Hyperventilation Induces Release of Cytokines from Perfused Mouse Lung

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Artificial mechanical ventilation represents a major cause of iatrogenic lung damage in intensive care. It is largely unknown which mediators, if any, contribute to the onset of such complications. We investigated whether stress caused by artificial mechanical ventilation leads to induction, synthesis, and release of cytokines or eicosanoids from lung tissue. We used the isolated perfused and ventilated mouse lung where frequent perfusate sampling allows determination of mediator release into the perfusate. Hyperventilation was executed with either negative (NPV) or positive pressure ventilation (PPV) at a transpulmonary pressure that was increased 2.5-fold above normal. Both modes of hyperventilation resulted in an approximately 1.75-fold increased expression of tumor necrosis factor α (TNFα) and interleukin-6 (IL-6) mRNA, but not of cyclooxygenase-2 mRNA. After switching to hyperventilation, prostacyclin release into the perfusate increased almost instantaneously from 19 ± 17 pg/min to 230 ± 160 pg/min (PPV) or 115 ± 87 pg/min (NPV). The enhancement in TNFα and IL-6 production developed more slowly. In control lungs after 150 min of perfusion and ventilation, TNFα and IL-6 production was 23 ± 20 pg/min and 330 ± 210 pg/min, respectively. In lungs hyperventilated for 150 min, TNFα and IL-6 production were increased to 287 ± 180 pg/min and more than 1,000 pg/min, respectively. We conclude that artificial ventilation might cause pulmonary and systemic adverse reactions by inducing the release of mediators into the circulation. von Bettmann AN, Brasch F, Nüsing R, Vogt K, Volk HD, Müller K-M, Wendel A, Uhlig S. Hyperventilation induces release of cytokines from perfused mouse lung.

Ventilation. For ventilation of the lungs with either positive or negative pressure a rotary vane compressor pump (VCM; Hugo Sachs Elektronik) was used. For positive pressure ventilation (PPV) a tube was connected to the trachea; switching a valve allowed us to change airflow direction through a venturi gauge and thereby to transform positive pump pressure into negative (with respect to the ambient atmosphere) chamber pressure (negative pressure ventilation, NPV). The venturi gauge was mounted within the chamber. Depending on the experimental design, end-expiratory, end-inspiratory and deep breath pressures were pre-set. Regular deep breaths (sighs) are common in vivo and help to prevent atelectasis. Breathing frequency was 90/min, inspiration time was 50% of each breathing cycle. The gas delivered by the ventilator was humidified by a frit. The frit and a pneumotachometer were positioned directly proximal to the tracheal cannula.

Perfusion. Lungs were perfused in a nonrecirculating manner with constant flow (generated by a peristaltic pump, Ismatec MS Reglo) at 1 ml/min, which corresponds to approximately 8% of the usual cardiac output of mice (3). A tygon tubing (inside diameter, 0.79 mm) led from the pump to the pulmonary artery cannula. Inside the warmed artificial thorax chamber the buffer was warmed by coiling the tubing around a cylinder. A bubble trap (tygon tubing: length, 0.5 cm; inside diameter, 0.25 cm) was placed directly before the pulmonary artery cannula. Another section of tygon tubing led directly from the chamber into the bubble trap to allow bolus infusions. A special pulmonary artery catheter made of stainless steel was used to avoid collapsing of vessel walls. Perfusion samples were drawn directly from the venous effluent cannula via a tubing connected to a syringe outside of the chamber. A fter leaving the chamber the effluent was directed into a pressure equilibration chamber, which in case of NPV was connected to the ventilation chamber. By this means, during NPV venous pressure follows pleural pressure, which has been suggested to be a most physiological way of perfusing isolated lungs (4, 5), which also helps to minimize edema formation (6). Because NPV and PPV have the same effect on interstitial pressure relative to pleural pressure (7), the pressure difference between interstitial and intravascular pressure (transmural pressure) is lower if the intravascular pressure is connected to chamber (pleural) and not atmospheric pressure.

