, Simmons RL, and Billiar TR.** Cytokines, endotoxin, and glucocorticoids regulate the expression of inducible nitric oxide synthase in hepatocytes. *Proc Natl Acad Sci U S A* 1993;**90**: 522-526.
23. **Belperio JA, Keane MP, Burdick MD, Londhe V, Xue YY, Li K, Phillips RJ, and Strieter RM.** Critical role for CXCR2 and CXCR2 ligands during the pathogenesis of ventilator-induced lung injury. *J Clin Invest* 2002;**110**: 1703-1716.
24. **Kim JH, Suk MH, Yoon DW, Lee SH, Hur GY, Jung KH, Jeong HC, Lee SY, Lee SY, Suh IB, Shin C, Shim JJ, In KH, Yoo SH, and Kang KH.** Inhibition of Matrix Metalloproteinase-9 Prevents Neutrophilic Inflammation in Ventilator-Induced Lung Injury. *Am J Physiol Lung Cell Mol Physiol* 2006.
25. **Imanaka H, Shimaoka M, Matsuura N, Nishimura M, Ohta N, and Kiyono H.** Ventilator-induced lung injury is associated with neutrophil infiltration, macrophage activation, and TGF-beta 1 mRNA upregulation in rat lungs. *Anesth Analg* 2001;**92**: 428-436.
26. **Zhang H, Downey GP, Suter PM, Slutsky AS, and Ranieri VM.** Conventional mechanical ventilation is associated with bronchoalveolar lavage-induced activation of polymorphonuclear leukocytes: a possible mechanism to explain the systemic consequences of ventilator-induced lung injury in patients with ARDS. *Anesthesiology* 2002;**97**: 1426-1433.
27. **Choudhury S, Wilson MR, Goddard ME, O'Dea KP, and Takata M.** Mechanisms of early pulmonary neutrophil sequestration in ventilator-induced lung injury in mice. *Am J Physiol Lung Cell Mol Physiol* 2004;**287**: L902-910.
28. **Peng X, Abdulnour RE, Sammani S, Ma SF, Han EJ, Hasan EJ, Tuder R, Garcia JG, and Hassoun PM.** Inducible nitric oxide synthase contributes to ventilator-induced lung injury. *Am J Respir Crit Care Med* 2005;**172**: 470-479.
29. **Razavi HM, Wang le F, Weicker S, Rohan M, Law C, McCormack DG, and Mehta S.** Pulmonary neutrophil infiltration in murine sepsis: role of inducible nitric oxide synthase. *Am J Respir Crit Care Med* 2004;**170**: 227-233.

