Pulmonary surfactant and inflammation in septic adult mice: role of surfactant protein A

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1Departments of Physiology and Medicine, Lawson Health Research Institute, University of Western Ontario, London, Ontario, Canada N6A 4V2; 2Division of Pulmonary and Critical Care, Department of Medicine, University of Cincinnati, Cincinnati, 45267; and 3Division of Pulmonary Biology, Children’s Hospital Medical Center, Cincinnati, Ohio 45229

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Malloy, Jaret L., Ruud A. W. Veldhuizen, Francis X. McCormack, Thomas R. Korfhagen, Jeffery A. Whitsett, and James F. Lewis. Pulmonary surfactant and inflammation in septic adult mice: role of surfactant protein A. J Appl Physiol 92: 809–816, 2002; 10.1152/japplphysiol.00628.2001.—Surfactant alterations, alveolar cytokine changes, and the role of surfactant protein (SP-A) in septic mice were investigated. Sepsis was induced via cecal ligation and perforation (CLP). Septic and sham mice were euthanized at 0, 3, 6, 9, 12, 15, and 18 h after surgery. Mice deficient in SP-A and mice that overexpressed SP-A were euthanized 18 h after surgery. In wild-type, sham-operated mice, surfactant pool sizes were similar at all time points, whereas in the CLP groups there was a significant decrease in small-aggregate surfactant pool sizes beginning 6 h after CLP. Interleukin-6 concentrations in bronchoalveolar lavage fluid from septic animals increased from 6 to 18 h after surgery. Identical surfactant alterations and concentrations of cytokines were observed in septic mice that were SP-A deficient or that overexpressed SP-A. In conclusion, alterations of pulmonary surfactant and alveolar cytokines occur simultaneously, 6 h after a systemic insult. In addition, we did not detect a role for SP-A in regulating surfactant phospholipid pool sizes or pulmonary inflammation in septic mice.

cecal ligation and perforation; surfactant aggregates; cytokines; in vivo; mouse

ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) is caused by both direct and indirect insults to the lung and is associated with an overall mortality of 40–60% (11). The most common cause of ARDS is sepsis, and this particular condition carries the highest mortality (~70%) (11). Sepsis represents a systemic response to a focus of infection, which ultimately becomes overwhelming and deleterious to the host. The pathophysiology of the lung injury associated with sepsis is complex and associated with increased expression of inflammatory mediators and cytokines (11), as well as alterations in the endogenous surfactant system. These latter changes include decreased surfactant lipid pool sizes and altered phospholipid and protein compositions (31). Recently, changes in the proportion of alveolar large- and small-aggregate surfactant lipids were observed relatively early in the course of the lung injury, before the development of significant lung dysfunction (22, 24). Although the significance of these early changes in the endogenous surfactant system is unknown, it is possible that they contribute to the initial pathophysiological stages of the lung injury.

Surfactant plays a role in the innate host defense of the lung in part by affecting uptake and killing of pathogens and influencing the release of inflammatory mediators from various cells within the lung (1, 2, 34). Surfactant protein A (SP-A), in particular, is thought to downregulate pulmonary inflammation, because mice deficient in this protein have increased inflammatory cytokine levels in response to an infectious insult compared with wild-type mice (7). It has also been shown that cytokines can increase or decrease surfactant lipid and/or surfactant protein levels (16, 30, 32). These data suggest that there may be a close relationship between the cytokine network and the endogenous surfactant system, particularly in the setting of acute lung injury. The role of SP-A in the regulation of surfactant homeostasis and its contribution to the biophysical function of surfactant are somewhat controversial at present, as reviewed by Hawgood and Pouclain (12). In vitro studies demonstrated that SP-A inhibited surfactant secretion, increased uptake, enhanced the stability of surfactant films, and mitigated the conversion of large aggregates to small aggregates within the alveolar space. However, in vivo studies have not supported a critical role of SP-A in surfactant function; SP-A−/− mice had no discernable abnormalities in surface activity or film stability, even after lung injury was induced (15, 17). Therefore, despite the seemingly limited contribution of SP-A to the biophysical function of pulmonary surfactant, this protein may well play a role in the setting of sepsis-induced lung injury by influencing the host’s inflammatory response.
and surfactant pool sizes via its influence on lipid metabolism.

