Determinants of surfactant function in acute lung injury and early recovery

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Mora, R., S. Arold, Y. Marzan, B. Suki, and E. P. Ingenito. Determinants of surfactant function in acute lung injury and early recovery. Am J Physiol Lung Cell Mol Physiol 279: L342–L349, 2000.—Relationships between lung function and surfactant function and composition were examined during the evolution of acute lung injury in guinea pigs. Lung mechanics and gas exchange were assessed 12, 24, or 48 h after exposure to nebulized lipopolysaccharide (LPS). Bronchoalveolar lavage (BAL) fluid was processed for phospholipid and protein contents and surfactant protein (SP) A and SP-B levels; surfactant function was measured by pulsating bubble surfactometry. Lung elastance, tissue resistance, and arterial-alveolar gradient were moderately elevated by 12 h after LPS exposure and continued to increase over the first 24 h but began to recover between 24 and 48 h. Similarly, the absolute amount of 30,000 g pelleted SP-A and SP-B, the phospholipid content of BAL fluid, and surfactant function declined over the first 24 h after exposure, with recovery between 24 and 48 h. BAL fluid total protein content increased steadily over the first 48 h after LPS nebulization. In this model of acute lung injury, the intra-alveolar repletion of surfactant components in early recovery led to improved surfactant function despite the presence of potentially inhibitory plasma proteins.

acute respiratory distress syndrome; lipopolysaccharide; apoprotein; pulsating bubble surfactometry; optimal ventilator waveform

SURFACTANT, a complex mixture of lipids and proteins, reduces surface tension at the air-liquid interface in the alveoli and small airways. In the absence of surfactant, surface tension forces exceed the counterbalancing tethering forces generated by adjacent lung tissue, leading to airway and alveolar instability and collapse. Prior studies (7, 16, 21) have demonstrated that the pathophysiology of acute respiratory distress syndrome (ARDS) is largely determined by abnormal surfactant function. Surfactant extracts from patients with ARDS demonstrate loss of surface activity, including an inability to achieve low (<5 dyn/cm) surface tensions during film compression, and loss of elastic behavior during surface area cycling (9, 16). Comparison of air- and fluid-filled pressure volume profiles in isolated lungs of animals with experimental ARDS indicates that increases in static elastic recoil after acute inflammation are due to abnormalities in surfactant function and not to changes in tissue elastic recoil (13). An in vivo animal study (1) has demonstrated surfactant dysfunction after capillary leakage due to chemical injury. To date, the biochemical and biophysical processes that relate inflammation to alterations in surfactant function are not well understood.

Several hypotheses have been proposed to account for the functional changes that occur in ARDS surfactant (9, 16, 23). Studies demonstrating decreased phospholipid and apoprotein contents in lung lavage samples from both patients with ARDS (9) and animal models (17) have suggested loss of surfactant through decreased production or accelerated destruction. Increased levels of oxidized lipids and serum proteins have been observed in lung lavage samples from animals with experimental ARDS (14) and from humans (22). Functional alterations in the affected surfactant included a decreased stability index, increases in minimum surface tension and compressibility, and delayed adsorption onto the air-water interface. These observations have led to efforts to restore normal surfactant function in ARDS by the addition of supplemental surfactant. However, the limited efficacy of surfactant replacement in restoring normal surfactant function (10) or improving clinical outcomes (2) among patients with ARDS remains puzzling.

This laboratory (15) has previously shown that endotoxin exposure with a combination of intravenous infusion and nebulization of lipopolysaccharide (LPS) in guinea pigs causes a reproducible pattern of acute lung injury (ALI) with many of the characteristics of ARDS. This was associated with surfactant dysfunction and a reduction in surfactant protein (SP) A levels in the surfactant of the injured animals (15). The studies presented here are aimed at a more extensive characterization of the relationship between alterations in lung mechanics, impairment of gas exchange, and alterations in lung surfactant in ALI produced by LPS exposure in guinea pigs. The results presented

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shed some light on the changes that occur in lung surfactant during early ALI and the early recovery phase.

