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Lung Contusion: Inflammatory Mechanisms and Interaction with Other Injuries

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Abstract

This article reviews current animal models and laboratory studies investigating the pathophysiology of lung contusion (LC), a common and severe condition in patients with blunt thoracic trauma. Emphasis is on studies elucidating cells, mediators, receptors and processes important in the innate pulmonary inflammatory response that contribute to LC injury. Surfactant dysfunction in the pathogenesis of LC is also discussed, as is the potential role of epithelial cell or neutrophil apoptosis. Studies examining combination injuries where LC is exacerbated by secondary insults like gastric aspiration in trauma patients are also noted. The need for continuing mechanism-based research to further clarify the pathophysiology of LC injury, and to define and test potential therapeutic interventions targeting specific aspects of inflammation or surfactant dysfunction to improve clinical outcomes in patients with LC, is also emphasized.

I. Introduction

Blunt chest trauma is involved in nearly one-third of acute trauma admissions to the hospital, and lung contusion (LC) is an independent risk factor for the development of acute lung injury (ALI), acute respiratory distress syndrome (ARDS), and ventilator-associated pneumonia (VAP) (8,35). The lung is also the second commonest organ involved in blast trauma-induced LC, which often has a peri-hilar distribution and carries a high risk of mortality (11). When LC injury leads to hypoxemia severe enough to meet the definition of ALI/ARDS, the prognostic and economic impacts are significant. These clinical syndromes continue to have very substantial overall mortality and morbidity despite significant advances in cardio-respiratory intensive care over the past several decades (38). In a 2004 study of trauma patients, the incremental hospital cost per patient with ALI or ARDS (\$36,713 or \$59,633, respectively) was much higher than for patients without ALI/ARDS (\$24,715) (54).

The pathophysiology of pulmonary contusion and blunt chest trauma includes inflammation, increased alveolocapillary permeability and pulmonary edema, ventilation/perfusion mismatching, increased intrapulmonary shunting, and a loss of compliance(9). A schematic showing selected pathophysiological aspects contributing to pulmonary contusion injury is given in Figure 1. Clinically, patients with pulmonary contusion display hypoxemia,

hypercarbia, and increased work of breathing of varying severity and duration (8), which are treated with supplemental oxygen and mechanical ventilation as indicated. There is frequently an inconsistent correlation between the volume of lung that is grossly contused in patients and the severity of clinical respiratory failure, indicating that cellular and sub-cellular injury and inflammation, as well as interactions with other insults occurring at or near the time of trauma, may be involved. The current review focuses on acute inflammatory mechanisms and the role of individual cell types in the evolution of LC injury in animal models. Surfactant dysfunction in LC injury is also discussed, along with potential interactions of contusion injury with secondary insults such as gastric aspiration and sepsis that commonly occur in trauma patients.

II. Animal models of lung contusion

Studies examining the pathophysiology and cellular/molecular mechanisms of pulmonary contusion require meaningful and reproducible animal models. Physiological models for LC injury have been developed in animal species ranging in size from rodents (rats and mice) (17–19,23,24,44–47,57) to larger species such as swine (10,16,29,36,40). Selected animal models of pulmonary contusion and their representative pathological characteristics relative to clinical features reported in retrospective studies in blunt chest trauma patients are summarized in Table 1.

Although large animal models of pulmonary contusion have important specific applications, they also can exhibit limitations that include high mortality, high cost, open chest trauma induction, the presence of penetrating as well as blunt trauma, and/or the existence of substantial concomitant cardiac trauma. Large animal models of LC are also somewhat limited in mechanistic investigations because of a lack of cell- and mediator-specific molecular probes and reagents, which are much more widely available for small animals like mice and rats. Pathophysiological studies in mice can also exploit the availability of transgenic models to probe specific gene-related effects in pulmonary contusion injury. Moreover, the use of small animal species in general requires substantially lower experimental expense than studies in large animal models. For all these reasons, a good deal of recent animal model research in the pulmonary contusion field has emphasized rodent models.