On the basis of this information, we hypothesized that surfactant changes would be closely associated with cytokine alterations during the initiation and progression of systemic sepsis in adult mice. Moreover, SP-A would play an important role in mediating not only the cytokine levels observed over the course of this disease but also the changes in surfactant pool sizes. To test this hypothesis, temporal changes in surfactant composition and cytokine levels within the lung over the 18-h course of systemic sepsis induced by cecal ligation and perforation (CLP) were characterized. The specific role of SP-A in this setting was also determined by utilizing mice deficient in SP-A as well as mice overexpressing rat SP-A under control of the SP-C promoter, resulting in an increased expression of the transgene in respiratory epithelial cells.

METHODS

Animals. Swiss Black SP-A−/− were developed from embryonic stem cells after disruption of the mouse SP-A gene by homologous recombination, as previously described (17). Lung-specific overexpression of rat SP-A in FVB/N mice was accomplished by using the human surfactant protein C promoter, as described previously (9). The rat SP-A transgene was bred into the null background by crossing the rat SP-A transgene bearing FVB/N mice (SP-A+/−,rSP-A) with Swiss Black SP-A−/− mice. Transgene positive (SP-A+/−,rSP-A) and transgene negative (SP-A−/−) littermates were used in this study. Wild-type animals consisted of the FVB/N genetic background and were purchased from Charles River Labs (St. Constant, PQ, Canada).

Animal model. Male and female mice weighing between 20 and 25 g (6–6 wk old) were anesthetized with an injection of ketamine and xylazine (0.25 mg/kg and 0.025 mg/kg ip, respectively). Septic animals were killed as described above and underwent identical lavage procedures as the wild-type animals. Animals were killed via an intraperitoneal injection of ketamine and xylazine, incising the abdominal cavity, and transecting the descending aorta. Immediately after death, the lungs were visualized, and a cannula (18-gauge) was secured in the trachea. A bronchoalveolar lavage was performed by infusing 1 ml of sterile 0.15 M saline into the lungs, which was withdrawn and the same bolus infused an additional two times and then collected. For each mouse that was utilized for surfactant analyses, bronchoalveolar lavage fluid (BALF) from a total of three lavage procedures was combined. This resulted in a total volume of ~2.5 ml for individual animals. In each mouse used for cytokine analyses, two bronchoalveolar lavage procedures were performed, and the BALF was combined for analysis (~1.6 ml).

SP-A−/− and SP-A+/−,rSP-A mice underwent both sham and septic surgical procedures, as described above, but were only killed at the 18-h time point after surgery. Similar to wild-type animals, separate groups of transgenic sham and septic mice were utilized for surfactant and cytokine measurements. Animals were killed as described above and underwent identical lavage procedures as the wild-type animals.

Lavage fluid analyses. There were no significant differences in the total volume of saline infused into the lungs or in the volume recovered after the lavage procedure among any of the experimental groups. In animals evaluated for surfactant measurements, the total recovered lavage was centrifuged at 150 g for 10 min to yield a pellet containing cellular material. One milliliter of the 150-g supernatant was removed and centrifuged at 40,000 g for 15 min to separate the large-surfactant-aggregate fraction (pellet) from the small-surfactant-aggregate fraction (supernatant) (21).

The total quantity of surfactant phospholipids was determined by phospholipid-phosphorous measurement from aliquots of the 150-g supernatant (total alveolar surfactant), 40,000-g pellet (large aggregates), and 40,000-g supernatant (small aggregates). Samples were initially extracted by using the method of Bligh and Dyer (4), and phospholipid-phosphorous levels were determined with the Duck-Chong phosphorus assay (8). Briefly, 100 μl of 10% magnesium nitrate in methanol were added to the extracted lipids. After drying, the samples were ashed in a fume hood on an electric rack for ~1 min. One milliliter of 1 M HCl was added, and the samples were covered and reheated for 15 min at 95°C. After cooling, a 66-μl aliquot of each sample was added to individual wells of a 96-well plate along with 134 μl of a dye consisting of 4.2% ammonium molybdate in 4.5 M HCl with 0.3% malachite green (1:3 vol/vol). The absorbency of the triplicate samples was read at 650 nm, and the phosphorous concentration was calculated by using a standard curve from 0.1 to 1.1 μg phosphorous on the sample plate.

