MINIREVIEW

Acute Lung Injury: Apoptosis and Signaling Mechanisms

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Acute lung injury (ALI) has been documented clinically following several pathological states such as trauma, septic shock and pneumonia. The histopathological characteristics, paired with the production of a number of cellular pro-inflammatory mediators, play a crucial role in the progression of ALI. During ALI, polymorphonuclear neutrophil (PMN)-mediated apoptosis is delayed by macrophages, possibly via effects on the Fas/FasL mediated pathway, leading to the accumulation of these cells at the site of injury and inflammation. The transcriptional regulation of NFκB, CREB, and AP-1 also regulates the pathogenesis of ALI. During sepsis and septic shock, we found evidence of infiltrating leukocytes in the alveolar spaces along with an increased number of TUNEL-positive cells in the lung sections. We also observed an increased expression of TRADD and Bax/Bcl_2 ratio at 7 days post-sepsis. In contrast, the NFκB/IκB ratio increased at 1 day post-sepsis. Together, these data provide evidence illustrating the induction of apoptosis in lung tissues subsequent to the onset of polymicrobial sepsis. The results support the concept that the upregulation of apoptosis following lung inflammation plays a crucial role in the development of acute lung injury and related disorders such as ARDS. Exp Biol Med 234:361–371, 2009

Key words: sepsis; apoptosis; neutrophils; cytokines; caspase-3

Introduction

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are inflammatory disorders of the lung that are caused by pneumonia, sepsis, trauma and/or aspiration (1). Both ALI and ARDS result from widespread lung inflammation and increased pulmonary vascular permeability (1). ALI is characterized by an abrupt onset of hypoxemia with the presence of diffuse pulmonary infiltrate (2). The disorder is defined as ARDS when the partial arterial pressure of oxygen [PaO_2]/fractional concentration of oxygen in the inspired air [F_IO_2] drops below 200. However, most of the interventional and epidemiological studies have considered the overall disorder as acute lung injury when it demonstrates a broad range of abnormalities ($PaO_2/F_IO_2 < 300$) (2). In addition, ALI and ARDS can be categorized on the basis of origin: about 55% of patient cases can be attributed to direct (or pulmonary) ALI arising from pneumonia or aspiration, while indirect (extrapulmonary) ALI arising due to sepsis and trauma can be seen in 20% of the patients (3).

ALI is a frequent complication following sepsis in critically ill ICU patients and is associated with high rates of morbidity and mortality (4, 5). According to the Acute Lung Injury Verification of Epidemiology (ALIVE) data report, ARDS/ALI affects about 7% of ICU patients, and approximately 54% of these patients develop full-blown ARDS within 24 h (5). A statistical analysis done in 1996 suggested that the mortality rate of ARDS patients has remained constant from 1967–1994 (6). In recent years, however, some investigators have reported an improvement in survival rates, mainly as a result of the implementation of new protective ventilatory strategies and drug therapies (7, 8). Even with these recent successes, there is still a tremendous need for continued research efforts utilizing multiple (biological, genomic and genetic) approaches to

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provide clarity to the underlying pathophysiological mechanisms of ALI. In this manuscript, we provide a brief overview of the pathophysiology and signaling mechanisms involved in the development of ALI.

Pathophysiology of ALI

Histopathological Evidence. Approximately 20% of the patients exhibiting severe sepsis develop lung dysfunction (9). The clinical presence of arterial hypoxemia and bilateral chest pulmonary infiltrate results into ARDS (9). The pathophysiological process is referred to as "diffuse alveolar damage" (DAD). DAD is not restricted to the sepsis syndrome since it also occurs during inhalational injury, aspiration of gaseous contents and X-irradiation damage. DAD proceeds through various stages, namely the exudative, regenerative and reparative phases. During the first week of the *exudative phase*, the lungs become dark red and heavy. There is alveolar wall congestion and expansion, edema, and red blood cells in the alveoli, coupled with damage to the type 1 epithelial cells and alveolar capillary endothelial cell damage. The late *exudative phase* is a classic and histologically identifiable stage often referred to as "shock lung." During this phase, alveolar collapse, hemorrhage and edema occur, along with a variable accumulation of neutrophils in the alveolar capillaries. The *regenerative phase* permits the recovery and healing of the lungs to its normal structure. The type 2 epithelial cells proliferate to replace the denuded epithelium. The epithelium may grow beneath the hyaline membrane, which is sloughed off, or over the membrane and later contributes to the development of interstitial fibrosis. In the patients in whom DAD has not been resolved through regeneration, a *reparative phase* ensues, characterized by local thrombosis, organization and local vascular remodeling (9).

