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Regional pulmonary inflammation in an endotoxemic ovine acute lung injury model

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Abstract

The regional distribution of inflammation during Acute Lung Injury (ALI) is not well known. In an ovine ALI model we studied regional alveolar inflammation, surfactant composition, and CT-derived regional specific volume change (sVol) and specific compliance (sC). 18 ventilated adult sheep received IV lipopolysaccharide (LPS) until severe ALI was achieved. Blood and bronchoalveolar lavage (BAL) samples from apical and basal lung regions were obtained at baseline and injury time points, for analysis of cytokines (IL-6, IL-1 β), BAL protein and surfactant composition. Whole lung CT images were obtained in 4 additional sheep. BAL protein and IL-1 were β significantly higher in injured apical vs. basal regions. No significant regional surfactant composition changes were observed. Baseline sVol and sC were lower in apex vs. base; ALI enhanced this cranio-caudal difference, reaching statistical significance only for sC. This study suggests that apical lung regions show greater inflammation than basal ones during IV LPS-induced ALI which may relate to differences in regional mechanical events.

Keywords

Lipopolysaccharide, LPS; endotoxin; acute lung injury (ALI); ARDS; inflammation; cytokines; functional CT imaging; specific compliance

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1. INTRODUCTION

Sepsis-induced acute lung injury (ALI), including its most severe form the acute respiratory distress syndrome (ARDS), is a common and frequently fatal disease triggered by a wide array of insults that lead to a severe refractory hypoxemia. Despite greater awareness and research efforts, mortality has stabilized around 30-40%, probably due to the complex and progressive pathophysiology and lack of effective therapies (Brun-Buisson et al., 2004; Rubenfeld et al., 2005). It is well known that inflammation is a central component of this complex pathophysiology. Inflammatory mediators (i.e. TNF- α , IL-6, and IL-1 β) have been widely studied in ALI, and have been found significantly increased in BAL fluid from septic ALI patients (Mokart et al., 2003; Pugin et al., 1996). Increases in plasma and bronchoalveolar lavage (BAL) levels of both IL-6 and IL-1 β have been identified as predictors of poor outcome in patients with septic or non-septic ALI (McClintock et al., 2008; Meduri et al., 1995; Parsons et al., 2005; Ware et al., 2010). Lung inflammation and resultant capillary leak and edema cause a decrease in lung compliance and increase in shunt fraction that compromises the ventilatory management of these patients. Lung strain triggers a local release of inflammatory mediators (Dos Santos and Slutsky, 2000; Halbertsma et al., 2010; Pinheiro de Oliveira et al., 2010; Simon et al., 2006) and impacts the release and effectiveness of alveolar surfactant (Hillman et al., 2011; Ito et al., 1997; Majumdar et al., 2012; Veldhuizen et al., 2002), potentially propagating the mechanical injury. In summary, mechanical ventilation is both lifesaving and an iatrogenic source of further alveolar inflammation and surfactant damage.

CT imaging has proven useful as a tool for exploring the pathophysiology of ALI. CT imaging techniques have increased our understanding of the diffuse and heterogeneous inflammatory process of the lung during ALI (Fernandez-Bustamante et al., 2009; Gattinoni et al., 2001; Kaczka et al., 2005), hypoxemia mechanisms (Easley et al., 2006), PEEP and recruitment maneuvers (Gattinoni et al., 2006; Vieira et al., 1998), and positioning (Galiatsou et al., 2006; Gattinoni et al., 1991). CT scans in patients and experimental ALI have demonstrated cranio-caudal (Gattinoni et al., 1986; Puybasset et al., 1998) and ventral-dorsal (Fernandez-Bustamante et al., 2009; Hedlund et al., 1985; Slutsky et al., 1984) gradients in aeration and recruitment, and a recent PET-CT study associated regional parenchymal stretch with regional inflammation (Bellani et al., 2011). However, to our knowledge no studies have explored whether regional differences in CT-derived measurements of regional lung mechanics reflect differences in underlying regional alveolar inflammation during septic ALI.

In this study we examined the cranio-caudal (apex vs. base) changes of: 1) inflammatory mediators (IL-6, IL-1 β) and surfactant content and composition in the bronchoalveolar lavage fluid; and 2) CT-derived parameters of regional aeration and lung mechanics (specific volume change, sVol, and specific compliance, sC), in a severe endotoxemic ALI ovine model. Our hypothesis was that CT-derived regional sVol and sC would reflect regional differences in pulmonary inflammation during endotoxemia and mechanical ventilation.

