Pulmonary Responses to Overventilation in Late Multiple Organ Failure

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Background: Patients with multiple organ failure (MOF) require mechanical ventilation for several days. The enormous significance of the ventilation strategy for the outcome of these patients is well appreciated. However, most studies have focused on the onset and the early phase of MOF. It was the aim of the current study to investigate the effect of ventilation in the course of MOF.

Methods: Using a model where mice develop MOF 7–14 days after intraperitoneal injection of zymosan, the authors analyzed lung functions, signaling pathways, and mediator release in response to protective ventilation (end-expiratory pressure 3 cm H₂O end-inspiratory pressure −10 cm H₂O) and overventilation (−22.5 cm H₂O) in isolated lungs ex vivo.

Results: On day 7, pulmonary compliance, pulmonary resistance, and tidal volume were normal, but vascular resistance was elevated compared with untreated animals. During ex vivo ventilation, these lungs showed enhanced nuclear factor-κB activation, Akt kinase phosphorylation, and cytokine release, and this was further aggravated by overventilation. After 14 days, zymosan-treated animals were characterized by pulmonary hypertension, reduced tidal volume, elevated pulmonary resistance, and increased mediator production. However, in these lungs, neither nuclear factor-κB activation nor cytokine production where enhanced by overventilation.

Conclusions: The zymosan model is characterized by pulmonary inflammation, diminished lung functions, and chronic hypertension. Mechanical ventilation with high distending pressures further augmented cytokine production in this chronic model of MOF only if it significantly augmented tidal volume. The authors speculate that these findings may be explained on the basis of different degrees of lung stretch.

MECHANICAL ventilation is a life-saving strategy for critically ill patients but suffers from important side effects, some of which resemble the symptoms of sepsis. This resemblance may at least in part be explained by the fact that both sepsis and overventilation trigger a similar set of proinflammatory mediators. Our previous studies with lungs from healthy mice have shown that overventilation leads to a cell-specific activation of several pathways, e.g., the phosphoinositide 3-OH kinase/Akt kinase/endothelial nitric oxide synthase pathway in endothelial cells, and nuclear factor-κB (NF-κB) activation with subsequent cytokine production in alveolar macrophages and alveolar type II cells.

An important focus of current research is to investigate the impact of mechanical ventilation strategies on inflammation in the course of multiple organ failure (MOF). So far, these so-called two-hit studies have capitalized on acute sepsis models, with endpoints from 2 h to maximally 24 h. The effect of different ventilation strategies on inflammatory responses during the late (chronic) phase of MOF is unknown.

In general, it is probably fair to state that chronic sepsis models have received comparatively little attention. In one increasingly popular model of MOF, called the zymosan-induced generalized inflammation (ZIGI) model, zymosan is injected intraperitoneally, to initiate surprisingly complex pathophysiologic alterations in those mice that survive the initial septic shock. Typically, the ZIGI model consists of three stages: an acute hyperinflammatory response that is followed by a phase of apparent recovery before MOF develops between days 7–14. In this stage, the lungs are characterized by progressive cytokine production, alveolitis, progressive fibrosis, and lung injury. The alterations in physiologic lung functions in this model have not yet been characterized. Apparently, the disease progress in this chronic model is driven by proinflammatory cytokines, because anticytokine strategies, blockade of nitric oxide synthase, inhibition of poly (adenosine diphosphate-ribose) synthetase, or other antiinflammatory strategies have been proven effective in reducing lung injury and MOF.

In the current study, we were particularly interested in the pulmonary responses during the chronic phase of MOF, 7 and 14 days after zymosan injection. To this end and to exclude extrapulmonary systemic effects, we prepared isolated lungs from control and zymosan-treated animals to study for the first time pulmonary artery pressure, pulmonary compliance, pulmonary resistance, neutrophil apoptosis, and activation of NF-κB and Akt kinase in this model. In addition, to gain insight into mechanisms of ventilator-induced lung injury in MOF, lungs from zymosan-treated mice were ventilated under different conditions, and cytokine release was assessed. Our findings show that the effect of ventilation depends on both the mechanical properties and the inflammatory state of the lungs, such that the effects of MOF and ventilation were additive on day 7 but not on day 14, where overventilation failed to activate NF-κB and cytokine release. However, the effect of overventilation depended also on the signaling pathway, because...
Akt kinase activation that was unaltered in MOF was increased by overventilation on all days.