Over the course of the last five years, three reproducible and functional closed-chest rat and murine models of LC injury have been developed (e.g., (17,19,24,44–47)). The rat model of isolated bilateral LC injury developed by Raghavendran and co-workers (44–47) involves dropping a cylindrical weight at a well-defined energy impact level onto a mobile lexon platform positioned over the thorax to minimize associated cardiac trauma. The first murine model developed for LC injury involved a unilateral chest impact using an ultrasonic blast wave (24). Recently, Hoth and colleagues (17,19) have published results in another closed-chest murine model, where an electrical cortical impactor was used to deliver a target energy level producing a uniform contusion to the right lung. The development of these small closed-chest rodent models has facilitated studies elucidating the roles and importance of specific cells (e.g., neutrophils), mediators, toll-like receptors, and surfactant dysfunction in LC injury as described in following sections.

III. The acute inflammatory response in LC injury

Acute lung injury in both humans and animal models is characterized by an intense inflammatory response in the pulmonary parenchyma. The innate inflammatory response triggered by direct or indirect insults to the lungs involves the recruitment of blood leukocytes, the activation of tissue macrophages, and the production of a series of different mediators including cytokines, chemokines, oxygen radicals, arachidonic acid metabolites and components of the complement and coagulation cascades (42,59). Although many specifics of the inflammatory response in LC injury remain to be clarified, it is clear that physiological

dysfunction in this condition is related in part to the significant acute inflammation that is present (47). As noted in the legend to Figure 1, the consequences of pulmonary inflammation in the context of LC-induced traumatic tissue injury include endothelial and epithelial cell disruption and increased alveolocapillary membrane permeability. These abnormalities in turn lead to alveolar edema, surfactant dysfunction, ventilation-perfusion mismatching, decreased lung compliance and volumes, and ultimately refractory arterial hypoxemia. This section highlights the importance of the acute inflammatory response in LC injury, with specific emphasis on the contributions of selected individual cell types and inflammatory mediators in pathogenesis.

A. Histopathological course of LC

Using the rodent model of bilateral LC developed in our laboratory (47), a detailed histopathological evaluation of the lung tissue at 8 min, 4 hr, and 12 hr post-contusion revealed diffuse areas of intra-alveolar hemorrhage with disruption of alveoli, along with interstitial hemorrhagic injury that frequently involved peri-hilar areas and extended to the visceral surface of the pleura. At 24 hr post-contusion, atelectasis was pronounced, and there were increased numbers of leukocytes (predominantly neutrophils) within the alveoli and interstitium (Figure 2A). At 48 hr, neutrophilic infiltration continued to be prominent, and alveolar lining tissue was thickened with an increase in alveolar macrophage infiltration and cellular debris (Figure 2B) (47). Similar histological findings in LC injury have been reported by others (17). At 7 days post-contusion, evidence of fibrosis especially around the bronchioles was also observed (47). This latter cellular feature may be related conceptually to pathology observed in the early fibroproliferative stage of human ARDS (32).

B. Role of neutrophils in LC

The importance of neutrophils in various forms of lung injury is well-established. For example in acid aspiration-mediated lung injury, which is considered later in terms of interactions with LC injury, neutrophils are a major contributor to airway and alveolar epithelial injury (14, 22). Neutrophil-induced lung injury is frequently oxidant-mediated, and may also involve impaired alveolar fluid transport function (34). However, neutrophil accumulation within the lungs is not always associated with injury (e.g., oleic acid lung injury is not strongly neutrophil-dependent despite the presence of increased numbers of these cells in the lungs) (21). To investigate the specific importance of neutrophils in LC injury, rats were treated with intravenous Vinblastine four days prior to blunt trauma-induced contusion (47). Rats given Vinblastine were evaluated for injury at 4 and 24 hr post-contusion, and met a prospectively defined limit of <5 PMN's per 100 leukocytes in peripheral blood smears at these times. Vinblastine-treated neutropenic rats had similar levels of albumin in BAL at 4 hr post-contusion compared to non-neutropenic rats (Figure 3). However, closed chest quasistatic P-V curves showed that the total lung volume at 40 cm H₂O was greater in neutropenic rats than in undepleted rats at this 4 hr time point (47). Moreover, at 24 hr post-contusion, neutropenic rats had significantly reduced albumin concentrations in BAL compared to non-neutropenic injured rats (478 ± 154 µg/ml versus $3,601 \pm 564$ µg/ml, $p < 0.001$) (Figure 3).

