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Reduction in alveolar macrophages attenuates acute ventilator induced lung injury in rats

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Abstract *Objective:* Alveolar macrophages are the sentinel cell

for activation of the inflammatory cascade when the lung is exposed to noxious stimuli. We investigated the role of macrophages in mechanical lung injury by comparing the effect of high-volume mechanical ventilation with or without prior depletion of macrophages. *Design and setting:* Randomized sham-controlled animal study in anesthetized rats. *Methods:* Lung injury was induced by 15 min of mechanical ventilation (intermittent positive pressure ventilation) using high peak pressures and zero end-expiratory pressure. The mean tidal volume was 40 ± 0.7 ml/kg. One group of animals was killed immediately after this period of volutrauma (HV), while in a second group normoventilation was continued for 2 h at a tidal volume less than 10 ml/kg (HV-LV). One-half of the animals were depleted of alveolar macrophages by pretreatment with intratracheal liposomal clodronate (CL₂MDP). *Measurements:* Arterial blood gas, blood pressure. After kill: lung static

pressure volume curves, bronchoalveolar fluid concentration for protein, macrophage inflammatory protein 2, tumor necrosis factor α , and wet/dry lung weight ratio (W/D). *Results:* During HV and HV+LV oxygenation, lung compliance, and alveolar stability were better preserved in animals pretreated with CL₂MDP. In both groups W/D ratio was significantly greater in ventilated than in nonventilated animals (4.5 ± 0.6), but the increase in W/D was significantly less in CL₂MDP treated HV and HV-LV groups (6.1 ± 0.4 , 6.6 ± 0.6) than in the similarly ventilated nontreated groups (8.7 ± 0.2 and 9.2 ± 0.5). *Conclusions:* Alveolar macrophages participate in the early phase of ventilator-induced lung injury.

Keywords Ventilator-induced lung injury · Volutrauma · Barotrauma · Macrophages · Clodronate · Inflammation

Introduction

The role of alveolar overdistension as an established mechanism of ventilator-induced lung injury (VILI) has become fairly well established [1]. Recent multicenter controlled, randomized clinical trials [2, 3] confirmed the findings of a prior study [4] that a low tidal volume ventilation strategy improves survival during mechanical ventilation for

acute respiratory distress syndrome (ARDS). Mechanical disruption of the alveolar capillary barrier obviously contributes to the lung injury. Previous studies from our laboratory indicate that the endothelial barrier actively controls the vascular permeability response to high airway and vascular injury even in presence of significant capillary rupture [5, 6]. In addition, elevated levels of proinflammatory cytokines and recruitment of activated neutrophils

and macrophages to the bronchoalveolar space have been reported in various animal models of VILI [7–9]. A similar inflammatory response is seen in human patients with ARDS who ultimately develop ventilator associated lung injury [10].

The early mechanisms by which lung overdistension leads to alveolar inflammation are unclear. Although activated neutrophils have an important role once the inflammatory cascade is well established, the initial steps leading to their activation and recruitment must depend upon the resident pulmonary cells population such as the endothelial and epithelial cells or alveolar macrophages. A number of studies have implicated the alveolar macrophages as the sentinel cell whose activation initiates lung inflammation after challenge with live bacteria, endotoxin or ischemia-reperfusion [11–15]. Among a variety of cultured respiratory cells subjected to cyclic strain, Pugin et al. [16] identified the lung macrophage as the main source for critical inflammatory mediators such as tumor necrosis factor (TNF) α , the chemokines interleukin (IL) 8 and 6 and matrix metalloproteinase 9.

The purpose of our study was to investigate the role of alveolar macrophage in a model of high tidal volume induced lung injury. We subjected adult rats to a mode of mechanical ventilation known to be injurious [1] and compared the degree of injury between animals with and without prior depletion of alveolar macrophages. Depletion of macrophages was achieved by intratracheal instillation of liposomes containing clodronate which has previously been shown to specifically eliminate alveolar macrophages in the adult rat [17]. The results of this work were previously presented in part at the 2002 meeting of Experimental Biology in New Orleans [18].

Methods

Animal preparation

This protocol was approved by our institutional animal care and use committee. Adult Sprague Dawley rats (weight range 340–460 g) were used for the study. Following anesthesia with intraperitoneal pentobarbital (45 mg/kg) a tracheotomy was performed. Thereafter, mechanical ventilation (intermittent positive pressure ventilation) was begun using an infant pressure and time cycled ventilator (Sechrist Model IV-100, Anaheim, Calif., USA). The inspired ventilatory gases were humidified and heated to body temperature. During mechanical ventilation animals were paralyzed with 0.1 mg/kg pancuronium.