Perfusion medium. RPMI 1640 cell culture medium (Biochrom, Berlin, Germany) was supplemented with 4% bovine serum albumin. In most experiments we used low endotoxin grade albumin (Serva, Heidelberg, Germany); however, in preliminary experiments we also used normal fraction V albumin (Serva). Values for osmolality of murine blood given in literature are in the range of 300–345 mOsm/kg for different mouse strains (8). In Balb/c mice we determined an osmolality of murine serum of 343 ± 10 mOsm/kg (n = 3). Therefore, the osmolality of the perfusion medium was adjusted by addition of NaCl to 335–340 mOsm/kg. A fter addition of all components the medium was sterile filtered, using the ZapCap S 0.2 CA System (Schleicher & Schuell, Dassel, Germany) and stored at 4°C. For perfusion purposes, the medium was heated to 37°C.

Animals and surgery. Specific pathogen free female Balb/c mice (22–30 g) from the animal house of our university were used as lung donors. Mice were anesthetized with 160 mg/kg body weight pentobarbital sodium (Nembutal; Wirtschaftsgenossenschaft Deutscher Tierärzte, Hannover, Germany). Subsequently, they were intubated and ventilated with 90 breaths/min room air with a tidal volume (V T) of approximately 200 μl. A fter laparotomy the diaphragm was removed. The animals were heparinized, exsanguinated and the abdomen was removed. A ligature was placed around the pulmonary artery and the aorta. The left atrium was cannulated, afterwards 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. Then, the thorax was removed and the chamber lid was closed. Negative pressure ventilation was started with chamber pressure oscillating between −2 and −10 cm H 2 O in order to achieve a V T of 200 μl. The final perfusion rate was 1 ml/min. Every 5 min a deep breath (−20 cm H 2 O) was initiated automatically (TCM; Hugo Sachs Elektronik).

Data sampling and calculation of lung mechanics. Arterial perfusate pressure relative to the top of the lungs was continuously monitored with a pressure transducer (Isotec™; Quest Medical, Dallas,
nulmonary compliance (C) were defined as percent of the mean of the NPV pressure by application of negative chamber pressure; under all conditions a tidal volume, pulmonary compliance, pulmonary resistance or pulmonary artery pressure did not become stable during this 60 min period of ventilated lungs were excluded from the present study (about 15%). After 60 min (at t = 0 min in the graphs), four different treatments were executed at random: Group 1 NPVlow: Continuation of the moderate NPV ventilation resulting in 200 μl Vt; Group 2 NPVhigh: 10 cm H2O peak transpulmonary inspiratory pressure by application of positive pressure ventilation; Group 3 NPVhigh: 25 cm H2O peak transpulmonary inspiratory pressure by application of passive pressure ventilation. Under all conditions a similar degree of end-expiratory transpulmonary pressure, i.e., 2 cm H2O, was maintained. All experiments were performed for an additional 150 min. Perfusate samples were taken every ten minutes and stored at −20°C. At the end of the experiment, small pieces of the lung parenchyma were used for RNA extraction, while others were immersed in 4% formaldehyde, dehydrated through a graded series of alcohol, embedded in paraffin and counterstained by hematoxylin/eosin.

When assessing 10 different mediators by t test or ANOVA there is a just-by-chance possibility of 40% (1-0.95; n = 10) that for one of them there is a significant effect (p < 0.05) of hyperventilation on mediator release. Therefore, the search for mediators that are released during hyperventilation was performed in two stages. In the first exploratory part we examined a broad spectrum of cytokines in order to form a hypothesis. In the second confirmatory part, this hypothesis was explicitly tested. It is emphasized that the data shown in Figure 3 and Figure 5 include the experimental data sets from both the exploratory (n = 4) and confirmatory (n = 3) part of this study. Statistical tests were only performed on the confirmatory data.

