Cytokine release, small airway injury, and parenchymal damage during mechanical ventilation in normal open-chest rats

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D’Angelo E, Koutsoukou A, Della Valle P, Gentile G, Pecchiari M. Cytokine release, small airway injury, and parenchymal damage during mechanical ventilation in normal open-chest rats. J Appl Physiol 104: 41–49, 2008. First published October 25, 2007; doi:10.1152/japplphysiol.00805.2007.—Lung morpho-functional alterations and inflammatory response to various types of mechanical ventilation (MV) have been assessed in normal, anesthetized, open-chest rats. Measurements were taken during protective MV (tidal volume (VT) = 8 ml/kg; positive end-expiratory pressure (PEEP) = 2.6 cmH2O) before and after a 2- to 2.5-h period of ventilation on PEEP (control group), zero EEP without (ZEEP group) or with administration of dioctylsodiumsulfosuccinate (ZEEP-DOSS group), on negative EEP (NEEP group), or with large VT (26 ml/kg) on PEEP (Hi-Vt group). No change in lung mechanics occurred in the Control group. Relative to the initial period of MV on PEEP, airway resistance increased by 33 ± 4, 49 ± 9, 573 ± 84, and 13 ± 4%, and quasi-static elastance by 19 ± 3, 35 ± 7, 248 ± 12, and 20 ± 3% in the ZEEP, NEEP, ZEEP-DOSS, and Hi-Vt groups. Relative to Control, all groups ventilated from low lung volumes exhibited histological signs of bronchiolar injury, more marked in the NEEP and ZEEP-DOSS groups. Parenchymal and vascular injury occurred in the ZEEP-DOSS and Hi-Vt groups. Pro-inflammatory cytokine concentration in the bronchoalveolar lavage fluid (BALF) was similar in the Control and ZEEP group, but increased in all other groups, and higher in the ZEEP-DOSS and Hi-Vt groups. Interrupter resistance was correlated with indexes of bronchiolar damage, and cytokine levels with vascular-alveolar damage, as indexed by lung wet-to-dry ratio. Hence, mechanical MV from resting lung volume causes mechanical alterations and small airway injury, but no cytokine release, which seems mainly related to stress-related damage of endothelial-alveolar cells. Enhanced small airway epithelial damage with induced surfactant dysfunction or MV on NEEP can, however, contribute to cytokine production.

IN AN EX VIVO MODEL of normal and lavaged rat lungs, ventilation with physiological tidal volumes (VT) from zero end-expiratory pressure (ZEEP) causes mechanical alterations with a significant increase of histologic injury scores in the terminal bronchioles and release of pro-inflammatory cytokines (5, 19, 24). Subsequently, it was shown that also in normal, anesthetized rabbits, mechanical ventilation at low lung volumes induces histological evidence of peripheral airway damage with a concurrent increase in airway resistance and lung elastance, which persist after restoration of normal end-expiratory volumes (6–8), and that these effects are caused by the abnormal stresses due to cyclic opening and closing of peripheral airways and increased surface tension (9). In contrast with the in vitro studies on rats, in the in vivo studies on rabbits there was no indication of an inflammatory response as assessed by the release of inflammatory cytokines, further suggesting that the so called “low volume injury” should be due to the mechanical stress related to cyclic opening and closing of small airways, hereafter referred to as tidal airway closure.

The discrepancy in the release of inflammatory cytokines could be, however, inherent to the models, i.e., in vivo vs. in vitro preparation. Differences in the extension of small airway involvement in tidal airway closure or degree of surfactant dysfunction and dependent noxious stress also provide alternative explanations. On the other hand, the discrepancy could be simply apparent: indeed, only tumor necrosis factor (TNF-α) was assessed in rabbits, and although the largest changes usually affect this cytokine (5, 25), it has been shown that release of pro-inflammatory cytokines can occur with little or no change in TNF-α levels (26).