The underlying mechanisms by which neutrophils contribute to increased LC injury severity are currently unclear. One potential mechanism relevant for neutrophils in other forms of acute pulmonary injury involves increased necrosis/apoptosis of cells in the alveolar epithelium (31,59). However, increased neutrophil accumulation in the lung parenchyma and airways following LC has recently been reported by Seitz et al (51) not to be associated with the increased apoptosis of type II cells that has been observed following blunt trauma (26). Further studies on the mechanism-based contributions of neutrophils to the pathogenesis and progression of LC injury are needed to define their role in this condition in more detail.

C. Role of the alveolar macrophages and peripheral blood monocytes in LC

Alveolar macrophages are considered to be primary scavengers of particulate matter that reaches the alveoli. Alveolar macrophages are also sentinel cells responsible for the recognition of antigens, and they have been implicated in the pathogenesis and/or resolution of many forms of acute pulmonary injury. For example, in sepsis-induced lung injury, the severity of protein leakage has been found to be dependent on the presence of functional alveolar macrophages and specifically on the production of inducible nitric oxide synthase by these cells (13). However, depletion of alveolar macrophages using liposome encapsulated clodronate does not prevent Fas-mediated lung injury in mice (4). The early leukocytic response in the first 24 hr following LC injury is predominantly neutrophilic, but it progresses to a largely monocytic response by 48 hr post-contusion (24,47). Although specific mechanisms of macrophage activation are not well understood in LC injury, there appears to be an augmented release of Th-2 cytokines by peripheral blood monocytes at 2 hrs post-contusion (41). In contrast, at 24 hr post-contusion, the release of cytokines from peritoneal macrophages, splenic macrophages, and splenocytes has been found to be significantly suppressed (41). Studies by Liener et al (26) suggest that increases in alveolar macrophage numbers at 48 hrs following thoracic trauma may be associated with increased type II cell apoptosis, suggesting a possible mechanistic role for the alveolar macrophage in the initiation or maintenance of LC injury (see following section). However, further studies on the specific timedependent mechanistic contributions of alveolar macrophages to LC injury are needed to clarify the roles and importance of these cells in pathophysiology more fully.

D. Apoptosis in LC

Over the past two decades, the importance of programmed cell death in the pathogenesis of ALI/ARDS and multiple organ failure has become well-recognized. Two major hypothesis that have been examined in animal models of lung injury and to a lesser degree in humans with ALI/ARDS have been categorized as the “neutrophil hypothesis” and the “epithelial hypothesis” (33). The former hypothesis suggests that apoptosis of neutrophils is the key feature in the resolution of inflammation, and that a persistent neutrophilic response will contribute to progressive inflammatory injury. The latter hypothesis suggests that alveolar epithelial injury and subsequent epithelial apoptosis is the hallmark of the pathogenesis of ALI/ARDS (33). In actuality, neutrophil-mediated and epithelial-mediated processes involved in lung injury are not mutually exclusive, and both increased alveolar epithelial apoptosis and decreased neutrophilic apoptosis may be important in the maintenance/progression of ALI/ARDS (31).

With regard to type II pneumocyte apoptosis in particular, studies in animals and humans have reported significant lung injury-induced apoptosis and dysfunction of this important lung cell type during acute inflammatory injury of various kinds (e.g., (2,26,51,56)). In terms of work directly relevant for LC injury, Leiner et al (26) have reported increased alveolar epithelial apoptosis following blunt chest trauma, and Seitz et al (51) have documented increased type II cell apoptosis at 48 hr following LC injury. In this latter study, reduced type II cell numbers in animals with blast wave-induced LC were associated with elevated levels of the apoptosis markers Caspase-3, Caspase-8, and Fas in the remaining population of these cells. These results suggested that the extrinsic Fas-Fas ligand pathway may be involved in type II cell apoptosis in LC injury (51). However the detailed mechanistic importance of type II cell apoptosis in the maintenance, progression, or resolution of LC injury is not fully-defined, and further work on this issue is needed in the future.