30. **Jacobs RF, Tabor DR, Burks AW, and Campbell GD.** Elevated interleukin-1 release by human alveolar macrophages during the adult respiratory distress syndrome. *Am Rev Respir Dis* 1989;**140**: 1686-1692.
31. **Frank J, Wray C, McAuley D, Schwendener R, and Matthay M.** Alveolar macrophages contribute to alveolar barrier dysfunction in ventilator-induced lung injury. *Am J Physiol Lung Cell Mol Physiol* 2006;**291**: 1191-1198.
32. **Hybertson BM, Lee YM, Cho HG, Cho OJ, and Repine JE.** Alveolar type II cell abnormalities and peroxide formation in lungs of rats given IL-1 intratracheally. *Inflammation* 2000;**24**: 289-303.
33. **Goggel R, Winoto-Morbach S, Vielhaber G, Imai Y, Lindner K, Brade L, Brade H, Ehlers S, Slutsky AS, Schutze S, Gulbins E, and Uhlig S.** PAF-mediated pulmonary edema: a new role for acid sphingomyelinase and ceramide. *Nat Med* 2004;**10**: 155-160.
34. **Roux J, Kawakatsu H, Gartland B, Pespeni M, Sheppard D, Matthay MA, Canessa CM, and Pittet JF.** Interleukin-1beta decreases expression of the epithelial sodium channel alpha-subunit in alveolar epithelial cells via a p38 MAPK-dependent signaling pathway. *J Biol Chem* 2005;**280**: 18579-18589.
35. **Altemeier WA, Matute-Bello G, Frevert CW, Kawata Y, Kajikawa O, Martin TR, and Glenny RW.** Mechanical ventilation with moderate tidal volumes synergistically increases lung cytokine response to systemic endotoxin. *Am J Physiol Lung Cell Mol Physiol* 2004;**287**: L533-542.
36. **Hollingsworth JW, Li Z, Brass DM, Garantziotis S, Timberlake SH, Kim A, Hossain I, Savani RC, and Schwartz DA.** CD44 Regulates Macrophage Recruitment to the Lung in Lipopolysaccharide-induced Airway Disease. *Am J Respir Cell Mol Biol* 2007.
37. **Hollingsworth JW, Whitehead GS, Lin KL, Nakano H, Gunn MD, Schwartz DA, and Cook DN.** TLR4 signaling attenuates ongoing allergic inflammation. *J Immunol* 2006;**176**: 5856-5862.
38. **Jiang D, Liang J, Fan J, Yu S, Chen S, Luo Y, Prestwich GD, Mascarenhas MM, Garg HG, Quinn DA, Homer RJ, Goldstein DR, Bucala R, Lee PJ, Medzhitov R, and Noble PW.** Regulation of lung injury and repair by Toll-like receptors and hyaluronan. *Nat Med* 2005;**11**: 1173-1179.
39. **Okutani D, Han B, Mura M, Waddell TK, Keshavjee S, and Liu M.** High-volume ventilation induces pentraxin 3 expression in multiple acute lung injury models in rats. *Am J Physiol Lung Cell Mol Physiol* 2007;**292**: L144-153.
40. **Santos CC, Zhang H, Liu M, and Slutsky AS.** Bench-to-bedside review: Biotrauma and modulation of the innate immune response. *Crit Care* 2005;**9**: 280-286.
41. **Fisher CJ, Jr., Dhainaut JF, Opal SM, Pribble JP, Balk RA, Slotman GJ, Iberti TJ, Rackow EC, Shapiro MJ, Greenman RL, and et al.** Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. Results from a randomized, double-blind, placebo-controlled trial. Phase III rhIL-1ra Sepsis Syndrome Study Group. *Jama* 1994;**271**: 1836-1843.
42. **Opal SM, Fisher CJ, Jr., Dhainaut JF, Vincent JL, Brase R, Lowry SF, Sadoff JC, Slotman GJ, Levy H, Balk RA, Shelly MP, Pribble JP, LaBrecque JF, Lookabaugh J, Donovan H, Dubin H, Baughman R, Norman J, DeMaria E, Matzel K, Abraham E, and Seneff M.** Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial. The Interleukin-1 Receptor Antagonist Sepsis Investigator Group. *Crit Care Med* 1997;**25**: 1115-1124.

43. **Matthay MA and Wiener-Kronish JP.** Intact epithelial barrier function is critical for the resolution of alveolar edema in humans. *Am Rev Respir Disease* 1990;**142**: 1250-1257.
44. **Ware LB and Matthay MA.** Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2001;**163**: 1376-1383.

FIGURE LEGENDS

Figure 1. The absence of IL-1 receptor 1 signaling decreases lung permeability to protein and pulmonary oedema in VILI. (**A**) Mice lacking IL1R1 (IL1R1KO) (n = 10) had less extravasation of radiolabeled albumin into the extravascular spaces of the lung compared with wild type mice in during high tidal volume ventilation (n = 10, *P < 0.05). Low tidal volume ventilation also resulted in slightly higher permeability in both groups, but differences were not statistically significant (P>0.05 compared with non-ventilated mice, n = 10 in each group). Permeability to albumin is expressed relative albumin flux compared with non-ventilated controls. (**B**) Pulmonary oedema as measured by excess lung water was significantly lower in IL1R1KO mice compared with wild type mice with high tidal volume ventilation (*P<0.05). There was a smaller increase in excess lung water in both high tidal volume groups, but the two groups were not different from each other.

Figure 2. Blocking IL-1 signaling with IL-1Ra in rats resulted in lower lung protein permeability and less pulmonary oedema during VILI. (**A**) Rats treated with IL-1Ra (n = 10) also had lower permeability to albumin compared with rats given saline (n = 11) during injurious ventilation (*P < 0.05). Data are expressed as the relative albumin flux compared with non-ventilated controls. (**B**) Pulmonary oedema measured as excess lung water was less severe in rats given IL-1Ra (*P < 0.05 compared with saline treated rats).

Figure 3. Blocking IL-1 signaling decreases lung injury severity. Representative histological sections (100X) from (**A**) IL-1Ra-treated rats and (**B**) saline treated rats after injurious mechanical ventilation. IL-1Ra infusion resulted less histological lung injury with fewer inflammatory cells (white arrowheads) and less oedema (black arrowheads) than saline infusion in the VILI model.

