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Differential roles of p55 and p75 tumor necrosis factor receptors on stretch-induced pulmonary edema in mice

Michael R. Wilson, Michael E. Goddard, Kieran P. O’Dea, Sharmila Choudhury, and Masao Takata

Department of Anaesthetics, Pain Medicine, and Intensive Care, Faculty of Medicine, Imperial College London, Chelsea and Westminster Hospital, London, United Kingdom

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Ventilator-induced lung injury plays a crucial role in the outcome of patients with acute lung injury. Previous studies have shown a role for the cytokine tumor necrosis factor-α (TNF) in stretch-induced alveolar neutrophil recruitment, but the involvement of TNF in stretch-induced pulmonary edema is unclear. We investigated the effects of TNF through its individual p55 and p75 receptors on early pulmonary edema formation during high stretch ventilation, before neutrophil infiltration. Anesthetized wild-type or TNF receptor single/double knockout mice were ventilated with high tidal volume (~38 ml/kg) for 2 h or until they developed arterial hypotension. Pulmonary edema was assessed by physiological parameters including respiratory mechanics and blood gases, and by lavage fluid protein and lung wet:dry weight ratio. No myeloperoxidase assay was performed. The results demonstrate a novel role for TNF signaling during VILI. Interestingly, we observed that pulmonary edema induced by high mechanical stretch, which occurred rapidly before neutrophil infiltration, was not attenuated in the TNF receptor double knockout mice (75), a finding independently reported by Yoshikawa and colleagues (78). Thus it has been generally considered that TNF is not involved in the development of early, neutrophil-independent, stretch-induced pulmonary edema (10, 78), despite the fact that TNF is already expressed within the same time frame during the course of VILI (28, 51, 74).

Although there is strong evidence that TNF is able to influence the progression of pulmonary edema by altering epithelial/endothelial cell functions, the predicted net effects of TNF on pulmonary edema formation is conflicting in the literature. For example, TNF may either promote pulmonary edema by increasing epithelial/endothelial permeability (30, 38, 44, 45) or oppose edema by enhancing alveolar fluid reabsorption (3, 13, 19). Such apparently conflicting effects of TNF may be related to the poorly understood processes of differential p55 and p75 receptor signaling. It is increasingly clear that the two TNF receptors can signal through different intracellular pathways and may induce different cellular responses (27, 33, 43, 71). In fact, recent reports have raised the possibility that the two TNF receptors may in some circumstances signal in a directly opposing manner, e.g., in models of retinal ischemic injury (18) and sepsis induced by cecal ligation and puncture (12).
We therefore hypothesized that differential signaling through the individual TNF receptors may have opposing effects on pulmonary edema development induced by high stretch ventilation, which could be masked by the absence of both receptors in TNF receptor double knockout mice. To address this, we investigated the development of pulmonary edema in mice lacking one or both of the TNF receptors and demonstrated for the first time that p55 signaling promotes edema development induced by high stretch ventilation, while this is seemingly opposed by signaling through the p75 receptor.