MATERIALS AND METHODS

Animal model. Male Hartley guinea pigs (weight 500–600 g; Charles River Laboratories, Needham, MA) were studied under an institutionally approved animal protocol. All were housed in a virus-free facility, were cared for in a similar fashion, and were studied within 2 wk of arrival from the distributor. The guinea pigs were randomized to either endotoxin [lipopolysaccharide (LPS); n = 18 total] or no treatment (control; n = 6). For LPS nebulization, 2.5 mg of Pseudomonas aeruginosa 10 LPS (Sigma, St. Louis, MO) were dissolved in 5 ml of normal saline and delivered via a nebulizer. The animals were then studied 12, 24, or 48 h after exposure. The LPS was suspended in saline by vortexing and then sonicated for 25 3-s cycles with a tip sonicator (Heat Systems-Ultrasonics, Farmingdale, NY) at maximal microprobe tip power. The solution was then administered at a continuous flow of 6 l/min via a DeVilbiss acrom nebulizer in a sealed chamber over a 15-min period. The nebulizer was thoroughly cleaned between administrations. Control animals, also referred to as time 0, were not subjected to nebulization.

The animals were anesthetized with intraperitoneal xylazine (5 mg/kg) and pentobarbital sodium (40 mg/kg), were tracheally cannulated, had a carotid arterial line placed for blood pressure and arterial blood gas measurements, and underwent a partial midsternal thoracotomy to allow for exposure of the pleural surfaces. The animals were mechanically ventilated at 60 breaths/min with a tidal volume of 5.1 ml/kg, a positive end-expiratory pressure of 3 cmH2O, and an inspired fraction of O2 of 21% with a Flexivent rodent ventilator (SCI-REQ, Montreal, PQ).

Lung mechanics. Lung mechanics were assessed by the optimal ventilator waveform (OVW) technique as described by Lutchen et al. (19). The OVW is a forced oscillatory volume signal consisting of six sine waves with frequencies between 0.5 and 14.25 Hz. These were selected according to a nonsum-nondifference criterion (26) that minimizes the nonlinear interactions between frequencies of the pressure output. The phase angles and amplitudes of the volume input are optimized so that the volume signal has a tidallike shape, which allows the measurement of lung impedance over a range of frequencies. The peak-to-peak volume was scaled to the same value as the tidal volume so that disruption to mechanical ventilation was minimal.

In these experiments, the airway opening pressure was low-pass filtered, and the flow was calculated from piston displacement. The Flexivent system delivered three complete OVW cycles (12 s) and discarded the first cycle to remove any transients in the calculation. Fast Fourier transforms of pressure and flow were calculated on overlapping segments of data. Lung input impedance (ZL) was determined at each of the six selected frequencies as the ratio of the cross-power spectrum of pressure and flow (Gpp) and the autopower spectrum of flow (GPP): ZL = Gpp/GPP. The calculated impedance was then fit to a mechanical impedance model to partition ZL into airway resistance (Rao), airway inerterance, tissue damping (G), and tissue elastance (H) according to Hantos et al. (11).

Lavage fluid collection and processing. After characterization of in vivo lung physiology, the animals received a lethal dose of intracardiac pentobarbital sodium (100 mg/kg) and underwent two whole lung lavages with 10 ml each (total of 20 ml) of warmed saline. The lavage return volume was measured, and the lavage fluid was centrifuged at low speed (450 g for 10 min at 4°C) to remove cells. The supernatant was stored under nitrogen at −20°C for subsequent surfactant isolation. The cell pellet was resuspended in 5 ml of red blood cell lysis buffer and brought up to the initial lavage return volume for total cell count by hemocytometry and determination of a differential count with rapid Wright’s stain.

Surfactant isolation and surface tension studies. Surfactant was pelleted by high-speed centrifugation (30,000 g for 45 min at 4°C). The crude pellet was resuspended in 0.5 ml of 0.15 M normal saline, and total phospholipid content was measured (25). The surfactant was dissolved in 5 mM CaCl2 in 0.15 M NaCl to a final concentration of 1 mg/ml, and interfacial properties were measured at 37°C with a pulsating bubble surfactometer (Electronetics, Amherst, NY). For equilibrium surface tension (γequil) measurements, a bubble was formed and observed without oscillations, and surface tension measurements were recorded at 30 s, 5 min, and 15 min. The 5- and 15-min values were in close agreement, and the 15-min value is reported as the γequil. Dynamic measurements were made at 20 cycles/min and a relative surface area change of 62% (standard settings) until stable readings (unchanging over 5 min) were achieved. Surface tension and hysteresis results represent those measured 15 min after pulsations were initiated (all samples had achieved steady state at this time). Static and dynamic calibrations were performed before and after each run with distilled H2O.