The aim of this study is that of establishing whether tidal airway closure with ventilation at low volumes induces an inflammatory response with release of inflammatory cytokines in normal lungs in vivo. The concentration of the pro-inflammatory cytokines and the concomitant functional and histologic alterations were, therefore, assessed in normal, anesthetized open-chest rats ventilated with physiological VT on ZEEP. To increase the amount of small airways involved in tidal airway closure and/or stress associated with this phenomenon, a group of open-chest rats was mechanically ventilated with physiological VT on NEEP, while another group was ventilated on ZEEP after having been treated with the aerosolized detergent dioctylsodiumsulfosuccinate to increase surface tension. Measurements were also obtained from a fourth group of open-chest rats ventilated with large VT on positive end-expiratory pressure (PEEP), because it is generally recognized that release of cytokines invariably occurs under this condition as a consequence of parenchymal overstretching (10, 12). Finally, measurements were performed in normal, untreated, open-chest rats subjected to “noninjurious” ventilation, i.e., prolonged mechanical ventilation with physiological VT on PEEP, these animals serving as the control group.

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METHODS

Thirty-five male Sprague-Dawley rats (weight range 380–460 g) were anesthetized with an intraperitoneal injection of a mixture of pentobarbital sodium (40 mg/kg) and chloral hydrate (170 mg/kg), after induction with diazepam (10 mg/kg). A metal cannula, connected to a pneumotachograph, and two polyethylene catheters were inserted into the trachea, jugular vein, and carotid artery, respectively. The animals were paralyzed with pancuronium bromide (0.1 mg/kg) and ventilated with a pattern similar to that during spontaneous breathing using a custom-made ventilator. Anesthesia and complete muscle relaxation were maintained with additional doses of the anesthetic mixture and pancuronium bromide. Adequacy of anesthesia was judged from the sudden increase in heart rate and/or systemic blood pressure. The chest was opened via a median sternotomy and a coronal cut made just above the costal arch, while application of PEEP prevented lung collapse.

Airflow (V) was measured with a heated Fleisch pneumotachograph no. 0000 (HS Electronics, March-Hugstetten, Germany) connected to the tracheal cannula and a differential pressure transducer (Validyne MP45, ±2 cmH2O; Northridge, CA). The response of the pneumotachograph was linear over the experimental flow range. Tracheal pressure (Ptr) and systemic blood pressure were measured with pressure transducers (8507C-2 Endevco, San Juan Capistrano, CA; Statham P23Gb, HS Electronics) connected to the side arm of the tracheal cannula and carotid catheter, respectively. There was no appreciable shift in the signal or alteration in amplitude up to 20 Hz. The signals from the transducers were amplified (RS3800; Gould Electronics, Valley View, OH), sampled at 200 Hz by a 12-bit analog-to-digital converter (AT MIO 16L-9; National Instruments, Austin, TX), and stored on a desk computer. Volume changes (ΔV) were obtained by numerical integration of the digitized airflow signal. Arterial blood PO2, PCO2, and pH were measured by means of a blood gas analyzer (IL 1620; Instrumentation Laboratory, Milan, Italy) on samples drawn at the end of each test session.

After completion of the surgical procedure, the rats were ventilated with a specially designed, computer-controlled ventilator (6), delivering water-saturated air from a high-pressure source (4 atm) at constant flow of different selected magnitudes and durations, while Ringer-bicarbonate was continuously infused intravenously at a rate of 4 ml·kg-1·h-1 and epinephrine occasionally administered to keep normal arterial blood pressure. A three-way stopcock allowed the connection of the expiratory valve of the ventilator either to the ambient (ZEEP) or to a drum in which the pressure was set at 2.5–2.8 (PEEP) or −3 cmH2O (NEEP) by means of a flow-through system. While animals were on PEEP, air was used to ventilate; on ZEEP and NEEP, oxygen (70–80%) mixtures were intermittently administered if needed to prevent marked, life-threatening hypoxia. However, all measurements were always performed during air breathing. Baseline ventilation consisted of a fixed VT (8 ml/kg), inspiratory and expiratory duration (0.25 and 0.5 s), and end-inspiratory pause (0.2 s). No intrinsic PEEP was present under any experimental condition, as evidenced by the absence of Ptr changes with airway occlusion at end expiration. During measurements, the ribs on the two sides and the diaphragm were pulled widely apart to prevent contact between lung and chest wall, except in their dependent parts.