Materials and Methods

Animals
All animal experiments were approved by the local animal care committee (Ministerium für Umwelt, Natur und Forsten, Kiel, Germany). Experiments were performed by using a total of 109 inbred C57BL/6 mice (from the animal house of our research center), aged 8–10 weeks, weighing 25–30 g.

Protocol
The animals were randomly divided into 12 groups according to the ventilation mode and the duration of the experiments (the experimental protocol is detailed in fig. 1). On day 0, mice received an intraperitoneal injection of a sterilized suspension of zymosan and paraffin oil in a concentration of 0.4 mg zymosan/g body weight. Previously, we have shown that this treatment results in a mortality of approximately 50% within 1 day. In the current study, the mortality was 39% (32 of 89). The survivors were studied on days 7 and 14. On each day, isolated perfused lungs were prepared, and the animals were perfused and ventilated for either 60 or 180 min under control conditions (10 cm H₂O distending pressure, see below) or with high distending pressure (22.5 cm H₂O). After 60 min, the lung tissue was moved. A ligature was placed around the pulmonary artery and the aorta. The left atrium was cannulated; afterward, the arterial cannula was inserted into the pulmonary artery formed by using a total of 109 inbred C57BL/6 mice (from the animal house of our research center), aged 8–10 weeks, weighing 25–30 g.

Isolated Perfused Mouse Lung Setup and Ventilation
Perfused lungs were prepared essentially as described before (IL-1 apparatus; Hugo Sachs Elektronik–Harvard Apparatus GmbH, March-Hugstetten, Germany). Briefly, anesthetized (pentobarbital, 160 mg/kg body weight) mice were intubated, placed in a water-jacketed (37°C) Perspex chamber (part of IL-1 apparatus), and ventilated using room air (fraction of inspired oxygen 0.2) with positive pressure at a frequency of 90 breaths/min, an inspiratory cycle time of 50%, and a tidal volume of approximately 200 µl. Intermittent deep breaths (25 cm H₂O) were automatically executed every 5 min. After laparotomy, the diaphragm was removed, the animals were heparinized and exsanguinated, and the abdomen was removed. A ligature was placed around the pulmonary artery and the aorta. The left atrium was cannulated; afterward, the arterial cannula was inserted into the pulmonary artery and fixed by the ligature. Lungs were perfused at an initial flow rate of 0.6 ml/min (Ismatec MS Reglo peristaltic pump; Isotec, Quest Medical, Dallas, TX) with sterile filtered RPMI 1640 medium–4% bovine serum albumin with low endotoxin content. Then, the thorax was removed and the chamber lid was closed. The final perfusion rate was 1 ml/min (venous pressure was cycled with the ventilation pressure to normalize transpulmonary pressure in the current study, venous pressure was not monitored, but previous measurements have shown that under control conditions it is approximately 0–2 cm H₂O). Perfusate samples were drawn directly from the venous effluent cannula via a tubing connected to a syringe outside of the Perspex chamber. Because the perfusion was nonrecirculating and flow was 1 ml/min, perfusate cytokine concentrations may also be interpreted as production/min.