2. METHODS

LPS-induced severe ALI was induced in a total of 22 anesthetized sheep. In 18 sheep we studied the composition of plasma and bronchoalveolar lavage (BAL) fluid at the apex and the base (2 samples per region) before ("Baseline") and after the LPS-induced ALI ("Injury"). The other 4 sheep underwent exactly the same animal preparation and injury protocol, but instead of BAL we performed whole lung inspiratory and expiratory CT images at the same baseline and injury time points. CT images were analyzed for mean

aeration of the whole lung and of apex and base regions, and regional tidal specific volume change ($sVol$) and specific compliance (sC) (Fuld et al., 2008) were calculated as a measure of biomechanical stretch during the respiratory cycle. Finally, to study the potential effect of dilution from repeated BALs at the same location, repeated BALs were performed in 3 additional uninjured sheep at the same apex and base locations at baseline and after 3h of mechanical ventilation (BAL Control group). Figure 1 shows a schematic description of the experimental protocol in the 3 groups.

All studies were performed by the same team; the CT imaging studies were performed at the University of Iowa Comprehensive Lung Imaging Center (iCLIC) while all other studies were performed in the research laboratory at the Johns Hopkins University School of Medicine.

2.1 Animal preparation

The animal protocol was approved by both the Johns Hopkins University and the University of Iowa Institutional Animal Care and Use Committees. Adult farm bred sheep weighing 25-45 kg were sedated with intramuscular ketamine (30 mg/kg) and anesthetized with halothane by mask. Sheep were instrumented with percutaneous external jugular central venous and pulmonary artery catheters, a femoral arterial catheter was placed via cut down, and a tracheotomy performed. Subsequent anesthesia was maintained with pentobarbital (1-3 mg/kg IV every hour and when indicated) and pancuronium (0.1 mg/kg and 0.5-1 mg hourly IV). Animals were mechanically ventilated with the following parameters: tidal volume (V_T) 10 ml/kg, frequency titrated for a target end-tidal CO_2 partial pressure (P_{ETCO_2}) 30-40 mmHg, 5 cmH₂O positive end-expiratory pressure (PEEP), and inspired oxygen fraction (FiO_2) 1.0. Mechanical ventilation was performed with a Drager Evita 2 ventilator (Drager Medical, Germany). Oxygen saturation (SaO_2), P_{ETCO_2} , airway pressure (Paw), heart frequency (f_H), arterial blood pressure (BP), and pulmonary artery pressure (PAP) were continuously monitored. Arterial and mixed venous blood samples were periodically obtained during the experiment for assessment of gas exchange per protocol. An infusion of lactated ringers (14 ml/kg/hr) was given for maintenance fluid replacement after an initial bolus of 40 ml/kg. PA temperature was maintained at 36°C with radiant heat lamps. At the end of the experiment, all animals were euthanized with an extra dose of pentobarbital followed by a bolus of saturated KCl.

2.2 Acute lung injury (ALI) model

Arterial blood samples were analyzed before the induction of ALI ("Baseline" time point) to assure the adequate initial health status of the animal, considering unacceptable any subject with a baseline arterial oxygen partial pressure to inspired oxygen fraction ratio (PaO_2/FiO_2) less than 400.

For induction of ALI, *E.coli* lipopolysaccharide 055:B5 (Sigma L4005, Sigma Chemical, St. Louis, MO) was intravenously infused at 10 mcg/kg/h and stopped when venous admixture (Qs/Qt) 40% and PaO_2/FiO_2 200 were obtained. ALI was considered established when these endpoints persisted for 20 minutes ("Injury" time point). Based on pilot studies designed to promote a uniform distribution of LPS in the pulmonary circulation, all subjects were positioned prone for the first 3 hours of LPS infusion and turned supine from that moment on or when injury endpoints were reached. This LPS administration protocol was chosen to achieve greater homogeneity of ventral/dorsal LPS vascular distribution and consequent injury, but the position changes confound interpretation of ventral/dorsal regional analysis of the resulting injury.

We compared parameters of regional (apex vs. base) lung inflammation and CT-derived lung aeration and lung mechanics before the induction of ALI (Baseline) and after ALI endpoints were reached (Injury).

2.3 Regional lung inflammation

Bronchoalveolar lavage (BAL) was performed in each sheep in the supine position at four lung locations: two in the left apex (gravitationally non-dependent and dependent segments) and two in the right base (gravitationally non-dependent and dependent segments), at baseline and injury time points. Each BAL was performed with 20mL 0.154M NaCl through the wedged tip of a custom extra-long Olympus bronchoscope. Concentrations of total protein, total surfactant, large and small aggregates of surfactant, IL-6 and IL-1 β were analyzed in the recovered fluid as described below. Arterial blood samples were also obtained for measuring plasma IL-6 and IL-1 β levels.

