# Pulmonary Cytokine Responses During Mechanical Ventilation of Noninjured Lungs With and Without End-Expiratory Pressure

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**BACKGROUND:** Positive end-expiratory pressure (PEEP) during mechanical ventilation may impose different degrees of stress on healthy lungs. On the assumption that stress is reflected by cytokine production, we performed a translational study investigating the effect of PEEP on bronchoalveolar and systemic mediator levels in isolated perfused mouse lungs (IPL) and in patients with healthy lungs.
**METHODS:** (Part I) IPL were ventilated with end-expiratory pressures of 0, 3, 6, or 10 cm $H_2O$ and end-inspiratory pressure (EIP) levels of 10 or 25 cm $H_2O$. Interleukin (IL)-6 and macrophage inflammatory protein-2 concentrations in the venous effluate were monitored. (Part II) Patients (nonsmokers) scheduled for elective otorhinolaryngology surgery (duration >90 min) were randomized to receive either ventilation with zero end-expiratory pressure or PEEP (10 cm $H_2O$). Mediators in bronchoalveolar lavage, nuclear factor κB, (NF-κB)-activation in alveolar macrophages and circulating systemic mediators were monitored. Control patients underwent bronchoalveolar lavage after intubation.
**RESULTS:** In the IPL, mediator concentrations increased with increasing end-expiratory pressure at an EIP of 10 cm $H_2O$, but decreased at 25 cm $H_2O$ EIP. In patients, bronchoalveolar IL-6, monocyte chemoattractant protein-1, and granulocyte monocyte-colony stimulating factor were increased by ventilation regardless of the PEEP level. IL-6 and IL-8 levels were moderately increased by PEEP but not zero end-expiratory pressure. Nuclear factor κB DNA binding activity in alveolar macrophages and systemic mediator levels did not change.
**CONCLUSIONS:** On the basis of the premise that cytokine levels may indicate mechanical stress, our findings indicate that even low tidal volume ventilation causes some stress. PEEP is beneficial at high inspiratory pressure, but imposes moderate stress at low inspiratory pressure.
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Mechanical ventilation is the most common supportive therapy for patients with acute lung failure.[1] However, the adverse effects of mechanical ventilation may affect patient outcome.[2–4] Ventilator-induced lung injury has been recognized as a risk for mechanically ventilated patients.[5]

Independent of their possible pathophysiological function, inflammatory mediators may serve as a sensitive index of pulmonary stress during mechanical ventilation. This hypothesis is also supported by findings in the ARDSnet study in which mortality correlated with cytokine levels, but not with physical measures of barotrauma.[6,7] It is now widely accepted that protective ventilation strategies, in particular ventilation with low tidal volume ($V_T$), attenuate mechanical stress and inflammation in patients with acute lung injury (ALI)/acute respiratory distress syndrome (ARDS).

Although most studies in this area have focused on patients with ALI, the majority of ventilated patients have healthy lungs and are ventilated in the course of surgery. The mechanical stresses that mechanical ventilation elicits in these patients are poorly defined.

One critical aspect of mechanical ventilation is the degree of positive end-expiratory pressure (PEEP). On the one side, PEEP prevents end-expiratory alveolar collapse and reopens atelectatic lung areas. On the other side, increasing PEEP in inhomogeneously injured lungs will first fill and thus (over)distend

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healthy lung areas, before atelectatic lung areas become recruited.[8] Thus, in patients with ALI, lung over-inflation resulting from elevated PEEP levels is common.[9,10] A major rationale for the clinical use of PEEP has been the atelectotrauma hypothesis, according to which the repeated opening and closing of alveolar units is associated with high and detrimental shear stress.[11,12] However, despite extensive experimental evidence supporting the use of PEEP,[13–15] the appropriate PEEP level in ARDS patients remains unknown. The ALVEOLI trial demonstrated similar outcomes in patients with ALI independent of the PEEP level applied.[16]

In the setting of mechanical ventilation during elective surgery, the best PEEP is even less clear. One clinical study failed to find an elevation of systemic cytokine levels after ventilation for 1 h with high $V_T$ (15 mL/kg) versus low $V_T$ (6 mL/kg) with zero end-expiratory pressure (ZEEP) or low $V_T$ with PEEP (10 cm $H_2O$) before surgery,[17] indicating that preoperatively none of these ventilator settings may cause additional stress in the lungs. In contrast, the use of protective ventilation including PEEP during esophagectomy with a period of one-lung ventilation[18] decreased the systemic proinflammatory release of cytokines and chemokines. Additionally, two recent studies with patients undergoing abdominal surgery showed that pulmonary inflammation can be reduced[19] and procoagulant alveolar changes can be prevented[20] by lower $V_T$ and PEEP.

The present study was performed to further clarify the effect of PEEP on the systemic and local release of immunomodulatory mediators in healthy lungs. We therefore performed a translational study investigating the effect of ZEEP and PEEP first in isolated perfused mouse lungs (IPL) and subsequently in patients scheduled for minor surgical procedures.

## METHODS

### Animal Study

The study was approved by the local animal care committee of Schleswig-Holstein (Kiel, Germany). Female BALB/c mice weighing 20–23 g were obtained from Charles River (Sulzfeld, Germany).

The mouse lungs were prepared as described previously.[21,22] Briefly, lungs were perfused in a non-recirculating fashion through the pulmonary artery at a constant flow of 1 mL/min with RPMI medium (BioWhittaker, Verviers, Belgium) containing 4% low endotoxin grade bovine albumin (Serva, Heidelberg, Germany). Under control conditions, the lungs were ventilated (using subatmospheric pressure) with transpulmonary pressures of 3 cm $H_2O$ (end-expiratory pressure, EEP) and 10 cm $H_2O$ (end-inspiratory pressure, EIP) at a rate of 90 breaths/min. $V_T$, pulmonary resistance, pulmonary compliance, and pulmonary artery pressure were continuously measured by standard procedures. We have previously shown that mediator release is the same during negative and positive pressure ventilation.[22] The advantage of negative pressure ventilation in our set-up is that physiological transmural pressure is maintained (this is discussed in detail in Ref. 22).