**METHODS**

**Animal preparation.** All protocols were approved by the United Kingdom Home Office in accordance with the Animals (Scientific Procedures) Act 1986, United Kingdom. We used wild-type (WT) male C57BL6 mice (Charles River) aged 10–18 wk, age-matched male p55<sup>−/−</sup> TNF receptor knockout (p55KO), p75<sup>−/−</sup> TNF receptor knockout (p75KO), or p55<sup>−/−</sup>/p75<sup>−/−</sup> TNF receptor double knockout (DKO) mice (generous gifts from Amgen, Thousand Oaks, CA) (43). The DKO mice were generated by crossing the p55KO and p75KO strains. The p55KO mice were C57BL6 inbred, whereas the p75KO and DKO mice were backcrossed onto their WT C57BL6 strain for five generations. Mice were anesthetized by intraperitoneal injection of 2.5 ml/kg Hypnorph (0.8 mg/kg Fentanyl, 25 mg/kg Fluanisone) and 2.5 ml/kg Midazolam (12.5 mg/kg). The surgical preparation has been described in detail previously (6, 20, 74, 75). Briefly, mice were ventilated via endotracheal tube using a custom-made mouse ventilator-pulmonary function testing system with O₂ (supplemented with 5% CO₂ during high stretch to prevent hypocapnia). The left carotid artery was cannulated for monitoring arterial blood pressure (BP) and blood gases and for saline infusion (0.4 ml/h). During instrumentation, artery was cannulated for monitoring arterial blood pressure (BP) and 5% CO₂ during high stretch to prevent hypocapnia). The left carotid artery was cannulated for monitoring arterial blood pressure (BP) and blood gases and for saline infusion (0.4 ml/h). During instrumentation, animals were ventilated with low stretch, i.e., tidal volume (VT) of 6–7 ml/kg, 2.5 cmH₂O positive end-expiratory pressure (PEEP), and respiratory rate (RR) of 120 breaths/min.

**Physiological measurements during high stretch ventilation.** Following physiological measurements to ensure stability of the preparation, mice were ventilated with high stretch, i.e., initial peak inspiratory pressure (PIP) set at 45–46 cmH₂O (mean VT₃ 38.5 ± 3.8 ml/kg; means ± SD), 0 PEEP, RR of 90/min. Immediately after the start of high stretch ventilation, a 200-μl saline bolus was given to aid with venous return. High stretch ventilation with the same constant VT₃ was continued either for 2 h or until imminent cardiorespiratory collapse was indicated by BP <45 mmHg (74), whichever occurred first. Airway pressure, gas flow, and mean BP were monitored continually throughout the experiments via transducers. Respiratory system resistance (Rₛ) and compliance (Cₛ) were determined by the end-inflation occlusion technique (15) throughout the ventilatory protocol. Blood gas analyses (~70 μl/sample, with bolus administration of saline to replace fluids) were carried out immediately after surgery, after 60 min of high stretch ventilation, and at the end of the protocol.

At the conclusion of ventilatory protocols, mice were euthanized by anesthetic overdose, and some animals were subjected to lung lavage as described previously (74). In these animals, protein concentration in lavage fluid was determined by the Bradford method (2) using a protein assay kit (Bio-Rad Laboratories, Hemel Hempstead, UK), and levels of TNF receptors (p55 and p75), macrophage inflammatory protein-2 (MIP-2), and keratinocyte-derived chemokine (KC) protein were measured using sandwich ELISA kits (R&D Systems, Abingdon, UK). Cells in lavage fluid were counted by hemocytometer, and differential cytology was performed on Diff-Quik-stained samples prepared by cytopsin (Shandon, Runcorn, UK). In nonlavedged animals, the right lung was removed, weighed, and dried at 60°C for wet: dry ratio determination. In some lavaged WT and p55KO animals, the lungs were removed, and MPO activity was quantified as described previously (6, 52). In brief, lung tissue was homogenized, sonicated two times, freeze-thawed three times, and centrifuged. The supernatant was mixed with O-dianisidine (Sigma-Aldrich, Poole, UK) and hydrogen peroxide, and changes in absorbance were measured over 3 min with the results expressed as optical density per minute.

**Permeability measurements during high stretch ventilation.** In a separate series of experiments, pulmonary endothelial epithelial permeability to a fluorescence-labeled albumin was assessed in each strain of mice during high stretch ventilation, using an adaptation of a previously described protocol (73). Alexa Fluor 594-conjugated albumin (Invitrogen) was infused into the right jugular vein of anesthetized instrumented mice, which were then ventilated with high stretch for 60 min. Animals were killed, lungs were lavaged, and blood was collected by cardiac puncture. Lung permeability was estimated by the ratio of lavage fluid: plasma fluorescent signal.

**Data analysis.** Data are expressed as means ± SD. Statistical analyses were carried out by t-tests, ANOVA with Bonferroni tests, general linear mixed model analysis, and Kaplan-Meier survival analysis with log-rank tests, using SPSS version 14.0 (Chicago, IL). A P value of less than 0.05 was considered significant.