Mechanical ventilation

Animals were mechanically ventilated using a peak inspiratory pressure (PIP) of 45 cmH₂O, zero positive

end-expiratory pressure (ZEEP), and inspiratory time and respiratory rate set at 0.8 s and 25 bpm, respectively. Mean delivered tidal volume was 40 ± 0.7 ml/kg. The animals were randomized to be killed 15 min after this short period of barotrauma (HV, $n = 14$) or continue to be mechanically ventilated for the following 2 h with reduced PIP sufficient to maintain normocapnea without delivering a tidal volume in excess of 10 ml/kg (HV-LV, $n = 14$). During the 2-h recovery PEEP was increased to 2 cmH₂O, and inspiratory time and ventilatory rate changed to 0.5 s and 60 bpm, respectively. The animals of this group were killed after 120 min this recovery period. All animals were ventilated with FIO₂ of 1.0.

Macrophage depletion

For both HV and HV-LV groups animals were randomized to receive either liposomal clodronate ($n = 7$) or liposomal phosphate-buffered saline (PBS) without clodronate ($n = 7$) as a control group. Liposomal clodronate (liposome-encapsulated dichloromethylene diphosphate, supplied by Dr. N van Roojen, Amsterdam, The Netherlands) was administered 48 h prior to mechanical ventilation. Animals were anesthetized with ketamine HCl (150 mg/kg, intraperitoneally). A small skin incision was made over the tracheal region and a suspension of Cl₂MDP liposomes in PBS (200 μ l liposome in a total volume of 1000 μ l) was insufflated intratracheally through a temporary intratracheal 24-G catheter. Preliminary experiments indicated that the number of bronchoalveolar lavage (BAL) macrophages was reduced by more than 70% 48 h after the tracheal instillation of liposomal clodronate (data not shown). The control sham group received liposomal PBS at the same time and by the same route.

Measurements

Arterial blood gases and blood pressure was monitored through a catheter inserted in the carotid artery. Tidal volume was measured at the beginning and the end of mechanical ventilation by a neonatal hot wire anemometer (LVM-1, InterMed Bear, Riverside, Calif., USA). Airway pressure was measured by a pressure transducer (Cobe, Arvada, Col., USA) and displayed on a Grass polygraph (Model 7 E, Quincy, Mass., USA). Immediately after kill the lungs were sequentially inflated and deflated in increments/decrements of 1.4 ml, each step being maintained for 60 s. From the resultant pressure volume recording we derived the static deflation lung compliance (volume at middeflation/pressure at middeflation) as well as an index of alveolar deflation stability: Gruenwald index [19] = $(2 \times \text{volume at } 5 \text{ cmH}_2\text{O} + \text{volume at } 10 \text{ cmH}_2\text{O}) / (2 \times \text{volume at } 35 \text{ cmH}_2\text{O})$. The right lung

was then lavaged repeatedly (three times) with 15 ml PBS (without Ca²⁺ and Mg²⁺, pH 7.4). The retrieved BAL fluid was analyzed for protein content by refractometer. BAL fluid cytokines [TNF- α and macrophage inflammatory protein (MIP) 2] were measured by immunoassay (Immunoassay Kit # KRC3011C and KRC1022, Biosource International, Camarillo, Calif., USA). The left lung was removed for wet/dry lung weight ratio (W/D ratio) and for whole lung tissue hemoglobin content [20]. To establish baseline for uninjured rats we determined baseline W/D lung weight ratios as well as BAL protein and cytokine concentrations in a similar size population of nonventilated rats ($n = 7$). Results are presented as mean \pm SEM. Statistical analyses was performed by analysis of variance using Scheffe's tests for post hoc analysis. Statistical significance was set at $p < 0.05$.

