Pulmonary and extrapulmonary acute lung injury: inflammatory and ultrastructural analyses

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Submitted 20 October 2004; accepted in final form 13 January 2005

Menezes, Sara L. S., Patricia T. Bozza, Hugo C. Castro Faria Neto, Andrea P. Laranjeira, Elnara M. Negri, Vera L. Capelozzi, Walter A. Zin, and Patricia R. M. Rocco. Pulmonary and extrapulmonary acute lung injury: inflammatory and ultrastructural analyses. J Appl Physiol 98: 1777–1783, 2005. First published January 13, 2005; doi:10.1152/japplphysiol.01182.2004.—To test whether pulmonary and extrapulmonary acute lung injury (ALI) of identical mechanical compromise would express diverse morphological patterns and immunological pathways. For this purpose, a model of pulmonary (p) and extrapulmonary (exp) ALI with similar functional changes was developed and pulmonary morphology (light and electron microscopy), cytokines levels, and neutrophilic infiltration in the bronchoalveolar lavage fluid (BALF), elastic and collagen fiber content in the alveolar septa, and neutrophil apoptosis in the lung parenchyma were analyzed. BALB/c mice were divided into four groups. In control groups, saline was intratracheally (it, 0.05 ml) instilled and intraperitoneally (ip, 0.5 ml) injected, respectively. In the ALIp and ALIexp groups, mice received E. coli lipopolysaccharide (10 μg it and 125 μg ip, respectively). The changes in lung resisitive and viscoelastic pressures and in static elastance, alveolar collapse, and cell content in lung tissue were similar in the ALIp and ALIexp groups. The ALIp group presented a threefold increase in KC (murine function homolog to IL-8) and IL-10 levels in the BALF in relation to ALIexp, whereas IL-6 level showed a twofold increase in ALIp. Neutrophils in the BALF were more frequent in ALIp than in ALIexp. ALIp showed more extensive injury of alveolar epithelium, intact capillary endothelium, and apoptotic neutrophils, whereas the ALIexp group presented interstitial edema and intact type I and II cells and endothelial layer. In conclusion, given the same pulmonary mechanical dysfunction independently of the etiology of ALI, insult in pulmonary epithelium yielded more pronounced inflammatory responses, which induce ultrastructural morphological changes.

pulmonary mechanics; cytokines; apoptosis

Although various causes of ALI result in similar pathologies in the late stage (2, 7, 22, 33), evidence indicates that the pathophysiology of early ALI may differ according to the type of the primary insult (22–24, 40, 42, 45). Data regarding respiratory mechanics and radiographic appearances suggest that there may be discernible differences in the pulmonary response to direct and indirect insults (8, 11, 13). In fact, differences in the initial insult, combined with underlying conditions, can result in the activation of different immune-inflammatory mechanisms. By understanding the range of pathways that leads to pulmonary dysfunction, it may be possible to assess several novel treatments in an attempt to modify lung injury.

The aim of the present study is to test whether pulmonary and extrapulmonary ALI of identical mechanical compromise would express diverse morphological patterns and immunological pathways. To that end, we developed BALB/c mice models of pulmonary and extrapulmonary ALI induced by Escherichia coli lipopolysaccharide (LPS) with similar functional changes. Considering the similar lung mechanical changes, we compared 1) the pulmonary morphology by using light and electron microscopy, 2) cytokine levels and neutrophilic infiltration in the bronchoalveolar lavage fluid (BALF), 3) elastic and collagen fiber content in the alveolar septa, and 4) neutrophil apoptosis in the lung parenchyma.

MATERIALS AND METHODS

Animal Preparation

A total of 56 BALB/c mice (20–25 g) were used. Twenty-eight animals were randomly assigned into four groups of seven animals each. In pulmonary and extrapulmonary control groups (Cp and Cexp, respectively), warm saline (0.9% NaCl) was intratracheally (it, 0.05 ml) instilled and intraperitoneally (ip, 0.5 ml) injected, respectively. In pulmonary and extrapulmonary ALI groups (ALIp and ALIexp, respectively), mice received LPS (E. coli, O55:B5, 10 μg in 0.05 ml it of saline/mouse and 125 μg ip in 0.05 ml of saline/mouse, respectively). These doses of E. coli LPS were able to yield a 1.5-fold increase in lung static elastance compared with control groups. For intratracheal instillation, mice were anesthetized with sevoflurane, a 1-cm-long midline cervical incision was made to expose the trachea, and LPS or saline was instilled with a bent 27-gauge tuberculin needle. The cervical incision was closed with 5.0 silk suture, and the mice were returned to their cage. The animals recovered rapidly after surgery.