Pro-Inflammatory Mediators. *Role of Activated Neutrophils and Cytokines During Acute Lung Injury.* Neutrophils (polymorphonuclear leukocytes, PMNs) play a vital role in the inflammatory responses present in sepsis, bronchopulmonary dysplasia and ALI (10–13); they are essentially the first responders in the host defense mechanism to counter infection. The balance of chemokines locally produced by macrophages and of those from distal or remote inflammatory sites is important to the direction of neutrophil migration to the lungs (14–16). In response to this chemical gradient, the neutrophils cross the endothelium and release numerous proteolytic enzymes and reactive oxygen species upon recruitment to the site of infection or inflammation. Paradoxically, this event also stimulates the release of macrophage-derived IL-10, a predominant anti-inflammatory modulator of the immune response (17–21). It has been suggested that the production of IL-10 by macrophages is a by-product after phagocytosis of the apoptotic neutrophils. In turn, IL-10 may subsequently suppress the additional cytokine production and phagocytic activity of these alveolar macrophages, allowing the neutrophils to serve in both pro- and anti-inflammatory capacities in this situation (17–19, 21–23).

In the polymicrobial sepsis model, a slight lung injury characterized by coagulation cascade occurs at 24 h post-cecal ligation and puncture (CLP) (24). This injury is associated with capillary congestion and perivascular cuffing, along with increased lung myeloperoxidase activity, an index of neutrophil sequestration (24). These results suggest that lung injury during sepsis is partially dependent upon the neutrophils and also upon other inflammatory cells such as monocytes/macrophages and lymphocytes.

Neutrophil Hypothesis, Depletion and Apoptosis. In the non-pathological immune response, PMNs targeted toward the lungs are cleared quickly once the invading pathogens have been eliminated. In contrast, the accumulation of activated PMNs in the lung tissue during severe lung inflammation/injury suggests that these cells play a significant role in the development of ALI. During ALI pathogenesis, the neutrophil function becomes dysregulated, leading to their sequestration in the lungs, progressing to associated tissue injury (25–27). Neutrophil retention has been observed early within the pulmonary capillaries that form a complex interconnected network of short capillary segments (28) in the lung parenchyma as well as in the bronchoalveolar lavage fluids (BALF) of ARDS patients (29). Because inflammation is closely linked to the pathogenesis of ALI, several inflammatory mediators (cytokines, chemokines and lipid mediators) further promote PMN recruitment (30, 31). In addition, the chemokines are critically involved in the activation and recruitment of PMNs; therefore, they contribute significantly to the harmful effects occurring at the organ level (32, 33).

It has been proposed that activated neutrophils in the lungs have an unusually prolonged half-life of 8 h due to delayed phagocytosis (or apoptosis) by the macrophages (34). According to this "neutrophil hypothesis," the switch from a proinflammatory to an anti-inflammatory environment is postponed (10, 35). In the septic patient, this delay appears to be related to the severity of sepsis since a progressive decrease in neutrophil apoptosis has been associated with the increased severity of sepsis (36). The delayed apoptotic response provides the PMNs with a longer life span, allowing them to accumulate at the local site of injury and inflammation (36, 37). This anti-apoptotic effect of ARDS upon the PMNs appears to be mediated through the granulocyte macrophage colony stimulating factor (GM-CSF) receptor (38).