Results

Arterial blood gases

As shown in Fig. 1, a significant decline in PaO₂ had occurred 15 min after the onset of high volume ventilation in the control groups without macrophage depletion. A further decline was observed after 120 min of low volume ventilation, and this was of significant larger magnitude in the animals without clodronate treatment. The changes in PaCO₂ are shown in Fig. 2. There were no differences between groups. At 120 min PIP and delivered tidal volume were similar between control and clodronate animals (20 ± 3 vs. 18 ± 2 cmH₂O and 8.3 ± 1 vs. 8.1 ± 0.6 ml/kg, respectively). Alveolar-arterial oxygen gradient was significantly higher at 120 min than at 15 min in the untreated control (473 ± 53

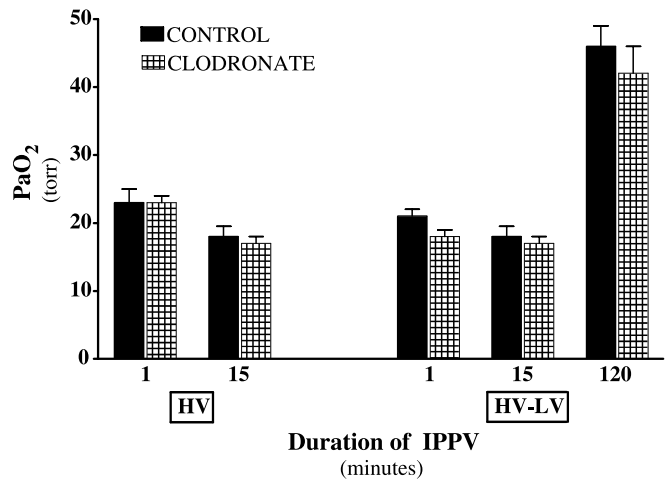


Fig. 2 PaCO₂ during mechanical ventilation

vs. 245 ± 34 torr, $p < 0.05$) and in the clodronate group (316 ± 40 vs. 160 ± 10 torr, $p < 0.05$). The difference in alveolar-arterial oxygen gradient between the two groups was significant at both times ($p < 0.05$). Figure 3 shows the systemic blood pressure, which was significantly decreased at 15 min and 120 min in the untreated control animals.

Static pulmonary mechanics and wet/dry lung weight ratio

Figure 4 demonstrates that high-pressure ventilated animals had further evidence of lung injury as the W/D lung weight was significantly greater than in the nonventilated population (4.5 ± 0.6 , $p < 0.01$). W/D ratios were signifi-

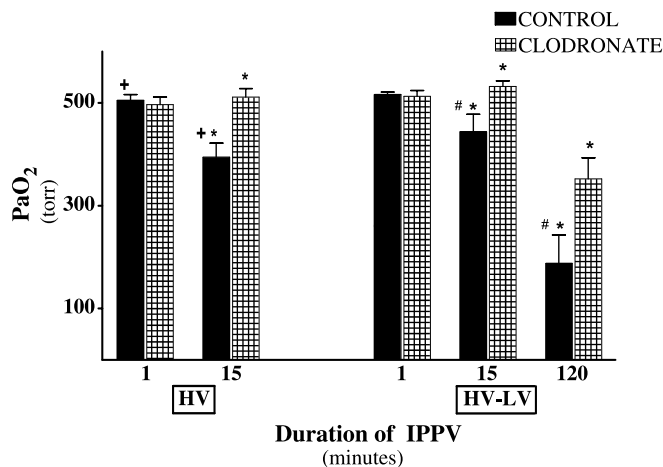


Fig. 1 PaO₂ during mechanical ventilation. * $p < 0.05$, control < clodronate, + 1' > 15', # 15' > 120'

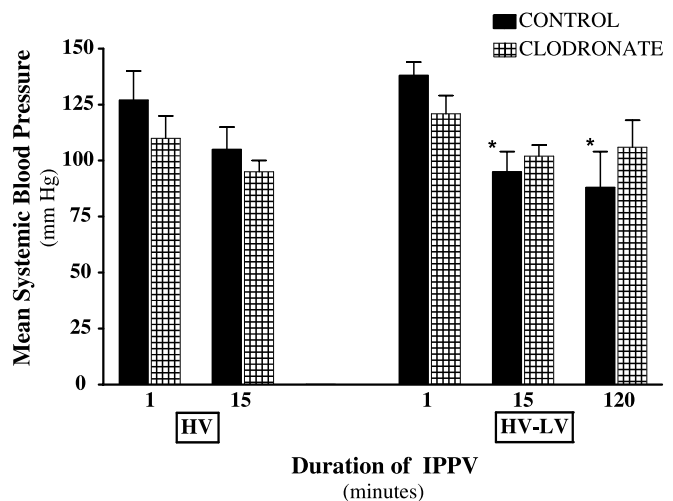


Fig. 3 Mean systemic blood pressure during mechanical ventilation. * $p < 0.05$ 1' > 15 and 120'