**RESULTS**

**Physiological parameters.** In WT animals, high stretch ventilation produced progressive increases in PIP during the 2-h ventilation protocol (Fig. 1). PIP was stable for 80–90 min but thereafter increased rapidly, whereas BP was well maintained throughout the protocol until the last 5–10 min of the experiment (when lung injury indicated by PIP became severe), consistent with our previous studies using similar mouse VILI models (6, 74, 75). We found that the patterns of PIP changes in response to high stretch were very different among the WT and TNF receptor knockout animals (P < 0.001, Fig. 2A). TNF receptor DKO mice showed similar PIP changes to WT animals with comparable kinetics. However, p55KO mice did not show any increases in PIP across the entire 2-h ventilation protocol. In contrast, p75KO mice displayed more rapid increases in PIP, suggesting a greater susceptibility of these mice to high stretch as described previously (74).

**Fig. 1.** High stretch-induced changes in peak inspiratory pressure (PIP) and blood pressure (BP) in wild-type mice. Mice were ventilated with high stretch ventilation at a constant tidal volume (VT); initial PIP 45–46 cmH₂O which corresponds to a VT of 38.5 ± 3.8 ml/kg for up to 2 h. PIP remained stable until 80–90 min and thereafter increased dramatically. BP showed a transient decline on initiation of high stretch ventilation, but thereafter was stable until a rapid decline that occurred within 5–10 min at the end of the experiment. N = 11 at the start of ventilation. Data points represent the mean of all surviving animals at each time point.

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to high stretch-induced injury. The PIP values at the end of the protocol (Fig. 2B) were similar in WT and DKO mice, but higher \((P < 0.05)\) in p75KO mice and lower \((P < 0.05)\) in p55KO mice, compared with both WT and DKO animals.

Consistent with these changes in PIP, high stretch ventilation produced alterations in respiratory mechanics (Fig. 3), including an increase in Rrs and a decrease in Crs compared with the start of the high stretch protocol. The increase in Rrs was similar in WT, DKO, and p75KO mice but attenuated \((P < 0.01)\) in p55KO mice compared with both WT and DKO animals. The decrease in Crs was similar in WT and DKO mice, greater in p75KO mice \((P < 0.01)\) vs. WT, and attenuated in p55KO mice \((P < 0.01)\) vs. WT and DKO.

Development of high stretch-induced lung injury was also associated with functional impairments in gas exchange (Table 1).

In WT, DKO, and p75KO mice, arterial \(\text{PO}_2\) levels decreased and \(\text{PCO}_2\) increased \((P < 0.01)\) in samples taken at the end of the protocol compared with those taken after 60 min of high stretch (when PIP changes were negligible). In contrast, \(\text{PO}_2\) and \(\text{PCO}_2\) did not significantly change in p55KO animals.

We have previously found in mice ventilated with high stretch that when BP falls \(< 45 \text{ mmHg}\) in conjunction with increased PIP, then cardiorespiratory collapse due to severe pulmonary edema follows soon after, associated with reduced organ perfusion and profound metabolic acidosis \((74)\). In the current experiments, we used mean BP \(< 45 \text{ mmHg}\) as a surrogate marker for mortality, which avoided any potential impact of such confounding variables on measured physiological and biochemical parameters, as demonstrated by a normal pH in all animals at the end of the experiments (Table 1). WT mice started to “die” at \(\sim 90\) min, and only \(\sim 25\%\) of the animals survived 2 h of ventilation (Fig. 4). This was similar in DKO mice, but occurred sooner in p75KO mice (after \(\sim 70\) min), and no animals in this group reached 2 h of ventilation. In contrast, all p55KO mice completed the full 2-h period. Statistical analysis indicated that the survival curves were different among the mouse strains, i.e., between p75KO vs.
DISCUSSION

The present study demonstrated that TNF signaling plays a major role in the development of stretch-induced pulmonary edema during VILI, but the two types of TNF receptor signal in apparently opposing manners. Historically, there has been considerable controversy regarding the involvement of TNF in the development of the two primary pathologies of VILI, namely pulmonary edema, which starts early in the course of VILI, and pulmonary inflammation, involving neutrophil influx into the alveoli, which occurs at later stages (10, 11, 49).

