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Interleukin-6 mediates pulmonary vascular permeability in a two-hit model of ventilator-associated lung injury

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Abstract

To test the hypothesis that IL-6 contributes to the development of ventilator-associated lung injury (VALI), IL-6-deficient (*IL6*^{-/-}) and wild-type control (*WT*) mice received intratracheal hydrochloric acid followed by randomization to MV (MV+IT HCl) or spontaneous ventilation (IT HCl). After 4 hr, injury was assessed by estimation of lung lavage protein concentration and total and differential cell counts, wet/dry lung weight ratio, pulmonary cell death, histologic inflammation score (LIS), and parenchymal myeloperoxidase (MPO) concentration. Vascular endothelial growth factor (VEGF) concentration was measured in lung lavage and homogenate, as IL-6 and stretch both regulate expression of this potent mediator of permeability. MV-induced increases in alveolar barrier dysfunction and lavage VEGF were attenuated in *IL6*^{-/-} mice as compared with *WT* controls, whereas tissue VEGF concentration increased. The effects of IL-6 deletion on alveolar permeability and VEGF concentration were inflammation-independent, as parenchymal MPO concentration, LIS, and lavage total and differential cell counts did not differ between *WT* and *IL6*^{-/-} mice following IT HCl+MV.

These data support a role for IL-6 in promoting VALI in this two-hit model. Strategies to interfere with IL-6 expression or signaling may represent important therapeutic targets to limit the injurious effects of MV in inflamed lungs.

Keywords

mechanical ventilation; pulmonary barrier dysfunction; acid aspiration; vascular endothelial growth factor (VEGF)

Introduction

Mechanical ventilation (MV) is a lifesaving therapy for critically ill patients with acute respiratory failure. However, excessive alveolar distention associated with mechanical ventilation may by itself promote acute lung injury. This ventilator-associated lung injury (VALI) is characterized by increased vascular permeability, increased production of

cytokines, chemokines, and growth factors, accumulation of neutrophils, and altered lung tissue mechanics (1-4).

One of the cytokines proposed to contribute to the development of VALI is interleukin 6 (IL-6) (5, 6). IL-6 is a multifunctional cytokine produced by a wide range of cell types (7, 8). Experimental evidence supports a role for IL-6 in promoting enhanced vascular endothelial permeability (9, 10) and inflammation (11, 12), thereby potentially augmenting acute lung injury. In addition, IL-6 has been shown to regulate expression of vascular endothelial growth factor (VEGF), a potent mediator of vascular leak, in a number of experimental models (13-15). In contrast, over-expression of IL-6 may have anti-inflammatory and anti-apoptotic effects (16), conferring benefit in oxidant-mediated lung injury models (17, 18).

The potential involvement of IL-6 in acute lung injury induced by MV is supported by data demonstrating that pulmonary and systemic IL-6 release is increased in animal models of VALI (19, 20), and lung epithelial cells exposed to cyclic stretch secrete IL-6 (5, 21). The degree of IL-6 production was proportional to tidal volume administered in both animal and human studies (22, 23), and higher concentrations of pulmonary or circulating IL-6 have been linked with increased severity of both experimental and human VALI (23-25). In contrast, a recently published study suggested that inhibition of hematopoietic cell-derived IL-6 may exacerbate airspace inflammation and alveolar barrier dysfunction in a murine model of MV-induced lung injury (26). These conflicting data highlight the complexity of IL-6 as a potential mediator of clinical VALI.