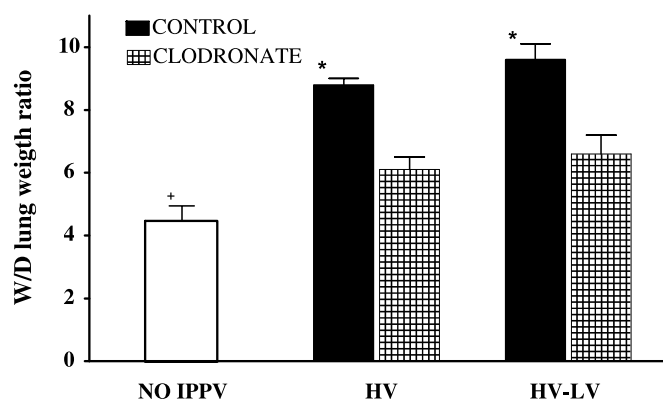


Fig. 4 Wet/dry lung weight ratio. * $p < 0.05$ control > clodronate, + $p < 0.01$ NO intermittent positive pressure ventilation < HV, HV+LV

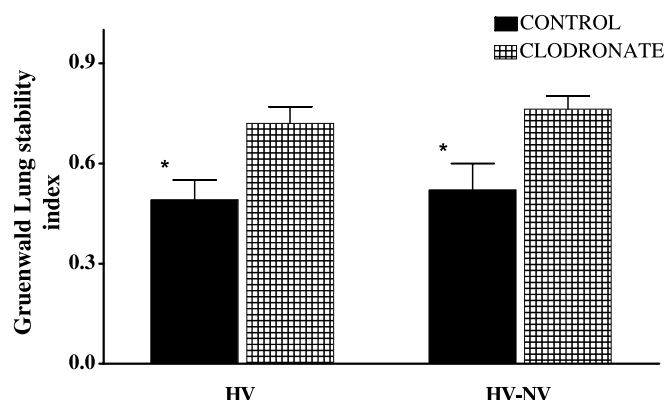


Fig. 6 Gruenwald Index of alveolar stability after mechanical ventilation. * $p < 0.05$ control < clodronate

cantly higher in the untreated controls ventilated for 15 and 120 min than in macrophage-depleted ventilated animals. Untreated ventilated controls had significantly lower static lung compliance and Gruenwald index of alveolar stability than the clodronate treated animals (Figs. 5, 6). Protein, MIP-2, and TNF- α concentrations in the BAL fluid are shown in Table 1. The protein concentration was similarly elevated in all ventilated animals. There was no difference in BAL cytokines concentration between the

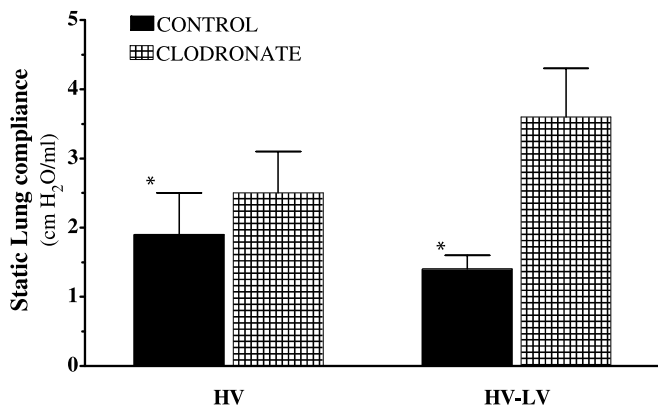


Fig. 5 Static lung compliance after mechanical ventilation. * $p < 0.05$ control < clodronate

control and the treated ventilated animals. Lung tissue hemoglobin concentration was similar for in animals.