Total protein recovery was also measured in the 150-g supernatant, by using the method of Lowry and colleagues (23) and bovine serum albumin as a standard.

Surfactant activity measurements. The biophysical function of the resuspended large-aggregate samples recovered from wild-type animals was performed by using a captive-bubble surfactometer. Because material recovered was limited, this analysis required separate groups of sham and septic wild-type animals and involved only the 18-h time point after their respective surgery. BALF obtained from three separate animals was combined for a single measurement of surface activity.

SP-A analysis. The presence of SP-A in the BALF of wild-type, SP-A−/−, and SP-A+/−,rSP-A mice was verified by Western blot. Briefly, 20 μl of BALF were subjected to a 10%
polyacrylamide protein gel electrophoresis containing 0.1% SDS by using the method of Laemmli (18). After electrophoresis, proteins were transferred to nitrocellulose by use of Bio-Rad transfer apparatus (Bio-Rad Laboratories, Missisauga, ON). Transfer was carried out at 100 V for 1 h. Western blot analysis was blocked overnight with 3% milk in 1 × PBS buffer at 4°C and incubated with a polyclonal rabbit anti-rat-SP-A antibody (a generous gift of Dr. Henk Haagsman, University of Utrecht, Utrecht, The Netherlands) in 1× PBS-0.2% normal rat serum for 1 h. Blots were then washed three times in 1 × PBS with 0.1% Tween-20 and incubated with the second antibody, horseradish peroxidase-labeled donkey-antirabbit sera. After enhanced chemiluminescent reagent development, the blots were exposed to X-ray film (Eastman Kodak, Rochester, NY). The enhanced chemiluminescence kit employed was purchased from Amersham Life Science (Little Chalfont, Bucks, UK) and used according to the instructions provided.

Cytokine analyses. For cytokine measurements in BALF, the total recovered lavage fluid from wild-type, SP-A−/−, and SP-A−/−:eSP-A mice was centrifuged at 150 g for 10 min to yield a pellet containing cellular material. The resultant supernatant was divided into 350-μl aliquots, frozen in liquid nitrogen, and stored at −70°C until further analysis. Measurements of tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-10 were performed by using commercially available ELISA kits that were specific for the mouse cytokines (R&D Systems, Minneapolis, MN). Measurements for each cytokine were performed simultaneously in triplicate by following the instructions provided by the supplier.

Cellular analyses. Cells from the pellet formed after the 150-g centrifugation procedure mentioned above were resuspended in 1 ml of Plasmalyte. To determine total cell count, cells were stained with trypan blue and counted in a hemocytometer under light microscopy. Differential cell counts were performed on cytospin preparations stained with Diff-Quick (Scientific Products, McGraw Park, IN) and counted in five random fields per slide under light microscopy.

Wet-to-dry ratios. Separate wild-type sham and CLP groups were utilized to determine wet-to-dry ratios. This analysis was performed on lungs removed from animals 18 h after their respective surgeries. Immediately after death, lungs were isolated, and the trachea, heart, and other tissue were carefully dissected and removed. The lungs were then weighed, placed in an oven at 60°C overnight, and then reweighed for comparison with their initial weight.

Statistics. Data are expressed as means ± SE. Values at the various time points over the 18-h experimental period for the sham and septic wild-type mice were compared by use of a two-way ANOVA. If a significant interaction was present, pairwise comparisons between groups at each time point were conducted by independent t-tests. To account for multiple comparisons, the level of significance was lowered to \( P < 0.02 \). To determine when significant differences occurred over the 18-h time line, a one-way ANOVA followed by a Dunnett’s post hoc test was used within the sham or septic groups, and \( P < 0.05 \) was considered significant. Values between the groups of transgenic animals at the 18-h time point were compared by using a two-way ANOVA followed by the Tukey’s test for multiple comparisons, and \( P < 0.05 \) was considered statistically significant.