#### Experimental Protocol

The lungs were first perfused and ventilated for 60 min under baseline conditions as described above. Subsequently, lungs were perfused and ventilated for an additional 150 min: three groups with a low EIP of 10 cm $H_2O$ and four groups with a high distending pressure of 25 cm $H_2O$. The mice were randomly allocated to one of the following seven groups: Group 10/0: lungs ventilated with 10 cm $H_2O$ EIP and 0 cm $H_2O$ EEP; Group 10/3: lungs ventilated with 10 cm $H_2O$ EIP and 3 cm $H_2O$ EEP; Group 10/6: lungs ventilated with 10 cm $H_2O$ EIP and 6 cm $H_2O$ EEP; Group 25/0: lungs ventilated with 25 cm $H_2O$ EIP and 0 cm $H_2O$ EEP; Group 25/3: lungs ventilated with 25 cm $H_2O$ EIP and 3 cm $H_2O$ EEP; Group 25/6: lungs ventilated with 25 cm $H_2O$ EIP and 6 cm $H_2O$ EEP; Group 25/10: lungs ventilated with 25 cm $H_2O$ EIP and 10 cm $H_2O$ EEP.

Perfusate concentrations of interleukin (IL)-6 and the chemokine macrophage inflammatory protein (MIP)-2 (corresponds to IL-8 in humans) were assessed by enzyme-linked immunosorbent assay every 30 min for 3 h (R&D systems GmbH, Wiesbaden, Germany). The detection limit for IL-6 and MIP-2 was 10 and 5 pg/mL, respectively.

### Clinical Study

#### Study Population

After the study was approved by the institutional ethics committee of the University of Lübeck and written informed consent had been obtained, patients scheduled for elective otorhinolaryngology surgery with expected minor systemic trauma (e.g., rhinoplastic surgery, total auricular reconstruction surgery) were enrolled in a prospective, randomized, controlled study. Patients were eligible if they were aged between 18 and 70 yr, classified with ASA status I/II, had an expected surgery time >90 min, and had a non-smoking history (minimum >1 yr). Exclusion criteria were a body mass index >35, inflammatory disease or elevation of leukocyte count, signs of pulmonary disease (pneumonia, chronic obstructive pulmonary disease, etc.), malignant disease, immunomodulation or immunosuppression therapy (e.g., steroid medication), and history of bronchoscopy, bronchoalveolar lavage (BAL), or ventilation therapy during the last 2 mo. Patients with an expected surgery time <90 min were assigned to a control group.

Anesthesia was performed using sufentanil, propofol, and rocuronium in body-weight-related doses. Administration of oxygen (fraction of inspired oxygen

[$F_{IO_2}$] 1.0) was performed before induction of anesthesia, and 100% oxygen was administered during ventilation via a facemask. After endotracheal intubation (7.5-mm tube) anesthesia was maintained with propofol and supplementary sufentanil at a dosage which did not allow spontaneous breathing.

Mechanical ventilation was performed in a volume-controlled mode with a $V_T$ of 8 mL/kg ideal bodyweight, an inspiratory/expiratory ratio of 1:2, an $F_{IO_2}$ of 0.5 and a respiratory rate adjusted to maintain $Paco_2$ between 35 and 45 mm Hg with a standard anesthesia ventilator. No vital-capacity maneuver to open collapsed lung areas was induced.

In addition to the standard monitoring, the study group underwent invasive arterial blood pressure monitoring; respiratory variables were monitored with the flow sensor placed at the proximal end of the endotracheal tube (Capnomac Ultima, Datex-Ohmeda, Duisburg, Germany).

### Clinical Study Protocol

Before anesthesia, the patients were randomly (computerized) assigned to receive either mechanical ventilation with ZEEP or with a PEEP of 10 cm $H_2O$. Arterial blood gases were analyzed 10 min after intubation, in 30-min time intervals, and before the BAL. At the end of surgery, 10 mL arterial blood samples were taken, and all patients underwent a fractionated BAL. Bronchoscopy (BF T20, Olympus, Tokyo, Japan) was performed via the endotracheal tube with the end of the scope wedged in the right middle lobe. Six 20-mL aliquots of sterile saline solution were instilled and gently aspirated. The first recovered aliquot was processed separately; the following five were pooled and placed on ice immediately.

In control patients ($n = 15$), the BAL was performed immediately after endotracheal intubation. During this study, each BAL was performed by the same investigator (T.M.). All patients with a positive microbiological test, a BAL recovery rate $\leq$30% or a ratio of macrophages $\leq$78% in the differential cell count, were excluded. The cut-off points were based on reference values for healthy non-smokers and were chosen to achieve comparability and homogeneity of the BAL results in the study groups.[23]

### BAL Cell Count and Differentiation

Microbiological analysis, alveolar cell viability check and differentiation, as well as nuclear protein extraction, were performed immediately after the BAL. The alveolar cells were separated from the BAL fluids (BALF) by centrifugation (150*g*, 10 min). The supernatant was stored at −80°C for further analysis. After this procedure, approximately $10^5$ cells/mL were separated from the cell pellet for cell count and Pappenheim's staining. Alveolar cells were counted, and the ratio of alveolar macrophages and leukocytes was calculated.

### Cytokine and Chemokine Determination

Blood samples of 10 mL were immediately centrifuged (1500*g*, 10 min), and the supernatants were stored at −80°C for IL-8 and MIP-1$\beta$ determination (BD Biosciences, San Diego, CA). Concentrations of cytokines and chemokines in BAL supernatants (granulocyte colony-stimulating factor [G-CSF], granulocyte-macrophage colony-stimulating factor [GM-CSF], interferon-$\gamma$, IL-1$\beta$, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, MIP-1$\beta$, monocyte chemoattractant protein-1 [MCP-1], and tumor necrosis factor-$\alpha$ [TNF-$\alpha$]) were simultaneously detected with an immunoassay (BioPlex, human17-Plex panel, Biorad Laboratories, Hercules, CA). The assay sensitivities were <10 pg/mL and the assay range was 1–32,000 pg/mL for all proteins determined.

### Nuclear Protein Extraction and Nuclear Factor-$\kappa$B Activation Assay

Nuclear extracts of alveolar cells and the NF-$\kappa$B activation assay were prepared and analyzed by electro mobility shift assay (EMSA) as described previously.[24] Cells were processed only if the total cell count in the BALF was $\geq 0.6 \times 10^6$ and the viability $\geq$90%. Since bacterial lipopolysaccharide (LPS) is a potent activator of NF-$\kappa$B in alveolar macrophages, we used LPS-stimulated alveolar macrophages as a positive control to calculate the percentage of DNA binding activity of NF-$\kappa$B.

## Statistical Analysis

The group size in the clinical study was powered to detect differences in IL-6 and IL-8 levels in BAL with $\alpha = 0.05$ and $\beta = 0.20$ (i.e., a power of 80%); 14 patients were required in each group.