Surfactant dysfunction was induced by means of 10% alcoholic solution of dioctylsodium sulfosuccinate (DOSS), (Aerosol OT, A
on PEEP (2.6 ± 0.02 cmH2O), separated by a 2- to 2.5-h period during which one of the following ventilation types was used: 1) baseline ventilation on ZEEP (ZEEP group); 2) baseline ventilation on NEEP of −3 cmH2O (NEEP group); 3) baseline ventilation on ZEEP after treatment with DOSS (DOSS-ZEEP group); 4) high volume (Vt = 26 ml/kg) ventilation on PEEP (Hi-Vt group); and 5) baseline ventilation on PEEP (Control group). When large Vt were used, the inspiratory (Ti) and expiratory duration were increased (1 and 2.9 s, respectively) to keep pulmonary ventilation nearly constant. Each group was made of seven animals, and the various types of experiment were done in random order.

Lung mechanics were assessed during the PEEP1 and PEEP2 periods and at the end of the period of test ventilation. Two types of measurements were carried out: 1) while keeping Vt at baseline values, test breaths were intermittently performed with different Vt and Ti in the range 0.25 to 3 s to assess lung mechanics at end inflation; and 2) while keeping Vt constant, test breaths were intermittently performed with different Vt to obtain the quasi-static inflation volume-pressure curve in the tidal volume range. End-inspiratory occlusions lasting 5 s were made in all test breaths, and repeated four to five times under each experimental condition. On PEEP, the lungs were inflated three to four times to Ptp of ~25 cmH2O before all measurements, and the expiratory valve was opened to the ambient for three to five expirations to measure the difference between the end-expiratory and the resting lung volume (ΔEELV), the latter being the volume at zero transpulmonary pressure.

Quasi static elastance (Est), interrupter resistance (Rint), which reflects airway resistance, and viscoelastic resistance (Rvisc) and time constant (tvisc) were assessed according to the rapid airway occlusion method, as previously described (6), while the ratio (Elow/Ebase) between Est with the lowest Vt (~1.3 ml/kg) and baseline Vt was taken as an index of amount of peripheral airways being involved in recruitment-derecruitment during tidal ventilation (9, 15).

After completion of the mechanics measurements, 1.5–2 ml of blood was drawn from the heart for the assessment of systemic release of cytokines and serum albumin concentration. The animals were killed with an overdose of anesthetics. The right lung was processed for histological analysis (see below). The left bronchus was cannulated and the left lung was removed, weighed immediately, lavaged with 4.3 ml/kg of normal saline in two aliquots, fluid recovery ranging from 40 to 50%, left overnight in an oven at 120°C, and weighed again to compute the wet-to-dry ratio (W/D). The effluents were pooled, centrifuged (Harrier 18/80, Sanyo Gallenkamp PLC, Loughborough, UK) at 2,000 rpm for 10 min, and the supernatant was frozen and stored at −20°C for subsequent assessment of cytokines and albumin concentration in broncho-alveolar lavage fluid (BALF).

Cytokine [TNF-α, IL-1β, IL-2, IL-6, IL-10, macrophage inflammatory protein (MIP)-2] analysis was carried out in duplicate in blinded fashion on BALF and serum using commercially available ELISA kits specific for rat (Quantikine, R&D Systems, Minneapolis, MN; Rat GRO/CINC-3 Assay Kit, IBL). Absorbance was read at 450 nm (correction wavelength set at 540 nm; Titertek Multiskan MCC, Flow Laboratories, Milan, Italy), background absorbency of blank wells being subtracted from the standards and samples prior to determination of the concentration. The lower limit of detection for those kits was 6.25, 15.6, 15.6, 62.5, 31.2, and 5 pg/ml, respectively, in which case concentration was assumed to be nil. The albumin concentration of the BALF supernatant and serum obtained shortly before lung lavage was determined with a clinical chemistry analyzer (Bayer ADVIA 2004, Jeol, Japan for Bayer Diagnostics Europe, Dublin, Ireland) at 596 nm using the BCG method (albumin reagent, Bayer, Tarrytown, UK) with bovine albumin as standard.