The exact mechanisms responsible for the decreased neutrophil apoptosis during ARDS and sepsis remain to be elucidated. However, one potential mechanism involves the activation of NFκB, with a concomitant reduction in the caspase-3 levels and of the mitochondrial membrane potential (37). The activation of the p38-MAPK signaling pathways (39), modulation of Mcl-1 (myeloid cell leukemia-1) (40) or mitogen-activated protein kinases (MAPK) may contribute to the neutrophil activation mechanisms

induced by various stimuli as well as modulate apoptosis (41–46). In particular, p42/p44 MAPK is involved in PMN apoptosis in response to treatment with LPS or GM-CSF, while the role of p38 MAPK remains controversial (39, 47–49). Nevertheless, the consensus seems to suggest that its activation may be a significant event in PMN spontaneous apoptosis.

A large number of neutrophils accumulate during ALI and are vital for the activation of other proinflammatory cytokines; however, their contribution to the severity of sepsis is not yet established. The analysis of these clinical observations could identify specific mechanisms for neutrophil apoptosis in sepsis-induced ARDS and provide new insights into the development of therapeutic strategies to improve the survival rate of patients with other inflammatory disorders in which neutrophils are implicated in the disease progression.

Role of Alveolar Macrophages in ALI. While the delayed apoptosis of recruited neutrophils is a crucial event in the development of ALI, alveolar macrophages (AMs) serve as the first line of defense in the lungs (50). These phagocytic cells are prolific secretory "factories" capable of regulating the inflammatory reactions in the lungs (51, 52) and are reported to be the principle mediators in the pathogenesis of septic shock (53). AMs reside in the alveoli and the alveolar ducts of the lungs and are unique in their survival within an aerobic environment. They actively phagocytize and kill invading airborne and blood-borne pathogens. In order to induce and potentiate the inflammatory and immune processes, AMs release cellular mediators such as tumor necrosis factor-alpha (TNF-α), macrophage-activating cytokine IFN-gamma, and eicosanoids (PGE2) during the initial phase of lung inflammation (54, 55).

A large amount of evidence suggests that AMs play an essential role in the regulation of the pro- and anti-inflammatory events during sepsis-induced ALI (56, 57). It has been reported that, in response to migratory signals, neutrophils cross the endothelium and stimulate the release of IL-10 ostensibly from the AMs (18–21), a phenomenon demonstrated in several independent investigations. In this scenario, the neutrophils act as both pro- and anti-inflammatory stimuli while interacting with the endothelial cells and immune cell populations. It has been demonstrated that the suppression of either the neutrophilic or macrophagic response reduces the indices of inflammation and lung injury following hemorrhage and subsequent septic challenge (58). Thus, both cell types are essential for the development of lung injury following shock and sepsis (58).

In contrast to the effect seen in the neutrophils, an increase in the percentage of apoptosis during sepsis has been observed in the AMs (59). This ALI-associated increase was evident as early as 3 h post-CLP induction, resulting in a significant decrease of AM numbers by 20 h post-CLP (59). This enhanced apoptosis and subsequent decrease in the AM number could be due to an inadequate supply of precursor monocytes from the peripheral circulation and could serve to compromise the antimicrobial defense of the lungs in septic patients (59).

Anti-Inflammatory Mediators. As the inflammatory reaction progresses during ALI, the main inhibitory gene products appear to be IL-10 and IL-13, T-cell-derived cytokines involved in immunomodulation and anti-inflammatory properties. These anti-inflammatory interleukins are powerful inhibitors of IκB-α hydrolysis that must occur prior to the activation of NFκB (60). Under these circumstances, the NFκB complex (p50, p65) remains interlocked with IκB-α, preventing its translocation into the nucleus and initiating the continued transcription of genes involved in inflammation, immune responses, cell proliferation and cell survival. Working in concert with these cytokines is secreted leukocyte protease inhibitor (SLPI), another regulatory protein generated during the acute inflammatory response and capable of inhibiting NFκB. SLPI inhibits the activation of NFκB by increasing the levels of cytoplasmic IκB-β, which also forms complexes with NFκB to inhibit its translocation to the nucleus. Thus, there are at least three potent anti-inflammatory regulators produced during an acute inflammatory response to the lungs (60).