### Statistical Analysis

A II data in the figures are given as mean ± SEM, whereas data in the tables and in the text are given as mean ± SD. In Figure 3 and Figure 5 the area under the curve (AUC) was calculated and subsequently the AUC data were analyzed by two-factor ANOVA with ventilation mode (PPV or NPV) and transpulmonary pressure (low or high) as the two factors. Values of p < 0.05 were considered statistically significant and the results are reported in Tables 1 and 2. In Figure 6 the mRNA levels were calculated as percent of the mean of the NPVlow control value at either 30 or 150 min. The standard errors shown in Figure 6 account for the error propagation that is necessary when di-
Characterization of the Isolated Perfused Mouse Lung

We developed a set-up for an isolated perfused mouse lung, which allowed us to measure physiological lung functions (Figure 1) and to study induction, synthesis and release of different mediators, such as cytokines. After a 60-min period for stabilization under control conditions (NPV\textsubscript{low}), we obtained the following physiological parameters: \( V_T \), 187 ± 26 \( \mu \)l; dynamic compliance \( C_{dyn} \), 0.022 ± 0.004 ml/cm H\(_2\)O; airway resistance, 0.45 ± 0.14 cm H\(_2\)O · s/ml; pulmonary artery pressure (PAP), 2.2 ± 1.5 cm H\(_2\)O.

Since we were interested in ventilation-induced release of mediators into the perfusate, we had to establish conditions in which under basal conditions only small amounts of mediators are produced. The most important factor in this respect was the composition of the perfusion medium. Figure 2 shows the effect of perfusion with two different buffers on the release of TNF\(_\alpha\). It can be seen that a buffer supplemented with standard albumin (concentration 4%) caused release of high amounts of TNF\(_\alpha\) into the perfusate. If low endotoxin grade albumin (4%) was used, only very low concentrations of TNF\(_\alpha\) were found.

Viability of control preparations was assessed as follows: (1) during four hours of perfusion and ventilation (NPV\textsubscript{low}) no measurable release of LDH into the perfusate was found (data not shown). (2) Tidal volume and dynamic pulmonary compliance decreased constantly by approximately 12% per hour (Figure 3), while airway resistance remained stable (not shown). Pulmonary artery pressure increased slightly with time (Figure 3). (3) By light microscopy, the lungs appeared undamaged. Most of the alveoli were slightly inflated and only small areas of noninflated alveoli were observed. No interstitial or alveolar edema were noted. In all groups, however, small amounts of edema around the larger vessels were found (not shown).
Hyperventilation

Initiation of hyperventilation by either NPV or PPV led to a sudden increase of \( V_T \) to 459 ± 59 \( \mu l \) (Figure 3). During the following 150 min of hyperventilation \( V_T \) decreased to 186 ± 81 \( \mu l \), while \( C_{dyn} \) decreased to 0.008 ± 0.0042 ml/cm H2O, which is about 50% of the corresponding value of control lungs at the same time point (0.014 ± 0.0054 ml/cm H2O). After switching from NPVlow to either PPVlow or PPVhigh, PAP increased by 6.9 ± 1.3 cm H2O and 9.9 ± 4.9 cm H2O, respectively. A small increase in PAP over time occurred regardless of the ventilation mode (Figure 3). The statistical analysis of these data (Table 1) showed that transpulmonary pressure, i.e., hyperventilation, had a significant effect on \( V_T \), \( C_{dyn} \), and PAP. There was no difference between NPV and PPV, except for PAP. No statistical interactions between ventilation mode and transpulmonary pressure were observed.

No LDH release into the perfusate was noted in any of the hyperventilated lungs. When such lungs from the hyperventilation groups were investigated by light microscopy (Figure 4), no alveolar edema and no rupture of the alveoli were noted. In NPVhigh and PPVhigh ventilated lungs a marked inhomogeneity of inflation of the alveoli was observed. A reas with over-distended alveoli (Figure 4B) were found next to areas with slightly or noninflated alveoli (Figure 4C). The alveolar septa within these areas appeared slightly thickened (Figure 4C). No marked differences were seen between NPVhigh and PPVhigh ventilated lungs and alveolar septa were intact in all lungs (Figure 4D).