Discussion

Alveolar macrophages are strategically located in the alveoli and airways to serve as sentinel cells to detect inhaled pathogens and mechanical stress. Activation of alveolar macrophages has been demonstrated to be critical to inflammatory lung injury in the presence of live bacteria, endotoxin, and ischemia reperfusion of the lung [12–15]. Cyclical stretch of cultured pulmonary macrophages has also been shown to initiate their activation and cytokine production [16]. The rapid activation of macrophages early on is suggested by the rapid decline in BAL macrophages during high volume ventilation. In preliminary studies we observed that the BAL macrophage count decreased within the first minute of such ventilation and was further reduced by more than 50% 5 min later (Fig. 7). The same inability to retrieve macrophages by BAL after ventilation with high inflation pressure ventilation has been reported by other both in vitro and in vivo [22, 23]. That this phenomenon is indicative of increase adhesion from activation [24] is suggested by the rapidity of the change and the report that high volume ventilation increases the expression of adhesion molecules Mac-1 and intercellular

Table 1 Bronchoalveolar lavage protein and cytokine concentration

	NO IPPV	HV Control	Clodronate	HV + LV Control	Clodronate
Protein (mg/ml)	0.5 \pm 0.2*	5.6 \pm 0.2	4.2 \pm 0.5	3.2 \pm 0.8	3.8 \pm 0.7
TNF- α (pg/ml)	112 \pm 62	7 \pm 20	42 \pm 52	71 \pm 28	108 \pm 64
MIP-2 (pg/ml)	316 \pm 117	372 \pm 143	443 \pm 174	370 \pm 183	360 \pm 166

* $p < 0.05$ NO intermittent positive pressure ventilation < HV, HV+LV

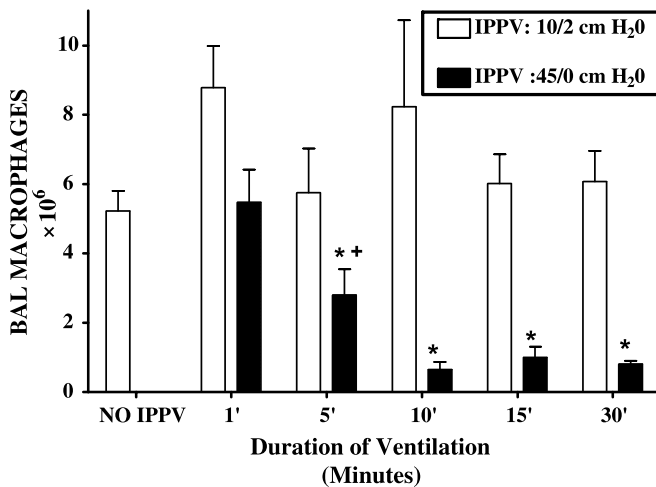


Fig. 7 Bronchoalveolar lavage yields of macrophages. Lavage was repeatedly ($\times 3$) performed at room temperature with a total of 30 ml PBS without Ca^{2+} and Mg^{2+} , pH 7.4). * $p < 0.01$ intermittent positive pressure ventilation (IPPV) 45/0 cmH_2O < no IPPV and IPPV 10/2 cmH_2O , $^+ p < 0.01$ 5' > 10, 15, 30'

adhesion molecule 1 in alveolar macrophages [22]. Previous studies have also shown that high volume ventilation induces an increased macrophage intracellular expression of GADD45 [25] and membrane expression of CD14 [26], both markers of activation.

Numerous studies [13–15, 17] have demonstrated that the tracheal instillation of clodronate encapsulated in liposomes induces apoptosis after being phagocytized by alveolar macrophage. Two days after clodronate treatment the number of alveolar macrophages was reduced by more than 70%. The present study demonstrates that intratracheal clodronate treatment significantly reduces the degree of lung injury produced by high tidal volume positive pressure ventilation. Ventilation with 45 cmH_2O PIP in adult rats leads rapidly to a state of respiratory failure from severe permeability pulmonary edema [1]. The reduced derangement of gas exchange and pulmonary mechanics that we observed after clodronate treatment are consistent with a reduced amount of pulmonary edema as confirmed by the differences in lung W/D weight ratio. W/D ratio increased by 85% (vs. nonventilated animals) in untreated animals and only by 35% only in the macrophages depleted rats. In clodronate-treated animals oxygenation status was unchanged after 15 min of high volume ventilation and the deterioration seen after 120 min of subsequent normoventilation in untreated animals was attenuated. A reduced surfactant dysfunction, possibly by reducing its “dilution” in the alveolar space [27, 28] is the most likely explanation for the better preserved oxygenation and lung compliance. Changes in the Gruenwald index, an index of alveolar instability at low lung volume [18], are also consistent with this hypothesis. Surfactant dysfunction

could also have been due to its inhibition from proteins leaked in the alveolar space. The increase in BAL protein concentration was of small magnitude in all groups, probably too low to inactivate endogenous surfactant [29]. The resolution of our method of protein measurement (by refractometer) was insufficient to detect significant differences for the range of small increases exhibited by the various ventilated groups.