After recording the total recovered BAL volume, aliquots were taken for inflammatory mediator (IL-1 β , IL-6) measurements. The rest of the BAL fluid was used for isolation of surfactant aggregates (Lewis et al., 1994). Briefly, samples were centrifuged at 150g for 10min to remove cell debris. The supernatant was separated, 1.5-3mL was aliquoted for assay of total surfactant (TS), and the remaining supernatant was spun at 40,000g for 15mins. The resultant pellet was resuspended in 1mL of 0.154M NaCl and frozen for analysis of large surfactant aggregates (LA), whereas the supernatant was reserved for measurement of small surfactant aggregates (SA). Aliquots of both sub-fractions, as well as the TS sample, were analyzed for phospholipid (PL), phosphorus, and protein assays.

Total protein analysis—Total protein in each recovered BAL sample was determined using the Micro-BCA protein assay kit on the TS samples following the manufacturer's instructions (Pierce Biotechnologies, Rockford, IL).

Cytokine analysis—IL-1 β and IL-6 levels were measured in the BAL and plasma samples. 1 mL aliquots of BAL and 1.5mL of blood were centrifuged at 2000 rpm for 10 minutes. The supernatant was aliquoted, flash frozen in dry ice, and stored at -80°C until IL-1 β and IL-6 enzyme-linked immunosorbent assays (ELISA) were performed. Ovine IL-1 β and IL-6 standards were purchased from the Animal Biotechnology Centre of the University of Melbourne. The mouse anti-ovine IL-6 (cat #MAB1004) and IL-1 β (cat # MAB 1001) monoclonal antibodies and the rabbit anti-ovine IL-6 (cat # AB 1889) and IL-1 β (cat # AB 1838) polyclonal antibodies were purchased from CHEMICON International.

Phospholipid analysis—Phospholipid analysis was performed in triplicate using the Bligh and Dyer (1) lipid extraction and modified Duck-Chong (2) phosphorous assay on aliquots from the TS, LA, and SA fractions. Briefly, samples were added to 1:2 methanol:chloroform in glass test tubes and the lipids were extracted in the chloroform phase after centrifugation. Magnesium nitrate (10% Wt/vol in methanol) was added, and the chloroform phase was evaporated under air in a heated water bath. The dried samples were ashed on an electric heating rack and allowed to cool. 1 mL of 1M HCl was added to each tube, covered with marbles, and heated for 15 min at 95°C on a heating block. Ninety-six-well plates were loaded with 66 μL of standard and each sample tube in triplicate before 134 μL of dye (3:1 of 4.2% molybdate in 4.5M HCl and 0.3% Wt/vol malachite green in water) was added. The absorbance was read at a wavelength of 650nm on the micro plate reader (Bio-Rad) and compared with phosphate standard.

2.4 CT-derived regional aeration and lung mechanics—4 sheep underwent exactly the same animal preparation and ALI model as described above, except without placement

of a pulmonary artery catheter to avoid radiological interference. Instead of obtaining BAL samples at baseline and injury time points, we acquired inspiratory and expiratory-gated CT images (120 kV, 250 or 180 mAS, and 1.5 or 1.2 mm slice thickness) of the entire lung in the supine position on a Siemens Somatom Sensation 16 or 64-slice scanner. Imaging was controlled by a computer running LabView software (National Instruments, Austin, TX) that allowed synchronization of the CT image acquisition with mechanical ventilation. By incrementing the table position half of the Z-axis image aperture after each inspiratory or expiratory acquisition, whole lung images at both lung volumes were compiled without stopping ventilation (Fernandez-Bustamante et al., 2009).

Volume CT images were analyzed using the Pulmonary Analysis Software Suite (PASS, University of Iowa Division of Physiologic Imaging) (Hoffman et al., 2003). PASS allows for semi-automated, supervised segmentation of the lung from surrounding structures and provides calculations of total volume, air volume, and tissue volume included in specified regions of interest (ROI), along with longitudinal and vertical volume and density profiles. ROIs can be defined to include the entire lung or selected sections of it. Whole lung and anatomically matched regions of the apex and base were studied in all sheep at end-inspiration and end-expiration. Corresponding inspiratory and expiratory apex and base regions were determined by matching distinct anatomic markings (ie., airways and blood vessels, see Figure 2) found in all image sets for a given animal and including all slices from that slice to the most cephalic (for the apex region) or the most caudal (for the base region) (Easley et al., 2009). Total, air, and tissue volumes were calculated with PASS for the whole lung and the selected apex and base segments in all animals. Mean lung density at end-inspiration and end-expiration of these selected regions was used for calculating the specific volume change (sVol) and specific compliance (sC) of the regions as an estimate of the changes in lung stretch and underlying tissue characteristics. The following formulas were used (Simon, 2000):

$$\begin{aligned} \text{sVol} &= (1000 * (D_{ei} - D_{ee})) / (D_{ee} * (D_{ei} + 1000)) \\ \text{sC} &= \text{sVol} / \Delta P \end{aligned}$$

where sVol is specific volume change, sC is specific compliance, D_{ee} and D_{ei} the end-expiratory and end-inspiratory mean density of the selected region in Hounsfield units (HU), and ΔP the tidal change in the whole lung inflation pressure (Plateau pressure - PEEP, cmH₂O). Note that estimating sVol from the density change is less sensitive to registration errors than using the measured volumes of these lung sub-regions (Fuld et al., 2008).