MEDIATOR RELEASE BY HYPERVENTILATION

Explanatory. To analyze possible effects of hyperventilation on the release of mediators from the lungs, we examined various mediators in the perfusate without predefined hypothesis. Only prostacyclin, TNF-\( \alpha \) and IL-6 were spontaneously released in detectable amounts into the perfusate. In contrast we found no detectable levels of IL-4, IL-10, interferon \( \gamma \), granulocyte-macrophage colony-stimulating factor, thromboxane, leukotriene B4 and prostaglandin E2. During hyperventilation, \( \alpha \)-thromboxane, an enzyme that elaborates PGH2, the precursor of prostacyclin, increased. Under no condition were any of the other mediators detected in the perfusate during hyperventilation (data not shown). In this exploratory part of our study no statistics were calculated. Please note that the data can be expressed as either pg/ml or pg/min, because the lungs were perfused at 1 ml/min in a nonrecirculating manner.

Confirmatory. In the confirmatory part of this study, we repeated the experiments of all four ventilation groups and measured only the three mediators prostacyclin, TNF-\( \alpha \) and IL-6 under the predefined hypothesis that hyperventilation increases the release of these mediators into the perfusate. Per fusate levels of prostacyclin, TNF-\( \alpha \) and IL-6 under hyperventilation regardless of whether lungs were ventilated by positive (PPVhigh) or negative pressure (NPVhigh) (Figure 5). These effects of hyperventilation on the release of mediators were significant, based on the area under the curve (Table 2). No statistical interactions between ventilation mode and transpulmonary pressure were observed.

Since the bioactivity of TNF-\( \alpha \) may be influenced by soluble TNF-\( \alpha \) receptors, we examined the perfusate concentrations of the murine soluble p55 and p75 TNF-\( \alpha \) receptors. Soluble p55 TNF-\( \alpha \) receptors were not detected under any condition. Small concentrations of soluble p75 receptors were present before initiation of hyperventilation (33 ± 18 pg/ml, \( n = 7 \)), but this value was not significantly (ANOVA) changed after 3 h of either normal ventilation (PPVlow: 41 ± 12, \( n = 4 \)) or hyperventilation (PPVhigh: 48 ± 23, \( n = 3 \)).

MEDIATOR EXPRESSION BY HYPERVENTILATION

Finally, we examined whether the enhanced release of prostacyclin, TNF-\( \alpha \) and IL-6 by hyperventilation was also reflected by an increased expression of mRNA. We measured the amounts of mRNA for TNF-\( \alpha \), IL-6 and cyclooxygenase-2, the enzyme that elaborates PGH2, the precursor of prostacyclin. Thirty and 150 min after the onset of hyperventilation we prepared tissue samples for PCR analysis from lungs of the NPVlow group as control group and from both hyperventilation groups, NPVhigh and PPVhigh.

### Table 1: Comparison of Physiological Measurements According to Type and Level of Ventilation

<table>
<thead>
<tr>
<th></th>
<th>AUC [VT (ml · min)]</th>
<th>AUC [Cdyn (ml · min/cm H2O)]</th>
<th>AUC [PAP (cm H2O · min)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPV low</td>
<td>32,190 ± 5,200 (7)</td>
<td>4.0 ± 0.8 (7)</td>
<td>202 ± 136 (7)</td>
</tr>
<tr>
<td>NPV high</td>
<td>58,850 ± 14,720 (7)</td>
<td>3.5 ± 0.9 (7)</td>
<td>560 ± 216 (7)</td>
</tr>
<tr>
<td>PPV low</td>
<td>36,310 ± 5,030 (7)</td>
<td>5.0 ± 0.4 (3)</td>
<td>1,192 ± 301 (6)</td>
</tr>
<tr>
<td>PPV high</td>
<td>58,680 ± 10,100 (7)</td>
<td>3.6 ± 0.5 (3)</td>
<td>1,967 ± 654 (7)</td>
</tr>
</tbody>
</table>