To determine whether IL-6 is a key mediator of increased pulmonary permeability in VALI, we utilized our previously described model that combines MV with an additional, clinically relevant inflammatory stimulus (low dose acid aspiration; IT HCl), to more closely model human disease. Subclinical aspiration has been estimated to occur in nearly one-fourth of critically ill, mechanically ventilated adults receiving enteral nutrition (27), firmly establishing the importance of a model such as ours. Prior data generated using this model demonstrated that MV of mice with high (17 ml/kg) tidal volume but no IT HCl did not cause lung barrier dysfunction, and that lung injury induced by MV+IT HCl was attenuated in animals ventilated with a low (6 ml/kg) tidal volume strategy. Increased pulmonary IL-6 expression was linked with exacerbation of lung injury in this two-hit model (24). In the current study we compared the effects of high tidal volume MV+IT HCl on measures of lung barrier dysfunction, inflammation, and cell death in IL-6 deficient (*IL-6^{-/-}*) mice and wild-type (*WT*) control animals. Because both IL-6 and ventilatory stretch may regulate VEGF expression, and concurrently increased IL-6 and VEGF have been linked with enhanced permeability in multiple clinical disorders (28-32), we quantified lavage and tissue concentration of VEGF as an additional outcome measure. Defining the role of specific mediators, such as IL-6, in the pathogenesis of MV-induced acute lung injury may help refine strategies to prevent VALI in critically ill patients with acute respiratory failure.

Methods

Protocol

The animal studies described were approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine. Eight-12 week old male IL-6 deficient mice (*IL-6^{-/-}*) and age-matched C57/B6 (*wild-type*; *WT*) control animals were purchased from Jackson Laboratories (Bar Harbor, Maine).

Administration of IT HCl and the MV protocol were performed as previously described (24), with minor modifications. After mice received intratracheal hydrochloric acid (HCl),

mechanical ventilation (MV) was initiated with a tidal volume (V_T) of 17 ml/kg, respiratory frequency of 120/min, FiO_2 of 0.4 and PEEP of 3 cm H_2O . After 4 hours of MV, mice were sacrificed under general anesthesia, and lung injury was assessed. In one set of animals ($n=6-7$ mice/group), the right hilum was ligated, the right lung was rapidly excised for measurement of wet/dry lung weight, and lavage was performed of the left lung. In a second group of mice ($n = 4-7$ mice/genotype), the right lung was snap frozen in liquid nitrogen after right hilar ligation, then the left lung was inflated with 0.5% low-melting agarose (25 cm H_2O), excised, and fixed in 4% paraformaldehyde for immunohistochemical evaluation.

To determine the independent effects of IT HCl in this two-hit model, separate groups of spontaneously ventilating mice of each genotype were intubated under short-acting inhalational anesthesia (2% isoflurane in room air). These mice were mechanically ventilated for 5 min during the administration of IT HCl, then were extubated to room air. After 4 hr, the mice were sacrificed for estimation of wet/dry lung weight and lung lavage parameters ($n=6$ /genotype), or freeze clamp lung biopsy and lung inflation for histology ($n=5-6$ /genotype), as described above. Additional control animals ($n=5$ /genotype) were sacrificed under general anesthesia without any endotracheal intervention or MV.

Measurements

Assessment of lung injury—Acute lung injury was assessed by measurement of lung wet/dry weight ratio, analysis of lung lavage protein concentration and total and differential cell count, and immunohistochemical estimation of cell death, using methods previously described (24, 33). Histologic assessment of inflammatory cell infiltration was determined by two investigators blinded to group assignments, using a semi-quantitative scoring system that was previously reported (34).

Measurement of MPO, IL-6, and VEGF protein concentration—Frozen tissue samples were solubilized by homogenization in lysis buffer containing Tris base, and protease and phosphatase inhibitors. Protein concentration was determined, then tissue MPO (Cell Sciences, Canton, MA) and both tissue and lavage IL-6 and VEGF protein concentrations were determined using commercially available sandwich ELISA kits (R&D Systems, Minneapolis, MN).

Statistical Analysis—Results shown represent mean $\pm$ SE. Differences between groups in lung wet/dry weight ratio, lavage protein concentration and cell counts, apoptotic index, histologic lung injury score, lavage and tissue mediator concentrations, and tissue MPO concentration were compared using two-way analysis of variance (genotype by treatment). Variables which were not normally distributed (wet/dry weight ratio, apoptotic index, tissue IL-6, VEGF and MPO concentration) were log transformed prior to statistical analysis. If significant interactions ($p < 0.05$) were identified, least-significant differences were calculated to allow comparison of genotype-specific treatment effects. When no significant interaction was identified, genotype-adjusted treatment effects were estimated using multiple linear regression. For mechanically ventilated animals, mean arterial and peak airway pressures during the 4 hour period of MV were compared by repeated measures ANOVA. When significant variance ratios were obtained, least-significant differences were calculated to allow comparison of individual group means. Differences were considered significant for $p < 0.05$.