RESULTS

Wild-type animals. Visual observations of both sham and CLP animals over the 18-h experimental period indicated that both groups of animals had fully recovered 6 h after their respective surgeries. These observations were similar to previous studies involving catheterized septic rats, which confirmed that arterial blood gases in those animals were normal 5 h post-CLP (24, 25). In the present study, sham animals remained active and responsive over the remaining 12-h monitoring period, whereas CLP animals were lethargic after 12 h. Their condition worsened after 18 h, at which time they were in obvious respiratory distress. Table 1 shows several pulmonary variables of sham and CLP wild-type mice measured 18 h after their respective surgeries. Despite the obvious physical differences observed between these two groups, there were no statistical differences with respect to total protein measured in the BALF, wet-to-dry ratios of isolated whole lungs, and surface tension-reducing properties of the recovered large-aggregate surfactant.

There were significantly greater numbers of cells measured in the BALF of the CLP animals 18 h after surgery compared with the sham animals, although differential cell counts were similar in the two groups. Again, these observations were similar to previous findings in adult rats rendered septic by the same procedure (24, 25). These previous studies also showed a relatively mild lung injury demonstrated by decreased oxygenation and morphological changes as well as an increased respiratory rate, increased heart rate, and elevated arterial lactate levels compared with sham-operated controls. Unfortunately, because of size limitations, these latter variables were unable to be accurately measured in mice.

Figure 1 shows the total surfactant phospholipid pool sizes measured in the BALF of the sham and CLP wild-type mice at each time point over the 18-h experimental period \((n = 6–8)\) animals/sham group per time point; \(7–8\) animals/CLP group per time point). For the sham groups, there were no significant differences in surfactant pool sizes at any time point after surgery. Septic animals exhibited a gradual decrease in total surfactant pools over the 18 h, which was statistically significant at the 18-h time point compared with the 0-h time point \((P < 0.05)\). Compared with their respective sham groups, total pool sizes in septic animals

Table 1. Pulmonary characteristics of wild-type sham and CLP groups 18 h after surgery

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<thead>
<tr>
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<th>Sham</th>
<th>CLP</th>
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<tr>
<td>Total BALF protein, mg/kg</td>
<td>10.3 ± 1.1</td>
<td>12 ± 1.3</td>
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<tr>
<td></td>
<td>((n = 10))</td>
<td>((n = 10))</td>
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<tr>
<td>Lung wet-to-dry ratio</td>
<td>5.0 ± 0.4</td>
<td>4.8 ± 0.5</td>
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<tr>
<td></td>
<td>((n = 8))</td>
<td>((n = 8))</td>
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<tr>
<td>Large-aggregate minimum surface tension, mN/m</td>
<td>7.5</td>
<td>7.5 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>((n = 2))</td>
<td>((n = 3))</td>
</tr>
<tr>
<td>Total BALF cell count, 10⁶ cells</td>
<td>0.5 ± 0.05</td>
<td>1.2 ± 0.3*</td>
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<td></td>
<td>((n = 8))</td>
<td>((n = 8))</td>
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<tr>
<td>Macrophages in lavage, % of total cells</td>
<td>97 ± 12</td>
<td>95 ± 19</td>
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<td></td>
<td>((n = 6))</td>
<td>((n = 6))</td>
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Values are means ± SE. CLP, cecal ligation and perforation; BALF, bronchoalveolar lavage fluid. *\(P < 0.05\) vs. sham group.
were significantly lower at the 9-, 15-, and 18-h time points ($P < 0.02$).

Figure 2 shows the large-aggregate and small-aggregate surfactant phospholipid pool sizes for wild-type sham and septic mice over the entire experimental period. The large-aggregate fraction (Fig. 2A) did not change over the 18 h for either the sham or CLP groups compared with the 0-h time point. Furthermore, there were no significant differences in large-aggregate pool sizes between sham and CLP groups at any time point over the 18 h. Measurement of small-aggregate pool sizes (Fig. 2B) in the sham groups revealed no differences over the 18-h experimental period, except for one isolated measurement at the 9-h time point ($P < 0.05$ vs. 0-h time point). In the CLP animals, there was an immediate and progressive decrease in small-aggregate pool sizes over the experimental period, which was statistically significant beginning at the 6-h time point compared with the 0-h time point ($P < 0.05$). These small-aggregate pool sizes in the CLP groups were also significantly lower than the respective sham group at the 6-h time point, and these differences continued throughout the 18-h experimental period ($P < 0.02$).

