Total extracellular surfactant is increased but abnormal in a rat model of gram-negative bacterial pneumonia

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Escherichia coli; pulmonary injury; phospholipid; surfactant dysfunction

PNEUMONIA DUE TO GRAM-NEGATIVE enteric bacilli is a serious disease, with high crude (20–60%) and attributable (10–20%) mortality rates (32, 53). More than 60% of nosocomial pneumonias are caused by gram-negative enteric bacilli with Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter sp., Acinetobacter, Serratia marcescens, and Proteus sp. being commonly isolated (6, 7, 24). Pneumonia is the second most frequent hospital-acquired infection (7), with $~ 300,000$ cases occurring per year (13, 32). In the United States, the estimated annual cost of nosocomial pneumonia due to gram-negative bacilli is greater than $1$ billion (53). An improved understanding of the pathogenesis of gram-negative infection and of the particular host functions affected may result in more efficacious treatment strategies.

A logical pulmonary host component that warrants further evaluation in gram-negative pneumonia is the pulmonary surfactant system. A significant body of research information has been generated on pulmonary surfactant and its function/dysfunction in health and disease (for review see Ref. 34). Lung surfactant is known to have abnormalities in activity or composition in various forms of acute lung injury. However, relatively little information is available on the effects of specific gram-negative infection on this critical host component (9). Pulmonary surfactant in vivo performs both biophysical and host-defense functions. This physiologically essential, phospholipid-rich material contains four surfactant proteins: surfactant protein (SP)-A, SP-B, SP-C, and SP-D. The first three of these proteins make important contributions to the biophysical function of lung surfactant. In addition, SP-A and SP-D have significant immunomodulating capability in enhancing alveolar macrophage function and promoting bacterial and viral clearance (33, 55). The present study examines levels of both phospholipids and surfactant proteins in bronchoalveolar lavage (BAL) from rats with acute lung injury from gram-negative pneumonia.

A well-defined extraintestinal pathogenic strain of E. coli (CP9) is used here as a model pathogen to induce pneumonia in adult rats (44). Our recent studies in this rat model of E. coli pneumonia have addressed inflam-
matatory pathophysiology (42), and the present work extends this to surfactant content and aggregation in BAL. Two physicochemical forms of lung surfactant, large and small aggregates, exist in the alveolar hypophase. The large-aggregate fraction of normal surfactant has the greatest surface activity and the highest levels of surfactant apoproteins (10, 12, 31, 39, 51, 56, 57). Relatively inactive, small aggregates of alveolar surfactant are preferentially taken up and recycled by type II cells in surfactant metabolism (10, 54, 57). Several studies have shown that the ratio and/or conversion of large to small surfactant aggregates is abnormal in acute lung injury in animals (16, 28, 38, 49) or patients with acute respiratory distress syndrome (ARDS) (9, 14, 19, 50). Aggregate changes are studied here in lavaged surfactant from rats with E. coli pneumonia, and in vitro surfactant cycling experiments examine whether bacterially derived factors may be mechanistically involved in the changes observed in surfactant aggregates in vivo.

MATERIALS AND METHODS

Pulmonary infection model. Acute lung injury was induced in Long-Evans rats by tracheal inoculation of E. coli organisms as described previously (42). The pertinent features of gram-negative pneumonia occur in this animal model, i.e., bacterial growth (or clearance), an ensuing inflammatory response, pulmonary damage, and progressive bacterial proliferation with subsequent death due to respiratory failure. Similar circumstances occur in untreated gram-negative pneumonia in humans. In brief, halothane-anesthetized, experimental rats (n = 21) received a tracheal challenge inocula (CI) of 1.1 × 10^7 (n = 6), 1.8 × 10^7 (n = 6), or 2.5 × 10^7 colony-forming units (cfu) (n = 9) of an extraintestinal pathogenic strain of E. coli (CP9). Previous experiments have established that higher CI result in fulminant injury and death, whereas lower CI generally result in bacterial clearance (42). Each experimental animal was prospectively assigned a time for subsequent sampling of surfactant by BAL. Twelve animals underwent sampling at 2 h, and nine at 6 h post-CI. One animal that died prematurely was excluded from analysis in the latter group. An additional group of control rats (n = 6) received halothane anesthesia and intratracheal instillation of saline, followed by physiological assessments and lavage. The rats were handled in accordance with the standards established by the U. S. Animal Welfare Act set forth in National Institutes of Health guidelines and by the University at Buffalo’s Institutional Animal Care and Use Committee.