2.5 Statistical analysis

All variables are shown as mean $\pm$ SEM unless otherwise indicated. BAL values from different regions were pooled together to show the total change of inflammatory mediators and surfactant components. Continuous variables were compared using Student's t-test, (paired t-test when appropriate, independent t-test for the rest) or ANOVA with Bonferroni post-hoc test (regional analysis). By convention, an α level of $p < 0.05$ was considered to be statistically significant. Statistical analysis was performed with SPSS 18.0 software.

3. RESULTS

3.1 Physiology

All sheep had comparable weight and baseline hemodynamic and respiratory parameters (Table 1). From baseline to injury there was a significant decrease in cardiac output, systemic blood pressure, arterial pH and oxygenation, P_{ETCO_2} and lung compliance, while

pulmonary artery pressures, shunt, respiratory dead space (measured by P_{ETCO_2} - $PaCO_2/PaCO_2$) and airway pressures (peak and plateau) significantly increased (Table 1). The LPS dose needed to achieve the severe ALI criteria was highly variable, with an average of 32.6 ± 10.9 mcg/kg, ranging from 11.5 mcg/kg to 44.5 mcg/kg. 6 animals (33.3%) met the required criteria within the first 3h and, therefore, received the whole LPS dose in the prone position. The other 12 animals (66.6%) received the last part of the needed LPS in the supine position.

No significant differences in oxygenation, respiratory, or hemodynamic parameters were observed in the animals undergoing whole lung CT images compared to the BAL animals. LPS dose and administered IV fluids were also comparable to those undergoing BALs.

BAL Control animals (undergoing BALs with no LPS administration but otherwise equal preparation, positioning and ventilatory management) had comparable physiology at baseline, with the following exceptions: these 3 animals had greater weights (42.4 ± 1.5 kg *vs.* 32.6 ± 4.0 kg, $p=0.001$) and therefore received greater tidal volumes (426.7 ± 15.3 mL *vs.* 338.1 ± 49.1 mL, $p=0.007$), and baseline temperatures were higher ($40.5 \pm 0.9^\circ\text{C}$ *vs.* $39.4 \pm 0.5^\circ\text{C}$ in study animals, $p=0.008$). No significant physiological changes were observed after 3h of mechanical ventilation and repeated BALs.

3.2 Regional lung inflammation

The BAL fluid recovery (20mL instilled at each of the four lung locations per animal) was significantly reduced at injury (7.1 ± 2.4 mL) compared to baseline (11.0 ± 5.7 mL) ($p=0.001$). Concentrations of BAL protein, and BAL and plasma IL-6 and IL-1 β significantly increased with the injury (Figure 3). IL-6 reached levels much greater in plasma than those in the BAL (note scale difference in Figure 3b graphs), while plasma and BAL IL-1 β were of similar magnitude (Figure 3c). In the apex *vs.* base analysis (Figure 4), BAL IL-6 increased homogeneously in both regions, while protein and IL-1 β levels were only significantly increased at the apex. At injury, BAL protein and IL-1 β cranio-caudal differences reached statistical significance.

In the BAL Control group no significant changes in BAL protein, BAL IL-1 β , plasma IL-6, or plasma IL-1 β were observed from baseline to 3h later (data not shown). There was, however, a significant increase in BAL IL-6 after 3 hours of mechanical ventilation.

3.3 Regional surfactant content and composition

A significant decrease of total surfactant content was observed in the recovered BAL fluid, from 6.8 ± 0.6 mcg PO_4 /mL at baseline to 4.0 ± 0.5 mcg PO_4 /mL at injury ($p=0.001$). Both surfactant components, large aggregates (LA) and small aggregates (SA), decreased significantly with endotoxemia. A greater decrease in SA than LA resulted in an increase in the LA/SA ratio (2.9 ± 0.3 at injury compared to 1.8 ± 0.5 at baseline), but this increase did not reach statistical significance ($p=0.054$). No regional differences (apex *vs.* base) were observed.

In the BAL Control group total surfactant content tended to increase over time (from 5.8 ± 0.9 at baseline to 8.0 ± 1.3 3h later), as well as LA/SA ratio (0.9 ± 0.2 to 1.4 ± 0.2 , respectively). None of these differences reached statistical significance. No regional differences were observed.