Role of Apoptosis During ALI

Lungs are complex organs that include different spatial arrangements of multiple cell types such as endothelium, epithelium, fibroblasts and inflammatory cells. The observation from *in vitro* studies that macrophages phagocytose apoptotic PMNs has led to the suggestion that enhanced PMN apoptosis may result in a blunted *in vivo* inflammatory response (61). This observation was then confirmed by quantitation of PMN numbers in the BALF of patients with ARDS or at risk of developing ARDS (62). In this study, Matute-Bello *et al*. further demonstrated that bronchoalveolar lavage (BAL) from ARDS patients decreased PMN apoptosis and prolonged the survival of normal human PMN cultured *in vitro*. This observation was attributed to the presence of anti-apoptotic factors such as GM-CSF and suggested that a lack of PMN apoptosis may serve to prolong the inflammatory process and predispose the patients to ARDS subsequent to ALI.

The Fas/FasL pathway appears to play an essential role in the apoptosis signaling system in alveolar epithelial injury during ALI and ARDS (63). The high levels of soluble Fas and FasL in BAL fluids correlated with the increased mortality rates of patients with ALI/ARDS (63, 64). These data agreed with the decreased lung injury observed in Fas or FasL-deficient mice after challenge with intrapulmonary deposition of the IgG immune complexes (65). Furthermore, the inhibition of caspase activity blunted the PMN-induced acute lung injury in wild-type mice (65). These collective data suggest that the Fas/FasL pathway may play an important role in apoptosis-mediated regulation for ALI.

Role of Transcriptional Factors in the Regulation of ALI

It has been established that NFκB is a critical transcriptional factor required for the maximal expression of many cytokines involved in the pathogenesis of ARDS. First discovered by Sen and Baltimore in 1986 (66), NFκB regulates the gene expression of major pro-inflammatory cytokines (TNF-α, IL-β), chemokines [macrophage inflammatory protein (MIP-2), cytokine-induced neutrophil chemoattractant (CINC)], and adhesion molecules (ICAM-1, E-selectin) (67), all of which play a major role in lung injury (68). Thus, NFκB activation is necessary for an intact host defense response such that an excessive activation of NFκB results in an exuberant inflammatory injury of lungs and other organs (67, 69, 70). Activated NFκB contributes to the accumulation of neutrophils and the expression of IL-1β, TNF-α and MIP-2 mRNAs in the lungs of endotoxemic or hemorrhagic mice (71). In addition to the enhancement of the immunomodulatory genes, NFκB plays an important role in apoptosis by regulating the expression of genes involved in cell death (72). Of note is that the activation of NFκB can decrease PMN apoptosis and subsequently increase the life span of these cells, an event that is a potential determinant of acute lung injury.

The activators of NFκB, such as TNF-α, IL-β and LPS, increase the cellular production of reactive oxygen species (ROS) by the mitochondria (73). The accumulation of neutrophils in the lungs leads to increased local concentrations of ROS and proinflammatory cytokines as a result of delayed apoptosis (62, 74, 75). In the experimental models of acute lung injury, secondary to hemorrhage or endotoxemia, NFκB is activated, and there is a decrease in the population of neutrophils (74). NFκB also interacts with a large number of selective heterologous transcription factors. One such transcriptional factor involved in the control of many inflammatory mediators is AP-1, a protooncogene product composed of c-Jun homodimers or heterodimers of c-Fos. AP-1 activation is considered to be an important first step in the chromatin remodeling process involved in the initial binding of transcriptional factors to a nucleosomal template (76). The interactions between AP-1 and NFκB do not always involve precise promoter/enhancer organization or require a κB element (77). However, an NFκB-dependent increase in AP-1 protein and mRNA levels in the plasma as well as the lungs following trauma has been observed, suggesting a direct interaction of NFκB with AP-1 (78). In hemorrhagic shock, the transcriptional mechanisms, activation of NFκB and CREB, are involved in regulating pulmonary cytokine expression (79). Because the binding elements for NFκB and CREB have been found in the enhancer/promoter regions of immunoregulatory cytokine genes for IL-1β and TNF-α, these binding elements have an important function in modulating the transcription of these genes (80–82). However, the precise roles of these two transcriptional factors and the involvement of NFκB in coordinating the control of inflammatory gene transcription during ALI remain to be elucidated.