For samples that underwent sucrose gradient centrifugation, the remaining portion of surfactant was brought to a total volume of 2 ml in saline and layered over 5 ml of 0.8 M sucrose. Contaminating proteins were pelleted by centrifugation (30,000 g for 30 min at 4°C), and purified surfactant was isolated as the pellicle at the saline-sucrose interface. The purified surfactant was washed, and interfacial properties were analyzed as before at 1 mg/ml with pulsating bubble surfactometry.

Phospholipid assay. The method of Stewart (25) was used. Fifty microliters of sample were added to glass tubes containing 2 ml of spectroscopic-grade chloroform. Two milliliters of 3.04% (wt/vol) ammonium ferrocyanate and 2.7% (wt/vol) ferric chloride hexahydrate in distilled H2O were added, and the mixture was vortexed for 1 min. Standards (0–100 µg/ml) were prepared with phosphatidylcholine in chloroform. The lower chloroform phase was withdrawn, and absorption was measured at 488 nm with a quartz cuvette.

Measurement of thio-barbituric acid-reactive substances. Lipid peroxidation products were measured following the procedure of Gillard et al. (8). Briefly, samples containing 1 mg/ml of phospholipid were added to a solution of 15% TCA, 0.375% thiobarbituric acid, 0.25 N HCl, and 0.01% butylated hydroxytoluene, heated to 95°C, and centrifuged, and the absorbance in the supernatant was measured at 535 nm. Malonaldehyde was used for standard calibration.

Total protein assay. The bicinchoninic acid spectrophotometric assay (Pierce, Rockford, IL) was used for protein determinations according to manufacturer’s specifications. Protein concentration is proportional to absorbance at 562 nm, with bovine serum albumin as a standard. Detection of protein content in surfactant pellets (at 1 mg/ml phospholipid concentration) was determined by solubilizing samples in 0.5% SDS and preparing standards in a solution of 1 mg/ml aqueous phosphatidylcholine to account for bicinchoninic acid binding by lipid.

Anti-approtein antibody development. SP-A was purified from calf lung surfactant with a modification of the method of
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McCormack et al. (20). Whole calf lung surfactant (10 mg in 1 ml) was injected into 45 ml of butanol on ice to precipitate the aqueous proteins. After 15 min, the samples were centrifuged at 10,000 \( g \) for 30 min, and the pellet containing SP-A and serum proteins was dried under nitrogen. The dried pellet was resuspended in 20 mM octyl glucopyranoside by repeated injection through a 25-gauge needle. The sample was ultracentrifuged for 60 min at 100,000 \( g \) at 4°C, and the pellet containing SP-A was resuspended in 5 mM Tris-HCl. Residual detergent was removed by microdialysis against a 5 mM Tris-HCl bath followed by passage over a Bio-Bead SM-2 (Bio-Rad) low-pressure chromatography column. Sample purity was verified by gel electrophoresis. Rabbit antisemur against SP-A was developed for our laboratory commercially (Tana Laboratories, Houston, TX).

SP-B was purified with a modification of the method of Beers et al. (4). SP-B purification from calf lung surfactant by the published protocol did yield a highly pure preparation of SP-B, which demonstrated a single band with a relative molecular weight of 6,000 with SDS-PAGE and Coomassie staining. However, when the preparation was tested for the presence of SP-A with Western blotting with anti-SP-A antibody, trace amounts of SP-A were found in several preparations. Because of concern about possible cross-reactivity of an antibody raised by this preparation, the SP-B purified by selective extraction was subjected to preparative electrophoresis with recovery by electroelution. The preparation yielded a single band with Coomassie staining and no detectable SP-A with anti-SP-A Western blotting. Rabbit antisemur against SP-B was developed for our laboratory commercially (Charles River Laboratories).