In the clinical domain, markedly lower pressures and volume are used than in our model of HV [2–4]. Nevertheless, similar degrees of over inflation as those associated with this model of VILI may be reached in localized part of a heterogeneously expanding diseased lung [30, 31]. More pertinent to the clinical realm was the HV-LV experimental group. In the latter a short period of severe barotrauma (45 cmH_2O PIP) was followed by mechanical ventilation at a modest level of PIP sufficient to deliver tidal volume less than 10 ml/kg. This experimental group was designed to detect later effects expected to develop if the activated macrophages triggered the development of an ensuing injurious proinflammatory cascade that may encompass neutrophils pulmonary sequestration and activation [32]. We observed only minimal evidence of an additional injury past the initial 15 min period of high PIP ventilation. Although oxygenation worsened less, and alveolar-arterial gradient gradient was lower in the macrophage-depleted group, the other parameters of lung injury were not changed significantly from the initial changes at 15 min.

Some experimental studies have concluded that injurious ventilation results in the production of many cytokines, major among them being $\text{TNF-}\alpha$ and IL-8 (or its equivalent MIP-2) [33, 34]. Such was not the case for others models of VILI in particular when the lungs had not been “primed” by underperfusion or endotoxin [35]. Likewise, we did not find elevated BAL level of $\text{TNF-}\alpha$, and MIP-2 was only moderately increased. It is possible that activated macrophages release cytokines that are removed by an intact pulmonary circulation, or that they release other cytokines than the one we measured. The acute effect of macrophage may also involved cytokine nondependent mechanisms such as release of products of arachidonic acid metabolism [36].

The reduced pulmonary edema seen after macrophage depletion could have been the result of a reduction in pulmonary blood flow. Systemic blood pressure did decline over the initial 15 min, but this was of modest magnitude, and there was no difference between the control and the clodronate-treated animals. It is therefore likely that macrophages themselves had a specific role in the in increased permeability and edema associated with cyclical lung overinflation. What are the possible mechanisms by which macrophages produce pulmonary edema? After short-term exposure to hyperoxia alveolar macrophages have been shown to increase their ability to generate O_2 radicals [37] so that hyperoxia may augment

the deleterious effect of high stretch ventilation [32, 38] ventilation. However, in preliminary studies we found the same degree of injury between HV animals ventilated with 100% or 21% FIO₂. Ventilator-associated injury may decrease the lung's ability to clear edema through decreased Na⁺K⁺ ATPase activity and the inactivation or decreased expression of pulmonary epithelium sodium channel (ENaC) [39, 40]. Following high tidal volume ventilation air space concentration of reactive nitrogen species is increased; the latter has been shown to reduce ENaC while nitric oxide synthase inhibition attenuates the ventilator-induced microvascular leak [40–42]. Bronchoalveolar macrophages are the major source of nitric oxide produced during the pulmonary inflammatory responses to either endotoxin or silica [43]. Activation of proinflammatory pathways associated with an increase in expression of nitric oxide synthase has also been shown to occur during volutrauma [44, 45] so that macrophages could be a major source of NO that would affect ENaC and reduce edema clearance. Therefore macrophage depletion would curtail NO production and the severity of the pulmonary edema through preservation of ENaC function. Overstretched lung epithelial cells has the capacity to produce IL-8 when mitogen-activated protein kinase proinflammatory

pathways are activated [46, 47]. Interactions between alveolar epithelial cells and alveolar macrophages have been shown to promote inflammatory responses to air pollution particles [46]. It is thus possible that IL-8 released by the stretched epithelial cells leads to early activation of macrophages that produce nitric oxide which inhibits ENaC [47].

Our study does not exclude a role for other pulmonary cells in the development of ventilator-induced lung injury. Many studies have demonstrated the important role of leukocytes, albeit in models in which mechanical ventilation was of significantly longer duration. Our study suggests that macrophages are activated even in the early phase of ventilator-induced lung injury when no other inflammatory cells seem to be yet involved. Recently Frank et al. [48] confirmed that depletion of alveolar macrophages reduces the lung increased lung endothelial and alveolar epithelial permeability associated with VILI and suggested that alveolar epithelial cell-macrophage interaction is required for the observed early macrophage activation. It remains to be demonstrated whether these activated macrophages are the major trigger for the ensuing activation of the proinflammatory cascade responsible for the pulmonary and general morbidity associated with mechanical ventilation.

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