Unless otherwise noted, all data were analyzed by SPSS® (Version 12.01., SPSS Inc., Chicago, IL). The data of the experimental study are shown as means ($\pm$SEM). The time course of the IL-6 and MIP-2 data was analyzed by repeated measurement ANOVA and orthogonal polynomials. Because the linear trend was highly significant ($P < 0.001$), the time course data were transformed with the linear coefficients (and the results log-transformed in case of heteroscedasticity before multiple comparisons were performed by the Waller–Duncan test). The data in Table 3 represent repeated measurements, of which only the first and the last are shown. These data were analyzed by mixed model analysis (JMP 6.0, SAS Institute, Cary, NC).

To analyze the BAL cytokine levels in the clinical study, the data were controlled by the Kruskal–Wallis test followed by two-sided Mann–Whitney tests; the *P* values were corrected for multiple comparisons according to the Shaffer procedure.

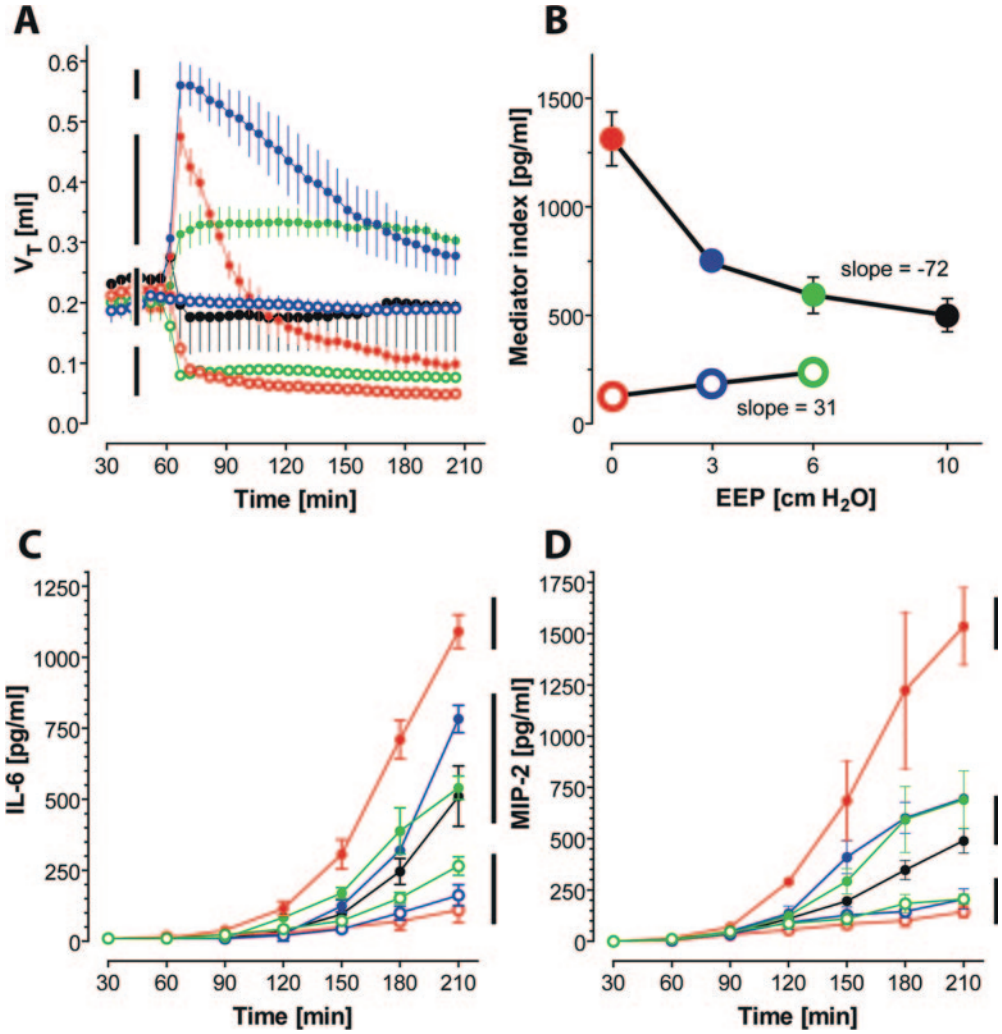


**Figure 1.** Isolated perfused mouse lung (IPL) model. (A) tidal volume ($V_T$), (B) mediator index, (C) interleukin (IL)-6, and (D) macrophage inflammatory protein (MIP)-2 perfusate concentrations of lungs ventilated with an end-inspiratory transpulmonary pressure (EIP) of either 10 cm $H_2O$ (open circles) or 25 cm $H_2O$ (closed circles). The end-expiratory pressure (EEP) was 0 cm $H_2O$ (red), 3 cm $H_2O$ (blue), 6 cm $H_2O$ (green), or 10 cm, $H_2O$ (black). The mediator index in (B) represents the mean of the maximum IL-6 and MIP-2 levels shown in (C) and (D), respectively. By linear regression, the slopes of the 2 lines were significantly ($P = 0.0002$) different from each other. Data are shown as mean ± SEM of 3–4 independent experiments. Statistically similar groups ($P < 0.05$) are indicated by the vertical lines on the right or left side of panels A, C, and D. The statistical grouping in panel B was similar to that in C and D (data not shown). The unit of the slope in panel B is $pg \cdot mL^{-1} \cdot cm\ H_2O^{-1}$.

## RESULTS

### Mouse IPL Experiments

For the first 60 min, all lungs were ventilated with a $V_T$ of approximately 200 μL. Lungs subsequently ventilated with low pressure (10 cm $H_2O$ EIP) and 0 or 6 cm $H_2O$ EEP had a stable low $V_T$ of about 100 μL during the treatment phase. In lungs ventilated with high distending pressure (25 cm $H_2O$) and low EEP (0 or 3 cm $H_2O$), $V_T$ decreased over time, although to different extents (Fig. 1A). Lungs of the 25/0 group became grossly edematous, as visible by translucent areas on the lung surface. Stable $V_T$ and no gross pulmonary edemas were observed in the other groups. However, in none of the groups did we detect elevated levels of the cytosolic enzyme lactate dehydrogenase in the lavage fluid (data not shown), excluding gross necrotic cell death.

Effluate perfusate IL-6 and MIP-2 concentrations were highest in the 25/0 group. They were about equally high in lungs ventilated with 25/3, 25/6, or 25/10, and they were low in all lungs ventilated with 10 cm $H_2O$ (Figs. 1C, D).