The negative pressure ventilation was started for all groups on baseline conditions (control): end-expiratory pressure −3 cm H₂O and end-inspiratory pressure −10 cm H₂O, resulting in a tidal volume of approximately 200 µl. After 60 min of baseline perfusion, overventilation was started: end-expiratory pressure −3 cm H₂O, end-inspiratory pressure −22.5 cm H₂O. During the experiment, arterial perfusate pressure, airflow velocity, and pressure inside the ventilation chamber were measured. Tidal volume (Vₜ), pulmonary resistance (R), and dynamic lung compliance (C) were calculated according to the law of motion (P = 1/C · Vₜ + R dV/dt), where P was obtained inside the chamber with a with a differential pressure transducer (Validyne DP 45-24; Northridge, CA), dV/dt (airflow velocity) was obtained with a pneumotachograph (type 0000), and V was obtained by electronic integration of dV/dt. These calculations were performed by the HSE Pulmodyn software (Hugo Sachs Elektronik–Harvard Apparatus GmbH) according to principles outlined by Amudr and Mcad: Compliance was calculated as Vₜ/(Pmax – Pmin) triggered if dV/dt was 0; resistance was calculated as ΔP/ΔVf 70% Vf during inspiration and expiration. Samples from the lung perfusate were taken every 30 min. The concentrations of tumor necrosis factor, macrophage inflammatory protein 2α, and interleukin 6 in the perfusate samples were measured by enzyme-linked immunosorbent assay. In another set of experiments, overventilation was stopped after 60 min, the lung was shock frozen in liquid nitrogen, and Akt kinase phosphorylation was determined by Western blot analysis and nuclear translocation of NF-κB by electromobility shift assay as described. To standardize these measurements over different gels, we prepared a standard from day 0 lungs (n = 4) that had been perfused and ventilated for 120 min and ran this standard on every gel.

Histology
One-micrometer sections were cut with a microtome and mounted onto Superfrost + slides (Gerhard Menzel, Glasbearbeitungswerk GmbH & Co. KG, Braunschweig, Germany). Sections were deparaffinized by incubation in xylene (2 × 10 min), 100% ethanol (2 × 10 min), 70%
ethanol (2 × 10 min), and demineralized water (1 min). Staining was performed by hematoxylin (5 min Mayer), double brief rinsing with water, and brief rinsing with 1% potassium acetate, water, and 0.1% eosin (90 s). Dehydration was then achieved by brief rinsing (1 min) in 80% ethanol (2×), 96% ethanol (2×), 100% ethanol (3×), and xylene (2×). Slides were mounted with Pertex and digitally photomicrographed by use of a Leica DM-LB microscope equipped with a Leica DFC 320 camera system (Leica Camera AG, Solms, Germany).

Slides were examined independently by two experienced lung pathologists blinded to the experimental grouping. A simple scoring system (score 0–3 with 3 = high) was used to describe the morphologic changes.

**Neutrophil Preparation and Apoptosis Measurement**

Bronchoalveolar lavage (BAL) and determination of apoptosis using flow cytometry was performed on days 7 and 14 in control and overventilated lungs after 120 and 240 min of perfusion and ventilation as described previously.19,28 Briefly, BAL was performed with 5 ml NaCl, 0.9%, in portions of 500 μl via tracheal incision. After centrifuging and resuspending in phosphate-buffered saline (Gibco, Paisley, United Kingdom), 100 μl cell suspension was incubated for 20 min with 10 μl anti-Ly6-G (murine neutrophil marker) for validation of the granulocyte gate. The pellet was resuspended in 4% paraformaldehyde and fixed for 20 min on ice. After washing, cells were incubated with equilibration buffer for 2 min on ice, centrifuged, and stained with the terminal deoxynucleotidyl transferase-mediated nick end-labeling (TUNEL) technique. For controls, cells were incubated with phycoerythrin-labeled isotype controls and 8 μl distilled water was substituted for TdT enzyme. Flow-cytometric analysis was performed on a FACSscan analyzer (Becton Dickinson, Heidelberg, Germany), and data acquisition and analysis were performed with CellQuest software (Becton Dickinson). Each measurement contained 10,000 or more cells in the granulocyte population as determined by forward/orthogonal light and positive Ly6-G expression. The following antibody
and detection kits were used: fluorescein isothiocyanate-labeled TUNEL, phycoerythrin-labeled isotype control, and anti-Ly6-G, all purchased from BD Pharmingen (Hamburg, Germany). Two distinct populations were detected after TUNEL staining, the one with the higher fluorescence intensity representing apoptotic cells.

Statistics

Univariate data were Box-Cox transformed (to alleviate heteroscedasticity) and analyzed by the all-pairwise comparisons procedure of Tukey (multcomp module, R: A language and environment for statistical computing, Vienna, Austria). Time course data of cytokine concentrations and tidal volume were analyzed as the area under the curve of the last 90 min of ventilation. After Box-Cox transformation, the area under the curve data were analyzed by mixed model analysis with ventilation strategy as a factor and the day after zymosan treatment as a covariate (nlme module, R). The effect of the ventilation strategy was estimated at each time point, and multiple comparisons were adjusted by the false discovery rate procedure.