E. Toll-like receptors (TLRs) in LC

Because of its direct interface with the external atmosphere, the respiratory epithelium is a crucial dynamic system in the regulation of innate host-defense. In addition to pathogen

recognition, toll-like receptors in the alveolar epithelium take part in the activation of various cytokines and acute phase proteins (e.g., (6,20). Specific roles of TLR-2 and TLR-4 in the pathogenesis of LC have recently been highlighted in murine models of pulmonary contusion (17,19). A study investigating LC in wild-type and TLR2(−/−) knock out mice suggested that lung injury, neutrophil accumulation in the BAL and serum levels of KC were all significantly reduced at 3hr in TLR2(−/−) mice compared to wild type mice. However no difference in mortality, serum levels of IL-6, or ICAM-1 levels were observed between these two types of mice (17). Hoth et al (19) have also reported studies in TLR4(−/−) and intracellular adaptor protein knockout mice [MyD88(−/−) mice] showing that lung injury severity parameters were reduced in these animals in conjunction with diminished levels of pulmonary neutrophils and reduced serum levels of KC and IL-6. Thus, in contradiction to reported findings in hyperoxia-induced and bleomycin-induced lung injuries (20,67), a deficiency of TLR-4 appears to be protective in LC injury. At present, the mechanisms underlying the disparate effects of TLR receptors in these various types of lung injury have not been completely elucidated.

F. Inflammatory mediators (cytokines, chemokines) in LC injury

All forms of inflammatory lung injury depend on cell-based actions and interactions mediated via soluble mediators including cytokines and chemokines (e.g., (38,52,59,60) for review}. Cytokines involved in chemotaxis (i.e., chemokines) have a documented importance in the initiation, maintenance, and resolution of acute pulmonary injury from multiple causes (52, 60). In closed chest LC-induced injury in rats, levels of neutrophilic CXC chemokines [cytokine-induced neutrophil chemoattractant (CINC-1/GRO/CXCL1) and macrophage inflammatory protein - 2 (MIP-2)] peak in BAL at 24 hr post-contusion (47) (Figure 4). This post-contusion time point correlates with increased neutrophil accumulation in the lung parenchyma and airways, and with increased total lung MPO activity as a marker of neutrophil activation. The concentration of the monocytic CC chemokine macrophage chemoattractant protein - 1 (MCP-1/CCL2) is also found to be increased in BAL at 24 hr post-contusion, and remains elevated at 48 hr post-contusion (47) (Figure 4). Levels of all three of these chemokines return to baseline at 7 days post-contusion. Concentrations of IL-6 in BAL are found to be significantly elevated at 24 hr post-contusion, and return to baseline at 7 days. In addition, levels of the pro-inflammatory cytokine IL-1 β in BAL are elevated at 24–48 hr post-contusion, and similarly decrease to baseline at 7 days post-contusion (47). Similar findings have also been reported in an open-chest model of lung contusion (18).

In terms of sub-acute as opposed to acute pathology relevant for LC injury, Bronchiolitis Obliterans Organizing Pneumonia (BOOP) is characterized by the proliferation of granulation tissue within small airways and alveolar ducts (12). BOOP has been observed in rats at 7 days post-contusion (47), and has also been reported in other forms of sub-acute lung injury in humans (12,43). T-cells have been implicated as important in BOOP-associated fibrosis (28), and these immune cells may contribute to the pathology and resolution of isolated lung contusion. MCP-1 activation of cells via the CCR2 receptor (a MCP-1 receptor) has also been implicated in the pathogenesis of granulomatous disease and Bronchiolitis Obliterans in lung transplant patients (3). Studies by Roseau et al (49) indicate that BAL levels of MCP-1 from patients with ARDS correlated with lung injury scores at 7, 14 and 21 days after the onset of ARDS. Additionally the increased levels of MCP-1 at 7 days had a direct relationship with mortality (49). This area of mechanistic importance of MCP-1 in the initiation and maintenance of acute lung injury is actively being explored in our laboratory.