Results of two-factor ANOVA

<table>
<thead>
<tr>
<th>Effect of ventilation mode (NPV versus PPV)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>0.605</td>
<td>0.146</td>
</tr>
<tr>
<td>&lt; 0.001</td>
<td>0.022</td>
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</tbody>
</table>

Shown are the area under the curve (AUC) data for tidal volume (VT), dynamic compliance (Cdyn) and pulmonary artery pressure (PAP) that were calculated from the curves shown in Figure 3. The data were analyzed by two-factor analysis of variance (ANOVA) with ventilation mode (i.e., PPV or NPV) and transpulmonary pressure (Pr, i.e., low or high) as the two factors. The p values calculated in the ANOVA are given; \( n = 3 \) was considered significant. Since there were no interactions between the factors, multiple comparisons were not performed. Data are given as mean ± SD, numbers in parentheses identify number of experiments. Please note that for technical reasons Cdyn during PPV was only calculated in the confirmatory part of the study (\( n = 3 \)).
The message for TNF$\alpha$ was up-regulated 30 min but not 150 min after the onset of hyperventilation (Figure 6), whereas IL-6 mRNA increased after 150 min (Figure 6). No change in COX-2 mRNA expression was observed.

DISCUSSION

Here we show that artificial mechanical ventilation leads to induction, synthesis and release of cytokines and eicosanoids from lung tissue. Since an experimental approach in vivo is limited by the small blood volume of about 2 ml per mouse as well as by the short half-life of circulating mediators, we chose the isolated perfused mouse lung to study this subject.

The Model of the Isolated Perfused Mouse Lung

Perfusion of murine lungs has only rarely been reported and was restricted mostly to toxicological investigations (e.g., ref. 14). Hitherto, ventilation and measurement of lung mechanics has not been reported in isolated mouse lungs. The set-up for the isolated perfused lung of the species mouse is an expansion of the one we have previously described in detail for the

Figure 4. Histology. (A) NPV$_{\text{low}}$, magnification $\times 6.3$; (B, C) NPV$_{\text{high}}$, magnification $\times 25$; (D) PPV$_{\text{high}}$, magnification $\times 25$. Lungs were prepared for light microscopy directly after end of experiments.
Major differences between the two set-ups are: (1) The murine lungs are not removed from the thorax cavity, but instead the whole animal is placed in the pressure chamber with an open chest. To avoid accumulation of water in the trachea this chamber is bent in a slightly sloping fashion. (2) Lungs are perfused at a constant flow rate of 1 ml/min. Though this represents only a small fraction of the normal cardiac output of mice (3), we chose this flow rate for mainly two reasons: to avoid high perfusate pressures during PPV\textsubscript{high} and to increase the concentration of metabolites secreted into the perfusate. (3) Like in rat lungs, in order to avoid interactions between blood-derived leukocytes and lung tissue we utilized a blood-free perfusion medium supplemented with albumin. To minimize release of TNF\textsubscript{x} (and also IL-6 and prostacyclin) we had to use low-endotoxin containing albumin (Figure 2). (4) When designing a lung perfusion system the following general considerations are of importance with respect to PPV and NPV (4, 5). With natural inspiration, the pulmonary artery pressure rises relative to pleural pressure and hence also to alveolar pressure. This relationship is maintained during NPV but not during PPV. On the other hand, in vivo left atrial pressure falls with pleural pressure as during PPV. However, in most negative pressure ventilation set-ups an artificially high venous pressure is created. This happens because the extravascular pressure is largely determined by the negative chamber pressure, whereas the intravascular pressure is connected to the ambient air. Such a set-up leads to high transmural pressures and favors edema formation (6). To circumvent this problem, an equilibration chamber (Figure 1) may be used that adds the oscillating chamber pressure onto the venous outflow pressure. Although we have not systematically investigated this subject, orientating experiments suggested that the presence of this chamber helps to minimize edema formation in our model.