NFκB-Independent Apoptosis Mechanisms. There are additional NFκB-independent pathways that may contribute to alterations in the lung neutrophil apoptosis after endotoxemia. It has been demonstrated that a member of the Bcl_2 family of proteins Mcl-1 (Myeloid Cell Leukemia-1) is associated with neutrophil survival during ALI (83–86). In addition, G-CSF and its receptors were found to be elevated in lung neutrophils after endotoxemia. The G-CSF levels are usually elevated in bronchoalveolar lavage specimens from patients with ALI and thus contribute toward a reduction in the neutrophil apoptosis (87, 88). It has been suggested that G-CSF may exacerbate the acute neutrophil-driven pulmonary inflammation (89), independent of NFκB. Therefore, even in the absence of NFκB, the up-regulation of the G-CSF receptors on the lungs might be capable of diminishing the neutrophil apoptosis (90).

Two tyrosine kinases, Src and Jak, also become rapidly activated in a LPS model of ALI (91). The Jak family of kinases plays a critical role in activating multiple downstream signaling pathways and is closely associated with the cytokine receptors. The Src family members are also known to participate in cytokine signaling and inflammatory responses (92, 93). These are also considered to be critical regulators of cell signaling in immune cells (94). In particular, the mechanism of endotoxin (LPS)-induced Src and Jak activation in the lungs appears to be multifactorial and changes over time as different cytokines and inflammatory mediators are elaborated (95). LPS has been shown to be a potent activator of these kinases in macrophages, and they are important for neutrophil and macrophage effector function such that their inhibition may confer protection by decreasing inflammatory cell migration and function (96, 97). Inhibition of these kinases also significantly decreases the production of the major proinflammatory cytokines TNF-α and IL-6 in both the serum and lungs of animals (98).

Although both of these tyrosine kinases have numerous downstream signaling effectors, they share common targets such as the signal transducer and activator of transcription (STAT) factors. Similar to NFκB, the STAT proteins regulate the expression of genes that are critical for inflammation and immune responses (99). In particular, STAT3 has been identified as an acute-phase response gene in the liver and plays a pivotal role in increased inflammatory cytokines, chemokines and inflammatory mediator expression (100, 101). In earlier studies, Severgnini *et al*. determined that STAT3, Src and Jak activation by LPS required the presence of reactive oxygen species (102). Thus, Src and Jak have been shown to be redox-regulated, as has LPS-induced signaling in certain instances (103, 104). This observation reiterates the need for an increased understanding of the cellular and molecular mechanisms in