G. Type II cell injury and surfactant dysfunction in LC

Alveolar type II cells are the primary cell type responsible for lung surfactant production and metabolism, and are also highly-important in regulating pulmonary inflammation and host defense (66). The potential importance of alveolar type II cell apoptosis in the maintenance/

progression of LC injury has been detailed in an earlier section, and discussion in this section focuses on surfactant-related abnormalities. Abnormalities in surfactant composition, content, or activity have been well-documented in BAL from patients with a number of forms of ALI/ARDS (e.g., (7,15,37) for review). Most relevant for LC injury is the study of Aufmkolk et al (1), which reported some abnormalities in surfactant lipid composition in BAL from trauma patients. Abnormalities in lung surfactant composition or content in LC injury may in part reflect type II cell injury or dysfunction, but this has not been studied specifically to date. However, significant surfactant activity deficits associated with increased alveolar plasma protein concentrations and consistent with direct inhibitor-induced surfactant dysfunction following LC have been demonstrated (46).

The study of Raghavendran et al (46) reported functionally-important reductions in both the surface activity and percentage content of large surfactant aggregates lavaged from rats with blunt trauma-induced LC (46) (Figure 5). The dynamic surface activity of large surfactant aggregates from rats with LC was significantly reduced in measurements on a pulsating bubble surfactometer, an instrument previously documented to assess overall surface tension lowering behavior under physical conditions directly relevant for the alveoli *in vivo* (37°C, 20 cycles/min, 50% surface area compression) (37). Results showed that surfactant activity deficits were most severe at 24 hr post-contusion, and improved towards normal over 48–96 hr in parallel with improvements in lung injury parameters (46). Additional experiments showed that large surfactant aggregates from rats with LC had high levels of incorporated plasma protein and altered phospholipid class distributions with decreased levels of PC and increased levels of lyso-PC at 24 hr post-contusion, consistent with direct interactions with these injury-induced inhibitors in the alveoli of injured rats (46).

The existence of surfactant dysfunction in LC injury has direct implications not only for pathophysiological understanding, but also for potential therapeutic interventions using exogenous surfactant replacement to mitigate lung injury (see (7,48,62,63) for reviews on surfactant therapy in ALI/ARDS). Benefits to lung function and/or survival following exogenous surfactant replacement therapy have been shown in children with ALI/ARDS (64, 65), as well as in term infants with acute respiratory failure from meconium aspiration and pneumonia (5,64). Clinical studies of surfactant therapy in adults with ALI/ARDS have been more problematic, but this in part reflects the limitations of the specific drugs used. In particular, two surfactant drugs (Exosurf® and Survanta®) (37) were tested in controlled trials in adults with ARDS in the 1990's, and found to have little or no beneficial effects. However, Exosurf® is now known to have very low activity, and is no longer used clinically in this country. Also, Survanta® has been shown to contain minimal levels of highly-active SP-B (37). Exogenous surfactants used to treat ALI/ARDS must have the maximum possible activity and inhibition resistance (7,48,62,63). Infasurf®, a bovine surfactant that has significant surface activity and an ability to overcome plasma protein-induced inhibition has recently been shown to improve respiratory function and survival in pediatric patients up to age 21 with direct pulmonary forms of ALI/ARDS (65). Tracheal instillation of Infasurf® in rats with blunt trauma-induced LC has also been shown by Raghavendran et al (46) to improve pulmonary mechanics at 24 hr post-injury, and surfactant therapy is also beneficial in swine with unilateral LC injury (53).