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All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guiding Principles in the Care and Use of Animals” approved by the Council of the American Physiological Society and the Institutional Animal Care and Use Committee of Carlos Chagas Filhos Biophysics Institute approved all protocols in this study. Twenty-four hours later, the animals were sedated with diazepam (1 mg ip) and anesthetized [pentobarbital sodium (20 mg/kg ip)], and a snugly fitting cannula (0.8 mm ID) was introduced into the trachea. Mechanical ventilation (model 683, Harvard Apparatus, South Natick, MA) with a frequency of 100 breaths/min, a tidal volume (Vt) of 0.2 ml, and a positive end-expiratory pressure of 5.0 cmH2O was applied. The anterior chest wall was surgically removed.

A pneumotachograph (1.5 mm ID, length = 4.2 cm, distance between side ports = 2.1 cm) was connected to the tracheal cannula for the measurements of airflow (V) and changes in lung volume (Vt) (31). The pressure gradient across the pneumotachograph was determined by means of a Validyne MP45–2 differential pressure transducer (Engineering Corporation, Northridge, CA). The flow resistance of the equipment (Req), tracheal cannula included, was constant up to flow rates of 26 ml/s and amounted to 0.12 cmH2O·ml⁻¹·s⁻¹. Equipment resistive pressure (= Req/Vt) was subtracted from pulmonary resistive pressure so that the present results represent intrinsic values. Tracheal pressure was measured with a Validyne MP-45 differential pressure transducer (Engineering Corporation). All signals were conditioned and amplified in a Beckman type R Dynograph (Schiller Park, IL). Flow and pressure signals were also passed through eight-pole Bessel filters (902LPF, Frequency Devices, Haverhill, MA) with the corner frequency set at 100 Hz, sampled at 200 Hz with a 12-bit analog-to-digital converter (DT2801A, Data Translation, Marlboro, MA), and stored on a microcomputer. All data were collected by using LABDAT software (RHT-InfoData, Montreal, PQ, Canada).

Measurement of Respiratory System Mechanics

Muscle relaxation was achieved with gallamine triethyliodide (2 mg/kg iv), and a constant-flow ventilator provided artificial ventilation (Samay VR15, Universidad de la Republica, Montevideo, Uruguay). Special care was taken to keep Vt (0.2 ml) and flow (V = 1 ml/s) constant in all animals to avoid the effects of different flows and volumes and inspiratory duration on the measured variables. Pulmonary mechanics were measured by the end-inflation occlusion method (3, 4). In an open-chest preparation, tracheal pressure reflects transpulmonary pressure. Pulmonary resistive (ΔP1) and viscoelastic/inhomogeneous (ΔP2) pressures, ΔPtot (ΔP1 + ΔP2), and static elastance were determined. Pulmonary mechanics measurements were performed 10 times in each animal. All data were analyzed using ANADAT data analysis software (RHT-InfoData).

Histology and Morphometry

Light microscopy. Heparin (1,000 UI) was intravenously injected immediately after the determination of respiratory mechanics. The trachea was clamped 10 min later at end expiration, and the abdominal aorta and vena cava were sectioned, yielding a massive hemorrhage that quickly killed the animals. Then, lungs were removed en bloc at functional residual capacity. Two investigators who were unaware of the origin of the material performed the microscopic examination.

To perform morphometric analysis of lung parenchyma, the right lung was quick-frozen by immersion in liquid nitrogen and fixed with Carnoy’s solution (32) and embedded in paraffin. Slices 4 μm thick were obtained by means of a microtome and stained with hematoxylin and eosin. Morphometric analysis was performed with an integrating eyepiece with a coherent system made of a 100-point grid consisting of 50 lines of known length, coupled to a conventional light microscope (Axioplan, Zeiss, Oberkochen, Germany). Volume fraction of collapsed and normal pulmonary areas was determined by point-counting technique, made at a magnification of ×400 across 10 random noncoincident microscopic fields (43). Polymorpho- and mononuclear cells and pulmonary tissue were evaluated at ×1,000 magnification. Points falling on tissue area were counted and divided by the total number of points in each microscopic field. Thus data were reported as the fractional area of pulmonary tissue (43). The same method was applied to determine the amount of polymorpho- and mononuclear cells.