We previously found that anti-TNF treatment attenuated both edema formation and neutrophilic inflammation in a rabbit model of VILI induced by saline lavage followed by injurious ventilation (24), but interpretation of this study is complicated by the intrinsic difficulties in such “two-hit” models to separate the effects of ventilation from those of preexisting lung injury (10, 75). In addition, the large degree of neutrophil infiltration in this model makes it impossible to distinguish between neutrophil-dependent and -independent effects. More recently, we have demonstrated in mouse models of VILI, induced

determined in lungs from WT and p55KO mice subjected to high stretch ventilation. There was no difference in MPO activity in samples from these mouse strains (Fig. 7), indicating that the protection from edema formation in p55KO mice was independent of the degree of neutrophil accumulation. In addition, no differences among any of the four strains were observed for the levels of neutrophil chemotactants MIP-2 (WT 19.8 ± 10.3, DKO 10.7 ± 10.4, p55KO 12.4 ± 7.1, p575KO 10.4 ± 6.7 pg/ml) or KC (WT 100 ± 89, DKO 26 ± 15, p55KO 62 ± 67, p75KO 67 ± 67 pg/ml) in lavage fluid, all of which supports the likelihood that the effects observed were independent of any influence of TNF on neutrophil recruitment. Finally, TNF receptor levels were determined in lavage fluid of WT mice. Compared with nonventilated mice, high stretch ventilation promoted dramatic increases in the levels of both p55 and p75 receptors in lavage fluid (Fig. 8).

The current study was performed in a mouse model of VILI by performing mechanical ventilation (24) and determining the resulting changes in various parameters. The authors found that TNF signaling was involved in the development of pulmonary edema and neutrophilic inflammation, as indicated by the increased levels of TNF receptors and chemotactants in the lavage fluid of WT mice. The observed protection from edema in p55KO mice was independent of the degree of neutrophil accumulation, indicating a role for TNF in mediating this effect. The authors also examined the role of TNF in the development of pulmonary inflammation, finding increased levels of TNF receptors and chemotactants in the lavage fluid of WT mice, indicating a role for TNF in mediating this effect.

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Pulmonary edema and permeability. To assess formation of pulmonary edema, we determined lavage fluid protein content and lung wet: dry weight ratio at the end of the ventilation protocols. The degree of pulmonary edema was similar by either measure in WT, DKO, and p75KO animals, but attenuated (P < 0.01) in p55KO mice (Fig. 5). To determine whether the observed protection from edema in p55KO mice was related to improved pulmonary endothelial/epithelial barrier function, translocation of fluorescence-labeled albumin from blood to alveolar space was measured in separate experiments after just 60 min of ventilation (Fig. 6). This early time point was used to perform measurements in the absence of potentially confounding factors such as deteriorating BP and PO2 that would occur in the later stages of the experiments. Even at this early stage before changes in PIP became apparent, the lung permeability was increased by high stretch ventilation in WT mice, which was similar in DKO and p75KO mice, but significantly lower in high stretch-ventilated p55KO animals (P < 0.05).

Pulmonary inflammation. The cells recovered by lung lavage contained negligible amounts of neutrophils in any mouse strain (WT 2.1 ± 2.4, DKO 0.4 ± 0.2, p55KO 0.7 ± 0.4, p75KO 0.7 ± 0.6%), indicating that the ventilation period was not long enough to produce significant alveolar neutrophil infiltration. As we have previously observed that neutrophils are sequestered within the lung vasculature at a very early stage of VILI before alveolar migration (6), MPO activity was

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purely by high stretch ventilation in the absence of preexisting lung injury, that pulmonary neutrophil recruitment was attenuated by blockade of TNF signaling (75). Here we provide the first in vivo evidence of a role for TNF receptor I (p55) signaling in promoting stretch-induced pulmonary edema formation. In contrast, mice lacking TNF receptor II (p75) signaling showed increased susceptibility to edema formation, indicating a protective role for this receptor.