Figure 3 shows cytokine concentrations in the BALF. TNF-$\alpha$ (Fig. 3A), IL-6 (Fig. 3B), and IL-10 (Fig. 3C) for sham and CLP wild-type mice ($n = 5–6$ animals/sham group per time point; $n = 7–8$ animals/CLP group per time point) were assessed over the 18-h experimental period. Sham animals had relatively low levels of TNF-$\alpha$ that did not significantly change over the experimental period. There was also no significant difference in TNF-$\alpha$ concentrations among the CLP groups over the same period, although there was a slight increase in TNF-$\alpha$ concentration in the 6-h CLP group. Statistical analysis revealed no significant difference in the concentration of TNF-$\alpha$ between the sham and CLP groups at any time point over the 18 h. IL-6 concentrations remained low in the sham groups throughout the entire experimental period and were not significantly different. CLP animals had a gradual increase in IL-6 levels that became significant at the 15- and 18-h time points after the CLP procedure compared with the 0-h time point ($P < 0.05$). This increased alveolar IL-6 concentration resulted in CLP groups having significantly higher IL-6 levels at the 6, 15, and 18-h time points compared with their respective sham groups ($P < 0.02$). IL-10 concentrations were not significantly different among the sham groups over the 18 h. There was only a significantly higher concentration of IL-10 in the 6-h CLP group compared with the 0-h time point ($P < 0.05$), which was also significantly higher than the respective sham group ($P < 0.02$).

$SP-A^{-/-}$ and $SP-A^{-/-},rSP-A$ animals. The presence of SP-A in the BALF recovered 18 h after surgery from wild-type, $SP-A^{-/-}$, and $SP-A^{-/-},rSP-A$ mice was verified by Western blot. There was no SP-A present in the $SP-A^{-/-}$ mice and a notably greater quantity of SP-A in the transgene-positive mice ($SP-A^{-/-},rSP-A$) compared with wild-type mice (data not shown).

Figure 4 shows the total surfactant phospholipid pool sizes 18 h after surgery for both the sham and CLP $SP-A^{-/-}$ and $SP-A^{-/-},rSP-A$ mice ($n = 5$ animals/sham group; $n = 7$ animals/CLP group). Although in general...
the measured phospholipid pool sizes in the transgenic animals were somewhat higher than in the wild-type animals, similar to the observations in wild-type animals there were significantly lower total surfactant pool sizes in the transgenic CLP mice compared with the transgenic sham animals \( (P < 0.05) \). There were no significant differences in total pool sizes between the two transgenic sham groups or between the two transgenic CLP groups.

Figure 5 shows the large- and small-aggregate phospholipid pool sizes measured at the 18-h time point after surgery for the four transgenic groups. Again, similar to findings in wild-type mice, there were no significant differences in large-aggregate pool sizes among the sham and CLP groups. Analysis of the small-aggregate pools revealed a significantly lower small-aggregate pool size in the CLP mice compared with the sham mice \( (P < 0.05) \). There were no significant differences in small-aggregate pools between the SP-A\(^{-/-}\) and SP-A\(^{-/-}\)rSP-A sham groups or between the SP-A\(^{-/-}\) and SP-A\(^{-/-}\)rSP-A CLP groups 18 h after their respective surgeries.

Figure 6 shows the concentrations of TNF-\(\alpha\) (Fig. 6A), IL-6 (Fig. 6B), and IL-10 (Fig. 6C), measured in the BALF of the four experimental transgenic groups at the 18-h time point \( (n = 5–7 \text{ animals/sham group}; n = 7 \text{ animals/CLP group}) \). There were no significant differences in TNF-\(\alpha\) and IL-10 levels among the four experimental groups at this time point. Similar to the wild-type mice, there were significantly elevated levels of IL-6 in the CLP animals compared with sham animals at the 18-h time point \( (P < 0.05) \); however, there were no significant differences in IL-6 concentrations between the two sham groups (SP-A\(^{-/-}\) and

Fig. 4. Alveolar surfactant phospholipid pool sizes for the sham and CLP groups deficient in surfactant protein (SP)-A (SP-A\(^{-/-}\)) and sham and septic animals that overexpress SP-A (SP-A\(^{-/-}\)rSP-A) at the 18-h time point after surgery. Values are means \( \pm \) SE; \( n = 5–7 \) mice/group. \# \( P < 0.05 \) all septic animals vs. all sham animals.