Results

To evaluate whether the dose and duration of IT HCl used in this two-hit model of VALI altered lung barrier dysfunction independently of MV, we compared lung wet/dry weight,

lavage cell counts and protein concentration, tissue MPO concentration, and lavage and tissue VEGF concentration in spontaneously ventilating *WT* and *IL6*^{-/-} mice 4h after administration of IT HCl, and compared results with measurements made in uninjured, spontaneously breathing animals of both genotypes. Shown in Table 1, total cell, macrophage, lymphocyte, and neutrophil counts in lung lavage all increased following IT HCl, but this increase was independent of mouse genotype ($p > 0.05$ for genotype-adjusted treatment effect). Acid aspiration without MV was associated with a significant increase in tissue MPO concentration ($p < 0.05$ for genotype-treatment interaction), and a trend for enhanced lavage neutrophilia ($p = 0.07$ for genotype-treatment interaction) in IT HCl-treated *WT* animals. Interestingly, airspace macrophage numbers were higher at baseline in *IL6*^{-/-} mice, and the effect of IT HCl on lavage macrophage counts was enhanced in these animals ($p < 0.05$ for genotype-treatment interaction). However, lavage protein concentration and lung wet/dry weight ratios were not altered by *IL6* deletion, and did not increase following this dose and duration of IT HCl in mice of either genotype when compared with uninjured animals. Similarly, lavage fluid and tissue homogenate concentrations of VEGF were not altered by *IL6* deletion, or following IT HCl in mice of either genotype.

To confirm that MV synergized with IT HCl to induce lung barrier dysfunction, and to determine the effects of *IL6* deletion in this two-hit model of injury, we next compared lung wet/dry weight, lavage cell counts and protein concentration, tissue MPO concentration, apoptotic index, and lavage and tissue VEGF concentration in *WT* and *IL6*^{-/-} mice subjected to 4h of MV after receiving IT HCl. Because effects of MV on systemic hemodynamics might confound assessment of acute lung injury, we continuously measured mean arterial pressure via a catheter placed in the carotid artery in all mechanically ventilated animals. Mean arterial pressure during MV did not differ significantly between anesthetized *IL6*^{-/-} and *WT* animals, averaging 54.3 ± 2.2 and 52.4 ± 2.4 mmHg during the 4 hr of MV, respectively. Similarly, peak airway pressure during MV did not differ between *WT* and *IL6*^{-/-} mice, averaging 22.9 ± 0.7 and 21.4 ± 0.4 mmHg throughout the duration of MV.

IL6 protein was below the limits of assay detection in all *IL6*^{-/-} mice. Shown in the top panel of Figure 1, lavage *IL6* concentration in *WT* mice did not significantly differ after IT HCl vs MV+IT HCl. In contrast, tissue *IL6* concentration (Figure 1, bottom panel) increased after MV+IT HCl when compared to IT HCl alone ($p > 0.05$). In order to determine whether *IL6* was essential for the development of vascular leak in VALI, we measured lavage protein concentration and lung wet/dry weight ratios in *IL6*^{-/-} mice following MV +IT HCl, and compared results with mechanically ventilated *WT* animals, and mice of both genotypes receiving IT HCl alone. Shown in the top panel of Figure 2, *IL6* deletion significantly attenuated MV-induced increases in lavage protein concentration in this two-hit model ($p > 0.05$ for genotype-treatment interaction). Similarly, there was a strong trend for attenuation of both enhanced lung wet/dry weight ratios (Figure 2 middle panel) and increased apoptotic index (Figure 2 bottom panel) in *IL6*^{-/-} mice treated with IT HCl+MV when compared with *WT* animals ($p = 0.07$ for both genotype-treatment interactions).