Results

Histology

At all time points and in all groups, lungs appeared partly emphysematous and showed few signs of atelectasis (table 1). The major differences occurred with respect to edema formation and inflammation. Except for the emphysema, lungs ventilated for 180 min with \(-10\) cm H\(_2\)O peak inspiratory pressure or \(-25\) cm H\(_2\)O on day 0 looked largely normal (figs. 2A and B and table 1). On day 7, lungs from zymosan-treated animals showed leukocytes in the vessels, but otherwise, lungs ventilated with \(-10\) cm H\(_2\)O looked similar to day 0 (fig. 2C), whereas some animals ventilated with \(-25\) cm H\(_2\)O showed alveolar edema (fig. 2D). On day 14, there was a similar degree of inflammation (leukocytes in the vessels, interstitium, and some alveoli, intermingled with a few macrophages) in both ventilation groups, but edema formation appeared slightly increased in the overventilated lungs (figs. 2E and F).

Apoptosis

On day 0 almost no neutrophils were present in the BAL fluid (data not shown). On day 7 approximately 4% and on day 14 approximately 10% of the neutrophils in the BAL fluid were apoptotic. The ventilation strategy had no effect on neutrophil apoptosis within the time frame of the experiment (fig. 3).

Lung Functions

Lung functions were determined after 55 min of perfusion and control ventilation (10 cm H\(_2\)O end-inspiratory pressure, 3 cm H\(_2\)O end-expiratory pressure). Both on day 7 and on day 14, lungs from zymosan-treated animals showed increased pulmonary artery pressure, indicating pulmonary hypertension (fig. 4). On day 14

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<th>Table 1. Histologic Scoring</th>
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<tr>
<td>10 cm H(_2)O</td>
</tr>
<tr>
<td>25 cm H(_2)O</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Day 0</td>
</tr>
<tr>
<td>Atelectasis</td>
</tr>
<tr>
<td>Emphysema</td>
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<tr>
<td>Inflammation</td>
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<td>Edema</td>
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The histologic appearance of three animals from each group was scored on a scale from 0 (no effect) to 3 (strong effect) by two independent observers who were blinded to the experimental groups. Shown are the mean score and, in parentheses, the averaged score of the two observers for individual animals.

only, tidal volume was decreased and pulmonary resistance was increased, whereas pulmonary compliance was not altered, suggesting moderate bronchoconstriction (fig. 4). Raising transpulmonary pressure to 22.5 cm H2O increased tidal volume to differing degrees in lungs ventilated on days 0, 7, and 14 (fig. 5); the largest increase in VT was noted on day 0, followed by day 7 and day 14.

**Signaling Responses and Cytokine Release**

The cytokine (tumor necrosis factor, interleukin 6, macrophage inflammatory protein 2α) release of control lungs (day 0) was comparable to previous reports. It was low during protective ventilation and increased during overventilation (fig. 6). With progressive MOF, the concentrations of these mediators during control ventilation increased compared with day 0 (fig. 6). Overventilation further increased mediator levels on day 7 but not on day 14 (fig. 6).

To summarize these data and to allow statistical analysis, we calculated the area under the curve of the cytokine concentrations shown in figure 6 during the last 90 min of perfusion and ventilation (fig. 7) and analyzed these data by mixed model analysis (table 2). This analysis clearly shows that cytokine production increased (significant slope in table 2) from day 0 to day 14 during protective ventilation, demonstrating the increasingly inflamed state of the lungs (figs. 7A–C). At the same time, however, there was a significant decrease in tidal volume during ventilation with high transpulmonary pressure (fig. 7D and table 2). Corresponding to these alterations in tidal volume, overventilation in-
creased cytokine concentrations on days 0 and 7 but not on day 14 (figs. 7A–C and table 2).

Nuclear translocation of NF-κB measured after 60 min of overventilation displayed a pattern similar to that seen with the cytokines: There was a trend toward increased activation from day 0 to day 14 ($P = 0.058$ for the slope), an increase by overventilation on day 0 (0.030), and a trend on day 7 ($P = 0.088$), but not on day 14 (fig. 7D and table 2).