Western blotting. Ten to fifty micrograms of purified whole surfactant from each sample (equal volumes from all pellets for comparative purposes) were applied to 12 or 15% SDS-polyacrylamide gels. Proteins were separated under reducing conditions, blotted onto polyvinylidene difluoride membrane, and analyzed with Western blot with primary antibodies raised to purified calf lung surfactant SP-A and SP-B. Cross-reactivity of these antibodies between the calf surfactant and guinea pig surfactant apoproteins was demonstrated during antibody development. Blots were developed with a streptavidin-biotin-peroxidase amplification system (Sigma) and subjected to quantitative densitometry with the Gel-Pro analyzer software package (Media Cybernetics, Silver Spring, MD).

Statistics. All data are presented as means ± SD for each treatment group unless otherwise specified. Data were analyzed by one-way analysis of variance and Levene’s test for homogeneity of variance. Comparisons between individual treatment groups were carried out by the Bonferroni method when the variances were homogeneous and by Dunnett’s T3 method when the variances were not homogeneous. Differences between groups were considered significant if \( P < 0.05 \).

RESULTS

Pathophysiological profiles of LPS-exposed animals. Guinea pigs exposed to LPS and studied at various time points after exposure demonstrated no obvious behavioral changes between the time of exposure and surgery. The animals moved freely about, with no clear alteration in their intake of liquid or food. Initial mean arterial blood pressures as assessed with the carotid arterial line were in the range of 50–60 mmHg, with no difference between the untreated and various LPS-treated groups.

Lung mechanics, however, were markedly altered by nebulized LPS exposure in these animals. Figure 1 plots the dynamic elastance and resistance components of total lung impedance versus input frequency for control animals and for animals 12, 24, and 48 h after LPS exposure. Tissue G, tissue H, and \( R_{aw} \) were calculated for each group as described in MATERIALS AND METHODS, and the results are summarized in Table 1. Tissue G and tissue H increased 10- and 6-fold, respectively, over the first 24 h after LPS exposure. Between 24 and 48 h, there was evidence of early recovery beginning to take place, with 10- and 4-fold falls from peak values in tissue G and tissue H, respectively. \( R_{aw} \) was increased 12 and 48 h after exposure yet appears to be unchanged from baseline in the 24-h treatment group.

Table 2 lists the gas-exchange parameters measured at various time points after LPS exposure shortly after the guinea pigs were placed on mechanical ventilation with room air. The gas-exchange values point to a progressive deterioration of oxygenation over the first 24 h after LPS exposure, with evidence of early recovery between 24 and 48 h.

Indexes of inflammation in LPS exposure. As shown in Table 3, administration of nebulized LPS produces a marked neutrophilic inflammatory response in the alveolar space. Neutrophil counts in the bronchoalveolar lavage (BAL) fluid peaked 12 h after exposure followed by a gradual decrease between 12 and 48 h. The numbers of other cells remained relatively stable over this time. As previously reported (15), lung histopathology revealed an interstitial and alveolar neutrophilic influx accompanied by exudative-appearing material and mild hemorrhage in the alveolar space.

BAL fluid biochemistry after LPS exposure. Figure 2 displays the phospholipid and protein concentrations in the surfactant pellets obtained from the BAL fluid by ultracentrifugation as a function of time after LPS exposure. In comparison with control samples, the phospholipid content of the surfactant pellets was diminished by ~50% by 12 h after LPS exposure and by ~75% at 24 h, the time point that corresponds to the most profound gas-exchange and ventilatory impairment. There is a modest but significant increase in pellet phospholipid between 24 and 48 h after LPS exposure, corresponding to the early recovery phase as defined by ventilatory mechanics and oxygenation.

In contrast to the phospholipid concentration, the pellet total protein concentration shows a relentless increase over the first 48 h after LPS administration and is increased ~12-fold 48 h after exposure compared with that in control samples. Previously published SDS-PAGE of protein recovered from the BAL fluid of LPS-exposed guinea pigs revealed a profile consistent with serum protein extravasation (15). The pattern observed here indicates that this process of extravasation proceeds over the first 48 h after exposure, even over the latter 24 h when the animals appear to be recovering on the basis of other parameters.
Surfactant function in LPS-exposed animals. A previous tissue mechanics study (15) of subpleural parenchymal strips harvested from guinea pigs similarly exposed to LPS revealed no significant difference in isolated tissue properties between control and LPS-exposed animals (15), pointing to an interfacial- or surfactant-mediated mechanism for physiological dysfunction. Table 4 lists means ± SD of both static and dynamic parameters for the various treatment groups.