Histological analysis. The right lung was fixed by intratracheal infusion of an 8% formaldehyde, 0.1% glutaraldehyde solution with the pressure maintained at 20 cmH2O for 24 h. Three blocks, ~1 cm thick, involving both subpleural and para-hilar regions, were obtained in each animal. Each block was processed through a graded series of alcohols and embedded in paraffin. From each block, sections of 5 μm thickness were cut and stained with hematoxylin-eosin. Histologic evaluation was performed by a single observer in a blind fashion, according to the procedure previously described in details (6, 7, 9).

The following measures were obtained using a computer-aided, image analysis system (IMAQ Vision for LabView, National Instruments): 1) the percent ratio of damaged (epithelial necrosis and sloughing) to total membranous bronchioles, the bronchiolar injury score (IS), as an index of small airway injury (19); and 2) the percent ratio of abnormal to total (normal and abnormal) bronchiolar-alveolar attachments and

Fig. 3. Average relationship between volume changes from the resting lung volume (ΔV) and quasi-static transpulmonary pressure obtained in the baseline tidal volume range (8 ml/kg) during mechanical ventilation on positive end-expiratory pressure at the beginning (PEEP1) and end of the experiment (PEEP2) or from a low EELV in the various groups of open-chest rats (indications as in Fig. 1).
the distance between normal attachments as indexes of airway-parenchymal mechanical uncoupling.

In addition, parenchymal and vascular injury was assessed by four parameters, focal alveolar collapse, perivascular and/or alveolar edema, recruitment of granulocytes to the air spaces, and hemorrhage (24), evaluated semiquantitatively with a four-grade scale (absent = 0; mild = 1; moderate = 2; marked = 3).

The study, which conforms to the American Physiological Society’s guidelines for animal care, was approved by Ministero della Salute, Rome, Italy.

Statistics. Analyses were performed using SPSS 11.5 (SPSS, Chicago, IL). Results from mechanical studies are presented as means ± SE. Comparisons among experimental conditions were performed using one-way ANOVA; when significant differences were found, the Bonferroni correction was made to determine significant differences between different experimental conditions. Results from cytokine assessments and histological studies are expressed as median and range, and the statistical analysis was done using the Mann-Whitney test. Multiple linear regression analysis was performed according to the least mean square method. The level for statistical significance was taken at \( P \leq 0.05 \).

RESULTS

Blood gasses and pH. During the initial period of ventilation on PEEP (PEEP1), the mean values of arterial PO2, PCO2 (PaO2, and PaCO2, respectively), and pH were similar for all groups of rats (Fig. 1). With ventilation at low end-expiratory lung volume, pHa decreased significantly in all groups, PaO2 decreased in the NEEP and ZEEP-DOSS group, while PaCO2 increased only in the ZEEP-DOSS group. On PEEP2, pHa was significantly reduced relative to PEEP1 values by the same amount in all groups of animals. While PaCO2 returned to the initial values on PEEP1 in all groups, PaO2 was significantly decreased only in animals treated with DOSS.

On PEEP1, the mean systemic blood pressure was similar in all groups of rats, averaging 80 ± 2 mmHg. It decreased during ventilation both with physiological \( V_t \) from low lung volumes (−11 ± 4 mmHg) and with large \( V_t \) from physiological lung volumes (−6 ± 4 mmHg), likely because of increased pulmonary vascular resistance and reduced left atrial filling. No significant differences in mean systemic blood pressure occurred between PEEP1 and PEEP2 in all groups.

Mechanics. During PEEP1, no significant differences occurred among the various groups for any mechanical parameter. Apart from a small nonsignificant increase of lung elastance, DOSS administration had no mechanical effects.