E. coli bacteria. A human bacteremic isolate of E. coli (CP9, O4/K54/H5) was used as the model pathogen for these studies (43). CP9 is a well-characterized extraintestinal pathogenic E. coli strain (ExPEC) (44). This bacterial isolate from human blood has been shown to be virulent in several in vivo animal models including the rat model of pulmonary infection (41, 42, 45). The CP9 isolate has undergone only two laboratory passages before being preserved at −30°C until analysis. The supernatant from the high-speed centrifugation, which contained small surfactant aggregates, was transferred to a 16-gauge angiocatheter inserted into the trachea and secured with a suture. Closed-chest pulmonary P-V curves (static lung compliance) during inflation and deflation with air were then measured with the tracheal catheter attached to a syringe pump (Harvard Apparatus, Holliston, MA) with an in-line pressure transducer. The timing of injection and the pressure in the system were continually recorded by a Macintosh 650 Quadra Computer (Apple Computer, Cupertino, CA) equipped with a data acquisition board (National Instruments, Austin, TX). Software written by the laboratory in LabVIEW 3.0 (National Instrument) was used to acquire data as the syringe injected air into the lungs at ~25 ml/min (precise injection rates were determined before each experiment). When the pressure reached 40 cmH₂O, or when the pressure began to increase dramatically, the syringe pump was reversed (26).

BAL procedures. Animals were euthanized at the designated time (1–2 or 6 h postbacteria or saline control). BAL was performed through a 16-gauge angiocatheter inserted into the trachea and secured with a suture. A standard lavage procedure with a constant total delivered volume of 50 ml of ice-cold normal saline was used in all animals. Lavage fluid was alternately injected and collected (5 × 10 ml aliquots) with a Harvard syringe pump. Recovered BAL was kept on ice, and the volume was recorded. BAL was then centrifuged at 200 g at 4°C for 10 min to remove cells, and the large aggregate surfactant fraction was obtained by centrifugation of the cell-free supernatant at 40,000 g at 4°C for 15 min. Pelleted large aggregates were resuspended in 2 ml of sterile normal saline and frozen at −30°C until analysis. The supernatant from the high-speed centrifugation, which contained small surfactant aggregates, was transferred to a fresh tube and also frozen at −30°C for analysis.

In vitro interaction between E. coli and surfactant. Calf lung surfactant extract (CLSE) containing all of the hydrophobic components of lavaged surfactant was used as a model natural surfactant for interactional studies with E. coli in vitro (35). An initial attempt was made to use rat surfactant obtained by BAL from uninfected animals, but sufficient amounts of reproducibly sterile surfactant could not be obtained. The same extraintestinal pathogenic E. coli strain (CP9) employed in in vivo studies was also used for in vitro surfactant experiments. CP9 was grown overnight in Luria-Bertani broth, resuspended in sterile 0.9% NaCl, and added to 12 ml of CLSE (1 mg/ml with 0.9% sterile NaCl) so that final concentrations consisted of either 1.0 × 10⁷ or 1.0 × 10⁸ with increased bacterial titer in BAL via serial 10-fold dilutions in sterile phosphate-buffered saline (pH 7.4) and plating on Luria-Bertani agar plates.

Physiological assessments of arterial oxygenation and lung pressure-volume compliance. Before lung mechanical studies and lavage, rats were placed in 100% oxygen for 5 min to facilitate absorption atelectasis. Halothane anesthesia was induced, a midline abdominal incision was made, and a blood sample was drawn from the descending aorta (using a 1-ml, heparinized syringe with 22-gauge needle). Arterial samples were obtained and assessed for partial pressure of arterial oxygen with a blood gas analyzer (ABL4; Radiometer America, Westlake, OH). The oxygen extraction ratio [arterial partial pressure of oxygen divided by the fraction of inspired oxygen (Pao₂/FIO₂)] was calculated as a measure of alveolar gas exchange efficacy. Pressure-volume (P-V) curves were obtained after absorption atelectasis in 10 infected animals and six controls. We flushed the lung vasculature by injecting 20 ml of 5 mM EDTA in normal saline (37°C) into the vena cava. The neck incision was reopened, and a 16-gauge angiocatheter was inserted into the trachea and secured with a suture. Closed-chest pulmonary P-V curves (static lung compliance) during inflation and deflation with air were then measured with the tracheal catheter attached to a syringe pump (Harvard Apparatus, Holliston, MA) with an in-line pressure transducer.
performed using an unpaired, two-tailed t-test with equal variance. All values are reported as means ± SE. P values ≤0.05 were considered significant.