In the current study, we have used a modification of our previous mouse model of high tidal volume VILI (6, 74, 75) in which the experimental protocol was designed specifically to detect development of pulmonary edema (as represented by changes in PIP, respiratory mechanics, gas exchange, lavage fluid protein levels, and lung wet:dry ratio) before neutrophil infiltration. The ventilator settings were chosen to induce substantial edema in WT animals within a 2-h period, which was not long enough to upregulate expression of neutrophil chemoattractants in lavage fluid or to produce intra-alveolar neutrophil recruitment, but sufficiently long to induce intrapulmonary expression of bioactive TNF, as demonstrated by ourselves (74) and others (8, 22, 28, 51, 61, 62, 67, 68) using similar protocols. This protocol thus enabled assessment of the effects of TNF signaling on stretch-induced edema formation independently from its effects on neutrophilic inflammation, whereas in more prolonged models such separation would be much more difficult.

Consistent with previous reports by ourselves and others (75, 78), high stretch-induced pulmonary edema was not attenuated in mice lacking both TNF receptors (DKO), which behaved very similarly to WT animals. In complete contrast, mice lacking solely the p55 receptor were strongly protected from the edema-promoting effects of high stretch, whereas p75KO mice were more susceptible than the other strains. Since the current protocol was designed to evaluate most of the injury parameters at the end of experiments when WT animals displayed substantial injury, it is well suited to detect protective effects, but much less sensitive to injurious effects (as virtually all animals in the control and increased susceptibility groups will be severely injured when measurements are taken). Presumably, for this reason, p75KO animals exhibited differences from WT animals only in some of the terminally measured parameters (PIP and Crs), but not in others (PO2, lavage fluid protein, and lung wet:dry weight ratio). However, analyses of the time course of injury parameters provided statisti-

![Figure 5](image_url.png)

Fig. 5. Pulmonary edema formation in WT and TNF receptor knockout mice assessed by lavage fluid protein content (A) (n = 7 for WT mice and 5 for each of the other strains) or lung wet:dry weight ratio (B) (n = 4 for each strain) at the end of high stretch ventilation protocols. *P < 0.01 vs. WT, †P < 0.01 vs. DKO by 1-way ANOVA with Bonferroni tests.

![Figure 6](image_url.png)

Fig. 6. Pulmonary endothelial/epithelial barrier permeability in WT and TNF receptor knockout mice assessed by lavage fluid/plasma ratio of Alexa Fluor 594-conjugated albumin, after 60 min of high stretch ventilation (before increases in PIP became apparent). Data from low stretch WT mice are shown as a dotted line for comparison. N = 7 for WT, and 5 for each of the other strains. *P < 0.05 vs. WT by 1-way ANOVA with Bonferroni tests.

Table 2. Baseline values of Rrs and Crs at the start of high stretch ventilation in WT and TNF receptor knockout mice