The functional integrity of the perfused mouse lungs used in this study is demonstrated by the following facts: (1) Lung mechanics and perfusate pressure changed only moderately during 4 h of perfusion and ventilation. While pulmonary resistance remained nearly stable during this time, tidal volume and dynamic pulmonary compliance decreased by 12% per hour. This figure compares fairly well to our experience with perfused rat lungs, where we noted a decrease in compliance of 4-8% per hour (15). The reason for this decline is unknown, but may be related to exhaustion of the intracellular surfactant stores, possibly as a result of the regular deep breaths (16). These regular deep breaths, however, are habitual in vivo and are necessary in perfused lungs to prevent atelectasis. (2) In histological sections, the lung structure appeared to be intact and no gross edema formation was observed. However, perivascular edema was detected around the big vessels, an observation that was also made in perfused rat lungs (16). (3) D uring NPV with a tidal volume of 200 μl typical for an anesthetized mouse, the spontaneous release of cytokines or eicosanoids into the perfusate was very low (Figures 2 and 5). The constant low release of the early cytokine TNF\textsubscript{x} from control lungs suggests that the lungs were neither infected nor primed by exposure to pyrogens such as endotoxin.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5}
\caption{Stimulation of TNF, IL-6 and 6-keto-PGF\textsubscript{1α} by hyperventilation. Perfusate samples were taken frequently and mediators measured by ELISA (TNF, IL-6) or BA (6-keto-PGF\textsubscript{1α}). NPV\textsubscript{low}, closed squares; NPV\textsubscript{high}, closed triangles; PPV\textsubscript{low}, open squares; PPV\textsubscript{high}, open triangles. All data are given as means ± SEM. For statistical evaluation of these data see Table 2.}
\end{figure}
realize that as a consequence of the so-called waterfall phenomenon, under Zone 2 conditions the driving pressure for flow is independent of the venous pressure (4, 5).

**Hyperventilation-induced Mediator Release**

During ventilation at low tidal volumes, only small amounts of prostacyclin, TNF-$\alpha$ or IL-6 were found in the perfusate of perfused mouse lungs. However, during hyperventilation pulmonary production of PG1, TNF-$\alpha$ and IL-6 was markedly increased. As judged by light microscopy, the mechanical stress applied by hyperventilation caused no gross physical damage to the lung tissue. This finding was important in so far as physical stress such as surgery, massaging the lung or stirring of chopped lung tissue may cause release of PG1 or IL-6 (20–22). Thus the absence of overt physical damage suggests that mechanisms other than tissue destruction must account for the release of mediators caused by hyperventilation. We noted, however, that continuous ventilation with elevated pressures caused formation of mild interstitial edema. We cannot decide whether this is responsible for the decline in tidal volume over time in these lungs. Since it is known that even brief periods of hyperventilation elicit surfactant releases into the alveolar space (23), one alternative possible explanation is that continued hyperventilation may exhaust the alveolar surfactant system (24). A further possibility, that does not exclude the other two possibilities, would be derecruitment of lung tissue; in view of the inhomogenous inflation of lung tissue this seems clearly possible.

The amount of mediator release was not different between hyperventilation during NPV or PPV. This was not surprising since at a given tidal volume (which was similar in both modes) the transpulmonary pressure difference must be independent of the ventilation mode. Interestingly, even though hyperinflation caused by NPV or PPV had opposite effects on pulmonary artery pressure, it still produced the same pattern of mediator release. We believe that this is an important observation, because it allows exclusion of changes in perfusion pressure as the cause for the mediator release. In line with this, others have shown that only changes in perfusate flow, but not in perfusate pressure or pulsatility increase PG1 levels in perfused lungs (25, 26). This is in agreement with the present data where switching from NPV$_{low}$ to PPV$_{low}$ increased perfu-