Collagenous [picrosirius-polarization method (30)] and elastic fibers [Weigert’s resorcin fuchsin method with oxidation (44)] were quantified in the alveolar septa. The alveolar septa quantification was carried out with the aid of a digital analysis system and specific software (Image-Pro Plus 4.1 for Windows; Media Cybernetics, Silver Spring, MD) under ×200 magnification. The images were generated by a microscope (Axioplan, Zeiss) connected to a camera (Sony Trinitron CCD, Sony, Tokyo, Japan), fed into a computer through a frame grabber (Oculus TCX, Coreco, St. Laurent, PQ, Canada) for offline processing. The thresholds for collagen and elastic fibers were established after enhancing the contrast up to a point at which the fibers were easily identified as either black (elastic) or birefringent (collagen) bands and kept constant during the measurements. The area occupied by fibers was determined by digital densitometric recognition. To avoid any bias due to sepal edema or alveolar collapse, the areas occupied by the elastic and collagen fibers, measured in each alveolar septum, were divided by the length of each studied septum. The results were expressed as the amount of elastic and collagen fibers per unit of septum length (μm²/μm).

Transmission electron microscopy. To obtain a stratified random sample, three slices of 2 × 2 mm were cut from three different segments of the right lung (upper, middle, and lower lobes) and then fixed with glutaraldehyde 2.5% and phosphate buffer 0.1 M (pH = 7.4) for 60 min at –4°C. The slices were then rinsed in phosphate buffer, postfixed with 1% osmic tetroxide in phosphate buffer for 30 min, and rewashed three times in phosphate buffer. Finally, the slices were dehydrated in an acetone series and then placed in a mixture of 1:1 acetone–Epon overnight before being embedded in Epon for 6 h. After fixation, the material was kept for 48 h at 60°C before being submitted to ultramicrotomy for transmission electron microscopy.

In Situ Detection and Quantification of Apoptotic Cells

For in situ detection of apoptosis at the level of a single cell, we used a method of end-labeling mediated by deoxyuridine triphosphate–transferase (TdT) (Boehringer Mannheim, Mannheim, Germany). This method involves the addition of deoxyuridine triphosphate (dUTP) labeled with fluorescein to the ends of the DNA fragments by the catalytic action of TdT. All the end-labeling experiments were performed several times so that the results for various tissue samples, including rat prostate, could be standardized. Thick paraffin sections (4–6 μm) were layered on glass slides. The tissue sections were deparaffinized with xylene and rehydrated with graded dilutions of ethanol in water. The slides were washed four times with double-distilled water for 2 min and immersed in TdT buffer (Boehringer Mannheim). Then TdT (0.5 U/ml) and fluorescein-labeled dUTP in TdT buffer were added to cover the section, and the samples were incubated in a humid atmosphere at 37°C for 60 min. For negative controls, TdT was eliminated from the reaction mixture. The sections were then incubated with antibody specific for fluorescein conjugated to peroxidase. The stains were visualized with a substrate system in which nuclei with DNA fragmentation stained brown. The reaction was terminated by washing the sections twice in phosphate-buffered saline. The nuclei without DNA fragmentation stained blue as a result of counterstaining with hematoxylin. Positive controls consisted of rat prostatic gland after castration. Three sections from each specimen were examined. A semiquantitative system was used to account for the degree of apoptosis. A five-point semiquantitative severity-based scoring system was used. The apoptotic findings were graded as...
RESULTS

There were no statistically significant differences in flow and volume among the groups (Table 1). $\Delta P_1$, $\Delta P_2$, $\Delta P_{tot}$, and static elastance were higher in ALIp and ALIexp than in Cp and Cexp, respectively (Table 1). The changes in lung mechanics were similar in ALIp and ALIexp groups.

Total and polymorphonuclear cell contents in lung tissue increased similarly from Cp and Cexp groups to ALIp and ALIexp, respectively. The fraction of alveolar collapse was higher in LPS groups than in control groups. There was no difference in alveolar collapse between ALIp and ALIexp groups (Table 2). Collagen fiber content was greater in ALIp and ALIexp than in Cp and Cexp groups. The amount of collagen fiber was already elevated 24 h after tissue damage independent of the etiology of lung injury. Elastic fiber content remained unchanged among the four groups (Table 2).