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<th>WT</th>
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<tr>
<td><strong>Baseline</strong></td>
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<tr>
<td>Rrs (cmH2O·ml⁻¹·s⁻¹)</td>
<td>1.6±0.2</td>
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<td>Crs (ml/cmH2O)</td>
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Values are means ± SD. N = 22 for WT mice, and 12-14 for DKO, p75KO, and p55KO mice. There were no significant differences among the strains for either parameter. Rrs, respiratory system resistance; Crs, respiratory system compliance.
rally significant evidence of an increased susceptibility in p75KO mice, i.e., PIP increased more rapidly and death occurred sooner with no animals surviving the full 2-h protocol. Rapidly developing, neutrophil-independent pulmonary edema induced by high stretch has traditionally been considered to be a result of mechanical disruption of the alveolar-capillary membrane, with little involvement of pulmonary inflammation. Although this may be the case with extremely acute (occurring in minutes) edema (9), it has been shown that certain inflammatory mediators such as TNF are upregulated within the alveoli sufficiently early in the course of VILI (74) to be involved in more gradually developing (occurring in hours) pulmonary edema. Our results clearly support an early involvement of inflammation in stretch-induced edema formation, with TNF playing a direct role. This occurred independently of its effect on neutrophil recruitment, as most apparent from the attenuated edema in p55KO mice despite similar lung MPO activity. The edema-promoting action of TNF in these experiments is mediated specifically through the p55 TNF receptor. The p55 receptor has historically been considered the predominant receptor for the cellular actions of TNF (71), with p75 proposed mainly as a modulator of p55 signaling, either cooperatively by ligand passing to p55 or negatively by the soluble receptor sequestering TNF and thus preventing binding to cell-associated p55 (32, 33, 71). However, if the only role for the p75 receptor was through modulating p55 signaling, we should expect TNF receptor DKO mice to be somewhat protected from injury as they lack both receptors, which was clearly not the case either here or as reported elsewhere (75, 78). Considering the increased susceptibility to high stretch ventilation in p75KO mice, and the fact that the DKO animals used were derived from crossing of the p55KO and p75KO animals and all three strains have a similar C57BL6 background, the data are better explained by p75 and p55 signaling having directly opposite effects, with p75 signaling impeding edema formation independently of p55. It is becoming clear that the two TNF receptors are indeed capable of signaling independently (27, 33, 43, 71), and directly opposing effects have been proposed in sepsis induced by cecal ligation and puncture (12) and in retinal ischemic injury (18), but not previously with relation to acute lung injury.

The mechanisms underlying the observed differential roles of the two TNF receptors are unclear, but there are several possibilities on which we may speculate. First, while TNF has been shown to induce impairment of endothelial and epithelial barrier function by promoting reorganization of the actin cytoskeleton (30, 38, 44, 45, 76, 77) predominantly through the p55 receptor (16), recent studies suggested that a tyrosine kinase pathway, Etk/BMX, activated by the p75 receptor (41), can improve epithelial barrier function by promoting a redistribution of actin filaments (23). Second, the aforementioned study of retinal ischemic injury (18) suggested that the protective effect of p75 signaling was mediated through the anti-apoptotic PkB/Akt pathway. High stretch ventilation has been demonstrated to activate Akt by phosphorylation (65), but the relevance of apoptosis to acutely progressing pulmonary edema in VILI is controversial. Third, the apparently protective effects of p75 TNF receptor signaling might be related to the stimulatory effect of TNF on alveolar fluid clearance (3, 13, 19), although potential involvement of each TNF receptor in this process has yet to be clarified. Fourth, TNF could be acting through downstream intermediates such as transforming growth factor-β or vascular endothelial growth factor, both of which have been implicated in pulmonary edema formation (26, 58) and may be upregulated/augmented by TNF (29, 59). Finally, it is possible that the recently described processes of plasma membrane wounding and turnover (70) may play some role in the acute pulmonary edema formation, but it is unknown whether TNF is involved in modulating these.