As shown in Table 4, the $\gamma_{\text{equil}}$ profiles among the groups were quite similar, with $\gamma_{\text{equil}}$ in the range of 22–23 dyn/cm. On the other hand, the dynamic interfacial profiles were markedly affected by LPS exposure. Over the first 24 h after exposure, the minimum surface tension ($\gamma_{\text{min}}$) increases ~20-fold, from a value of <1 dyn/cm to an average of 20 dyn/cm, and the maximum surface tension ($\gamma_{\text{max}}$) increases 2-fold, from ~30 to 59 dyn/cm. The surfactometry curves themselves revealed a substantial distortion of the surface tension-surface area curve, consistent with loss of appropriate hysteretic behavior. Between 24 and 48 h after exposure, there was a moderate improvement in dynamic surfactant function, with $\gamma_{\text{min}}$ decreasing from 20 to 14 dyn/cm and $\gamma_{\text{max}}$ decreasing from 59 to 41 dyn/cm.

![Fig. 1. Total lung resistance (Res; A) and elastance (B) for control animals (●) and for animals at 12 (○), 24 (▲), and 48 (●) h after lipopolysaccharide (LPS) exposure. Inset: results for the control and 12- and 48-h groups in magnified form. Freq, frequency.](image)

<table>
<thead>
<tr>
<th>Table 1. Lung mechanics after LPS exposure</th>
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<tr>
<td>Control</td>
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<tr>
<td>Post-LPS</td>
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<tr>
<td>12 h</td>
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<tr>
<td>24 h</td>
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<tr>
<td>48 h</td>
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</table>

Values are means ± SD. LPS, lipopolysaccharide. *Significant difference from control and 12- and 48-h damping and elastance, $P < 0.005$. †Difference between 24- and 48-h resistances approached significance, $P = 0.066$. 
Selected surfactant samples from animals 24 h post-LPS exposure were subjected to sucrose gradient centrifugation to remove the bulk of contaminating plasma proteins. Sucrose gradient purification resulted in an improved dynamic profile, with a reduction in $\gamma_{\text{min}}$ from 20 dyn/cm to the 5–10 dyn/cm range and a reduction in $\gamma_{\text{max}}$ from 59 dyn/cm to the 35–40 dyn/cm range. However, it was evident from simple observations that the dynamic profiles of these purified samples did not match those of the normal samples from control animals. These results suggested that although serum protein contamination is likely playing a role in altering surfactant function in this model, it cannot account for all of the dysfunction seen. Also consistent with this observation is the finding that surfactant samples from animals 48 h post-LPS exposure exhibit better function than those at 24 h postexposure despite the presence of higher total protein levels in the pellet (Fig. 2). These considerations led to a search for other possible alterations of lung surfactant in this model, including changes in surfactant apoproteins or constituent phospholipids.

Alterations in surfactant apoproteins. The levels of SP-A and SP-B in the surfactant pellets of animals in the various treatment groups were assessed by Western blotting and quantitative densitometry as described in MATERIALS AND METHODS. Figure 3 shows changes in the relative level of SP-A and SP-B as a function of time after LPS exposure. Adjusted for the phospholipid level, SP-A levels in the surfactant pellets remained relatively constant over the first 24 h after LPS administration, whereas SP-B levels decreased by 55% over the first 24 h and increased to 220% of baseline values in the recovery period between 24 and 48 h. As shown in Fig. 3B, there was a substantial diminution in the levels of both SP-A and SP-B in the surfactant pellet over the first 24 h after LPS exposure. By 24 h after exposure, the levels of SP-A and SP-B had decreased by 75 and 85%, respectively, relative to the control values.

Western blots of SP-B for control surfactant and at various time points after LPS exposure revealed not only the decreases in SP-B levels described above but also the emergence of a larger molecular weight form of SP-B resistant to the reducing conditions used for the electrophoresis in LPS-exposed animals. For purposes of the densitometry measurements reported in Fig. 3, both molecular weight forms of SP-B were counted when they appeared.

Alteration in surfactant phospholipid profile. To measure the extent of lipid peroxidation occurring in the surfactant samples as a consequence of LPS exposure, thiobarbituric acid assays were performed on all surfactant samples. The results are shown in Table 5. There was no increase in detectable aldehyde products of lipid peroxidation in samples from LPS-exposed animals as opposed to those from control animals. The positive control value shown in Table 5 represents the basal level of lipid peroxidation observed in control animals.