There was an increment in IL-6, KC, and IL-10 levels in the BALF 24 h after the E. coli LPS-induced ALI group presented a threefold increase in KC and IL-10 in relation to ALIexp group, whereas IL-6 was approximately doubled in the ALIp group (Table 3). In ALIp group, BALF analysis also demonstrated an intense neutrophilic infiltration in the alveolar space (Table 4).

Neutrophil apoptosis was more pronounced in the ALIp group (2.6 ± 0.4%) than in the ALIexp group (0.9 ± 0.1%). The detection of apoptotic cells by in situ end labeling of fragmented DNA was supported by the fact that pathological criteria for apoptosis were also met at electron and light microscopic view.

Intratracheal instillation of LPS (ALIp group) was followed by extensive injury of alveolar epithelium, swollen and fragmented type I and II cells (lamellar bodies in the alveolar space), intact capillary endothelium, hyaline membrane formation, and apoptotic neutrophils in electron microscopy (Fig. 1). In contrast, electron microscopy from ALIexp group (intraperitoneal injection of LPS) showed interstitial edema formation and preservation of lung epithelial and endothelial layers. Type III collagen fiber appeared early in the course of ALIp and ALIexp groups.

DISCUSSION

The present work disclosed that, given the same pulmonary mechanical changes, direct insult to the pulmonary parenchyma in ALI induced by intratracheal instillation of E. coli LPS yielded more pronounced inflammatory responses than indirect insult induced by intraperitoneal LPS administration. The direct insult resulted in ultrastructural morphological changes accompanied by an increase in cytokine levels and neutrophilic infiltration in the BALF, thus indicating a distinct pathogenic mechanism.

Table 1. Mechanical parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>Flow, ml/s</th>
<th>Volume, ml/s</th>
<th>$\Delta P_{tot}$, cmH$_2$O</th>
<th>$\Delta P_1$, cmH$_2$O</th>
<th>$\Delta P_2$, cmH$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cp</td>
<td>1.02 ± 0.01</td>
<td>0.19 ± 0.01</td>
<td>1.19 ± 0.01</td>
<td>0.14 ± 0.00</td>
<td>1.05 ± 0.01</td>
</tr>
<tr>
<td>ALIp</td>
<td>1.04 ± 0.01</td>
<td>0.20 ± 0.01</td>
<td>2.71 ± 0.09*</td>
<td>0.17 ± 0.00*</td>
<td>2.54 ± 0.07*</td>
</tr>
<tr>
<td>Cexp</td>
<td>1.01 ± 0.01</td>
<td>0.19 ± 0.01</td>
<td>1.88 ± 0.00</td>
<td>0.15 ± 0.00</td>
<td>1.03 ± 0.00</td>
</tr>
<tr>
<td>ALIexp</td>
<td>1.04 ± 0.01</td>
<td>0.19 ± 0.01</td>
<td>2.70 ± 0.11*</td>
<td>0.17 ± 0.00*</td>
<td>2.54 ± 0.13*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 7 animals each group. Flow and volume were not statistically different among groups. $\Delta P_{tot}$, $\Delta P_1$, and $\Delta P_2$ were significantly different from Cp and Cexp (Table 1).