The effects of *IL6* deletion on VEGF concentration in lung lavage and tissue homogenate were determined following MV+IT HCl vs IT HCl alone in *WT* and *IL6*^{-/-} mice. Shown in the top panel of Figure 3, MV+IT HCl led to a significant increase in lung lavage VEGF concentration in *WT*, but not *IL6*^{-/-}, mice ($p > 0.05$ for genotype-treatment interaction). In contrast, tissue VEGF concentration (bottom panel of Figure 3) increased following MV+IT HCl in *IL6*^{-/-} mice when compared with *WT* control animals ($p > 0.05$ for genotype-treatment interaction).

Next we measured the effects of IL-6 deletion on airspace and tissue inflammation after MV +IT HCl, by quantifying lung lavage total and differential cell counts, tissue homogenate MPO concentrations, and histologic inflammation scores. Recovery of lung lavage fluid averaged 83±1%, and did not vary among groups. Shown in Table 2, total BAL cell count did not differ between *WT* and *IL6*^{-/-} mice (p=0.3 for genotype-treatment interaction). Although the airspace inflammatory profile differed between *WT* and *IL6*^{-/-} mice after IT HCl alone, differences in lavage differential cell counts were not present in mice subjected to MV (p = 0.05 for genotype-treatment interaction). Similarly, tissue MPO concentration and histologic inflammation score were not altered by IL-6 deletion after MV+IT HCl (Figure 4; p=0.31 and 0.45 for genotype-treatment interactions, respectively), although both measures of tissue inflammation increased after MV when compared with IT HCl alone (p = 0.05 for genotype-adjusted treatment effect).

Discussion

Despite accumulating literature regarding the potential harmful effects of MV on both lung and systemic organ function, specific pathways contributing to the development of VALI are incompletely understood. In the current study, we sought to establish whether IL-6 was a critical mediator of VALI, by assessing whether *IL6*^{-/-} mice were protected from the development of MV-induced pulmonary barrier dysfunction in a clinically relevant, two-hit injury model. The dose of IT HCl used in these experiments was titrated to prime the lung for the development of MV-induced injury, without causing significant permeability enhancement independently of high tidal volume MV.

High tidal volume MV has been associated with increased concentration of IL-6 in lung lavage, tissue homogenate and/or the systemic circulation (19, 20, 22, 23). The current data confirm that lung parenchymal IL-6 concentration increased significantly in *WT* mice after MV+IT HCl when compared with IT HCl alone, whereas IL-6 concentration in the airspace following acid aspiration was not altered by the addition of MV. To determine whether IL-6 played a causative role in the generation of VALI in this model, we compared the effects of MV+IT HCl in *WT* and *IL6*^{-/-} mice. Baseline permeability did not differ between uninjured, spontaneously ventilating, *IL6*^{-/-} mice and their *WT* controls. Lung barrier function was also not significantly altered by acid aspiration alone in spontaneously ventilating *WT* or *IL6*^{-/-} mice. Notably, however, increased alveolar-capillary permeability was significantly attenuated in *IL6*^{-/-} mice following MV+IT HCl. There was also a trend towards attenuation of increased lung water and cell death in mechanically ventilated *IL6*^{-/-} animals (p=0.07). Differences in lung injury between groups were not explained by alteration of hemodynamic parameters, as mean arterial pressure did not differ between mechanically ventilated *WT* and *IL6*^{-/-} mice. Along with published reports of an association between worsened clinical outcome and plasma IL-6 concentration in patients with ARDS (22, 23, 25), these data support a causative role for IL-6 in MV-induced alveolar barrier dysfunction.