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**Table 2**

**Comparison of Mediator Release According to Type and Level of Ventilation**

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>AUC</th>
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<tr>
<td></td>
<td>TNF-$\alpha$</td>
<td>IL-6</td>
</tr>
<tr>
<td></td>
<td>(ng · min/ml)</td>
<td>(ng · min/ml)</td>
</tr>
<tr>
<td>NPV low</td>
<td>1.9 ± 1.4</td>
<td>19 ± 13</td>
</tr>
<tr>
<td>NPV high</td>
<td>31 ± 11</td>
<td>141 ± 97</td>
</tr>
<tr>
<td>PPV low</td>
<td>1.0 ± 0.6</td>
<td>11 ± 5</td>
</tr>
<tr>
<td>PPV high</td>
<td>25 ± 19</td>
<td>56 ± 14</td>
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Results of ANOVA

- Effect of ventilation mode (NPV versus PPV): No
- Effect of Ptp (low versus high): Yes

<table>
<thead>
<tr>
<th>p Value</th>
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<tr>
<td>0.600</td>
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<table>
<thead>
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<td>0.019</td>
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</table>

Shown are the area under the curve (AUC) data for TNF-$\alpha$, IL-6 and 6-keto-PGF$_{1\alpha}$ that were calculated from the curves shown in Figure 5 (confirmatory). The data were analyzed by two-factor analysis of variance (ANOVA) with ventilation mode (i.e., PPV or NPV) and transpulmonary pressure (Ptp, i.e., low or high) as the two factors. The $p$ values calculated in the ANOVA are given; $p < 0.05$ was considered significant. Since there were no interactions between the factors, multiple comparisons were not performed. Data are given as mean ± SD, $n = 3$. 

Our data with the low endotoxin versus usual albumin supplemented buffer support this interpretation. These findings demonstrate the suitability of our experimental setting to study the relationship between the respiratory system and mediators of the immune system.

**Physiological Alterations during Hyperventilation**

Perfusion and ventilation of lungs can occur under three paradigmatic conditions, referred to as Zone 1 to Zone 3, where flow rate depends on the relative magnitude of pulmonary artery, alveolar and pulmonary vein pressure. Of these three parameters we know only PAP from direct measurements. A vascular pressure can be estimated from tracheal pressure, which in case of homogenous inflation is a good estimator of mean alveolar pressure (17). Venous pressure was not measured, but can be inferred by taking into account the height of the venous outflow (which was 1 cm above the hilum of the lung) and, in the case of NPV, also the chamber pressure (which by the equilibration chamber was added to the venous pressure for the reasons outlined above). From these considerations the following mean pressures were derived (in cm H$_2$O): NPV$_{low}$: 2 (PAP) > 0 (alveolar pressure) > −4 (pulmonary venous pressure); NPV$_{high}$: 1 > 0 > −11; PPV$_{low}$: 15 > 5 > 1; PPV$_{high}$: 17 > 11 > 1. Therefore, all our lungs were in Zone 2 (PAP > alveolar pressure > venous pressure). Thus, our conditions approach the clinical situation during mechanical ventilation by PPV, which as a result of the elevated alveolar pressure increases the amount of lung in Zone 2 (18). The changes observed in pulmonary artery pressure after initiation of hyperventilation have been described before (4, 5), although most studies were performed under conditions of static inflation. Performing hyperventilation in Zone 2 lungs during PPV raises alveolar pressure and as a consequence of this the alveolar capillaries are compressed which in turn increases vascular resistance. In Zone 2 lungs during NPV, mean alveolar pressure during hyperventilation remains unchanged, and expanding the lungs increases the transmural pressure on extraalveolar and corner vessels, which finally results in diminished vascular resistance (19). Moreover, it is important to realize that as a consequence of the so-called waterfall phenomenon, under Zone 2 conditions the driving pressure for flow is independent of the venous pressure (4, 5).
Hyperventilation and Cytokine Release