RESULTS

Total lavage phospholipid and protein after bacterial challenge. BAL was obtained from 21 experimental rats challenged with 1.1 × 10⁷–2.5 × 10⁷ cfu of CP9 and from six control rats challenged with saline alone. Total phospholipid in BAL and total protein in BAL (an indicator of alveolar-capillary wall integrity) were measured. Total phospholipid in BAL from infected animals ranged from 0.4 to 4.1 mg (using a conversion of 0.75 mg/μmol), and total BAL protein levels ranged from 4.06 to 180 mg (Fig. 1). Total BAL phospholipid from control animals was 1.01 ± 0.03 mg, whereas total BAL protein was 2.25 ± 0.29 mg (Table 1). There was a significant correlation between increasing total phospholipid and increasing total protein in BAL (R² = 0.9081, P < 0.0001; Fig. 1). Thus in this rat model of gram-negative pneumonia, total BAL phospholipid (a common marker for extracellular lung surfactant) increased as the severity of the alveolar-capillary leak increased.

Pulmonary surfactant aggregate abnormalities above a threshold of pulmonary injury. Alterations in surfactant aggregate subpopulations in BAL from infected rats were also found to be related to the extent of lung injury (Table 1, Fig. 2). There were no significant changes in the total BAL phospholipid levels between infected and control animals as a total group compared with controls. However, a clear increase in the S/L ratio was observed in the subset of infected animals with a level of total BAL protein >50 mg (Table 1, Fig. 2). Mean S/L ratios were 0.94 ± 0.08 in control animals (n = 6) and 0.99 ± 0.07 in experimental animals (n = 12) with <50 mg of total protein in BAL (Table 1). In contrast, experimental animals with >50 mg total protein in BAL (n = 9) had

![Fig. 1. The relation between total phospholipid and total protein in bronchoalveolar lavage (BAL) from rats with gram-negative pneumonia. Each point represents values from the BAL from a single animal obtained either 2 or 6 h postbacterial challenge. The line shows the significant correlation between total BAL phospholipid with increasing total BAL protein (R² = 0.9081, P < 0.0001).](image-url)
Table 1. Oxygenation and BAL composition and surfactant aggregates in control and experimental rats

<table>
<thead>
<tr>
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<th>P_{AOO}/FiO_2</th>
<th>BAL Protein, mg</th>
<th>BAL Phospholipid, mg</th>
<th>S/L</th>
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<tr>
<td>Control animals</td>
<td>330 ± 19</td>
<td>2.25 ± 0.29</td>
<td>1.01 ± 0.03</td>
<td>0.94 ± 0.08</td>
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<td><em>E. coli</em> animals</td>
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<tr>
<td>&lt;50 mg protein</td>
<td>310 ± 40</td>
<td>17.1 ± 4.0</td>
<td>1.23 ± 0.12</td>
<td>0.99 ± 0.07</td>
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<tr>
<td>&gt;50 mg protein</td>
<td>119 ± 27*</td>
<td>104 ± 13*</td>
<td>2.77 ± 0.24*</td>
<td>2.57 ± 0.68*</td>
</tr>
</tbody>
</table>

All values are expressed as means ± SE. For controls, n = 6 for bronchoalveolar lavage (BAL) protein, BAL phospholipids, and small-to-large surfactant aggregate ratio (S/L); n = 5 for P_{AOO}/FiO_2. For *E. coli* (<50 mg protein), n = 12. For *E. coli* (>50 mg protein), n = 9 for BAL protein, BAL phospholipids, and S/L ratio; n = 8 for P_{AOO}/FiO_2. *P < 0.001 compared with control; †P = 0.015 compared with control.