There are several potential pathways by which IL-6 deletion might attenuate pulmonary barrier dysfunction in VALI. First, IL-6 deletion can attenuate neutrophil recruitment to sites of tissue injury (35), suggesting that the absence of IL-6 could blunt permeability by limiting granulocyte-mediated tissue damage. We do not believe that this mechanism is likely to explain our findings for the following reason. Although macrophage counts in lung lavage fluid from control *IL6*^{-/-} mice were higher than in *WT* animals, the airspace inflammatory response to MV was similar in mice of both genotypes. Furthermore, parenchymal MPO concentration and LIS did not differ between mechanically ventilated *WT* and *IL6*^{-/-} mice, and acid aspiration without MV had no significant adverse effects on lung barrier function. Although inflammation modulates pulmonary vascular permeability in many experimental

models of lung injury, there are also published examples of leukocyte-independent pulmonary barrier dysfunction (36-39), and it is well documented that neutropenic patients are not protected from the development of ARDS (40). Interestingly, our data suggest that MV and IT HCl synergized to promote IL-6-independent tissue, rather than airspace, inflammation. This unexpected finding is consistent with a prior report that lung MPO content increased, but lavage total cell and neutrophil counts decreased, in another two-hit VALI model (IP LPS + MV) (41), and is supported by recent evidence that high stretch MV enhanced monocyte and neutrophil recruitment and activation in the pulmonary microcirculation (42). Although not previously reported for lung injury, prior studies in a model of bacterial meningitis suggested that IL-6 mediated inflammation and permeability may be independently regulated (43).

Because IL-6 can promote TNF α -mediated apoptosis *in vitro* (44), another potential explanation for the protective effects of IL-6 deletion is that pulmonary parenchymal cell death is attenuated when MV-induced IL-6 production is reduced. Inhibition of either pulmonary epithelial or endothelial cell apoptosis might result in a more intact permeability barrier. We have not excluded this possibility in our model, although the MV-induced increase in pulmonary apoptotic index seen after MV+IT HCl was not statistically significantly reduced in the absence of IL-6. Finally, our experiments corroborate the findings of several prior studies demonstrating that recombinant IL-6 administration directly increased permeability of pulmonary endothelial cell monolayers *in vitro* (9, 10, 45, 46) and enhanced pulmonary vascular permeability *in vivo* (47). Our data may be most consistent with a direct effect of IL-6 deletion on inhibition of MV-induced pulmonary vascular barrier dysfunction.

The results of our current study are supported by published data that *IL6*^{-/-} mice were less susceptible to alveolar barrier dysfunction following ozone exposure (48) and acute kidney injury (49), as well as a prior report demonstrating that *IL6*^{-/-} mice had lower wet/dry lung weights than *WT* controls in another acid aspiration injury model that included MV (50). In contrast, a recently published study by Wolters et al (26) demonstrated that alveolar barrier function of *IL6*^{-/-} and *WT* mice comparably increased following 3 hr of *in vivo* MV alone. MV-induced barrier disruption and tissue neutrophil accumulation in *WT* mice were, however, exacerbated by IL-6 neutralizing antibody administration. These investigators used bone marrow chimera experiments to demonstrate that loss of hematopoietic cell-derived IL-6 augmented MV-induced barrier leak in this single-hit VALI model. The two-hit model used in our study differs in several regards, including duration of MV, specific mechanical ventilatory parameters used (tidal volume and PEEP levels), and most notably, the use of another, pro-injurious stimulus prior to the institution of MV. The discrepancies between our data and those reported by Wolters et al (26) reinforce the complexity, and likely context-dependence, of IL-6-mediated signaling in differing models of VALI, and suggest that the cellular source of IL-6 could be a critical determinant of its downstream effects.

IL-6 and ventilatory stretch both can regulate *in vitro* expression and/or release of vascular endothelial growth factor (14, 51), a potent mediator of vascular barrier dysfunction. Prior data from our laboratory and others demonstrated an association between increased VEGF and enhanced permeability in animal models of ischemia-reperfusion-, LPS-, and VEGF overexpression-mediated acute lung injury (24, 52-56), and increased IL-6 and VEGF have been linked with vascular leak in several systemic clinical disorders (29-32). Conversely, other published reports suggest that increased VEGF attenuated acute lung injury in animal models of hyperoxic-, intestinal ischemia-reperfusion- and LPS-induced lung damage (57-59), and human ARDS (60, 61). Few animal or human studies of acute lung injury have simultaneously measured BAL and tissue VEGF concentrations. The present study demonstrates that VEGF concentration in lung lavage fluid and whole lung homogenate did