Table 2. Morphometrical parameters and cellularity in lung parenchyma

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal Area, %</th>
<th>Alveolar Collapse, %</th>
<th>Total Cells</th>
<th>PMN, %</th>
<th>MN, %</th>
<th>Collagen Fiber, $\mu$m$^2$/mm$^2$</th>
<th>Elastic Fiber, $\mu$m$^2$/mm$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cp</td>
<td>88.5 ± 1.6</td>
<td>11.5 ± 1.6</td>
<td>13.1 ± 1.8</td>
<td>91.0</td>
<td>9.0</td>
<td>0.01 ± 0.001</td>
<td>0.33 ± 0.01</td>
</tr>
<tr>
<td>ALIp</td>
<td>26.3 ± 1.6*</td>
<td>73.7 ± 1.6*</td>
<td>29.0 ± 0.6*</td>
<td>22.0*</td>
<td>78.0*</td>
<td>0.04 ± 0.01*</td>
<td>0.36 ± 0.02</td>
</tr>
<tr>
<td>Cexp</td>
<td>91.8 ± 1.7</td>
<td>8.2 ± 1.7</td>
<td>8.5 ± 0.6</td>
<td>92.9</td>
<td>7.1</td>
<td>0.02 ± 0.001</td>
<td>0.35 ± 0.01</td>
</tr>
<tr>
<td>ALIexp</td>
<td>24.5 ± 2.5*</td>
<td>75.5 ± 2.5*</td>
<td>30.5 ± 1.3*</td>
<td>19.9*</td>
<td>80.1*</td>
<td>0.05 ± 0.01*</td>
<td>0.36 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE of 7 animals each group. Data were gathered from 10 random, noncoincident fields per mouse. In Cp and Cexp groups, saline was instilled (0.05 ml it) and injected (0.5 ml ip), respectively. In ALIp and ALIexp groups, mice received E. coli LPS (10 $\mu$g it and 125 $\mu$g ip, respectively). Total cell, total cellular fractional area; PMN, polymorphonuclear cell fractional area; MN, mononuclear cell fractional area. *Significantly different from Cp and Cexp groups (P < 0.05).
There is a general belief that ALI is the extreme form of a spectrum of lung injury caused by a uniform mechanism that is independent of the precipitating disease (1). This assumption mainly originates from pathology studies, which have consistently indicated that the lung response to injury is stereotyped, with transition from acute alveolar capillary damage to a late proliferative phase, quite independently of the initial cause. Unfortunately, most of the studies report late or terminal events, and pathological features of early phases of ARDS such as interstitial edema and alveolar collapse are not easily recognized. Another limitation in assessing the possible differences between ALI resulting from pulmonary disease and that resulting from extrapulmonary disease is the determination of the severity of the lung injury, yielding the comparison between the two conditions unwarranted. Furthermore, it is possible that direct and indirect insults coexist in the same subject, making it difficult to evaluate these two entities separately. In the experimental models of ALI used in this study, the animals breathed spontaneously. However, all previous studies dealing with pulmonary and extrapulmonary ALI investigated conditions in which mechanical ventilation was initiated (11, 17, 23). The institution of mechanical ventilation might induce and/or exacerbate lung injury, leading to misinterpretation of the data (21, 24, 26, 29). In this context, Kim and coworkers (21) observed that pulmonary ARDS would be more vulnerable to ventilator-induced lung injury, leading to more severe sequelae compared with extrapulmonary ARDS. In addition, in our models of ALIp and ALIexp, pulmonary mechanical changes were similar independently of the etiology of ALI. By these criteria, the severity of lung dysfunction did not differ between ALIp and ALIexp groups ($P < 0.05$); significantly different from ALIp.

### Table 3. Cytokine levels in the bronchoalveolar lavage fluid

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-6, ng/ml</th>
<th>KC, ng/ml</th>
<th>IL-10, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cp</td>
<td>0.29±0.06</td>
<td>0.53±0.08</td>
<td>0.64±0.08</td>
</tr>
<tr>
<td>ALIp</td>
<td>2.95±0.05*</td>
<td>4.57±0.32*</td>
<td>1.39±0.16*</td>
</tr>
<tr>
<td>Cexp</td>
<td>0.21±0.26</td>
<td>0.17±0.05</td>
<td>0.15±0.06</td>
</tr>
<tr>
<td>ALIexp</td>
<td>1.33±0.14+</td>
<td>1.38±0.29+</td>
<td>0.50±0.14+</td>
</tr>
</tbody>
</table>

Values are means ± SE of 7 animals in each group. In Cp and Cexp groups, saline was instilled (0.05 ml it) and injected (0.5 ml ip), respectively. In ALIp and ALIexp groups, mice received *Escherichia coli* LPS (10 μg it and 125 μg ip, respectively). IL-6, interleukin 6; KC, interleukin 8 function homolog; and IL-10, interleukin 10 levels in the bronchoalveolar lavage fluid. *Significantly different from Cp and Cexp groups ($P < 0.05$); †significantly different from ALIp.