Fig. 5. LA and SA phospholipid pool sizes for the sham and CLP groups at the 18-h time point after surgery. Values are means \( \pm \) SE, \( n = 5–7 \) mice/group. \# \( P < 0.05 \) all CLP animals vs. all sham animals.
SP-A

in the CLP mice compared with the sham mice (P < 0.05). There were significant differences in the percentage of alveolar macrophages recovered in the BALF of the four transgenic groups at the 18-h time point after surgery for both sham and septic groups. 

A significant decrease in small-aggregate phospholipid pools accounted for the overall change in total surfactant pool sizes observed in mice undergoing the CLP procedure. These relatively “early” alterations of the endogenous surfactant system in response to the CLP procedure support the hypothesis that surfactant alterations are not merely a consequence of severe lung injury but may play a role in the initial stages of the injury. Small aggregates represent the nonfunctional component of alveolar surfactant and are the metabolic conversion products of the superiorly functioning large-aggregate forms. Surfactant metabolism involves synthesis and secretion of the lipoprotein large-aggregate forms from alveolar type II cells that are converted into small-aggregate forms within the alveolar space. Small aggregates are cleared from the air space by type II cells or alveolar macrophages (36). The decreased small-aggregate pools observed after the CLP procedure in both rats and mice may have occurred via either a decrease in large-aggregate conversion within the air space or an increase in small-aggregate clearance from the air space in the septic animals. Because large-aggregate pool sizes did not change over the course of sepsis in the present study, it is unlikely that surfactant-aggregate conversion was altered in these animals. On the other hand, the increased number of alveolar macrophages present within the air space of animals undergoing the CLP procedure suggests there may have been an increased clearance of small aggregates compared with sham animals. It is also possible that greater amounts of small aggregates were taken back up into type II cells for reutilization or clearance in the septic animals, given that a recent study utilizing a similar model to induce systemic sepsis documented abnormally large changes in surfactant pool sizes were observed in both species, and the changes in surfactant pool sizes were identical. Moreover, despite the inability to obtain adequate samples for blood-gas measurements to confirm hypoxemia and lung dysfunction in mice, these animals did have evidence of lung inflammation as reflected by the increased number of cells and inflammatory cytokines recovered from their air spaces. Severe pulmonary edema was not present, however, given that total alveolar protein measurements and wet-to-dry ratio values were similar in the sham and septic groups.

Table 2. Total cell counts and percentage of macrophages recovered in the BALF

<table>
<thead>
<tr>
<th></th>
<th>SP-A&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>SP-A&lt;sup&gt;−/−,rSP-A&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>CLP</td>
<td>Sham</td>
</tr>
<tr>
<td>Total cell count, 10&lt;sup&gt;6&lt;/sup&gt; cells</td>
<td>0.25 ± 0.02</td>
<td>1.28 ± 0.1*</td>
</tr>
<tr>
<td>Macrophages, % of total cells</td>
<td>95.2 ± 1.0</td>
<td>93.4 ± 2.6</td>
</tr>
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Values are mean ± SE; n = 5 for sham groups and n = 7 for CLP groups that are deficient in surfactant protein A (SP-A<sup>−/−</sup>) and that overexpress SP-A (SP-A<sup>−/−,rSP-A</sup>). Total cell counts and percentage of macrophages recovered in the BALF 18 h after respective surgery are given. *P < 0.05 CLP animals vs. sham animals.
lamellar bodies within the type II cells of these animals (10). More elaborate in vivo metabolic studies are required to further explore the mechanisms responsible for the surfactant pool size changes observed over the course of sepsis.