The Pearson correlation coefficient between IL-6 and MIP-2 concentration was 0.80 ($P = 0.001$). Because of this significantly high correlation, and since we were interested in analyzing the general effect of ventilation on mediator release, we used the mean of the maximum IL-6 and MIP-2 concentrations of the individual experiments to calculate a mediator index. Regardless of the EEP level applied, the mediator index was highest in lungs ventilated with 25 cm $H_2O$ compared with 10 cm $H_2O$ EIP ($P < 0.001$) (Fig. 1B). Elevation of EEP in the group ventilated with low EIP (10 cm $H_2O$) resulted in an increasing mediator index

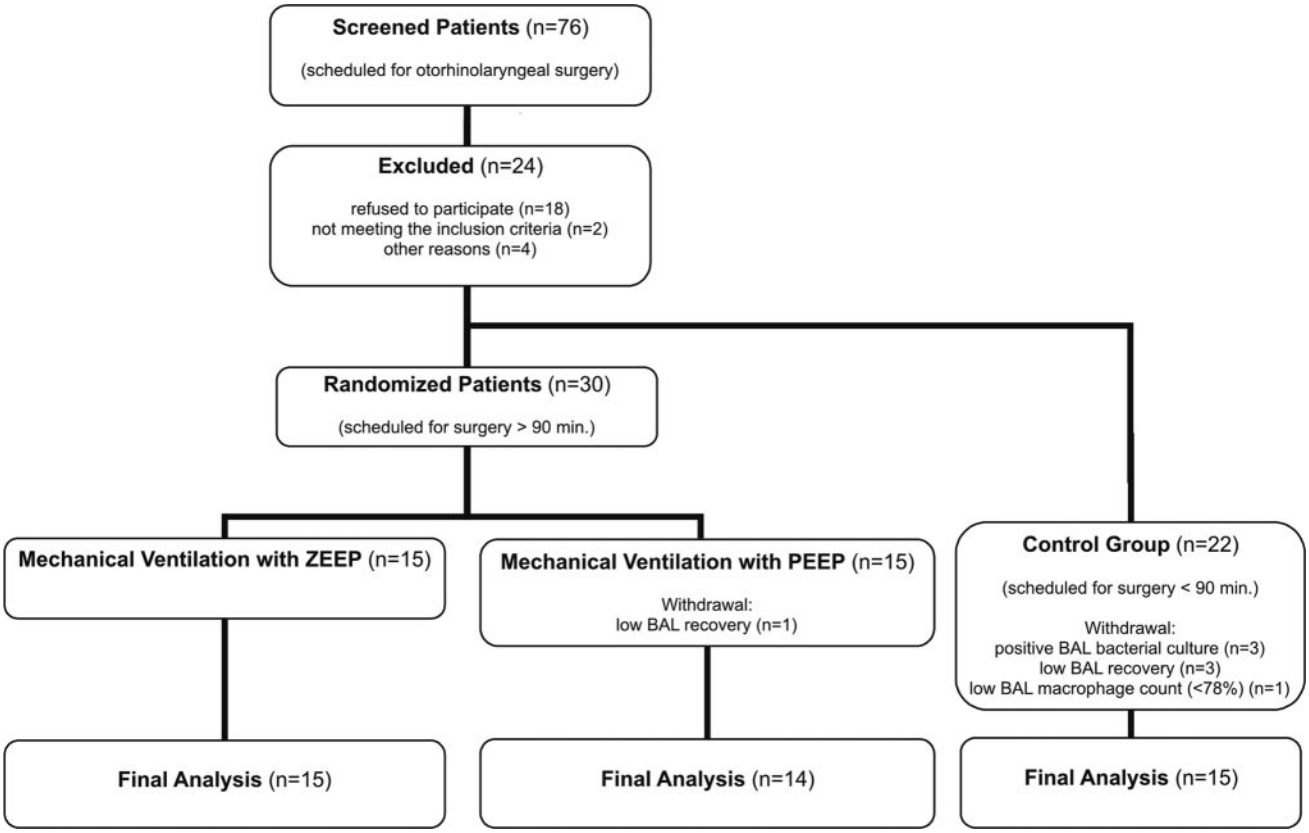


**Figure 2.** CONSORT diagram. BAL = bronchoalveolar lavage; PEEP = positive end-expiratory pressure (10 cm $H_2O$); ZEEP = zero end-expiratory pressure (0 cm $H_2O$).

**Table 1.** Baseline Characteristics of Individuals Studied

| | Control patients | ZEEP | PEEP (10 cm $H_2O$) |
|---|---|---|---|
| No. patients | 15 | 15 | 14 |
| Age (yr) | 35 (±10.0) | 35 (±12.4) | 39 (±15.3) |
| Sex, f/m | 8/7 | 7/8 | 6/8 |
| BMI (kg/m$^2$) | 25.2 (±3.5) | 24.2 (±3.5) | 24.1 (±3.9) |
| ASA, I/II | 13/2 | 12/3 | 12/2 |
| Leukocytes (10$^9$/L) | 6.68 (±1.3) | 6.00 (±1.1) | 6.06 (±1.0) |
| Duration of ventilation (min) | — | 147.5 (90–320) | 155.2 (95–252) |

Data are expressed as mean (±SD) or median (range).

There were no significant differences among the groups.

ZEEP = zero end-expiratory pressure; PEEP = positive end-expiratory pressure; BMI = body mass index; ASA = American Society of Anesthesiologists.

**Table 2.** Bronchoalveolar Cell Recovery and Differential Cell Count from Bronchoalveolar Lavage (BAL)

| | Control patients | ZEEP | PEEP (10 cm $H_2O$) |
|---|---|---|---|
| No. patients | 15 | 15 | 14 |
| Recovery (mL) | 65.6 (±13.5) | 73.6 (±9.1) | 70.5 (±11.5) |
| Total cells (10$^6$) | 1.77 (±0.1) | 1.93 (±0.97) | 1.28 (±0.61) |
| Macrophages (%) | 92 (±6) | 93 (±4) | 92 (±6) |
| Neutrophils (%) | 1.8 (±1.7) | 1.5 (±1.5) | 2.5 (±3.6) |
| Eosinophils (%) | 0.5 (±0.6)* | 0.17 (±0.2) | 0.12 (±0.2) |
| Basophils (%) | 0.02 (±0.07) | 0.03 (±0.07) | 0.02 (±0.06) |
| Lymphocytes (%) | 4.9 (±3.8) | 5.8 (±3.3) | 5.6 (±4.5) |
| Microbiological analyses | Culture neg. | Culture neg. | Culture neg. |

Data are expressed as mean (±SD).