Ventilation at low EELV increased Rint, Est, and Rvisc in all groups of animals, while rvisc decreased significantly in the NEEP and ZEEP-DOSS group only (Fig. 2). Relative to PEEP1, Rint increased by 53 ± 53, 769 ± 98, and 1,035 ± 186%; Est by 319 ± 14, 363 ± 35, and 449 ± 31%; and Rvisc by 118 ± 12, 142 ± 26, and 212 ± 16% in the ZEEP, NEEP, and ZEEP-DOSS group, respectively. The quasi-static inflation V-P curve (Fig. 3), which on PEEP was concave toward the pressure axis, became sigmoid in the ZEEP and NEEP group, and convex toward the pressure axis in the ZEEP-DOSS group.

As a consequence, \( E_{low}/E_{base} \) increased markedly during ventilation at low EELV (Fig. 2), and significantly more in the ZEEP-DOSS and NEEP group than in the ZEEP group; to the extent that the increase in \( E_{low}/E_{base} \) reflects tidal recruitment of lung units, the latter should have been larger in the ZEEP-DOSS than in the NEEP and ZEEP group. Indeed, cardiac artifacts in the Ptr records were always present during the occlusion at end inspiration, but absent at end expiration in four and two animals of only the ZEEP-DOSS and NEEP group, respectively.

With the same end-expiratory \( P_{tp} \) (2.6 ± 0.02 cmH2O), the EELV was similar on PEEP1 and PEEP2, except in the ZEEP-DOSS group where it decreased from 3 ± 0.2 to 1.8 ± 0.2 ml (Fig. 3). The inflation V-P in the \( V_t \) range resumed the initial shape in all groups: as a consequence, \( E_{low}/E_{base} \) did not differ significantly between PEEP1 and PEEP2 (Fig. 2).

Prolonged ventilation on PEEP had no mechanical effects in animals ventilated with physiological \( V_t \) (Control group), while in those ventilated with large \( V_t \) (Hi-\( V_t \) group), Rint and Est were significantly increased (13 ± 3 and 20 ± 3%; \( P < 0.001 \)). After restoration of PEEP in animals previously ventilated at low EELV, Rint and Est remained elevated in all groups, Rvisc only in animals treated with DOSS, while rvisc returned to control values in all groups (Fig. 2). Relative to PEEP1, Rint increased by 33 ± 4, 49 ± 9, and 573 ± 84% and Est by 19 ± 3, 35 ± 7, and 248 ± 12% in the ZEEP, NEEP, and ZEEP-DOSS group, respectively, while Rvisc increased by 79 ± 15% in the ZEEP-DOSS group.

![Fig. 4. Cytokine levels (median) in serum and bronchoalveolar lavage fluid (BALF) assessed at the end of the final period of mechanical ventilation on positive end-expiratory pressure (PEEP2) in the various groups of open-chest rats (indications as in Fig. 1). TNF, tumor necrosis factor; IL, interleukin; MIP, macrophage inflammatory protein. Values significantly different from those of the Control group: *\( P < 0.05 \); **\( P < 0.01 \).](http://jap.physiology.org/)
Cytokines. Figure 4 shows the absolute levels of inflammatory and anti-inflammatory cytokines in serum and BALF for the various groups of rats, IL-2 concentration being below detectable levels under all circumstances. The lowest levels of inflammatory cytokines were found in the Control group. In the ZEEP group, serum and BALF concentration of inflammatory cytokines did not differ significantly from that in the Control group. In the other groups of rats, the concentration of inflammatory cytokines in BALF was significantly higher than that of the Control group, while that of the anti-inflammatory cytokine IL-10 was significantly increased in the ZEEP-DOSS group only. TNF-α levels did not differ among the NEEP, ZEEP-DOSS, and Hi-Vt groups, whereas the concentration of MIP-2, IL-1β, and IL-6 was significantly higher in the ZEEP-DOSS than in the NEEP group, but similar in the ZEEP-DOSS and Hi-Vt group. In general, cytokine levels in serum paralleled those in BALF: except for TNF-α, there was in fact a significant correlation between serum and BALF concentrations (Fig. 5).