The expression of TNF receptors within the lung and the affinity of each receptor to different forms of TNF likely play a role in determining which receptor TNF binds and activates during VILI. Both p55 and p75 receptors have been demonstrated to be present in lung tissue (14, 37), although the precise localization is unclear. Some studies suggest that alveolar epithelial cells express p55 only (37, 42, 47), whereas others demonstrate epithelial expression of both receptors (4, 31). The affinity of p75 receptor to soluble TNF at low
concentrations is much greater than that of p55 (66), and it has been suggested that membrane-bound TNF signals mainly through p75 receptor (21). Therefore, during the course of high stretch-induced injury, TNF may signal initially through “protective” p75 (either by rapidly upregulated membrane TNF on TNF-expressing cells or by low local concentrations of soluble TNF) but later through “deleterious” p55 as concentrations of soluble TNF increase. In addition, shedding of TNF receptors within the alveoli would also influence TNF signaling. We demonstrate here for the first time that soluble TNF receptors of both types are dramatically increased in lavage fluid by high stretch ventilation. The physiological effects of this, as well as the cell sources, are as yet undetermined. Theoretically, increased soluble TNF receptor levels could either impede TNF signaling or prolong its biological half-life, and it is not yet clear whether the increased receptor levels in our study represent a protective or deleterious process. Increased soluble TNF receptor levels in both bronchoalveolar lavage and plasma have, however, been reported following nonprotective ventilation in ARDS patients and have been associated with worse outcome (42, 48).

In the current study, the V_T used to produce VILI was much greater than would be used in the clinical setting in humans. However, the results should still give important insights into the pathophysiology of VILI, as the V_T employed is similar to those used in previous in vivo and ex vivo animal studies in the literature (9, 25, 50, 61), which have substantially contributed to our understanding of the mechanisms of VILI. Moreover, as has been pointed out (54), the lung stretch induced by such volumes may not be dissimilar from that experienced clinically, as 40 ml/kg delivered to a healthy mouse lung may approximate the stretch induced by 10 ml/kg delivered to an injured human ARDS lung with only 25% aerated lung capacity. Finally, it may be entirely inappropriate to directly compare the absolute values of either V_T or inspiratory pressure between mice and humans, as the mouse respiratory system is more compliant than other species with very different pressure-volume curves, such that intact mouse lungs can be temporarily inflated to pressures above 60 cmH_2O, relating to a V_T of 60–70 ml/kg, without reaching a traditionally defined total lung capacity or producing morphological damage (56).

Formation of permeability pulmonary edema is an important hallmark of VILI and ALI, determining the impairment of lung function and crucially influencing the clinical course of the diseases. Our findings indicate that in the current model of rapidly evolving VILI, TNF signaling strongly mediates stretch-induced pulmonary edema independently of its effects on neutrophil recruitment. As with all small animal models, the conditions used to create the model represent a balance between clinical relevance, practicality, and addressing the question posed, and therefore limitations may exist in extrapolating our results to more slowly evolving VILI models and/or clinical situations. In particular, since neutrophil infiltration is usually associated with VILI/ALI pathology in patients, the precise clinical implication of such neutrophil-independent effects of TNF signaling has yet to be determined. However, it is possible that the direct effects of TNF on lung permeability could be additive/synergistic with neutrophil-mediated effects in vivo. Moreover, the direct effects of TNF may play an important role in certain conditions of VILI and ALI, as evidenced by the observations that ALI can develop even in neutropenic patients (40) and that neutrophil recruitment per se does not necessarily induce ALI (34). Our results provide an important notion that TNF signaling affects stretch-induced pulmonary edema in a complex fashion, having direct effects on lung permeability that may be either positive or negative, in addition to generally negative indirect effects mediated by neutrophils in later stages.

The results of the present study may have further implications for the treatment of VILI and potentially ALI of other etiologies if differential TNF signaling is a common phenomenon. The demonstration of differential roles of TNF receptors suggests that total blockade of TNF signaling may be counterproductive for the treatment of VILI and that more targeted strategies may be necessary, such as specific blockade of p55 or upregulation of p75 signaling. Which of these targets would be most appropriate for intervention may vary during the progression of lung injury and depend on the importance of pulmonary edema in the pathophysiology at that stage. Our results may offer insights to reevaluate the generally negative outcome of previous studies of sepsis and ALI utilizing total TNF signaling blockade strategies (e.g., anti-TNF antibodies, TNF knockout, or TNF receptor DKO mice), as TNF involvement may have been masked by such differential receptor signaling.

GRANTS

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REFERENCES

TNF RECEPTOR SIGNALING IN HIGH LUNG STRETCH