There were no significant differences among groups except the ratio of the eosinophils in the group control patients (* $P < 0.05$) compared with the ZEEP and PEEP groups.

ZEEP = zero end-expiratory pressure; PEEP = positive end-expiratory pressure.

($P = 0.034$), whereas during ventilation with high EIP (25 cm $H_2O$) the mediator index decreased ($P = 0.003$) (Fig. 1B). The slopes of the two curves were different from each other ($P = 0.0002$).

## Clinical Study

### Patients' Characteristics

Of the 76 screened patients, 15 were finally analyzed in the control group, 15 in the PEEP group, and 14 in the ZEEP group (Fig. 2). The clinical characteristics of all groups are given in Table 1. The groups were equal in age, sex, body mass index, physical status, and blood leukocyte count. The duration of ventilation before BAL was between 90 and 320 min, with no significant differences between the study groups.

### BAL Variables

BAL recovery rates as well as total and differential cell counts were within the normal range and did not significantly differ between the groups. Cell ratios between groups were equal (Table 2). In the microbiological analysis, all BALF samples were negative.

**Table 3.** Ventilatory and Hemodynamic Data

| | | | ZEEP | | PEEP 10 cm $H_2O$ | |
|---|---|---|---|---|---|---|
| | G | *T* | T1 | T2 | T1 | T2 |
| PIP, cm $H_2O$ | * | — | 13.8 ± 2.0 | 14.0 ± 2.0 | 21.9 ± 2.9 | 22.4 ± 1.6 |
| $Paw_{mean}$, cm $H_2O$ | * | — | 11.6 ± 2.3 | 11.5 ± 2.2 | 17.3 ± 4.2 | 18.5 ± 2.3 |
| PEEP, cm $H_2O$ | * | — | 0.9 ± 0.6 | 1.0 ± 0.8 | 10.1 ± 1.0 | 9.9 ± 0.8 |
| RR, /min | — | * | 10.4 ± 1.2 | 9.3 ± 1.6 | 10.4 ± 1.7 | 9.9 ± 1.2 |
| $V_T$, mL | — | — | 578 ± 91 | 577 ± 91 | 570 ± 98 | 550 ± 90 |
| MV, L | — | * | 5.9 ± 1.0 | 5.3 ± 1.5 | 5.8 ± 1.2 | 5.4 ± 1.1 |
| $C_{dyn}$, mL/cm $H_2O$ | † | * | 57.4 ± 10.5 | 54.4 ± 9.4 | 67.0 ± 14.2 | 61.0 ± 11.7 |
| $Etco_2$ | — | — | 33.3 ± 3.7 | 32.5 ± 2.9 | 34.5 ± 5.0 | 33.6 ± 4.1 |
| arterial pH | † | — | 7.43 ± 0.02 | 7.43 ± 0.04 | 7.43 ± 0.04 | 7.39 ± 0.03 |
| $Pao_2$, mm Hg | — | — | 240 ± 60 | 241 ± 42 | 267 ± 56 | 255 ± 42 |
| $Paco_2$, mm Hg | — | — | 36.0 ± 4.5 | 35.5 ± 4.4 | 37.0 ± 4.4 | 36.8 ± 3.7 |
| $AaDo_2$, mm Hg | — | — | 71.4 ± 48.0 | 79.6 ± 52.7 | 46.3 ± 52.7 | 56.3 ± 38.5 |
| MAP, mm Hg | — | * | 71.3 ± 11.2 | 84.9 ± 14.1 | 77.2 ± 9.7 | 78.4 ± 12.0 |
| Heart rate, bpm | — | ‡ | 64.7 ± 11.6 | 63.7 ± 10.3 | 70.9 ± 15.0 | 58.9 ± 26.6 |
| BT, °C | ‡ | * | 36.0 ± 0.4 | 36.1 ± 0.5 | 36.6 ± 0.6 | 36.3 ± 0.6 |

Data are expressed as mean (±SD).

No interactions between time and group effect were observed.

T1 = 10 min after intubation; T2 = before bronchoalveolar lavage (BAL); PIP = peak inspiratory pressure; $Paw_{mean}$ = mean airway pressure; PEEP = positive end-expiratory pressure; RR = respiration rate; $V_T$ = tidal volume; MV = minute volume; $C_{dyn}$ = dynamic compliance; $Etco_2$ = end-tidal carbon dioxide; $Pao_2$ = arterial oxygen tension; $Paco_2$ = arterial carbon dioxide tension; $AaDo_2$ = alveolar-arterial oxygen difference; MAP = mean arterial blood pressure; bpm = beats per minute; BT = body temperature (rectal). G (group), effect of test PEEP vs ZEEP; *T* (time), change over time.

* $P < 0.001$; † $P < 0.05$; ‡ $P < 0.01$.

### Ventilatory and Hemodynamic Variables

Peak inspiratory pressure, mean airway pressure, and pulmonary compliance were all higher in the PEEP group. Minute volume, pulmonary compliance, and heart rate decreased over time, whereas mean arterial blood pressure increased. No differences in gas exchange variables were observed. All patients were normothermic throughout the study (Table 3).

### Concentrations of Chemokines and Cytokines

Compared with control patients, both PEEP- and ZEEP-ventilated patients had increased BALF levels of IL-6, MCP-1, and GM-CSF, with no differences between these two groups (Fig. 3). However, if, in analogy to the mouse lung study, IL-6 and IL-8 levels were combined by calculating their means, this mediator index was moderately higher in the PEEP group (Fig. 4). Other cytokines determined in the BAL (interferon-$\gamma$, IL-1$\beta$, IL-2, IL-4, IL-5, IL-7, IL-10, IL-12, IL-13, IL-17, TNF-$\alpha$) were below 10 pg/mL in all individuals (data not shown). Serum concentrations of IL-8 and MIP-1$\beta$ were not detectable in any group.

### NF-$\kappa$B Activation

The DNA binding activity of NF-$\kappa$B was analyzed in BAL cells from 21 patients (ZEEP group, $n = 10$; PEEP group, $n = 11$). Nine BALs had to be excluded from NF-$\kappa$B analysis due to a cell viability ≤90%. There was no significant difference in NF-$\kappa$B activation (Fig. 5) between the ZEEP- and PEEP-ventilated groups.