IV. Interactions of lung contusion with other pulmonary injuries

One of the more vexing clinical aspects of blunt trauma-induced LC is that there is frequently not a clear correlation between the volume of affected lung and the severity and duration of hypoxemia (8). Patients with small contusions (seen only on CT scan) may exhibit more severe hypoxemia than those with large contusions (visible on chest x-ray). Such observations are consistent with the importance of cellular and sub-cellular factors in the pathophysiology of

LC injury, as described in previous sections. In addition, however, experiments with both rat and murine models of isolated LC indicate that hypoxia is effectively reversed by about 48 hr (24,47), which is not the case in a non-trivial subset of patients with this clinical diagnosis. This raises the possibility that interactions with other aspects of trauma-induced pathology may be occurring to exacerbate respiratory deficits in these individuals.

One potential contributor of this kind is gastric aspiration, which is known to be a major cause of direct pulmonary ALI/ARDS (27,30). Patients are at increased risk for gastric aspiration at the time of trauma, particularly if they experience a brief loss of consciousness or have associated risk factors such as recent food or alcohol intake (27,30). Many cases of gastric aspiration are unwitnessed or unreported, and aspiration pneumonitis is often a diagnosis of exclusion in patients without other known causes of respiratory compromise (27,30). Raghavendran et al (44) recently investigated the effects of concurrent gastric aspiration and LC in comparison to the component injuries alone. Four groups of Long-Evans rats were studied: LC alone, gastric aspiration (acid and gastric particles) alone, LC + gastric aspiration, and uninjured controls. Rats given LC + gastric aspiration had lower mean PaO₂/FiO₂ ratios compared to LC alone at 24 hr, and higher BAL albumin concentrations compared to either LC or gastric aspiration alone (44) (Figure 6). Rats with LC + gastric aspiration also had had more severe inflammation than rats given LC alone based on increased levels of PMN's in BAL at 5 hr, increased whole lung myeloperoxidase (MPO) activity at 5 and 24 hr, and increased levels of inflammatory mediators in BAL (TNF α IL-1 β , and MCP-1 at 5 and 24 hr; IL-10, MIP-2, and CINC-1 at 5 hr) (44). This study concluded that concurrent gastric aspiration can exacerbate permeability lung injury and inflammation associated with LC, and that unwitnessed gastric aspiration had the potential to contribute to more severe forms of LC injury associated with ALI/ARDS and pneumonia in patients with thoracic trauma (44).

In an investigation of effects of an added septic challenge on LC injury, Perl et al (41) reported that mortality was increased when mice were subjected to cecal ligation and puncture at 24 hrs following LC compared to the septic challenge alone. The authors additionally reported that severe immunosuppression evidenced by reduced cytokine production from both splenic and peritoneal macrophages following initial LC injury could have been important in the increased mortality observed when the subsequent septic challenge was added (41).

V. Future Directions

Mechanistic investigations of the inflammatory response and cellular and surfactant abnormalities following pulmonary contusion have been significantly enhanced by the establishment of reproducible small animal models. Current work has established a functional role for neutrophils in the inflammatory response contributing to LC injury, and an important aspect for future investigation involves studies aimed at defining the mechanistic importance of other cell types (e.g., alveolar epithelial cells and the alveolar macrophages) in more detail. Future work also needs to address and clarify the importance of signaling via TLR-2 and -4 in LC injury, and extend interpretations to other receptors such as TLR-3 and associated ligands. The roles and importance of fibroblast proliferation and the pathogenesis of fibrosis during the healing/resolution phase of inflammatory LC injury also need further study, and may have broader applications for fibroproliferative ALI/ARDS and chronic lung disease outside of the trauma field. Studies examining specific interactions between pulmonary fibroblasts and alveolar epithelial cells, as well as the effects of TGF- β during LC injury progression, are examples of important aspects that could be addressed in future work of this kind. To date there is no available data on gene expressions, proteomics or bio-informatics with respect to LC in either human subjects or animal models. An understanding of the functional genomics for LC needs to be pursued at both animal and human levels, as comprehensively discussed for other lung diseases in this supplement(61).