Figure 4. IL-1Ra decreases airspace neutrophil chemokine levels and airspace neutrophils in rat VILI. Rats treated with IL-1Ra (n=5)had (**A**) significantly lower BAL fluid levels of the neutrophil chemokine CXCL1 (GRO-α/KC) (*P < 0.05 compared with saline-treated group, n=4). CXCL1 levels were higher in both ventilated groups compared with non-ventilated controls. (**B**) There were also fewer BAL neutrophils (PMN)(dark bars) in rats treated IL-1Ra compared with rats treated with saline only. The total BAL cell counts in each group were similar; however, there were more macrophages (Mac)(light bars) in the BAL fluid from IL-1Ra treated rats (*P < 0.05 compared with the saline treated group). There were significantly fewer macrophages in both ventilated groups compared with non-ventilated controls. In all groups, there was a similar small percentage of lymphocytes (0-4%) in the BAL fluid (not shown).

Figure 5. IL-1β impairs alveolar epithelial type II monolayer barrier function *in vitro*. (**A**) Incubating primary rat type II cells with recombinant rat IL-1β for 24h decreased transepithelial electrical resistance (TEER, Ohm/cm^2) in a dose-dependent fashion (*P < 0.05 compared with 0 ng/ml control) (three replicates of 6 wells each for each group) (**B**) IL-1β (10 ng/ml for 24h) increased alveolar epithelial type II cell monolayer permeability measured as the flux of 4 kD FITC-dextran from the apical compartment to the basolateral compartment (*P < 0.05 compared with media control). Data are mean and SEM.

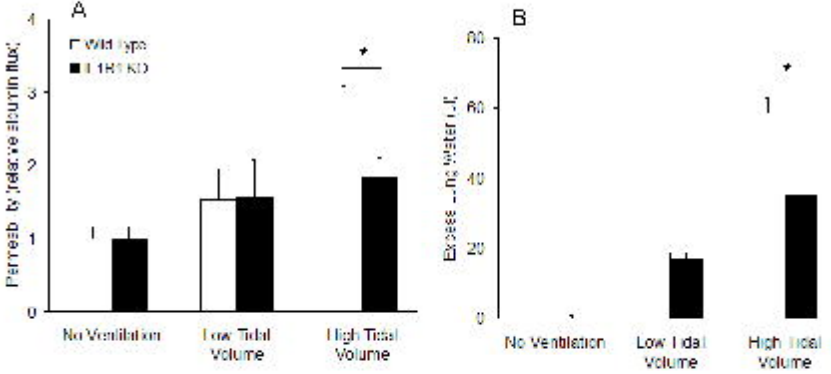

A
Wild type
AT1R KO
No Ventilation
Low Tidal Volume
High Tidal Volume
B
No Ventilation
Low Tidal Volume
High Tidal Volume

Figure 1

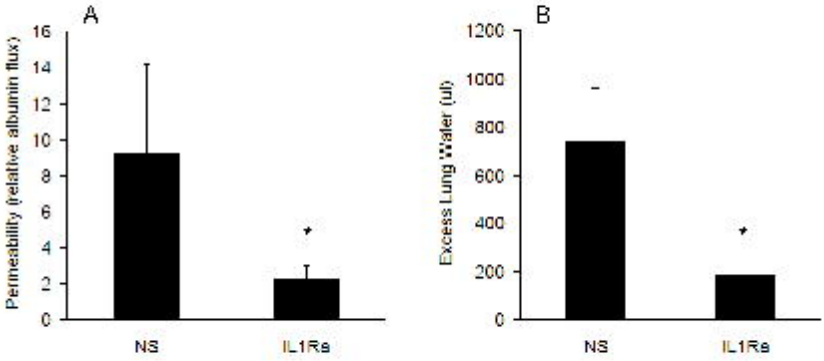

A
Permeability (relative albumin flux)
16
14
12
10
8
6
4
2
0
NS
IL1Rs
B
Excess Lung Water (ul)
1200
1000
800
600
400
200
0
NS
IL1Rs

Figure 2

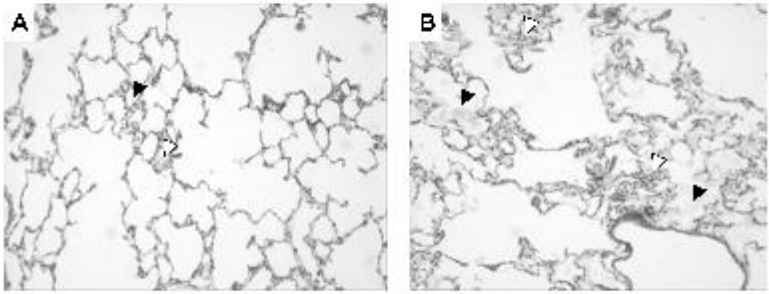
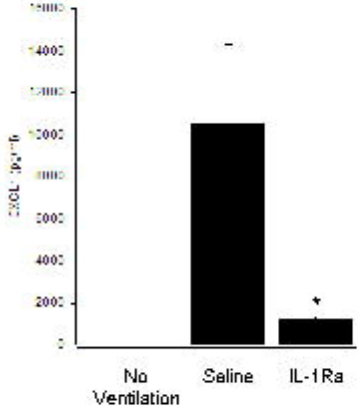