### Table 4. Cellularity in the bronchoalveolar lavage fluid

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Cells $\times 10^6$</th>
<th>PMN, %</th>
<th>MN, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cp</td>
<td>2.3±0.4</td>
<td>9.5</td>
<td>89.7</td>
</tr>
<tr>
<td>ALIp</td>
<td>8.4±1.6*</td>
<td>74.8*</td>
<td>25.2*</td>
</tr>
<tr>
<td>Cexp</td>
<td>2.2±0.7</td>
<td>1.8</td>
<td>97.9</td>
</tr>
<tr>
<td>ALIexp</td>
<td>2.1±0.4</td>
<td>1.2</td>
<td>98.8</td>
</tr>
</tbody>
</table>

Values are means ± SE of 7 animals in each group. In Cp and Cexp groups, saline was instilled (0.05 ml it) and injected (0.5 ml ip), respectively. In ALIp and ALIexp groups, mice received *Escherichia coli* LPS (10 μg it and 125 μg ip, respectively). Total cellularity, percentage of PMN and MN cells in the bronchoalveolar lavage fluid. *Significantly different from Cp and Cexp groups ($P < 0.05$).
the presence of altered alveolar capillary barrier. Although we did not measure intra-abdominal pressure in our animals, we believe that there is no increase because the time lag between the induction of lung injury by intraperitoneal injection of E. coli LPS and the measurement of lung mechanics was only 24 h and we observed neither intra-abdominal distension nor ascites at the moment of functional analysis. Thus the comparison of our model with the mechanical studies in humans with extrapulmonary ALI is unwarranted. Furthermore, although the authors usually described indirect insult related to abdominal causes, in other situations such as cardiac surgery, trauma, or meningitis the precise identification of the pathogenetic pathway is somewhat questionable. Our experimental models showed that the damage of direct insult is primarily focused on the alveolar epithelium, whereas in indirect injury it lies on the vascular endothelium.

Light microscopy analyses showed similar increment in alveolar collapse and tissuecellularity 24 h after the induction of the lesion (Table 2). However, in analyzing the electron microscopy in ALIp, an extensive injury of alveolar epithelium, swollen and fragmented type I and II cells (lamellar bodies in the alveolar space), intact capillary endothelium, ductal hyperdistension, neutrophil recruitment into the alveolar space, proliferation of fibroblasts into the alveolar septa, and the presence of collagen fiber type III and hyaline membranes were observed. ALIexp group presented interstitial edema and the presence of collagen type III and intact types I and II cells. On the contrary, Hoelz and colleagues (17) described the morphological differences between pulmonary lesions in ARDS originating from pulmonary and extrapulmonary ARDS patients and observed a predominance of alveolar collapse, fibrinous exudates, and alveolar wall edema in pulmonary ARDS. The morphological difference between pulmonary and extrapulmonary ARDS was mainly quantitative in extent and distributed according to the underlying disease. However, the study by Hoelz and colleagues used biopsy tissues of ARDS patients and presented important limitations: 1) the large heterogeneity of pulmonary parenchyma alterations, 2) the difficulty in obtaining lung tissue in patients in acute phase, 3) the underlying diseases taking different courses until death, 4) the lesion associated with ventilator-induced lung injury and pneumonia even though the initial event yields to an extrapulmonary ARDS. In this context, our murine model of pulmonary and extrapulmonary ALI bypasses these drawbacks because we can control the time of analysis (earlier or later in the course of lung injury) and the animals breathed spontaneously.

Collagen fiber content increased similarly 24 h after tissue damage independent of the etiology of lung injury (Table 2), indicating that the biochemical processes implicated in collagen synthesis are indeed able to react very quickly to the aggression (37, 38). In addition, type III collagen fiber was identified with electron microscopy. It has been described that type III collagen fiber, which is more flexible and susceptible to breakdown, appears early in the course of lung injury (36). The present study showed that elastic fiber content did not increase early in the course of lung injury both in ALIp and ALIexp. The absence of elastosis in this model of ALI could be attributed to the intensity of lung injury (37, 39). Rocco and colleagues (38) observed that with a high dose of paraquat (25 mg/kg) elastogenesis appears early in the course of lung injury, and with 10 mg/kg there is a late increment of elastic fiber content. Therefore, there is a degree of lung injury responsible for the beginning of elastogenesis independently of the time course of ALI. Additionally, the mechanism triggering fibrogenesis seems to be different from that of elastogenesis (39).