Histology. Scores of bronchiolar epithelial injury (IS) and airway-parenchymal uncoupling (% normal bronchiolar-alveolar attachments, distance between normal attachments) are shown in Table 1 for all groups. Epithelial injury of small airways and airway-parenchymal uncoupling, i.e., abnormal bronchiolar-alveolar attachments, were more prominent in rats subjected to mechanical ventilation at low lung volume than in those ventilated on PEEP only, the injury score being in turn significantly higher in the NEEP and ZEEP-DOSS groups, in which it was similar, than in the ZEEP group. Some degree of airway-parenchymal uncoupling occurred, however, in the Hi-Vt group too, as the number of abnormal bronchiolar-alveolar attachments, but not the distance between normal attachments, was significantly higher than that in the Control group. Damage of cartilaginous airways was never observed.

Scores of parenchymal and vascular injury are shown in Table 2. No parenchymal and vascular damage occurred in rats of the Control and ZEEP group, while in the NEEP group one animal showed mild focal alveolar collapse, two animals showed recruitment of granulocytes to the air space, and three animals showed mild to moderate perivascular and peribronchial edema. In contrast, the lungs of all animals in the ZEEP-DOSS and Hi-Vt group were presenting various combinations of parenchymal and vascular injuries, that were more prominent in rats treated with DOSS, particularly interstitial and alveolar edema.

Measurements of lung W/D ratio and ratio of BALF to serum albumin concentration (ABALF/ASER) reported in Table 2 further support the morphologic evaluation of lung edema. The W/D ratio was similar in the Control and ZEEP group; higher, although not significantly, in the NEEP group; and significantly increased in the Hi-Vt and even more in the ZEEP-DOSS group. The ABALF/ASER ratio was also similar in the Control, ZEEP, and NEEP group; relative to Control group values, it was significantly increased in the Hi-Vt and even more in the ZEEP-DOSS group, indicating that alveolar edema developed mainly in the latter group.

DISCUSSION

In normal, open-chest rats prolonged mechanical ventilation with physiological end-expiratory and tidal volumes (PEEP group) did not cause mechanical changes (Figs. 2 and 3) and essentially no signs of lung injury (Tables 1 and 2), in line with the results obtained in normal rabbits (6, 7). On the other hand, prolonged mechanical ventilation from the resting lung volume (ZEEP group) with physiological tidal volumes caused histolog-
Injury score in each group divided by the maximal possible sum, i.e., total attachments with tidal airway closure that occur in normal small airway epithelium and rupture of alveolar-bronchiolar pulmonary vascular injury (Tables 1 and 2). Hence, damage of inflammatory cytokine production, as indicated by the similarity of mechanical ventilation (7–9), occurred in the absence found in normal, open-chest rabbits subjected to the same ex vivo rat models (5, 19, 25), these functional and morphological alterations differed in part between the NEEP and ZEEP-DOSS group. According to E_{low}/E_{base} values (Fig. 2), the extent of tidal airway closure should have been in fact greater in the NEEP and ZEEP-DOSS groups than in the ZEEP group. Indeed, both the histological (Tables 1 and 2) and mechanical alterations (Figs. 2 and 3) were also greater, and multiple linear regression analysis shows that R_{int} was significantly correlated with indexes of bronchiolar injury (Fig. 6), besides lung W/D ratio. The nature of the histological alterations differed in part between the NEEP and ZEEP-DOSS group. According to E_{low}/E_{base} values (Fig. 2), the extent of tidal airway closure should have been similar in animals of the NEEP and ZEEP-DOSS group, and, in fact, the histological damage of small airways (Table 1) and the mechanical alterations occurring during ventilation at low lung volumes (Fig. 2) were also similar. On the other hand, substantial parenchymal and pulmonary vascular damage occurred in all animals of the ZEEP-DOSS group, probably because of higher surface tension, with