3.4 Regional CT imaging analysis

The whole lung image analysis showed an overall decrease in air volume (20%) and air fraction (from around 63% to 55%) with injury, partially replaced by an increase in tissue

volume (~10%) (Table 2), resulting in a modest decrease in total lung volume of about 9%. The apex presented greater changes than the base in air, tissue and total volumes.

At the apex, the end-inspiratory and end-expiratory aeration decreased to a similar degree (~15.5%) from baseline to injury (Table 2) and therefore sVol was unchanged. At the base, end-expiratory aeration showed a slightly greater decrease than end-inspiratory aeration from baseline to injury (9.6% compared to 6.4%, respectively). The base sVol therefore increased at injury, but differences in the base sVol from baseline to injury, or between apex and base sVol at baseline or injury, did not reach statistical significance (Figure 5a).

Baseline specific compliance (sC) was lower in the apical regions than the basal ones but did not reach statistical significance. This was consistent with the greater starting degree of parenchymal expansion of the apex (baseline apex air fraction $75.6 \pm 4.1\%$ vs. $58.7 \pm 3.6\%$ for base). sC tended to decrease in all anatomically-matched regions from baseline to injury, although this decrease was not statistically significant. The slightly greater decrease in sC at the apex compared to the sC reduction at the base enhanced the baseline sC cranio-caudal difference, making the apex significantly less compliant than the base at injury (Figure 5b).

4. DISCUSSION

In this study we explored whether CT-derived measurements of regional lung mechanics would reflect regional pulmonary inflammation as measured by localized BAL. In a severe ovine LPS-induced ALI model we observed increased cranio-caudal (apex vs. base) differences in bronchoalveolar lavage levels of protein and IL-1 β that paralleled an increase in apex-base CT-derived specific compliance (sC) differences.

These results suggest that the apex experiences a greater alveolar inflammation than the base in a sepsis-like lung injury model that may be related to its regional mechanical experience.

The severe LPS-induced ovine ALI model used in this study has been characterized in a previous study (Fernandez-Bustamante et al., 2009). The physiologic results and wide range of LPS dose needed to reach injury criteria, with the associated animal positions (prone for 3h, supine if needed afterwards), were comparable to our previous findings and did not seem to affect the following inflammation characterization.

As expected, endotoxemia increased IL-6 and IL-1 β in plasma and BAL (Headley et al., 1997; Lopez-Aguilar et al., 2010; Simons et al., 1996). IL-6 levels were 500-fold higher in plasma than in the BAL with no regional differences in BAL levels. This extremely high IL-6 plasma/BAL ratio probably reflects the systemic inflammatory reaction secondary to intravenous endotoxemia and, taken with the much lower and homogeneous regional BAL IL-6 levels, may suggest an extra-pulmonary origin of the BAL IL-6. In contrast, we did observe regional differences in the BAL concentration of protein and IL-1 β . Apical BAL protein and IL-1 β concentrations were significantly greater, compared to the basal values, after injury. Unlike IL-6, plasma and BAL concentrations of IL-1 β were of comparable magnitude, and together with the similar regional differences seen in BAL protein levels, may suggest that BAL IL-1 β is somehow related to the magnitude of alveolar inflammation and capillary damage. The systemic vs. pulmonary origin of BAL IL-6 and IL-1 β , however, would need to be confirmed since multiple mechanisms may be involved (capillary leakage, active transport).

In our model, regional inflammation may have been influenced by the regional effect of systemic endotoxemia and by the ventilatory strain with moderate tidal volumes (10mL/kg) (Altemeier et al., 2004). Whether this greater inflammation at the apex, compared to the base, is related to an increased LPS exposure from apical pulmonary perfusion during prone

administration or to the effect of apical over-distention is difficult to know. In case of greater apical perfusion, however, one could argue that if the IL-6 originated systemically then the BAL levels would be expected to rise proportionally to regional perfusion, thus suggesting perfusion differences cannot account for the cranio-caudal differences observed in IL-1 β . In our previous study with this injury model, perfusion of the apex and base were not significantly different after injury (Fernandez-Bustamante et al., 2009). Previous findings also suggest a relationship between pulmonary IL-1 β and lung overdistention. Copland *et al* (Copland et al., 2003) found an increase in IL-1 β RNA and intracellular protein in the lungs of adult rats submitted to high (25mL/kg) tidal volume ventilation for 30 and 90min respectively. Caruso *et al* (Caruso et al., 2003) observed greater IL-1 β levels in nondependent lung regions, compared to dependent regions, in rats ventilated with either 6 or 24mL/kg for 1h. In our study, no differences were detected when dependent and nondependent BAL samples were analyzed independently. The position changes in our experimental protocol (prone position for a more homogeneous LPS distribution in the pulmonary circulation, then supine for bronchoscopy or CT imaging) may have played a role in the vertical homogeneity of inflammatory mediators. Caruso's vertical IL-1 β findings in a non-septic model may suggest that IL-1 β could be related to the degree of regional strain and epithelial damage. It is accepted that nondependent and apical alveoli are of a larger volume but on a less compliant portion of their pressure-volume curve than dependent and basal alveoli (Marcucci et al., 2001; Treppo et al., 1997). Although exposed to less ventilation than nondependent/basal regions, Caruso's results suggest that that IL-1 β release may be more related to the average than cyclic lung strain. Our regional specific compliance results, with the sepsis-like lung injury amplifying the cranio-caudal sC distribution and showing significantly lower sC at the apex compared to the base, may support this mechanically-enhanced regional inflammation.