Table 2. Gas exchange after LPS exposure

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>pH Control</th>
<th>P_{O_2} mmHg Control</th>
<th>P_{CO_2} mmHg Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.44 ± 0.05</td>
<td>82.1 ± 5.5</td>
<td>46.2 ± 1.6</td>
</tr>
<tr>
<td>12 h</td>
<td>7.47 ± 0.18*</td>
<td>55.3 ± 29.7†</td>
<td>19.9 ± 12.7†</td>
</tr>
<tr>
<td>24 h</td>
<td>7.29 ± 0.11*</td>
<td>40.3 ± 15.6</td>
<td>72.2 ± 8.1‡</td>
</tr>
<tr>
<td>48 h</td>
<td>7.43 ± 0.06</td>
<td>44.8 ± 10.6</td>
<td>51.1 ± 13.0</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Significant difference between 12- and 24-h groups, $P = 0.042$. †Significant difference between 12- and 24-h P_{O_2}, $P = 0.013$. Control P_{O_2} significantly different from 24- and 48-h P_{O_2}, $P < 0.001$ and 0.011, respectively. Difference between control and 48-h P_{O_2} approached significance, $P = 0.058$. Difference between 12- and 24-h P_{O_2} approached significance, $P = 0.078$. ‡Control arterial-alveolar gradient significantly different from 12- and 24-h gradient, $P = 0.054$ and < 0.001, respectively.

Table 3. BAL fluid cell counts after LPS exposure

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Macrophages</th>
<th>Eosinophils</th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>430 ± 14</td>
<td>34 ± 12</td>
<td>13 ± 6</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>12 h</td>
<td>419 ± 141</td>
<td>202 ± 89</td>
<td>122 ± 37</td>
<td>2,747 ± 32</td>
</tr>
<tr>
<td>24 h</td>
<td>173 ± 56</td>
<td>201 ± 140</td>
<td>71 ± 26</td>
<td>1,713 ± 50</td>
</tr>
<tr>
<td>48 h</td>
<td>275 ± 55</td>
<td>207 ± 94</td>
<td>60 ± 18</td>
<td>1,433 ± 63</td>
</tr>
</tbody>
</table>

Values are means ± SD in no. of cells × 10^3/ml.

Table 4. Surfactometry characteristics of various treatment groups

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>$\gamma_{\text{min}}$ Control</th>
<th>$\gamma_{\text{max}}$ Control</th>
<th>$\gamma_{\text{equil}}$ Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.00 ± 0.00</td>
<td>33.00 ± 2.83</td>
<td>22.80 ± 1.00</td>
</tr>
<tr>
<td>12 h</td>
<td>14.33 ± 5.32</td>
<td>42.50 ± 11.08</td>
<td>22.87 ± 1.18</td>
</tr>
<tr>
<td>24 h</td>
<td>20.03 ± 1.18</td>
<td>59.14 ± 3.08</td>
<td>23.87 ± 1.20</td>
</tr>
<tr>
<td>48 h</td>
<td>14.33 ± 6.09</td>
<td>40.67 ± 13.62</td>
<td>22.90 ± 0.89</td>
</tr>
</tbody>
</table>

Values are means ± SD in dyn/cm. $\gamma_{\text{min}}$: Minimum surface tension; $\gamma_{\text{max}}$: maximum surface tension; $\gamma_{\text{equil}}$: equilibrium surface tension. Control $\gamma_{\text{min}}$: significantly different from 12-, 24-, and 48-h $\gamma_{\text{min}}$, $P < 0.05$. Control $\gamma_{\text{max}}$: significantly different from 24-h $\gamma_{\text{max}}$, $P < 0.001$. Significant difference between 24- and 48-h $\gamma_{\text{max}}$, $P = 0.001$.
control surfactant samples that were exposed to oxidizing conditions in vitro, with subsequent thiobarbituric acid measurements. Clearly, there is a substantial population of readily oxidizable lipid in these samples, and the end products are readily detectable with the assay system employed.