On a clinical level, therapeutic strategies to improve oxygenation and alter the inflammatory response in trauma patients with LC continue to be a challenge. A few studies have suggested improvements with intravenous corticosteroids or antioxidants in animal models (e.g., (55)), but precise randomized studies in trauma patients have not yet been carried out. Similarly, improvements in lung function with exogenous surfactants in LC have been noted in animal models (46,53), but this has not been translated to clinical studies in patients with LC. The presence of surfactant dysfunction in LC injury provides a specific rationale for more detailed studies not only with active animal-derived surfactants like Infasurf®, but also new synthetic surfactant preparations such as those containing phospholipase-resistant components (39,58). In addition, combination therapies for lung injury that combine exogenous surfactant therapy with other interventions targeting specific aspects of inflammatory pathology are an important area for future animal model and clinical research (48). Despite recent advances in understanding inflammatory mechanisms that contribute to LC pathogenesis, mortality and morbidity remain high in patients who develop ALI/ARDS following this direct pulmonary insult. Continuing animal and clinical research to facilitate the translation of mechanism-based interventions into the realm of beneficial clinical therapies needs to be aggressively pursued.

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Figure 1. Schematic of selected changes in inflammation and permeability following lung contusion (LC)

The diagram depicts the onset of selected aspects of acute inflammation and permeability injury associated with blunt trauma-induced LC. Direct traumatic insult to the lungs generates an innate inflammatory response that includes the recruitment and activation of blood leukocytes, the activation of lung tissue macrophages, and the production of multiple mediators such as cytokines and chemokines. Neutrophils contribute significantly to the severity of inflammatory LC injury, and are activated at least in part via Toll-like receptors (TLRs) such as TLR 2 and 4 in the epithelium. Inflammatory mediators in LC are also released by alveolar type II cells and other resident pulmonary cells. This inflammatory response, in conjunction with direct

LC-induced tissue injury, damages the barrier integrity of the alveolocapillary membrane and increases epithelial cell apoptosis/necrosis. Plasma proteins and other substances in permeability edema enter the alveoli and inactivate (inhibit) lung surfactant, exacerbating respiratory deficits. LC injury can also induce fibroblast activation and proliferation, although the mechanistic contributions of these cells to the progression of acute inflammatory injury are unclear. See text and subsequent sections for details.

A

B

Figure 2. Histopathology of tissue injury in rats with lung contusion

Panel A: Lung tissue section (10X) at 24 hr post-contusion stained with hematoxylin-eosin (H&E) showing neutrophils, edema, and red blood cells in the air spaces. Panel B: H&E-stained lung tissue section (10X) at 48 hr post-contusion showing thickening of the alveolar lining, increasing neutrophilic infiltrate and cellular debris.

Figure 3. Effects of neutrophil depletion on the severity of lung contusion injury based on albumin concentrations in bronchoalveolar lavage (BAL)

Neutrophils were depleted by intravenous Vinblastine prior to LC, and injury severity based on BAL albumin levels was evaluated at 4 hr and 24 hr post-contusion. Neutrophil depletion significantly reduced lung injury as described in the text. Data are Mean $\pm$ SEM for N=6 at each time point. * $p < 0.001$ compared to undepleted, contused rats.

Figure 4. Concentrations of chemokines in BAL from rats with evolving lung contusion injury
Concentrations for MIP-2, CINC-1 and MCP-1 (pg/ml) in cell-free BAL were measured by ELISA. Numbers of rats were N=9 at each time point except 12 hrs (N=7) and 7 days (N=8). Chemokine values for uninjured control rats (N = 9) were: CINC-1 (13.1 ± 1.5 pg/ml), MIP-2 (undetectable), and MCP-1 (28.0 ± 3.0 pg/ml). * $P < 0.001$ compared to uninjured controls.