a mean S/L ratio of 2.57 ± 0.68 (P = 0.015 compared with controls). Increased BAL protein in these rats reflected increased lung injury rather than changes in recovered lavage volume, which were similar in all animals (mean percent volume recoveries in BAL were 92% for controls, 85% for rats with <50 mg protein, and 83% for rats with >50 mg protein). Using 50 mg of total protein in BAL protein as a threshold, we found a significant correlation (R^2 = 0.885, P < 0.0001) between increasing S/L ratio and alveolar-capillary membrane integrity (total protein in BAL) (Fig. 2). Therefore, despite the observed increase in total BAL phospholipid with protein content in lavage, the surfactant became increasingly dysfunctional once a critical threshold of lung injury occurred.

SP-A, SP-B, SP-C, and SP-D in BAL after bacterial challenge. Levels of SP-A, SP-B, SP-C, and SP-D were measured by Western blotting in BAL from rats at 2 h (n = 3) or 6 h (n = 3) after challenge with CP9. Relative to control animals (n = 3), SP-A, SP-B, and SP-D were significantly increased in the large aggregate fraction at 2 h (179 ± 14, 229 ± 24, 295 ± 32% relative to control) and at 6 h (444 ± 40, 291 ± 75, 442 ± 38% relative to control) (Fig. 3A). Differences in SP-C content in the large aggregate fraction were less pronounced and varied with time (172 ± 35% at 2 h and 36 ± 15% at 6 h relative to control) but were not statistically significant. Values for SP-A and SP-D in the small aggregate fraction of BAL from experimental animals were significantly increased relative to controls at 2 h (268 ± 24 and 295 ± 47%) and 6 h (311 ± 0.05).

Fig. 2. The relation between the small-to-large (S/L) aggregate ratio and total BAL protein in the rat model of gram-negative pneumonia. Each point represents aggregate ratio and total protein in BAL from a single animal obtained either 2 or 6 h postbacterial challenge. The solid line demonstrates there was no correlation between the S/L and total BAL protein when the BAL protein was <50 mg. The dotted line demonstrates the significant correlation between S/L ratio and total BAL protein when the latter was >50 mg (R^2 = 0.8845, P < 0.0001).

Fig. 3. Changes in surfactant proteins in S/L aggregate fractions of BAL from rats with gram-negative pneumonia. A: large aggregate fraction; B: small aggregate fraction. Levels of surfactant protein (SP)-A, SP-B, SP-C, and SP-D were measured by Western blotting in BAL from experimental rats at 2 or 6 h after challenge with *E. coli* (CP9) or from uninfected control rats. Levels of SP-A, SP-B, and SP-D relative to control were significantly increased in the large aggregate fraction at 2 h and at 6 h. Levels of SP-A and SP-D were significantly increased at 2 and 6 h in the small aggregate fraction (SP-B and SP-C were not measured in the small aggregate fraction). Data are expressed as percentage of its respective control and are represented as means ± SE (n = 3 for all groups). *P < 0.05 compared with respective, uninfected control.
110 and 373 ± 43%; Fig. 3B). Values for SP-B and SP-C were not measured in the small aggregate fraction.

Physiological effects of E. coli on lung injury in vivo. We assessed the physiological status of rats with E. coli pneumonia by measuring arterial oxygenation and P-V compliance. The mean oxygen extraction ratio (PaO₂/FIO₂) was unchanged in E. coli-challenged experimental animals in which the total BAL protein was <50 mg (n = 12) compared with saline-challenged control animals (n = 5; 330 ± 19 vs. 310 ± 40; Table 1). In E. coli-challenged experimental animals in which the total protein in BAL was >50 mg (n = 8), the mean PaO₂/FIO₂ was significantly reduced (119 ± 27, P < 0.001 compared with controls). Pulmonary P-V loops were also performed in a subset of animals to assess alterations in compliance, and representative curves are shown in Fig. 4. In E. coli-challenged experimental animals in which the total BAL protein was <50 mg (n = 5), opening pressure increased but total lung volumes were unchanged from saline-challenged control animals (n = 6). Total lung volumes in these experimental animals being unchanged from controls is consistent with normal lung surfactant function. The increased opening pressure may be due to atelectasis induced by interstitial edema from mild inflammation. In contrast, E. coli-challenged experimental animals in which the total protein in BAL was >50 mg (n = 5) had increased opening pressures with substantially diminished lung volumes (Fig. 4). Although the mechanism for decreased lung compliance in this latter group may be multifactorial, it is consistent with abnormal lung surfactant activity from the measured increase in S/L aggregate ratios described in Pulmonary surfactant aggregate abnormalities above a threshold of pulmonary injury.