not differ between uninjured, spontaneously ventilating *WT* and *IL6*^{-/-} mice, and was not altered by acid aspiration alone in animals of either genotype. The finding that tissue VEGF concentration was higher in mechanically ventilated *IL6*^{-/-} animals when compared with *WT* controls suggests that IL-6 does not directly regulate VEGF expression in this model. However, alveolar barrier dysfunction following MV+IT HCl in *WT* mice was linked with higher concentrations of VEGF in lung lavage fluid and lower concentrations of tissue VEGF, as compared with *IL6*^{-/-} animals. There are several possible explanations for these findings. First, IL-6 could promote VEGF production by airway epithelial or inflammatory cells, or augment MV stretch-induced release of VEGF into the alveolar space. Alternatively, it is possible that increased lavage VEGF in mechanically ventilated *WT* mice reflects alveolar barrier disruption and non-selective leak of VEGF into the airspace, or differential protease activation with cleavage of VEGF from the extracellular matrix. Reduced parenchymal VEGF concentration has been associated with both enhanced permeability and alveolar epithelial cell death in other animal (62) and human (63) lung injury models. Increased VEGF concentration in the lung parenchymal compartment of *IL6*^{-/-} mice could thereby contribute to preservation of alveolar-capillary barrier function. Additional studies will be required to explore whether stretch-induced changes in VEGF production or release are augmented by IL-6, and how IL-6, ventilatory stretch, and VEGF interact to alter lung barrier function in VALI.

Conclusions

The results of the current study demonstrate that genetic deletion of IL-6 significantly attenuated alveolar-capillary barrier disruption in a two-hit model of VALI. The protective effects of IL-6 deletion were not associated with differences in MV-induced airspace or parenchymal inflammation, although we cannot exclude the possibility that macrophage or monocyte subpopulations differ in mechanically ventilated *IL6*^{-/-} and *WT* mice. Beneficial effects of IL-6 deletion on lung barrier function were linked with reduced VEGF in the alveolar space and enhanced levels of tissue VEGF. Altogether, these data suggest that strategies to interfere with IL-6 expression or signaling may represent an important therapeutic target to limit the injurious effects of mechanical ventilation in the inflamed lung. Because IL-6 inhibition using neutralizing antibodies may promote tissue neutrophil accumulation and alveolar permeability after injurious MV of normal mouse lungs (26), additional experiments will be necessary before considering strategies to systemically inhibit IL-6 in human studies of ARDS.

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Figure 1.

IL-6 protein concentration measured in lung lavage fluid (**top panel**) and whole lung homogenate (**bottom panel**). In *WT* mice, MV for 4 hr after acid aspiration augmented expression of IL-6 in whole lung homogenate, but had no effect on lavage IL-6 concentration, when compared to IT HCl alone. IL-6 protein was below the limits of assay detection in lung lavage fluid and tissue homogenate from *IL6*^{-/-} mice. Mice receiving IT HCl alone are represented by black bars, while animals in the MV+IT HCl groups are shown by gray bars. Values shown represent mean + standard error for 5-6 animals/group.

Figure 2.

Lung lavage protein concentration (**top panel**) after MV+IT HCl was significantly higher in *WT* mice when compared with *IL6*^{-/-} mice after MV+IT HCl, or mice of either genotype receiving IT HCl alone. There was a trend for attenuation of enhanced lung wet/dry weight ratio in mechanically ventilated *IL6*^{-/-} mice as compared with *WT* controls (**middle panel**). There was a similar trend for reduced apoptotic index (**bottom panel**) after MV+IT HCl in *IL6*^{-/-} mice when compared with *WT* controls. Mice receiving IT HCl alone are represented by black bars, while animals in the MV+IT HCl groups are shown by gray bars. Values shown represent mean ± standard error for 4-7 animals/group.