Figure 5. Content and surface activity of large surfactant aggregates in cell-free BAL from rats at various times following lung contusion (LC)

In both panels of the figure, box plots display the 25th and 75th quartiles of the data and the bars indicate the range. The horizontal bar within each quartile box denotes the median, and the filled square symbol the mean (n=6 rats in each group). Significant differences from specific groups are indicated by: *uninjured, #LC 24 hr, †LC 48 hr, §LC 72 hr. Differences were considered significant if $p < 0.0025$ to adjust for multiple comparisons and maintain a family-wise α error < 0.05 . Panel A: The fraction of total BAL phospholipid that sedimented as large surfactant aggregates by centrifugation at $12,000 \times g$ for 30 min was depleted in LC injury at all time points except 96 hr compared to controls, with the greatest decrease in large aggregate

content found at 24 hr post-LC. Panel B: The minimum surface tension after 20 min of pulsation on a bubble surfactometer (37°C, 20 cycles/min, 50% area compression) was elevated in LC injury at all time points compared to uninjured controls, with the greatest elevation occurring at 24 hr post-LC.

Figure 6. Severity of lung contusion, gastric aspiration, and the combination injury of contusion plus aspiration based on albumin concentrations in BAL

Rats given lung contusion (LC), aspiration of combined acid and small gastric particles (GA), or both (LC+GA) were sacrificed at 5 or 24 hr post-injury, and concentrations of albumin in BAL were determined by ELISA ($\mu\text{g/ml}$, mean $\pm$ SEM, $n = 6-11$). Kruskal-Wallis (rank sum) statistical analysis was performed on data at each time point, and inter-group comparisons were made with a Bonferroni correction for multiple comparisons such that $p < 0.0083$ was considered significant (family-wise α error = 0.05). * $p < 0.00001$ compared to uninjured controls; # $p < 0.006$ compared to LC alone; & $p < 0.00006$ compared to GA alone.

Table 1
Selected animal models of lung contusion (LC) and pathological findings relative to humans

	Mode of injury	Pulmonary histopathology	BAL Cellularity (Quantitative & Qualitative)	Hypoxemia	BAL Albumin	P-V Compliance
Swine (Moomey et al (36) and Davis et al (10))	Captive gun bolt with ongoing mechanical ventilation (open chest)	Alveolar hemorrhage and atelectasis	Not reported	Immediate and persists for 8 hr; further progression not reported	10–20 fold increase in BAL protein	Not reported
Rat model (Wang et al (57))	Swinging pendulum (combined cardiac +lung contusion) - force 1.7 – 6 J	Alveolar bleeding and atelectasis	Not reported	Not reported	Not reported	Not reported
Mice (Knofel et al (23))	Blast wave from compressed air	Alveolar disruption with hemorrhage	Not reported	Early hypoxia that peaks at 6 hr, and correlates with the force of the blast wave	Not reported	Not reported
Rat model (Raghavendran et al(45))	Weight dropped onto a platform with a protective shield to prevent cardiac injury (2.2, 2.45 J)	Alveolar disruption with hemorrhage; Significant neutrophil infiltration by 24 hr, with minimal associated cardiac injury.	Significant numbers of WBC's in BAL, predominant neutrophilic in nature, peaking at 24hr.	Severe hypoxia that improves by 48 hr, and correlates with impact energy.	Peaks at 48 hr, and correlates with impact energy.	Reduced, and correlates with impact energy; LP(lower inflection point) is present.
Mouse model with cortical contusion impactor (Hoth et al(17,19))	Electrical device used to deliver a blow to the right chest with a velocity of 5.8m/sec.	Alveolar disruption with hemorrhage; Significant neutrophil infiltration by 24 hr	Significant numbers of WBC's in BAL, predominant neutrophilic in nature, peaking at 24hr	Hypoxia peaking at 24 hr	Not reported	Not reported
Human studies(Retrospective) (8,25,50)	Most common is motor vehicular accidents, but also blast trauma and other causes	Alveolar disruption hemorrhage and, progression in some to ARDS	Not reported	Hypoxia that improves over 2–3 days or can progress to respiratory failure over a period of days.	Not studied	Reduced, with LP present; improves with PEEP and alveolar recruitment maneuvers.