Our current hypothesis to explain the hyperventilation-induced mediator release is that it is caused by the overdistention of lungs. Overdistention may lead to activation of stretch-activated ion channels. Such channels have been described in epithelial cells (27), endothelial cells (28) and alveolar macrophages (29). Human alveolar macrophages, i.e., a source of TNFα, contain a stretch-sensitive potassium channel (29). TNFα derived from alveolar macrophages may then activate alveolar type II cells to produce IL-6 (30). Alternatively, since many forms of stress elicit IL-6 production, stretching itself may be the stimulus for IL-6 release. A further alternative is that hyperinflated lungs become partly atelectatic (suggested by the histology as well as the decline in tidal volume in hyperventilated lungs); this could increase shear stress (31) which in turn might elicit IL-6 release. The additional findings that not only the protein but also the message for TNFα and IL-6 were elevated at 30 and 150 min respectively, suggest that induction/stabilization of mRNA may be involved in the hyperventilation-induced release of cytokines from lung tissue.

The source of prostacyclin is also unknown, but stretching of fetal rat lung cultures (20) and of cultured endothelial cells (32) resulted in prostacyclin production, which again may be related to activation of ion channels (28). Prostacyclin was produced almost instantly after switching from low to high volume ventilation, which is unlikely for an enzyme induction mechanism. In line with this, we did not find induction of cyclooxygenase-2 mRNA in lung tissue. A rapid release of prostacyclin in response to hyperventilation is in line with previous findings (33). Ventilation at birth is a known stimulus for PGI release into the pulmonary vein (34). Also, ventilation of perfused rat lungs at higher frequencies increased release of PGI into the perfusate (35). Ventilation-induced release of pulmonary PGI may have clinical implications, since the systemic hypotension complicating mechanical hyperventilation is attributable in large measure to the release of vasodilator agents (33).

In the present study we have not checked for the bioactivity of TNFα, IL-6, or prostacyclin. However, we found no increase in the concentration of soluble TNFα receptors that may reduce TNFα bioactivity. For IL-6 no endogenous inactivator is known; the soluble IL-6 receptor appears to be rather activating than inactivating (36). Also, for prostacyclin no endogenous inhibitor is known; however, it is an unstable compound that quickly forms 6-keto-PGF1α. Taken together, at present we have no reason to believe that either of the mediators released by hyperventilation was not bioactive.

In intensive care, artificial ventilation is used in order to maintain adequate blood oxygenation. This requires frequent ventilation with high pressures. High pressure will cause opening of collapsed and atelectatic, but also overdistension of compliant lung areas (37). Such overdistension creates a condition similar to ventilation of certain lung areas with higher volumes, analogous to the experimental system described in the present study. Extrapolated to the clinical situation, the following pulmonary and systemic consequences may arise: In the lung, release of TNFα may promote inflammatory responses, e.g., accumulation of neutrophils. Release of mediators such as TNFα, IL-6 and prostacyclin into the circulation may cause vasodilatation, systemic hypotension and a systemic inflammatory response including fever even before pulmonary lesions can be recognized. In fact, it is a frequent observation that in ventilated patients such responses occur without signs of infection, e.g., positive blood cultures (38). When extrapolating the present data to the clinical situation, however, caution should be exercised, since obviously various differences between our model and the in vivo situation exist, such as species differences, the absence of innervation, artificial buffer instead of blood or the absence of an underlying

Figure 6. Induction of mRNA by hyperventilation. Tissue probes for detection of mRNA for TNFα (A), IL-6 (B) and COX-2 (C) by PCR were prepared 30 min (left group of bars) or 150 min (right group of bars) after initiation of hyperventilation. NPVlow: filled bars; NPVhigh: hatched bars; PPVhigh: open bars. All bars represent means ± SEM of three independent preparations given as the percentage of NPVlow at either 30 or 150 min. *Statistically significantly different from NPVlow at p < 0.05.
lung disease. Therefore, it will be important to investigate the clinical consequences of mediator release during artificial ventilation and to find out whether it contributes to the development of the inflammatory response syndrome.

References