Association of increased total protein and S/L ratio with increased bacterial titer in BAL. The titer of the E. coli challenge strain CP9 was measured in BAL fluid. A comparison of bacterial titers in BAL fluid with total protein in BAL is depicted in Fig. 5. Higher bacterial titers were found in animals with increased total protein in BAL. Eight of nine animals with a total BAL protein of >50 mg had a bacterial titer of >4.0 × 10⁶ cfu, whereas all 12 rats with a total BAL protein <50 mg had a bacterial titer equal to or below this value. The mean bacterial titer in animals with a BAL protein >50 mg was 3.6 × 10⁷ ± 1.4 × 10⁷ cfu (range = 7.7 × 10⁵–1.3 × 10⁸ cfu). In contrast, the mean bacterial titer in rats with BAL protein <50 mg was 1.9 × 10⁶ ± 3.0 × 10⁵ cfu (range = 2.4 × 10⁵–4.0 × 10⁶ cfu). Rats with higher bacterial titers also had higher S/L ratios. Seven of ten BAL fluids with an S/L ratio >1.2 (mean = 2.54 ± 0.60, range = 1.2–6.8) had a bacterial titer of >4.0 × 10⁶ cfu (mean = 3.0 × 10⁷ ± 1.4 × 10⁷ cfu, range = 2.4 × 10⁵–1.3 × 10⁸). However, only 2 of 11 BAL samples with an S/L ratio of <1.2 (mean = 0.87 ± 0.04, range = 0.66–1.04) had a bacterial titer of >4.0 × 10⁶ cfu (mean = 3.5 × 10⁶ ± 1.2 × 10⁶, range = 7.3 × 10⁵–1.3 × 10⁷). Thus both alveolar-capillary leak and an increase in S/L ratio were associated with increasing bacterial titers. However, it remained unclear
whether these effects were directly mediated by *E. coli* (CP9) or were a result of the ensuing host response. *E. coli* effects on S/L surfactant aggregate after cycling in vitro. The increase found in the S/L ratio after *E. coli* challenge in the rat pneumonia model may be caused by bacterial factors, host factors, or a combination of both. To help determine whether a direct bacterial effect on surfactant aggregate conversion might be involved, the *E. coli* strain CP9 was mixed in vitro with an extracted natural surfactant preparation (CLSE), and the S/L ratio was studied after repetitive cycling for 0, 12, and 24 h (Table 2, Fig. 6). A significant bacteria-associated conversion of CLSE from large to small aggregates was found to occur at cycling times of both 12 and 24 h. Conversion to small aggregates is increased when it was mixed with CP9 bacteria and cycled in vitro for 12 or 24 h. Data represent means + SE (see Table 2 for number of each group). *P < 0.05 compared with bacteria-free control at respective time point.

times (24 h compared with 12 h). These findings are consistent with one or more bacterially derived factors playing a role in the increased S/L ratio in BAL in the gram-negative rat pneumonia model.

**DISCUSSION**

The results of this study show that significant alterations in the amount and aggregation of pulmonary surfactant in lavage are present in an in vivo rat model of gram-negative pneumonia. Extracellular pulmonary surfactant in BAL based on total phospholipid and surfactant apoprotein content (SP-A, SP-B, and SP-D) was increased at 2 and 6 h after tracheal inoculation with an extraintestinal strain of *E. coli* (CP9) (Table 1, Figs. 1 and 3). Increased levels of total BAL phospholipid were highly correlated with the extent of alveolar-capillary injury reflected in increasing levels of total BAL protein (Fig. 1). In addition, the S/L ratio in BAL was significantly increased in infected rats in which a threshold of lung injury was reached (total BAL protein >50 mg) (Fig. 2). This indicates that conversion of large to small aggregates, a marker inversely proportional to surfactant function, increased in injured animals. Measurements of pulmonary P-V mechanics show reduced lung volumes and compliance in rats with total BAL protein >50 mg, consistent with reduced lung surfactant activity. Arterial oxygenation was also reduced in infected rats above this threshold of pulmonary damage relative to control rats or infected rats with total BAL protein <50 mg (Table 1).