Activation of Akt kinase, i.e., phosphorylation at Ser-473, showed a different picture: There was no change during the time course of the zymosan model, and overventilation always induced a similar degree of activation (fig. 7F).

### Discussion

Acute lung injury and MOF frequently persist over several days or even weeks. Experimentally, in terms of acute lung injury or MOF, anything beyond 1 day has been difficult to study, and the ZIGI model is currently the only long-term model available, exhibiting MOF after 7–14 days.15,17 The current study details for the first time the time courses of changes in lung functions, neutrophil apoptosis, and signaling components (Akt kinase, NF-κB). In addition, we have shown how ventilator-induced proinflammatory cytokine responses are modulated in lungs from animals with MOF.

### Model Critique

For two reasons, the final analysis was performed *ex vivo*, i.e., in isolated perfused lungs prepared from mice with MOF: First, pulmonary artery pressure, which turned out to be increased in chronic sepsis, is difficult to measure *in vivo*. Second, we were interested in observing the pulmonary response itself, uncomplicated by the contribution of other organs (e.g., nervous system or blood), to examine the release of mediators specifically from chronically inflamed lungs. Another advantage of this model is that it permits multiple sampling from the perfusate as opposed to the *in vivo* situation, where, without volume management, only one to maximally three blood samples can be drawn from one mouse. It should be noted that in the current setting of negative pressure ventilation, tidal volume depends on the mechanical properties of the respiratory system, because in contrast to the *in vivo* situation, no changes in other parameters such as breathing frequency, respiratory cycle time, or thorax compliance are possible. In the current study, the perfused lungs showed signs of emphysema; however, because its extent was comparable in

### Table 2. Statistical Analysis of the Data Shown in Figure 7

<table>
<thead>
<tr>
<th></th>
<th>AUC C TNF</th>
<th>AUC C IL-6</th>
<th>AUC C MIP-2</th>
<th>AUC C NF-κB</th>
<th>AUC C P-Akt</th>
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<tr>
<td>OV vs. control</td>
<td></td>
<td></td>
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<tr>
<td>Day 0</td>
<td>0.0084</td>
<td>0.0001</td>
<td>0.0076</td>
<td>0.0304</td>
<td>0.0385</td>
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<tr>
<td>Day 7</td>
<td>0.0811</td>
<td>0.0023</td>
<td>0.0121</td>
<td>0.0880</td>
<td>0.0004</td>
</tr>
<tr>
<td>Day 14</td>
<td>0.3690</td>
<td>0.9675</td>
<td>0.6159</td>
<td>0.6320</td>
<td>0.0087</td>
</tr>
<tr>
<td>Slope C</td>
<td>0.0003</td>
<td>0.0066</td>
<td>0.0466</td>
<td>0.0639</td>
<td>0.1010</td>
</tr>
<tr>
<td>Slope OV</td>
<td>0.4689</td>
<td>0.8029</td>
<td>0.7189</td>
<td>0.2573</td>
<td>0.5777</td>
</tr>
<tr>
<td>Slope C vs. slope OV</td>
<td>0.0148</td>
<td>0.0048</td>
<td>0.0864</td>
<td>0.0712</td>
<td>0.3000</td>
</tr>
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The area under the curve (AUC) of tumor necrosis factor (TNF), interleukin-6 (IL-6), and macrophage inflammatory protein-2α (MIP-2α) release during the last 90 min of perfusion and the densitometric data from nuclear factor-κB (NF-κB) electromobility shift assays and P-Akt kinase immunoblots were analyzed by mixed model analysis. The first three rows show the effect of overventilation (OV) at each day. Rows 4 and 5 indicate the probability that the slope of the lines for controls (slope C, squares in fig. 7) or overventilation (slope OV, circles in fig. 7) are equal to zero; thus low $P$ values suggest a strong change from day 0 to day 14. Row 6 (slope C vs. slope OV) gives the probability that the slopes of the two lines in each of the different panels of figure 7 are equal; as an example, in case of an additive effect at all time points, the slope would be equal.
control and overventilated lungs, we do not believe that this affected the conclusions of this study.