## DISCUSSION

The aim of the study was to monitor proinflammatory cytokine responses as an estimate of the pulmonary stress in lungs without preexistent injury during mechanical ventilation with and without PEEP. In the experimental study, the effect of EEP on IL-6 and MIP-2 perfusate concentrations depended on the EIP: at high distending pressures cytokine release was increased by lowering EEP, but at low distending pressures, cytokine release was decreased. A similar tendency was observed in the clinical trial, where ZEEP was associated with a lower mediator index. Another noteworthy observation was the fact that mechanical ventilation by itself caused a modest inflammatory response.

### The Experimental Study

The mouse IPL model allows continuous measurement of inflammatory mediators and makes it possible to study (extreme) ventilation strategies without side effects such as a decrease in arterial blood pressure or infiltration of leukocytes that may confound interpretation of the results *in vivo*. This is particularly important when measuring cytokine concentrations, which may be affected by alterations in blood volume, by products from sequestered leukocytes, and by hepatic/renal elimination. Moreover, to establish true cytokine kinetics, lungs were always perfused with a flow of 1 mL/min, implying that the ordinates in Figures 1B–D can also be read as cytokine production per minute. Another distinct advantage of this model is that, in contrast to the *in vivo* situation, lungs can completely collapse at ZEEP since pleural pressure can be nullified, thus maximizing potential shear stress due to opening and reopening of alveolar units.

In our analysis, we introduce the concept of a mediator index for ventilatory stress, i.e., the mean of the IL-6 and the MIP-2/IL-8 concentrations. Both mediators are particularly sensitive to ventilatory

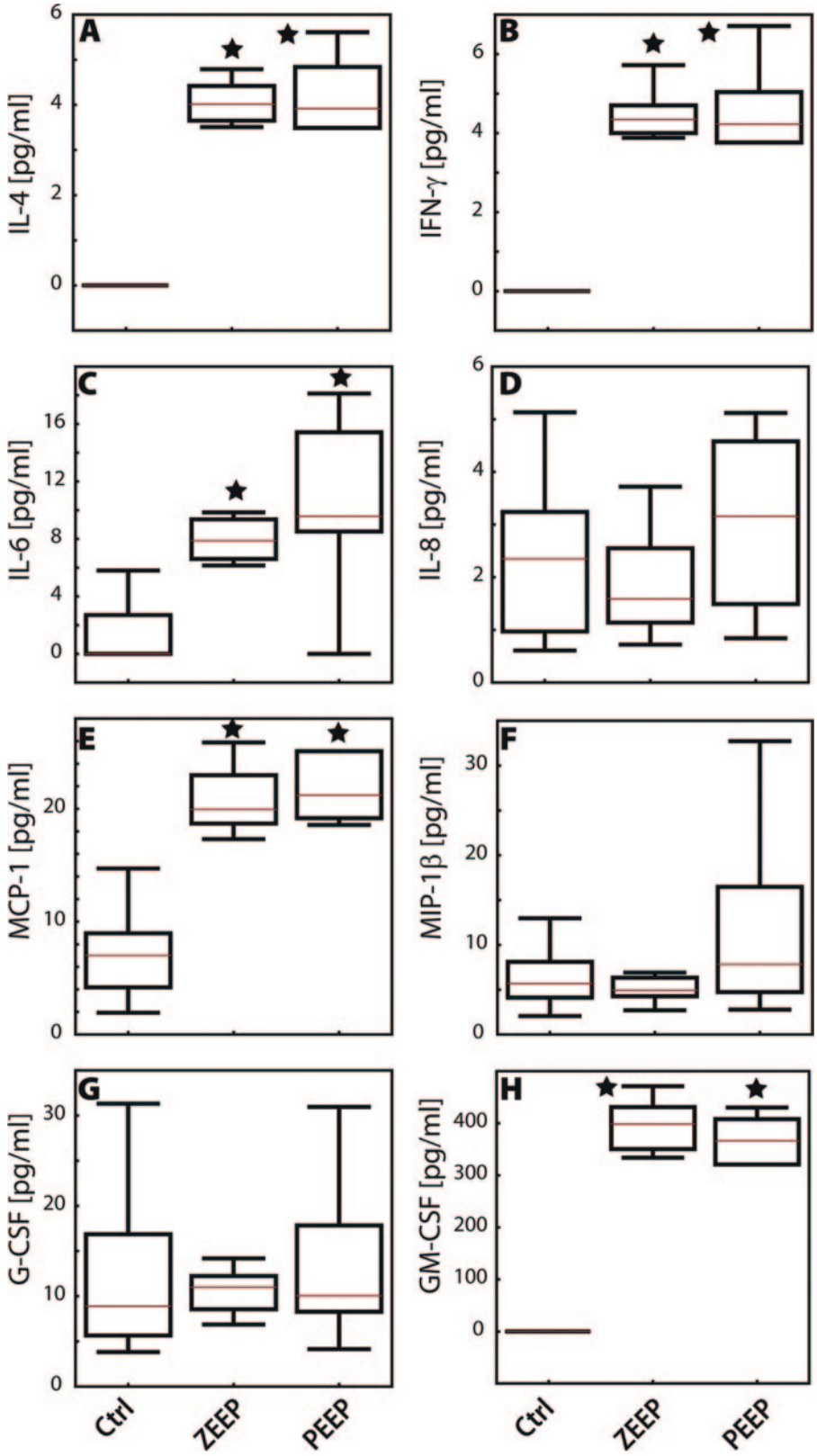


**Figure 3.** Mediator concentrations in bronchoalveolar lavage (BAL) fluid of control and study patients [zero end-expiratory pressure (ZEEP) vs positive end-expiratory pressure (PEEP)]. (A) interleukin (IL)-4; (B) interferon (IFN)-γ; (C) IL-6; (D) IL-8; (E) monocyte chemoattractant protein (MCP)-1; (F) macrophage inflammatory protein (MIP)-1β; (G) granulocyte-colony stimulating factor (G-CSF); (H) granulocyte-monocyte colony stimulating factor (GM-CSF). The box plots show the median and the upper and lower quartiles; the whiskers indicate the 10th and 90th percentiles. $n = 14–15$ per group. ⋆, $P < 0.05$ vs control.

stress, but as stress indicators they do not appear to be redundant, as their production is clearly regulated by different mechanisms.[25] Therefore, averaging their production may provide a useful, sensitive and nonredundant index of ventilatory stress, as was demonstrated first by the findings in the perfused lung and then confirmed in the clinical trial.