Levels of SP-A, SP-B, and SP-D in large surfactant aggregates in lavage were increased by 179–295% at 2 h postinfection and 291–444% at 6 h postinfection relative to control (Fig. 3). In small aggregates, SP-A and SP-D were increased by 268–295% at 2 h and 311–373% at 6 h postinfection relative to control. Levels of SP-C in large aggregates were increased by 172% at 2 h but decreased to 36% relative to controls at 6 h. However, in contrast to SP-A, SP-B, and SP-D, these differences were not statistically significant. The relevance, if any, of these less-pronounced changes in SP-C is unclear. Although the concentrations of surfactant apoproteins and phospholipids in lavage were elevated (except for SP-C at 6 h) and SP-A is known to increase the resistance of lung surfactant to protein inhibition, surfactant activity as reflected in P-V mechanical measurements was still abnormal (Fig. 4). This implies that detrimental effects on surfactant activity by se-
rum components and/or related increases in the S/L aggregate ratio in lavage were not overcome by the higher surfactant phospholipid and apoprotein levels. Increases in SP-A, SP-B, and SP-D in response to pulmonary infection with E. coli could be due to release from damaged tissue. However, given the known host-defense roles of SP-A and SP-D (33, 55), a physiological increase in response to E. coli is another possibility. Interestingly, all three of the surfactant proteins that were consistently increased in lavage in the rat model of gram-negative pneumonia are synthesized not only in type II cells but also in bronchiolar epithelial cells such as Clara cells (for review see Refs. 15, 33, 34). The present study did not address specific cellular production or message expression for surfactant proteins.

Surfactant abnormalities have been demonstrated in several animal models of ARDS-related acute lung injury (for review see Refs. 19, 25, 29, 34). A decreased content of large surfactant aggregates in lavage has also been reported in animals and humans with acute lung injury or ARDS (9, 14, 16, 19, 28, 38, 49, 50). In vitro studies here suggest that alterations in the surfactant aggregate ratio in BAL from infected rats might be due in part to the actions of one or more bacterially derived factors. When CLSE was mixed with CP9 in vitro, an increase in the S/L aggregate ratio was found after 12 and 24 h of repetitive cycling compared with surfactant not exposed to bacteria (Table 2, Fig. 6). CLSE does not contain SP-A or SP-D, which may affect the conversion of large-to-small aggregate fractions during in vitro cycling. Nonetheless, this in vitro observation is consistent with the in vivo findings of similar S/L ratio changes in lavaged surfactant in severely infected animals. However, the correlation of increased S/L ratios in surfactant with high BAL protein in injured rats (Fig. 2) suggests that aggregate-related changes were also influenced by host factors. Possible contributors include interactions of alveolar surfactant with substances in edema or present in the lungs in the inflammatory response. A variety of endogenous substances such as plasma proteins, cell membrane lipids, free fatty acids, proteases, and phospholipases are able to inhibit lung surfactant activity in vitro (17, 20–23, 37, 52). Specific mechanistic factors contributing to surfactant dysfunction and aggregate changes in gram-negative pneumonia need to be addressed in detail in future studies.

Several studies on lavaged surfactant from humans and animals with bacterial pneumonia have demonstrated decreases as opposed to increases in lavage phospholipids and/or surfactant proteins (2, 3, 14, 27, 49). Pulmonary instillation of endotoxin has also been reported to decrease BAL phospholipid levels in animal models (46, 48), whereas phospholipid in BAL from rats with E. coli pneumonia has been reported to be slightly higher, but not significantly different, from controls by Song et al. (47). Our work found lavage phospholipid levels to be elevated in animals above a threshold injury marker of ≥50 mg total protein in BAL. This protein level is an arbitrary figure that is dependent on the lavage protocol used. A uniform 50-ml total volume of normal saline was employed in lavage in all animals studied (five repetitive doses of 10 ml instilled by a syringe pump attached to a tracheal cannula). Recovered volumes of lavage fluid were similar for the three groups of rats analyzed.