Shortcomings of this model are the exclusion of neurally mediated lung injury (e.g., Chavolla-Calderon et al.30), the relatively short duration (4 h) of ventilation, and the difficulty to extrapolate lung injury in the ex vivo situation to animals with an intact thorax. Therefore, the focus of the current study was on activation of proinflammatory responses rather than on lung injury.

In theory, the cytokine release in our model could at least partly be explained by decompartmentalization, e.g., transfer of cytokines from the alveolar space to the pulmonary circulation.31 Although currently we cannot completely exclude such a mechanism, there are several reasons to assume that overventilation increases perfusate mediator levels independent of decompartmentalization (maybe by basolateral release from alveolar type II cells): First, the histologic data on day 0 show intact epithelia and endothelia also in overventilated lungs. Second, end-expiratory pressure, which was used throughout the entire study, is known to decrease decompartmentalization.31,32 Third, exogenous surfactant prevents ventilator-induced decompartmentalization,32 but in our model it increases cytokine perfusate levels.33 Finally, perfusate cytokine levels during overventilation were higher on day 7 than on day 14, when lung injury was most severe. It seems unlikely that the barrier properties increased on day 14.

Having noted these limitations, however, we would like to point out that important conclusions from the perfused mouse lung model such as a contribution of cytokines and chemokines to ventilator-induced lung injury26,34 or an increased pulmonary artery pressure in erythropoietin-transgenic mice35 have been confirmed in vivo (e.g., Hasegawa et al.,36 Belperio et al.,37 Wilson et al.).

**Pathophysiologic Changes in the Course of This Model**

A remarkable finding was the presence of pulmonary hypertension 7 and 14 days after zymosan injection. Pulmonary hypertension in acute lung injury and MOF is an important and difficult to treat condition,38 and a model of chronic pulmonary hypertension for this condition has not yet been described. The current findings suggest that the zymosan model may be useful to study chronic pulmonary hypertension in sepsis. The other pathophysiologic changes in lung functions were visible only on day 14, but not on day 7. The decrease in tidal volume is best explained by the increased airway resistance. Given the histologic appearances, the lack of a change in compliance may seem surprising; however, the fact that measurements of dynamic compliance are less sensitive than histologic analysis is in line with previous findings.17

Former studies had described increased levels of plasma tumor necrosis factor in the chronic phase of the zymosan model.17,19 Our findings suggest that a substantial part of the circulating tumor necrosis factor may be derived from the lungs. In addition, our findings show the release of other mediators, in particular interleukin 6 and the potent chemokine macrophage inflammatory protein 2α also from the lungs. These findings are in line with an increased pulmonary cytokine gene expression on day 9.40 Activation of NF-κB has previously been

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**Fig. 7.** Cytokine levels, tidal volume, nuclear factor-κB (NF-κB) activation, and Akt kinase phosphorylation in isolated perfused mouse lungs on days 0, 7, and 14 ventilated with low (closed squares, control) or high (closed circles, overventilation) transpulmonary pressure. The cytokines tumor necrosis factor (TNF-A), interleukin 6 (IL-6; B), and macrophage inflammatory protein (MIP-2α; C) as well as the tidal volume (E) are expressed as the area under the curve of the last 90 min of ventilation. Nuclear translocation of NF-κB (D) was measured after 60 min with electromobility shift assays and P-Akt kinase after 60 min by immunoblot (F). Data are mean ± SEM from 3–9 experiments and were analyzed by mixed model analysis. *P < 0.05 versus control ventilation at that day. # Control values increase significantly (P < 0.05 for slope) over time. § Overventilation values decrease significantly (P < 0.05 for slope) over time.
MECHANICAL VENTILATION AND MOF

Our findings show that nuclear translocation of NF-κB increased in parallel with proinflammatory mediator production. A recent study demonstrated that blocking of NF-κB exerted protective effects in the zymosan model.\(^{25}\) Akt, a kinase that in some conditions may regulate NF-κB,\(^{42,43}\) was not activated, indicating that in the ZIGI model, Akt kinase activity and NF-κB are not causally related.