Steinberg et al (Steinberg et al., 1999) observed heterogeneity in total protein concentration (TP) and cell counts in BAL samples comparing the right middle lobe and the lingula of individual patients with ARDS, with 43% of patients exhibiting 2-fold or greater differences in TP between sides. The comparison with our findings results is challenging since patients included in that study were of heterogeneous nature (patients with or at risk of ARDS secondary to severe sepsis or severe trauma).

We observed in our ALI model a decrease of total surfactant content. The apparent discrepancy in our injury model between the decreased surfactant content at the apex and the concomitant increase in apical BAL protein was unexpected. Although not fully understood, different timing of capillary leakage and surfactant changes or different regional susceptibility to the BAL-related inflammation might have contributed in our findings. In terms of surfactant composition, we found an increase of the percentage of surfactant large (more active in surface tension reduction) aggregates (or increased LA/SA ratio), due to a larger decrease in SA than LA. These results are comparable to those observed 24h after cecal ligation and puncture (CLP) in septic rats with and without lung injury (Malloy et al., 1997). However, a decrease in the LA/SA ratio has been observed after 48h in a CLP-induced septic sheep model (Lewis et al., 1994), and also in humans with systemic inflammatory response syndrome (SIRS) or sepsis during the first 7 postoperative days (Raymondos et al., 1999). The different findings could be related to the time course of surfactant changes after systemic endotoxemia combined with mechanical ventilation (Ikegami and Jobe, 2002; Jobe and Ikegami, 2001). Our results suggest an early, more pronounced reduction in small (less active) aggregates (early increase in LA/SA), and a slower fall in large (more active) aggregates (late decrease in LA/SA ratio). This time course is consistent with surfactant composition findings in mice studied at different time points after CLP (Malloy et al., 2002). In this study, Malloy et al. found that the concentration of SA was decreased early, reaching statistical significance 6h after CLP compared to the sham

group, while the amount of LA did not significantly change over the 18h after CLP. In any case, our results can be considered an effect of endotoxemia but not to mechanical ventilation or the dilution effect from repeated BALs, since our control animals (that did not receive LPS) presented a non-significant increase in total surfactant content and LA concentration, with a slight decrease in SA concentration and increased LA/SA ratio. Although none of the described changes in this control group reached statistical significance, similar results have been observed in previous studies as an effect of mechanical ventilation (Truscott et al., 2010).

The decrease in aeration of whole lung CT images was comparable to our previous results in this endotoxemic ALI ovine model (Fernandez-Bustamante et al., 2009). The cranio-caudal aeration gradient has been described before (Gattinoni et al., 1986; Puybasset et al., 1998). Instead of density-based aeration, in this study we focused on specific compliance (sC), which better reflects the underlying regional tissue properties without the bias of the regional size and changing global tidal volume, although it is limited by assuming a uniform distribution of intrapleural pressure (Simon, 2000). sC is an ideal tool for comparing lung regions, such as apex and base, with known different radiological density (aeration) and inflation degree (regional tidal volume). Accurate measurements of regional intrapleural pressure would obviously be ideal but is not feasible. In our study, sC showed non-significant lower regional compliance at the apex than the base in healthy animals, and a tendency for sC to decrease in both regions with LPS injury. This is not surprising, since a reduction in whole lung compliance is one of the most typical features of ARDS (Artigas et al., 1998; Lamy et al., 1976). The enhancement in the cranio-caudal difference of sC with the injury matched the distribution of alveolar protein and IL-1 β .

CT imaging also provides information about both local volume strain (sVol, change in volume/initial volume) and regional distension (density in HU or air fraction AF). The apex regions are more distended at baseline (end-expiratory AF 76% vs 59% for base, Table 2) and undergo a volume strain of about 10% at baseline compared to almost 30% for the base (see baseline sVol change of 0.10 in apex compared to 0.30 in base, Figure 5a). This difference may be due to the lower compliance of the distended apex parenchyma, the restrictive nature of the apical chest wall (which would result in higher apical intrapleural pressures), or most likely both. After injury, both the apex and base are less distended (end-expiratory AF 64% vs. 53%, respectively). The sVol at the apex does not change after injury, implying the regional volume change has fallen in proportion to this fall in end-expiratory volume, whereas the sVol at the base shows an (albeit statistically non-significant) increase, implying the regional tidal volume has increased or decreased proportionately less than the end-expiratory volume. The end-inspiratory or maximal distension is lower in both regions after injury than before, and neither region would be classified as overdistended using the usual criteria (end-inspiratory density <900 HU or AF>90%) (Vieira et al., 1998).