Our finding that maximizing the tidal swing (25/0 group) increased ventilation-induced cytokine release is in line with previous studies, showing that the combination of high ventilation pressures with ZEEP is detrimental and a strong stimulus for mediator production.[26,27] The 25/0 group clearly differentiated itself from the other groups; it was the only group in

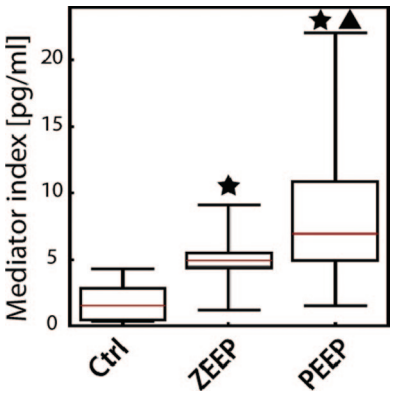


**Figure 4.** Mediator index in bronchoalveolar lavage (BAL) fluid of control and study patients [zero end-expiratory pressure (ZEEP) vs positive end-expiratory pressure (PEEP)]. The mediator index was calculated as the mean of interleukin (IL)-6 and IL-8 from Figures 3C and D. The box plots show the median and the upper and lower quartiles; the whiskers indicate the 10th and 90th percentiles. $n = 14–15$ per group. ⋆, $P < 0.05$ vs control; ▲, $P < 0.05$ vs. ZEEP.

which the lungs became grossly edematous, it had the most dramatic decrease in $V_T$, and it had the highest IL-6 and MIP-2 concentrations. These observations suggest that the mechanism responsible for the high mediator release in this group may not only be related to the maximal stretch, but also to increased capillary permeability and/or tissue destruction. This in turn may result from the high shear stress generated during repeated opening and reopening of atelectatic areas (atelectotrauma)[14] and/or from deformation of the alveolar epithelium.[28] These mechanisms could further augment perfusate concentrations by destroying the compartmentalization that separates the alveolar from the systemic compartment.[29,30] In addition, tissue destruction might lead to release of preformed mediators. The inverse correlation between PEEP and cytokine release in lungs ventilated with 25 cm $H_2O$ EIP is in line with the conventional interpretation that adding PEEP progressively reduces atelectotrauma and stabilizes the lung.

Somewhat unexpectedly, we found that PEEP by itself and at low inspiratory pressures, lack of PEEP did not augment mediator release, but rather reduced it. In this respect, it is particularly instructive to compare the 10/3 group with the 25/10 group. Although both lungs had similar $V_T$ (Fig. 1), perfusate concentrations of IL-6 and MIP-2 were significantly higher in lungs in the 25/10 group. These findings may indicate that a critical stimulus of ventilator-induced mediator release is the degree of lung distension, since the overall lung distension must clearly have been greater in the 25/10 group than in the 10/3 group. Thus, the effects of PEEP may be dual: PEEP may decrease cytokine release by reducing atelectotrauma, but increase it by augmenting pulmonary strain.

### The Clinical Trial

To confirm these findings in the clinical setting, we designed a prospective, randomized trial in patients with healthy lungs who were scheduled for minor surgery. Despite several differences between the human and the experimental study (e.g., nonphysiologic lung perfusion, buffer instead of blood), one commonality was the pressures required to ventilate human and murine lungs.

Our data from the clinical study show the typical variations of respiratory mechanics and gas exchange variables in anesthetized nonobese patients.[31] PEEP-ventilated patients had a higher compliance, which may be explained if atelectatic areas, that develop after induction of anesthesia and ventilation with 100% oxygen,[32] become partly recruited. However, in both the PEEP and the ZEEP group pulmonary compliance decreased over time, suggesting that even a PEEP of 10 cm $H_2O$ is not sufficient to entirely prevent progressive collapse of lung areas. Gas exchange variables did not significantly change during ventilation with PEEP, corroborating the results of other studies using nonobese patients.[31]

In line with the experimental findings, ventilation with ZEEP did not increase the pulmonary production of IL-6, IL-8, or other inflammatory mediators; the same is true for cell counts and the number of proinflammatory cells in BAL. Remarkably, however, in the clinical study, increasing PEEP increased the mediator index (median of IL-6 plus IL-8 values), just as in the experimental part of this study. However, it should be kept in mind that, because of the dilution involved, the concentrations in the BAL do not represent the actual local concentrations. Another caveat is that we did not perform an initial recruitment maneuver and did not monitor regional ventilation, e.g., by computed tomography analysis. The possible role of regional hyperinflation respectively atelectasis of healthy lung areas as demonstrated by Terragni et al.[10] therefore remains unknown. Because adding a fixed PEEP level may produce alveolar hyperinflation and/or recruit actelectatic areas, the mechanisms leading to the PEEP-related increase in the mediator index cannot be fully explained.

In contrast to the hypothesis of cyclic collapses of lung areas during ZEEP ventilation in inflamed lungs,[12,14,33] and also in contrast to the experimental data at high distending pressures, the cytokine profile we obtained from healthy human lungs does not suggest any additional intrapulmonary stress in the ZEEP group. Unfortunately, the effect of shear stress on mediator release from pulmonary epithelial cells has hardly been investigated. Most evidence is indirect and comes from studies where the interpretation of altered mediator levels is confounded by decompartmentalization.[29,34] By analyzing early steps of the ventilation-induced pulmonary inflammatory cascade in a rabbit IPL model, Kirchner et al.[35] found that ventilation of the lungs with ZEEP or high PEEP was