The levels of inflammatory cytokines (IL-6, KC, and IL-10) in the bronchoalveolar lavage fluid were quantified and correlated with lung function and histology (light microscopy). Although functional and morphological pulmonary changes were similar independently of the etiology of ALI, direct insult yielded more pronounced inflammatory responses, e.g., the ALIp group presented a threefold increase in KC and IL-10 levels in the BALF in relation to ALIexp, whereas IL-6 was apparently doubled in the ALIp group. Thus it is important to differentiate between direct and indirect pathophysiological pathways, because the underlying mechanisms seem to be different in the two conditions, at least during the early phase, and this may influence the approach to treatment.

Activated neutrophils play a major role in mediating the microvascular and lung tissue damage in ARDS. IL-8 is described as the main chemotactic factor for neutrophils in the blood and BALF of ARDS patients (9, 20). Moreover, accumulating evidence has implicated high levels of IL-8 in the BALF as a reliable biomarker of poor outcome prediction in ARDS (5, 19). Levels of KC (the murine function homolog to IL-8) positively correlated with neutrophil recovered from the BALF in our experimental settings. Indeed, significantly higher levels of neutrophil influx and KC were observed in the BALF in the course of pulmonary ALI inflammation compared with extrapulmonary ALI. In addition to neutrophils recovered from the BALF, in LPS-induced ALI led to a significant increase in the number of neutrophils infiltrating the lung parenchyma compared with control animals. Interestingly, no differences were observed in the number of infiltrating neutrophils when the lung parenchyma from pulmonary and extrapulmonary ALI were compared, thus suggesting that activated neutrophils in the lung are better correlated with the pulmonary mechanical dysfunction. The signals and mechanisms that determine normal cell turnover and influence apoptosis and necrosis in lung injury need to be further elucidated. Recent evidence has indicated an important role for neutrophil apoptosis in the pathogenesis and/or resolution of ALI (28). Studies in humans and animals suggest that neutrophil life span is modulated early in ARDS through the action of cytokines; IL-8 was demonstrated to inhibit neutrophil apoptosis (1, 12, 27). Engulfment of apoptotic cells by phagocytes is thought not only to remove the dying cell from the tissues but also to provide protection from local damage resulting from release or discharge of proinflammatory substances (16). Moreover, ingestion of apoptotic cells actively suppresses the production of proinflammatory cytokines in a mechanism dependent on transforming growth factor-β, and PGE2 production (10, 18), resulting in accelerated resolution of inflammation, and has a protective role in the mortality and lung injury in models of ALI (18, 41). In our study, we observed that the amount of apoptotic neutrophils was higher in pulmonary than in extrapulmonary ALI, although the animals had the same degree of pulmonary mechanical dysfunction. Further studies would be necessary to verify whether differences could be observed in later time points in terms of pulmonary mechanical dysfunction or in inflammatory resolution when pulmonary and extrapulmonary ALIs are compared.
In conclusion, given the same pulmonary mechanical dysfunction independently of the etiology of ALI, direct insult yielded more pronounced inflammatory responses, which induced ultrastructural morphological changes. These models allow for the understanding of the mechanisms regulating the inflammatory and fibroproliferative responses. Although an exaggerated inflammatory response underlies the pathogenesis of pulmonary ALI at the early phase, the amount of collagen was similar in pulmonary and extrapulmonary ALI, suggesting that inflammation and fibrosis could be dissociated.

The distinction between pulmonary and extrapulmonary ALI is not always clear and simple, and the observation of some overlapping in pathogenic mechanisms and morphological alterations may be frequent. Thus we need to strongly reconsider ARDS as a consistent response to its diverse etiology. It is our proposal that despite similar functional changes, ALI is not always clear and simple, and the observation of certain pathogenic mechanisms in pulmonary and extrapulmonary ALI would lead to a more precise and efficient clinical management. Mice and humans clearly share many basic physiological processes; nonetheless, each animal model should be viewed as one component of the process for studying human disease and not viewed in isolation nor extrapolated directly to the clinical setting.

ACKNOWLEDGMENTS

We express our gratitude to Antônio Carlos de Souza Quaresma and Alaine Prudente for skillful technical assistance.

GRANTS

This research was supported by The Centers of Excellence Program (PRONEX-MCT and MCT/FAPERJ), The Brazilian Council for Scientific and Technological Development (CNPq), São Paulo State Research Supporting Foundation (FAPESP), Rio de Janeiro State Research Supporting Foundation (FAPERJ), and PTB (International Scholar from Howard Hughes Medical Institute).

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