Figure 3.

Top panel: Lung lavage VEGF increased significantly after MV+IT HCl in *WT* mice when compared to *WT* mice receiving IT HCl alone, and *IL6*^{-/-} mice receiving IT HCl alone or MV+IT HCl. **Bottom panel:** In *WT* mice, MV for 4 hr after acid aspiration did not alter VEGF concentration in whole lung homogenate when compared with IT HCl alone. In contrast, tissue concentration of VEGF was significantly higher in mechanically ventilated *IL6*^{-/-} animals. Mice receiving IT HCl alone are represented by black bars, while animals in the MV+IT HCl groups are shown by gray bars. Values shown represent mean $\pm$ standard error for 5-6 animals/group.

Figure 4.

Deletion of IL-6 did not alter MV-induced increases in tissue MPO concentration (**panel A**), or histologic evidence of inflammation (LIS) (**panels B and C**). Mice receiving IT HCl alone are represented by black bars, while animals in the MV+IT HCl groups are shown by gray bars. Values shown represent mean $\pm$ standard error for 5-6 animals/group.

Table 1

MV-independent effects of IT HCl.

	WT		IL6 ^{-/-}	
Outcome	Uninjured	IT HCL	Uninjured	IT HCL
Lavage:				
Total cells ($\times 10^4$)	5.65 $\pm$ 0.49	11.17 $\pm$ 1.33	7.89 $\pm$ 1.11	14.21 $\pm$ 1.24
Macrophages ($\times 10^4$)	5.55 $\pm$ 0.46	7.35 $\pm$ 1.19	7.80 $\pm$ 1.10 *	13.5 $\pm$ 1.01 *
Neutrophils ($\times 10^4$)	0.06 $\pm$ 0.03	3.73 $\pm$ 1.56 *	0.02 $\pm$ 0.01	0.62 $\pm$ 0.20
Lymphocytes ($\times 10^2$)	4.02 $\pm$ 0.75	9.1 $\pm$ 3.0	6.44 $\pm$ 1.65	11.5 $\pm$ 1.7
Tissue MPO (μ g/mg)	16.3 $\pm$ 2.7	32.7 $\pm$ 2.2 *	15.4 $\pm$ 1.5	18.4 $\pm$ 2.7
Lavage protein (pg/ml)	327 $\pm$ 79	370 $\pm$ 25	288 $\pm$ 46	375 $\pm$ 20
Lung wet/dry weight	4.52 $\pm$ 0.05	4.67 $\pm$ 0.12	4.49 $\pm$ 0.08	4.49 $\pm$ 0.02
Lavage VEGF (pg/ml)	48.2 $\pm$ 5.7	52.4 $\pm$ 3.1	48.8 $\pm$ 4.0	54.6 $\pm$ 10.1
Tissue VEGF (pg/mg)	402 $\pm$ 34	394 $\pm$ 9	399 $\pm$ 20	388 $\pm$ 40

*
p<0.05 for genotype-treatment interaction

Table 2

Lung lavage cell counts: IT HCl vs MV+IT HCl.

	WT		IL6 ^{-/-}	
	IT HCl	MV+IT HCl	IT HCl	MV+IT HCl
Lavage:				
Total cells ($\times 10^4$)	11.17 $\pm$ 1.33	9.47 $\pm$ 1.44	14.21 $\pm$ 1.24	10.17 $\pm$ 1.21
Macrophages ($\times 10^4$) [*]	7.35 $\pm$ 1.19	9.31 $\pm$ 1.43	13.5 $\pm$ 1.01	8.90 $\pm$ 1.97
Neutrophils ($\times 10^4$) [*]	3.73 $\pm$ 1.56	0.06 $\pm$ 0.02	0.62 $\pm$ 0.20	0.02 $\pm$ 0.01
Lymphocytes ($\times 10^2$)	9.1 $\pm$ 3.0	10.4 $\pm$ 2.6	11.5 $\pm$ 1.7	6.9 $\pm$ 3.4

^{*}
p<0.05 for genotype-treatment interaction