Since the BAL data showed that greater inflammation and protein leak evolved in the apex, we could speculate that one should look at the lung mechanical changes predisposing to injury (eg., baseline) rather than as a consequence of injury. In that context, it was unexpected that, in the presence of circulating LPS, the apex would experience a greater degree of injury because there was lesser predisposing baseline volutrauma (regional volume strain of 10% compared to 30% in the base) and less atelectrauma (end-expiratory AF of 76% vs. AF 59% in the base). It would be interesting to examine whether intraregional mechanical heterogeneity and dependent/non-dependent differences might further influence the development of regional inflammation and eventual injury.

There are several potential pitfalls in our study: although this large animal model of sepsis-related ALI shares multiple aspects with the human ALI/ARDS syndrome (hemodynamic, respiratory mechanics, etc), other features may not be comparable (presence of pulmonary intravascular macrophages, acuity) (Matute-Bello et al., 2008; Winkler, 1989). Also, we used moderate tidal volumes and high FiO_2 , in an attempt to maximize the inflammatory signal, and this may limit the relevance when protective ventilation is used in patients. We used airway pressures and, thus, our measurements reflect respiratory system mechanics; while regional intrapleural pressures would better identify regional changes in lung mechanics and parenchymal properties, we at least would not expect chest wall properties or the distribution of pleural pressure to change within an animal over the course of an experiment, but this important limitation suggests caution in interpreting our results. In this study, the baseline shunt fraction was surprisingly elevated (~22%) but comparable with our previous study (Fernandez-Bustamante et al., 2009). Since their oxygenation fulfilled the required $\text{PaO}_2/\text{FiO}_2$ 400 criteria, they were included in the study, but could have affected the observed inflammatory findings. Finally, the small number of subjects in the imaging and BAL control groups may have influenced our results and should be further confirmed.

In summary, we compared regional CT-derived measurements of lung aeration and lung mechanics with alveolar inflammatory mediators and surfactant composition. Our findings of greater levels of alveolar protein and IL-1 β along with changes in CT-derived specific compliance, specific volume change, and aeration in the apical regions, compared to the bases, in a LPS-induced ALI model suggest that lung regional mechanical events impact the development of regional inflammation.

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HIGHLIGHTS

- We study regional inflammation and CT-derived specific compliance in an ALI model.
- IV LPS significantly increases alveolar and plasma cytokines.
- Lung apex shows greater alveolar protein and IL-1 β and lower specific compliance.

Figure 1.
Schematic description of experimental groups.

Figure 2. Representative CT slices with landmarks used for apex and base ROIs selection. The Apex and Base ROIs were comprised of all lung slices from the matched index slice (left) to the most cranial or most caudal extent of the images, respectively (right, in coronal section).

Figure 3.

Effect of endotoxemia on the concentration of protein (Figure 3.a), IL-6 (Figure 3.b) and IL-1 β (Figure 3.c) in the bronchoalveolar lavage (BAL) and blood samples. All samples from different regions and animals were pooled together for this whole lung analysis. Note the difference in BAL and plasma IL-6 concentrations, but the similarity in IL- β BAL/plasma levels.

Figure 4. Regional analysis (apex vs. base) of BAL protein (Figure 4.a), IL-6 (Figure 4.b) and IL-1 β (Figure 4.c) concentrations. 2 BAL samples from each region (2 apical, 2 basal) per animal were pooled together for this regional analysis. IL-6 levels significantly increased in both apex and base with the injury, while protein and IL-1 β concentrations increased at the apex but not at the base. At injury time point, apical levels of protein and IL-1 β were significantly greater than the basal ones.

Figure 5.

Representation of regional sVol (Figure 5.a) and sC (Figure 5.b), calculated as described in the Methods section. Baseline sVol and sC were lower at the apex than at the base. With the injury, sVol increased at the base and was unchanged at the apex. sC slightly decreased with the injury in both regions. The cranio-caudal difference in sC was statistically significant at injury ($p=0.039$). None of the other sC comparisons (apex vs. base at baseline, baseline vs. injury in both regions) or any sVol comparisons were significantly different.

Table 1

Demographics and physiology data from the 18 animals that underwent LPS-induced ALI and bronchoalveolar lavages (BAL). Data are presented as Mn±SD.