Another interesting observation was the increased amount of neutrophil apoptosis (10%) in animals with MOF. This is readily explained by the fact that apoptosis is the critical step in the removal of neutrophils that become sequestered in the lungs during the disease process.\(^{44,45}\) This observation, however, is in some contrast to observations in the BAL fluid of patients with adult respiratory distress syndrome where low (median below 3%) percentages of apoptotic neutrophils have been observed up to day 21.\(^{46}\) The ventilation strategy had no effect on neutrophil apoptosis, but clearly 3 h is a very short period of time to affect apoptosis, although Imai et al.\(^{48}\) reported altered apoptosis of pulmonary and renal cells in a similar time.

**Effects of Overventilation in MOF**

Our findings reflect both model (zymosan)-dependent and ventilator-dependent effects. Model-dependent effects include the pulmonary hypertension, the histologic signs of inflammation, and the increasing amounts of cytokines released during control ventilation at later time points. Ventilator-dependent effects include slightly increased edema formation, activation of cytokine release, NF-κB translocation, and Akt kinase phosphorylation.

The effect of overventilation in chronically inflamed lungs was difficult to predict, because there are at least three different possible outcomes: (1) The effects of pulmonary inflammation and overventilation are additive as shown for the case of instillation of LPS or acid followed by high tidal volume ventilation.\(^{47,48}\) (2) The lungs are primed (e.g., by leukocyte sequestration or activation of common pathways) and show an exaggerated (synergistic) mediator response as was demonstrated for overventilation during acute endotoxemia.\(^{11}\) (3) The lungs have become less responsive either by mechanisms related to altered mechanical properties of the lungs or through induction of protective mechanisms such as induction of heat shock proteins.\(^{10,49}\)

In our study, the effect of ventilation in chronically inflamed lungs changed with the degree of lung injury: The effect of high-pressure ventilation on inflammatory responses (cytokine induction, NF-κB) was additive to that of lung injury when injury was mild (day 7), but was absent when lung injury was severe (day 14) and the high-pressure ventilation did not further increase tidal volume. Therefore, on day 14, measurements were made under conditions of reduced stretch due to the decrease in the tidal volume. From these findings, it is tempting to speculate that cytokine release is related to pulmonary stretch, but certainly it is difficult to predict whether ventilation with the same tidal volume on day 14 as on day 7 would have induced the same increase in cytokines. Nonetheless, the inability of heavily injured lungs to release mediators in response to overventilation may also explain the lack of correlation of lung injury with inflammation in other studies of ventilator-induced lung injury.\(^{50}\) From our findings, we hypothesize that mechanical ventilation with the same supraphysiologic pressure stimulates cytokine production only if it leads to a concomitant increase in tidal volume and stretch of compliant (healthy) alveoli. A similar correlation as observed here was observed in surfactant-treated lungs, where cytokine production correlated with tidal volume rather than ventilation pressure.\(^{33}\)

While tidal volume, NF-κB activation, and cytokine release all followed the same pattern with a decrease at day 14 (relative to day 7), activation of the Akt kinase occurred to a similar extent at all time points. These findings suggest that the ventilation-induced activation of Akt kinase and NF-κB occurs by separate and independent mechanisms. Previously, we have shown that during overventilation, Akt kinase activation occurs primarily in endothelial cells, where it leads to increased nitric oxide formation,\(^{3}\) whereas NF-κB becomes activated in alveolar type II cells and alveolar macrophages, where it stimulates cytokine gene expression.\(^{3}\) Taken together, our findings suggest that the mechanical effects of ventilation on pulmonary vessels on the one hand and cells of the alveoli on the other are sensed or transduced by different mechanisms. Further studies will be required to identify these mechanosensors.

In summary, MOF in the ZIGI model is associated with pulmonary inflammation, loss of pulmonary functions, and pulmonary hypertension. The latter finding suggests the first model of chronic pulmonary hypertension in MOF. Patients with MOF depend on mechanical ventilation, but their heterogeneous lung injury renders these lungs sensitive to ventilator-induced lung injury. This was also noted in the current study, because overventilation of lungs from mice with MOF showed increasing amounts of pulmonary edema. In addition to physical lung injury, ventilator-induced release of proinflammatory mediator release may further augment the underlying lung injury. We hypothesize that this ventilator-induced mediator release depends the degree of pulmonary distension and not on the degree of lung injury.

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