Table 2. Indexes of parenchymal and vascular injury after 2–2.5 h of mechanical ventilation with various ventilatory strategies

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<td>5.34±0.09*</td>
<td>7.6±0.7*</td>
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Ventilation with physiological tidal volume (8 ml/kg) on Control group, ZEEP group, NEEP group, or ZEEP-DOSS group, and ventilation with large tidal volume (26 ml/kg) on positive end-expiratory pressure only (Hi-Vt group). Lung wet-to-dry ratio (W/D) and percent ratio of BALF to serum albumin concentration (ABALF/ASER %). Injury score: 0 = absent, 1 = mild, 2 = moderate, 3 = marked. A rough evaluation of group average alveolar granulocytes is obtained as the sum of number of rats time maximal injury score. Significantly different from Control group: *P < 0.05.

Fig. 6. Relationships between R_{int} measured at the end of the experiment (PEEP2) and bronchiolar injury score in the various groups of rats (see key to symbols). Numbers are B (slope) coefficient ± SE of multiple linear regression with bronchiolar injury score, abnormal bronchiolar-alveolar attachments, and wet-to-dry ratio as independent variables.
marked interstitial or alveolar edema, while these alterations were present in mild degree only in two animals of the NEEP group (Table 2). In this connection, it should be noted that the more negative airway pressure (−7 to −10 cmH₂O) applied to a few rats not included in the study, rapidly caused marked, deadly lung edema. After restoration of PEEP and repeated recruitment maneuvers, airway collapse and tidal airway closure were eliminated in all groups, as shown by F_tidal/F_base values being similar to those on PEEP1 (Fig. 2), but because of lung edema, fewer units were being ventilated in the ZEEP-DOSS than in the NEEP group, as indicated by the end-expiratory volume on PEEP2 being reduced in the former group and normal in the latter group (Fig. 3). Development of lung edema in the ZEEP-DOSS group is further supported by the high values of the W/D ratio (Table 2) and reduced diffusing capacity, as shown by the significant fall in arterial oxygen pressure (Fig. 1). This should explain the significantly greater mechanical changes occurring on PEEP2 in the ZEEP-DOSS than in the NEEP and ZEEP group (Fig. 2), with the additional contribution from higher surface tension in the ZEEP-DOSS group.

Further reduction of the end-expiratory volume with negative airway pressure or augmentation of surfactant dysfunction at low volume with DOSS administration increased small airway epithelial damage relative to ventilation on ZEEP (Table 1), and, in contrast with the ZEEP group, both the NEEP and the ZEEP-DOSS group exhibited significantly higher concentration, relative to that of the Control and ZEEP group, of inflammatory cytokines in BALF and serum (Fig. 4). This suggests that greater damage and shedding of small airway epithelia and more extensive lesion of bronchiolar-alveolar attachments with enhanced tidal airway closure in the NEEP and ZEEP-DOSS group can eventually induce biotrauma. The discrepant inflammatory response to ventilation at ZEEP observed in the present in vivo and previous in vitro studies (5, 25) could be, therefore, related to greater susceptibility of the in vitro rat lung to noxious stimuli and/or presence of reparative processes in the in vivo lung. In this connection, it is of interest that ventilation with markedly negative airway pressure eventually induced a cytokine response in isolated mouse lung (3). Small airway injury does not seem, however, to represent the main contributor to the inflammatory reaction that occurred with ventilation at low lung volume in normal rat lungs in vivo. Indeed, the release of inflammatory cytokines was substantially greater in the ZEEP-DOSS than in the NEEP group, except for TNF-α levels, which did not differ significantly (Fig. 4), despite the fact that indexes of small airway injury were similar (Table 1). On the other hand, small airway injury was markedly more pronounced in the ZEEP-DOSS than the Hi-Vt group (Table 1), while the concentrations of pro-inflammatory cytokines were not significantly different (Fig. 4). Furthermore, studies performed on isolated, nonperfused lungs (5, 25) have missed indicating airway epithelium as the main source of inflammatory cytokines, although airway and alveolar epithelial cells were recognized effectors of inflammation (17, 23). It was, instead, hypothesized (25) that activated leukocytes were responsible for the observed cytokine release, on the basis of a significant number of leukocytes being retained even in lungs perfused with saline for several hours (22).