**DISCUSSION**

Exposure of guinea pigs to nebulized LPS at the concentrations employed produces a substantial influx of neutrophils into the alveolar space by 12 h after LPS exposure. This is followed by ALI, which is histologically similar to the exudative phase of ARDS (5), 24 h after LPS exposure. Finally, there is evidence of early recovery between 24 and 48 h after LPS exposure. The acute inflammatory process is marked by capillary leakage, resulting in plasma protein extravasation into the alveolar space, a severalfold increase in lung tissue H, and profound gas-exchange abnormalities. The most striking change in lung mechanics produced by LPS exposure is an increase in tissue H, most likely due to increased surface tension with concomitant airway closure and alveolar collapse. This increase in tissue H in LPS-treated animals corresponds to a dramatic decrease in static compliance as assessed by conventional quasi-static pressure-volume curves (data not shown). Tissue G shows a dramatic 15-fold increase at 24 h vs. the control value, whereas $R_{aw}$ appears to undergo a complex series of changes over time.

Compared with the control value, $R_{aw}$ first increases in the 12-h group by ~50%, then decreases by ~50% at 24 h, and finally increases again by ~100% at 48 h. The apparent decrease in $R_{aw}$ in the 24-h group is most likely a consequence of the development of mechanical time-constant inequalities, or heterogeneities, in the lung. The model used to fit $Z_L$ data for the purposes of this paper is a single-compartment model. Yet whereas the lung behaves as a homogeneous one-compartment system in control or healthy animals, it tends to behave as a multicompartment system in the setting of injury or disease. Heterogeneity will be most marked in the lungs of the most injured animals in the 24-h group. When the single-compartment model is fitted to the data from injured animals, time-constant inequalities are folded into an artificial increase in G and a decrease in $R_{aw}$ (18) to compensate for the increased frequency dependence of lung resistance at low frequencies. Thus we estimate that the true increase in tissue G is probably similar to that in H. Lung heterogeneity induced by injury not only results in an increase in average $R_{aw}$ but also leads to ventilation-perfusion mismatch.

The combination of ventilation-perfusion mismatch and atelectasis produced the highest arterial-alveolar gradient in the 24-h group. At the time that blood gas measurements were made, all animals were sedated and mechanically ventilated to the same weight-adjusted minute ventilation. Therefore, an increase in $PCO_2$ in the 24 and 48 h post-LPS animals is consistent with an increase in the alveolar dead space quotient as a consequence of injury. Whether nebulized LPS produces an increase in CO$_2$ production that contributes to this change cannot be determined based on the available data. The mild respiratory acidosis seen only in the 24-h animals under anesthesia and mechanical ventilation suggests that these animals had a higher spontaneous minute ventilation when awake. The effects

**Table 5. TBAR free radical products in surfactant**

<table>
<thead>
<tr>
<th>Sample</th>
<th>TBAR concentration, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.253 ± 0.079</td>
</tr>
<tr>
<td>12-h LPS</td>
<td>0.236 ± 0.077</td>
</tr>
<tr>
<td>24-h LPS</td>
<td>0.219 ± 0.013</td>
</tr>
<tr>
<td>48-h LPS</td>
<td>0.280 ± 0.121</td>
</tr>
<tr>
<td>Oxidized</td>
<td>2.340 ± 0.291</td>
</tr>
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</table>

Values are means ± SD. TBAR, thiobarbituric acid.
fects of such relative hyperpnea would have been to normalize pH, reduce Pco₂, and produce some improvement in PO₂. The ability of guinea pigs to survive with such appallingly low PO₂ values may be due in part to the relative high avidity of their hemoglobin for oxygen: even with PO₂ values in the 42- to 46-mmHg range, oxygen saturation remained in the 80-85% range.

Alteration in lung mechanical properties in LPS-treated guinea pigs can be accounted for by an alteration in interfacial properties, which, in turn, result from dysfunction of lung surfactant. The degree of physiological dysfunction and surfactant dysfunction observed closely paralleled each other both during the development of lung injury over the first 24 h after LPS exposure and during early resolution between 24 and 48 h after LPS exposure. This animal model serves as a useful system for examining the mechanisms leading to and the consequences stemming from surfactant dysfunction during early ALI secondary to inflammatory processes such as sepsis.