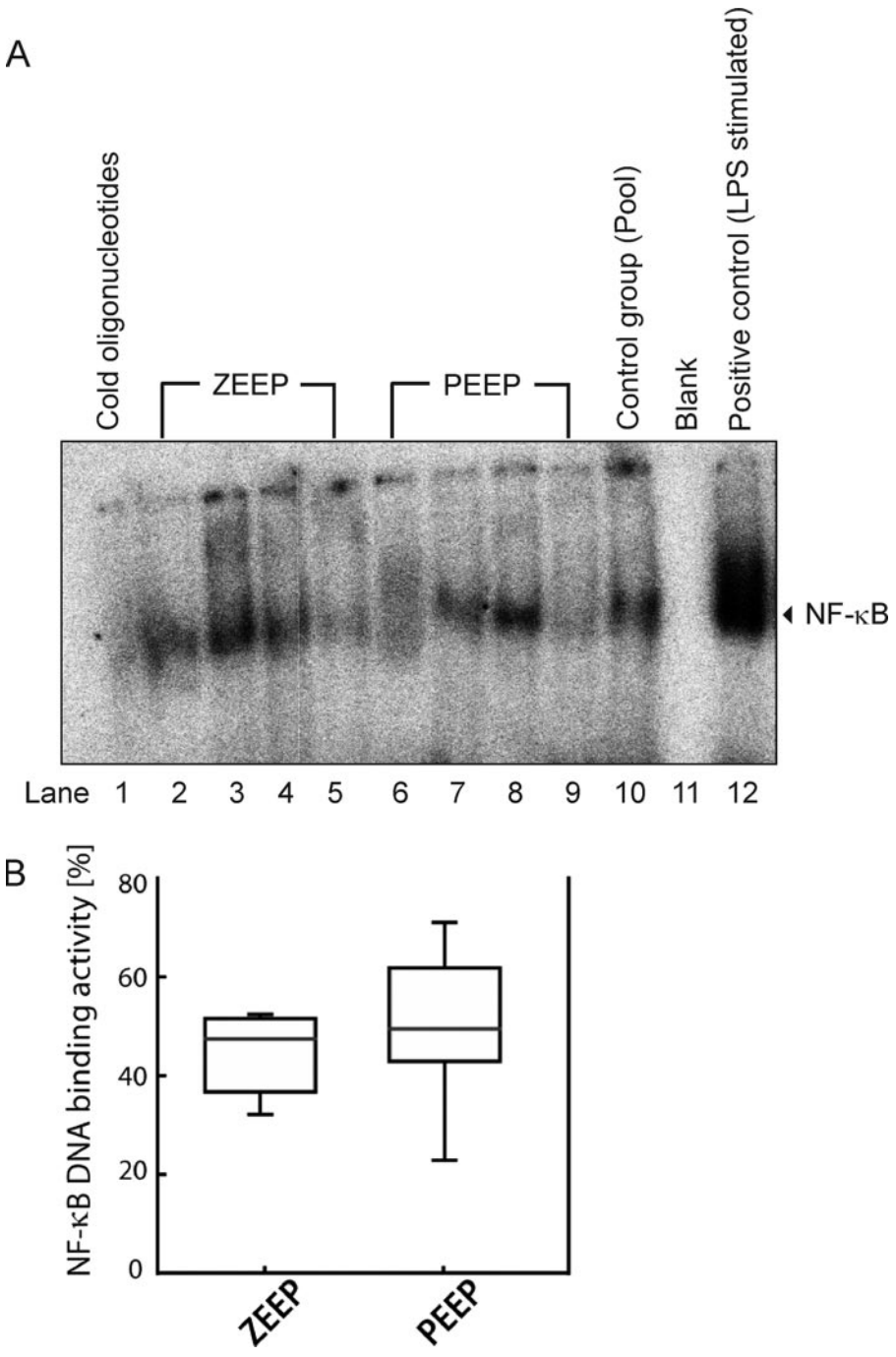


**Figure 5.** (A) Nuclear factor (NF)-κB DNA binding activity in alveolar cells. Lane 1, cold oligonucleotides (negative control); lanes 2–5, representative results from the zero end-expiratory pressure (ZEEP) group ($n$ = 4); lanes 6–9, representative results from the positive end-expiratory pressure (PEEP) group ($n$ = 4); lane 10, control group (pool, $n$ = 4); lane 11, blank; and lane 12, lipopolysaccharide (LPS)-stimulated alveolar cells (positive control). (B) Densitometric intensity of NF-κB DNA binding activity in alveolar cells of study patients ($n$ = 21) expressed as percent of control (*in vitro* cell activation by LPS-stimulated alveolar cells).

associated with the highest activation of NF-κB and activator protein 1 in whole lung tissue. Others demonstrated activation of NF-κB elicited by cyclic pressure stretching in ventilated human macrophages[36] or perfused mouse lungs.[21] However, our analysis of NF-κB activation in lavage alveolar cells from the clinical study failed to observe any differences between ventilation with PEEP or ZEEP. These findings corroborate our conclusion that any proinflammatory stimulus imposed by PEEP (10 cm $H_2O$) or its lack during perioperative ventilation is low. As a caveat, we cannot exclude that lavage alveolar cells were prestimulated, because DNA binding activity of NF-κB was nearly 50% of an *in vitro* stimulation of alveolar cells with LPS.

We would like to emphasize that in the present study we took care to systematically exclude bacterial infections of the respiratory tract in order to avoid false-positive results. Thus, the increased levels of IL-6, MCP-1, and GM-CSF in both the PEEP- and ZEEP-ventilated groups compared with control conditions suggest that mechanical ventilation by itself (not the intubation) is a mild proinflammatory stimulus by itself similar to what has been reported in children.[37] Possible explanations are induction by surgery, anesthesia, ventilation *per se*, or any combination. It is increasingly being recognized that type and duration of clinical anesthesia during surgery may influence the expression of proinflammatory responses.[38–40] Therefore, it is important to note that in

our study all groups had comparable times of propofol anesthesia. Bregeon et al.[41] demonstrated increased MCP-1, TNF-$\alpha$, and IL-1$\beta$ gene transcription in alveolar cells from healthy rabbit lungs that had been mechanically ventilated for 6 h. Similarly, in rats, Haitsma et al.[42] observed increased alveolar MIP-2 levels after 90 min and 4 h of ventilation with 3 cm $H_2O$ PEEP and 13 cm $H_2O$ EIP. In addition, Plötz et al.,[37] enrolling infants subjected to diagnostic cardiac catheterization and ventilated over a 2 h period with sevoflurane and 10 mL/kg $V_T$ with a PEEP of 4 cm $H_2O$, observed elevated TNF-$\alpha$ and IL-6 levels in tracheal aspirate. These results support the present findings that any form of mechanical ventilation may activate cytokine responses in the lung.

In conclusion, mechanical ventilation appears to be a (weak) proinflammatory stimulus by itself. Alterations of EEP caused small changes in pulmonary cytokine production in isolated mouse lungs at low EIP and in healthy human lungs ventilated with $V_T$ of 8 mL/kg during minor surgical procedures. We conclude that, in contrast to high $V_T$, at low $V_T$ ZEEP ventilation *per se* is not a stressor for healthy lungs. However, there may be specific situations, such as one-lung ventilation during esophagectomy[18] or abdominal surgery of long duration,[19,20] that require special attention.

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