Figure 3


No Ventilation
Saline
IL-1Ra

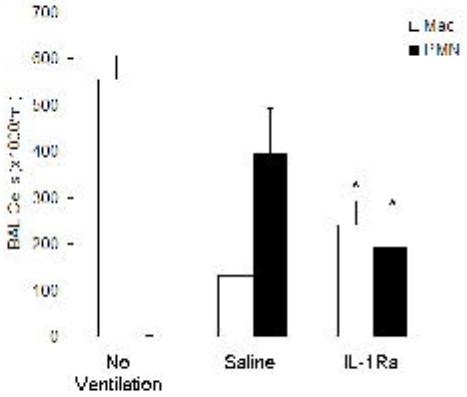

Mac
PMN
No Ventilation
Saline
IL-1Ra

Figure 4

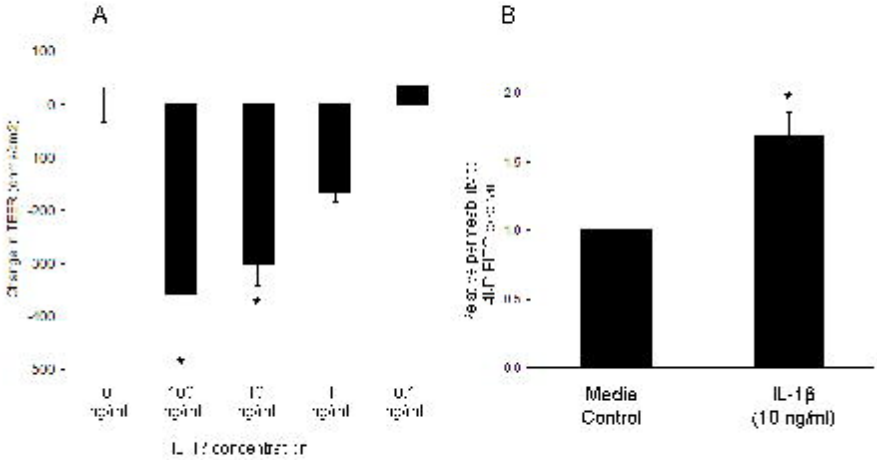

A
B
100
0
100
200
300
400
500
0
ng/ml
1
ng/ml
10
ng/ml
Media
Control
IL-1β
(10 ng/ml)

Figure 5

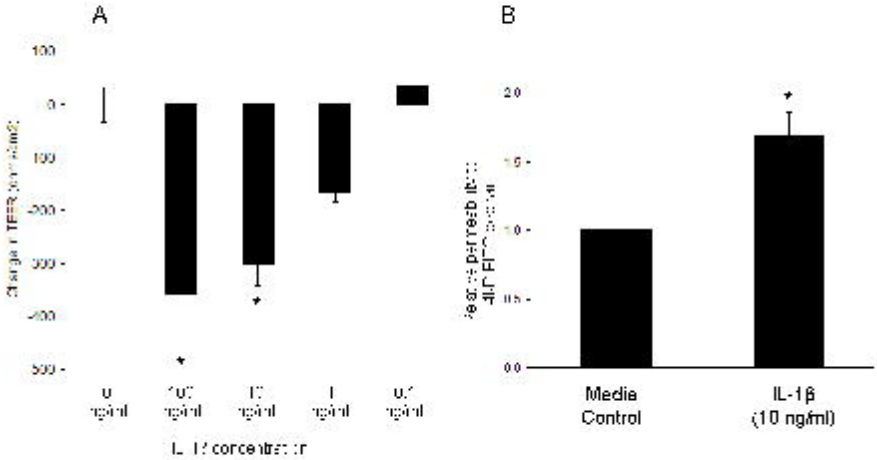

A
B
100
0
100
200
300
400
500
0
ng/ml
1
ng/ml
10
ng/ml
Media
Control
IL-1β
(10 ng/ml)

Figure 5