![Fig. 7. Relationships between lung wet-to-dry ratio and cytokine concentration in bronchoalveolar lavage fluid obtained in the various groups of rats (see key to symbols). Numbers are B (slope) coefficient ± SE of multiple linear regression with wet-to-dry ratio, bronchiolar injury score, and abnormal bronchiolar-alveolar attachments as independent variables.](http://jap.physiology.org/)
Despite bicarbonate administration, arterial pH decreased substantially throughout the period of mechanical ventilation (Fig. 1), reflecting the development of metabolic acidosis. Studies on cultured cells have provided conflicting results concerning the effects of pH on cytokine production (16). In the five groups of animals, however, pH did not differ significantly on PEEP1, nor did its decrease from PEEP1 to PEEP2. Hence, whatever the influence of pH might have been, it cannot be responsible for the differences in the release of inflammatory cytokines that occurred with the various ventilatory strategies (Fig. 4).

Cultured lung epithelial and endothelial cells subjected to mechanical stress eventually produce inflammatory mediators (17, 20, 27). In the present animals, release of inflammatory mediators occurred during ventilation at both low (ZEEP-DOSS and NEEP group) and high lung volumes (Hi-Vt group). Common to mechanical ventilation with large tidal volumes from physiological end-expiratory lung volume and mechanical ventilation with physiological tidal volumes from low end-expiratory lung volume is surfactant depletion and alteration of surface forces (1, 2, 28, 29), leading to alveolar instability, small airway collapse with dependent gas trapping or atelectasis, tidal airway closure, and regional overdistension that depend on the ventilatory mode, and are exaggerated in the presence of artificially induced surfactant dysfunction (ZEEP-DOSS group). Hence, the uneven, abnormally high stresses that develop at the alveolar and vascular level should cause increased epithelial and endothelial permeability (11, 13), microvascular stress failure (14) and eventually interstitial or alveolar edema. Abnormal shear stress on endothelial cells, capillary stress failure, and disruption of the alveolar-capillary membrane can lead to the exposure of adhesion molecules and/or release of chemotactic factors, causing recruitment and activation of granulocytes (4, 20, 27), which could in turn represent the main contributors to cytokine production (10). In fact, a rough parallelism occurred between levels of MIP-2, a chemotactic factor, in BALF of all groups (Fig. 4) and corresponding semi-quantitative evaluations of alveolar granulocytes (Table 2), although the absence of their phenotypic characterization might render the relevance of this relationship questionable. This sequence of events, which has been proposed to explain the mechanisms of ventilator-induced lung injury in acute respiratory distress syndrome (21) could have in fact occurred in the present open-chest rats, as indicated by the significant correlation between the levels of inflammatory cytokines in BALF of all animals and the corresponding lung W/D ratios, taken as an index of the mechanical deformations responsible for vascular damage and, hence, interstitial or alveolar edema (Fig. 7). In contrast, no correlation was present between levels of inflammatory cytokines in BALF and indexes of small airway injury. The link between stress-induced vascular injury and inflammatory response is further supported by the significant correlation between cytokine concentration in serum and BALF that was observed in the present animals (Fig. 5), because this type of connection implies, in fact, loss of compartmentalization.

In conclusion, the present study has shown that in normal rat lungs 1) the histologic damage of the small airways due to tidal airway closure during mechanical ventilation with physiological tidal volumes from the resting lung volume does not cause an inflammatory response characterized by release of inflammatory cytokines; 2) more extensive small airway alterations with enhanced tidal airway closure due to negative airway pressure or surfactant dysfunction eventually result in cytokine release; and 3) the main cause of the pulmonary and systemic inflammatory reaction with injurious modes of mechanical ventilation should be represented by stress-related damage of endothelial and alveolar epithelial cells leading to development of lung edema.

REFERENCES