The major function of surfactant has traditionally been believed to be related to its biophysical properties, that being to lower surface tension and maintain alveolar stability. There is growing evidence that surfactant also plays a role in the development of pulmonary inflammation, possibly by influencing the synthesis and/or secretion of inflammatory cytokines. The specific in vivo relationship between the surfactant system and the inflammatory cytokine network is not completely understood. Few studies have been conducted to date and are limited to examining changes in the surfactant system after extremely high concentrations of a specific cytokine were instilled directly into the lungs of normal animals (32). This situation may be quite different than the effects of endogenously produced cytokines on the pulmonary surfactant system. Unfortunately, there are no studies examining this relationship in a model that adequately reflects a human condition in which both systems are perturbed. Analyses of cytokine concentrations in the BALF recovered from animals at various time points over the 18-h period after CLP revealed that IL-6 levels were increased as early as 6 h and continued to increase over the remaining 12 h. In general, IL-6 is thought to represent a proinflammatory cytokine and has been correlated with overall mortality in patients with ARDS (26, 27). In addition, serum IL-6 was also shown to be higher in a group of ARDS patients managed with a “higher stretch” mode of mechanical ventilation reflecting higher tidal volumes compared with a “low-stretch” mode. These changes were associated with a greater mortality in the former group (6). However, recent studies have also reported that IL-6 has potent anti-inflammatory and protective properties. Overexpression of IL-6 in the lung markedly enhanced survival and decreased alveolar-capillary protein leak in mice exposed to 100% oxygen over several days (35). Further investigation is required to determine the specific role of the increased alveolar IL-6 in septic mice.

TNF-α, a proinflammatory cytokine, has been shown to increase relatively early in the inflammatory response, with peak levels being extremely transient (33). Indeed, it is possible in the present study that the highest level of TNF-α was missed within the 3-h measurement intervals in the animals undergoing the CLP procedure. Because TNF-α has been shown to decrease surfactant lipid metabolism and to downregulate the expression of surfactant associated proteins (3, 29, 30, 32), our results do not preclude TNF-α as having a significant role in the surfactant alterations observed in this model. Future studies, possibly utilizing transgenic animals, are required to probe the relationship between specific cytokine changes and surfactant alterations in vivo.

To investigate the specific role of SP-A in the development of sepsis, animals deficient in SP-A and animals overexpressing this protein were utilized. SP-A has been shown in vitro to inhibit surfactant secretion and enhance surfactant uptake by alveolar type II cells (12), although the impact of SP-A on surfactant metabolism in vivo is somewhat controversial. For example, administration of exogenous lipids and SP-A into the lungs of normal rabbits demonstrated increased secretion of lipid within the air space compared with lipids administered without SP-A, results opposite to the in vitro data (28). In addition, animals deficient in SP-A had no alterations in lipid metabolism when evaluated with the use of radiolabeled surfactant precursors (14). Utilizing both SP-A-deficient and SP-A-overexpressing mice, our data showed that SP-A had no effect on surfactant pool sizes in normal mice or the surfactant pool size changes observed in animals undergoing the CLP procedure. Our results are consistent with results reported by Ikegami and colleagues (13, 15) in which SP-A−/− animals were stressed with exercise or when acute lung injury was induced by subcutaneous N-nitroso-N-methylurethane administration. No differences in surfactant metabolism were noted in those animals compared with wild-type animals. On the basis of this information, together with our study, we conclude that SP-A has no essential role in regulating surfactant phospholipid pool sizes, either in normal animals or in the setting of acute lung injury.

On the other hand, previous in vivo studies involving similar SP-A-knockout mice to those used in the present study showed increased concentrations of both TNF-α and IL-6 in the BALF compared with wild-type animals in response to an infectious pulmonary insult (5, 19, 20). Administration of exogenous SP-A to these animals prevented these changes, indicating that SP-A has an important role in the host’s inflammatory response to these insults (5, 19, 20). Findings in the present study revealed similar levels of inflammatory cytokines and total number of cells in the BALF 18 h after CLP in mice both deficient in and overexpressing SP-A. Therefore these results are not consistent with the previous studies, suggesting that SP-A may have no effect on the development of lung inflammation when induced by a systemic insult such as sepsis, as opposed to the more direct infectious insults such as those induced by bacterial or viral inoculation.

In summary, alterations of alveolar surfactant and increased alveolar cytokine levels were demonstrated relatively early after CLP in adult mice. SP-A did not play a role in alterations of the surfactant system in this model, suggesting that SP-A has limited impact on surfactant metabolism in vivo. SP-A-deficient mice are susceptible to infection and inflammation after intratracheal challenge with viral or bacterial pathogens, demonstrating the important role of SP-A in innate host defense of the lung. In contrast, present findings do not support the role of SP-A in the modulation of pulmonary inflammation induced by systemic sepsis.

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