One potentially important factor in assessing surfactant changes in BAL in gram-negative pneumonia is the specific infectious organism causing injury. The extraintestinal pathogenic strain of E. coli (CP9) used as the pathogen here was chosen for several reasons. E. coli is one of the commonly isolated agents in nosocomial gram-negative pneumonia (32). However, not all strains of E. coli are equivalent from a pathogenic perspective. Only extraintestinal pathogenic strains possess the relevant virulence traits that enable them to cause gram-negative pneumonia. Thus the use of laboratory, commensal, and intestinal pathogenic strains of E. coli for these studies would be inappropriate (44). As shown previously (42), the rat model of gram-negative pneumonia employed in this study possesses the critical features of human infection. Of the animal studies on surfactant changes in gram-negative pneumonia noted above, only Song et al. (47) used E. coli organisms, and no details on the specific strain or its virulence have been reported. Likewise, the use of endotoxin administration to simulate bacterial pneumonia is not directly comparable with infection with live organisms.

Another important factor in lavage assessments in gram-negative pneumonia concerns the timing of measurements relative to the initiation of injury and the degree of lung injury found in that model. Even for a given organism and strain, the extent of bacterial growth and the level of associated pulmonary leak has a direct impact on the magnitude of lung injury (Fig. 5). Surfactant was harvested by lavage within 6 h postinfection in the present study, but was obtained at 24 h in the study of Vanderzwan et al. (49) and at 48–72 h by Song et al. (47). The situation is even more complex in human studies, where it is impossible to be sure of the exact duration of infection before BAL. However, most if not all patients reported in the literature have certainly been infected for ≥24 h (14, 18, 40). The heterologous mix of organisms and levels of infection that inevitably occur in human studies greatly complicate data comparisons. The effects of antibiotics, mechanical ventilation, and higher-than-ambient concentrations of inspired oxygen are also potential confounding variables. The use of specific bacterial strains of known virulence and titer, along with well-defined assessments over time, will facilitate data comparisons in future animal model studies.

Some combination of increased production, increased secretion, or decreased turnover can be postulated to account for the increased lavage phospholipid and surfactant protein levels observed here. The increase in the ratio of S/L surfactant aggregates in lavage from infected rats also is consistent with changes in surfactant processing or metabolism in vivo. Interactions with bacterially derived lipopolysaccharide or other bacterial factors could directly affect the reuptake,
synthesis, or secretion of surfactant by type II pneumocytes. In addition, the rapid in vivo conversion of secreted surfactant to the inactive small aggregate form could have resulted in a secondary secretory signal to the type II pneumocyte. Extraintestinal pathogenic E. coli like CP9 have outer membrane proteases and phospholipases that are known to be produced by other pulmonary pathogenic bacteria (8, 36). As noted earlier, multiple host-derived substances, such as plasma proteins, cell membrane lipids, free fatty acids, reactive oxidants, proteases, and phospholipases, are known to inhibit surfactant activity, and they may also affect its metabolism or aggregate processing. The correlation of alveolar-capillary membrane injury (high BAL protein) with increased S/L aggregate ratio and phospholipid/apoprotein levels in lavage suggests that host factors as well as bacterial factors contribute to surfactant changes in E. coli pneumonia.

In summary, the findings here have several important implications. First, the demonstration of increased levels of total lavage phospholipid and SP-A, SP-B, and SP-D levels in E. coli pneumonia is novel and emphasizes the need for organism-specific studies at defined times during the progression of acute lung injury. Mechanistic information is currently lacking on changes in the metabolism and aggregate processing of surfactant during gram-negative pneumonia and needs investigation in future studies. Second, the demonstration that gram-negative pneumonia includes surfactant aggregate dysfunction that increases with the severity of lung injury (increased total BAL protein) is important in pathophysiological understanding. This is also true of our finding from in vitro cycling studies that one or more bacterially derived factors may contribute to large aggregate depletion in E. coli pneumonia. Despite limited success in the use of surfactant replacement outside of the neonatal arena, the potential clearly exists for this therapy in lung injury syndromes such as gram-negative pneumonia. Additional studies are warranted to establish specific mechanisms contributing to surfactant dysfunction and alteration in gram-negative pneumonia.

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