Number of subjects (Total)	18		
Weight (kg)	32.6 ± 4.0		
Total dose of LPS (mcg/kg)	32.1 ± 10.9		
IV fluids (mL/kg)	107.3±30.5		
Mechanical ventilation duration (min)	232.8±65.3		
	Baseline	Injury	p level
Temperature (C)	39.4±0.5	38.7±3.1	0.460
Heart frequency (b/min)	138.8±27.3	133.5±32.3	0.651
Mean arterial pressure (mmHg)	116.5±10.0	82.1±15.8	0.001
Mean pulmonary artery pressure (mmHg)	14.2±5.4	26.7±5.7	0.001
Pulmonary artery occlusion pressure (mmHg)	7.5±4.9	8.6±4.6	0.449
Central venous pressure (mmHg)	6.4±5.5	7.5±4.6	0.580
Cardiac output (L/min)	5.9±0.9	4.5±1.4	0.003
PaO ₂ (mmHg) (FiO ₂ 100%)	458.0±32.1	72.4±27.9	0.001
Pa _{co2} (mmHg)	39.8±7.6	46.9±10.2	0.024
pH	7.41±0.05	7.27±0.09	0.001
Qs/Qt (%)	22.3±4.5	60.1±12.3	0.001
Exhaled tidal volume (mL)	338.1±49.1	334.7±47.8	0.838
Respiratory frequency (breaths/min)	19.6±1.4	19.1±2.3	0.521
P _{ETco2} (mmHg)	38.8±6.0	32.3±6.7	0.004
Peak airway pressure (cmH ₂ O)	17.9±3.8	35.1±5.4	0.001
Plateau airway pressure (cmH ₂ O)	12.6±2.3	25.3±8.1	0.001
Respiratory system compliance (ml/cmH ₂ O)	47.1±11.3	19.6±9.4	0.001

(IV=Intravenous; LPS=lipopolysaccharide; PaO₂=arterial O₂ partial pressure; PaCO₂=arterial CO₂ partial pressure; Qs/Qt=shunt fraction; P_{ETco2}= End-tidal CO₂ partial pressure)

Table 2

Regional aeration and specific compliance (sC) of apex and base at baseline and injury time points. 4 sheep underwent the same experimental endotoxemic ALI protocol except the bronchoalveolar lavages (BAL). Whole lung end-inspiratory and end-expiratory CT images were obtained at baseline and injury time points. Anatomically matched regions of apex and base were selected. Table shows the air, tissue and total volumes of the whole lung and the selected regions at end-expiration and end-inspiration (data shown as $\text{Mean} \pm \text{SEM}$).

	Baseline			Injury		
	Whole lung	Apex	Base	Whole lung	Apex	Base
Air volume	1,297.2±85.			1,035.6±128		
ee (mL)	7	185.2±14.3	603.2±37.7	.2	119.4±29.6	520.1±41.5
(% change)				-21.1±5.0	-35.3±16.2	-13.9±3.7
ei (mL)	1,643.8±81.	216.3±18.3	783.8±24.9	1,300.4±108	151.6±39.5	717.2±33.3
(% change)	2			.4*	-29.6±17.9	-8.4±4.1
				-21.3±2.8		
Tissue volume						
ee (mL)	760.2±56.8	61.7±10.5 f	425.9±37.1	835.6±50.6	71.1±23.2	458.9±35.3
(% change)				10.8±6.5	5.5±19.4	8.3±5.0
ei (mL)	802.6±61.0	64.5±10.7	439.9±33.0	918.7±56.9	76.9±20.0	480.3±35.6
(% change)				15.0±4.4	12.7±13.7	9.2±1.1
Total volume	2,057.4±130		1,029.0±65.	1,871.2±140		
ee (mL)	.8	246.9±24.4	1	.1	190.5±39.1	979.0±63.3
(% change)				-9.2±1.3	-24.5±13.7	-4.7±3.4
ei (mL)	2,446.3±130	280.8±28.5	1,223.7±48.	2,219.1±153	228.6±46.0	1,197.5±66.
(% change)	.9		5	.5	-19.6±14.7	4
				-9.5±1.9		-2.2±3.1
Air Fraction						
ee (%)	63.1±1.4	75.6±2.1	58.7±1.8	54.9±3.5	63.9±8.1	53.1±2.2
(% change)				-13.1±5.0	-15.8±9.7	-9.6±2.5
ei (%)	67.3±1.2	77.5±1.8	64.1±1.5	58.4±1.6*	65.8±7.4	60.0±0.9
(% change)				-13.1±2.2	-15.3±8.7	-6.4±1.2

(ee=end-expiratory; ei=end-inspiratory; % change = ((Injury Volume/Air Fraction - Baseline Volume/Air Fraction) / Baseline Volume/Air Fraction) × 100

* p<0.050 between baseline and injury)