The inflammation produced by nebulized LPS appears to alter surfactant metabolism in a variety of ways. The most obvious is a reduction in the size of the surfactant pellets recovered from BAL fluid, which corresponds to a reduction in the most active large-aggregate surfactant fraction. At the same time, there is a significant extravasation of plasma proteins into the alveolar space secondary to capillary leak. The reduction in surfactant SP-A level mirrors the overall decrease in surfactant phospholipid in the large-aggregate fraction. SP-B behaves differently, however, in that large-aggregate surfactant becomes relatively deficient in SP-B as a consequence of LPS exposure. SP-B is not only critical to surfactant function under normal conditions (28) but also appears to confer on surfactant a degree of resistance to the deleterious effects of plasma proteins (6, 24, 28).

The cumulative effect of these processes accounts for the majority of surfactant dysfunction and physiological dysfunction observed in these animals over the first 24 h. Between 24 and 48 h after LPS exposure, there was a recovery in the levels of surfactant phospholipids, SP-A, and particularly SP-B. Surfactant harvested 48 h after exposure revealed an improvement in function observed in vitro despite the persistence of significant plasma protein contamination in the surfactant pellet. Lipid peroxidation per se did not appear to occur to any measurable degree in these samples (see Table 5).

The small-aggregate fraction of alveolar surfactant was not studied in these experiments, and, therefore, we cannot comment on potential changes in its function or composition. The nature of the larger molecular weight form of SP-B seen by Western blot in the LPS-exposed animals remains to be determined. Possible candidates include covalently formed aggregates of SP-B caused by free radical mechanisms or the presence of biosynthetic precursors of SP-B being released into the alveolar space in the setting of alveolar injury. Finally, the extent to which residual dysfunction in the sucrose-purified surfactant pellet can be corrected for by apoprotein replacement or other reconstitution approaches in vitro remains to be determined.

It is likely that a variety of mechanisms are contributing to surfactant dysfunction within the alveolar space in this model of ALI. The ability of both whole serum and albumin to inhibit surfactant function has been shown to be dependent on surfactant phospholipid concentration (12). The presence of surfactant apoproteins has been observed to protect surfactant from inhibitory effects of proteins (6, 24, 28). In clinical observations, decreased BAL fluid phospholipid content and SP-B have been reported among patients with ARDS (9).

The reasons for the limited efficacy of surfactant replacement strategies that have thus far been employed in ALI and ARDS are not well understood. A better understanding of this process will be needed to devise more effective approaches to the restoration of surfactant function in critically ill adults. The findings of this study have several potential implications for surfactant replacement strategies. Adequate surfactant replacement in this model of ALI will require that surfactant containing adequate amounts of both phospholipid and apoprotein components be delivered to the most affected lung segments in sufficiently high concentrations to overcome the inhibitory effects of plasma proteins and other unidentified factors. The application of a recruitment maneuver or positive end-expiratory pressure to open up collapsed areas of the lung before surfactant administration may therefore enhance the efficacy of surfactant replacement.

To the extent that there may be ongoing degradation of surfactant apoproteins in the areas of most intense inflammation, supranormal quantities of apoproteins may need to be included in the surfactant replacement mixture to act as a reserve and to supplement existing surfactant in those alveoli. On the basis of the results observed in this animal model, one would predict that a non-apoprotein-containing surfactant delivered by aerosolization would have limited clinical efficacy in ARDS as has been reported (2). On the other hand, a hydrophobic apoprotein-containing surfactant delivered by instillation in supraphysiological quantities might well have a positive impact in ARDS as has also been reported (10).

The presence of inhibitors to surfactant function in the alveolar space in ALI or ARDS points to the potential utility in performing alveolar lavage before surfactant replacement. Such a maneuver might remove those inhibitors, the effects of which are not readily overcome by increasing apoprotein and phospholipid concentrations at the interface, from the alveolar space. The potential utility of a lavage step before surfactant administration has been demonstrated in a piglet model of saline lavage-induced ALI (3) and in a limited clinical study in patients with ARDS (27).

The issues surrounding ALI, physiological dysfunction, surfactant dysfunction, and surfactant replacement are highly complex, and the clinical importance of understanding them is quite clear. It is our hope that
the establishment of a small-animal model of ALI and surfactant dysfunction secondary to LPS administration, coupled with a detailed multipronged examination of the underlying processes, will make a contribution to our understanding of ARDS.

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