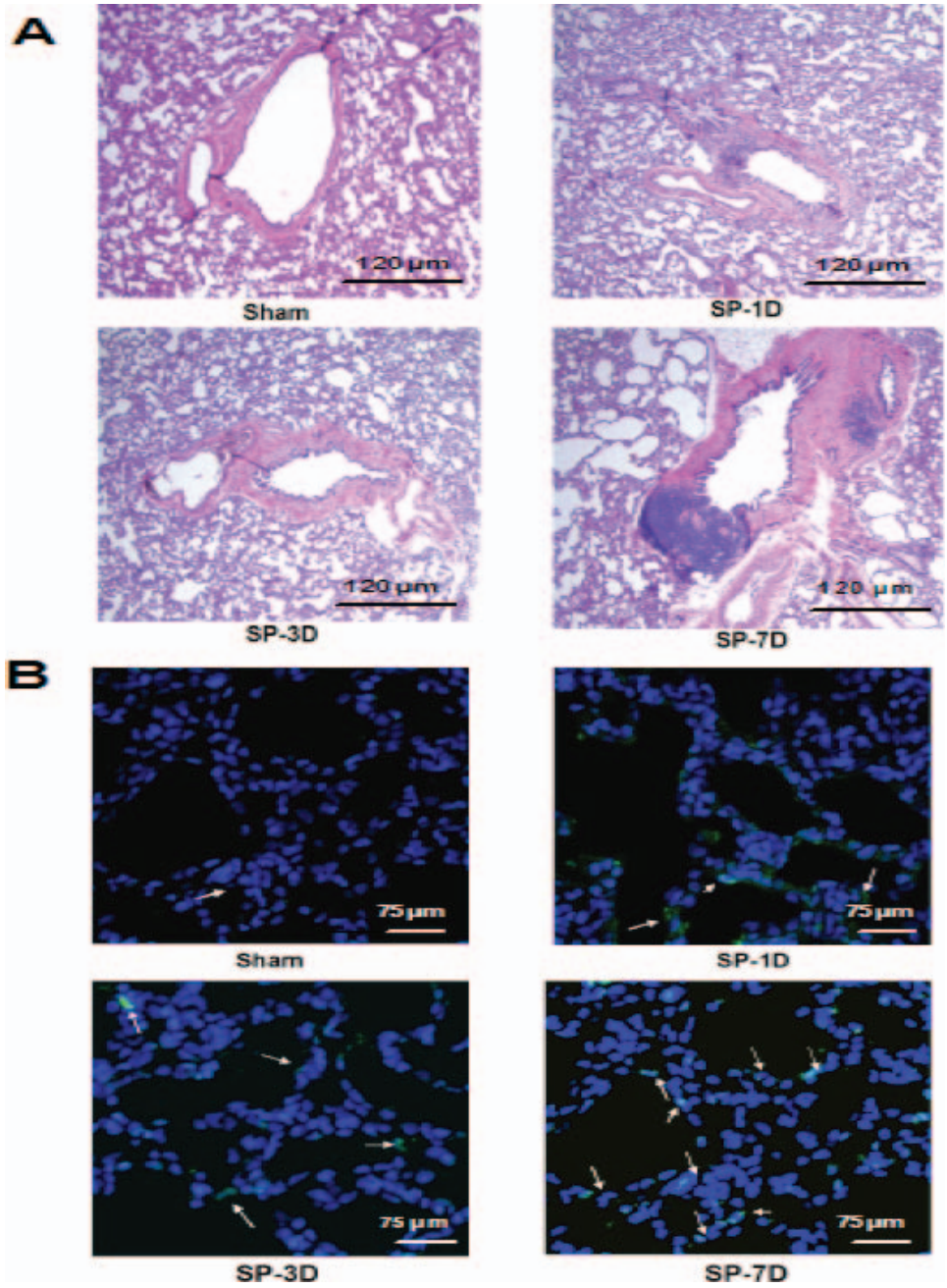

Figure 1. Microscopic images (magnification, ×5, A) and photomicrographs (magnification, ×40, B) of lung tissue sections representing histology and DNA fragmentation using BrdU TUNEL Kit (Invitrogen), respectively, in the sham, 1- (SP-1D), 3- (SP-3D), and 7-day (SP-7D) post-sepsis groups. Immunohistochemistry performed on paraffinized sham and septic lung tissues and visualized using confocal microscopy. TUNEL positive cells exhibit green fluorescence to indicate the DNA breaks (488 nm) in the nuclei (blue fluorescence with TO-PRO, 633 nm). A color version of this figure is available in the online journal.

ALI is crucial for the development of novel effective treatment strategies.

Sepsis-Induced Lung Injury

A major complication evident in traumatized patients with sepsis is a progressive impaired organ function, primarily in the lungs. Approximately 30% of the septic patients in Intensive Care Units develop lung dysfunction (105), with pulmonary dysfunction being one of the most common findings in septic patients. Moreover, these patients are susceptible to respiratory tract infections, presumably due to insufficient immune defense mechanisms and overwhelming lung injury. Despite extensive experimentation and model development, the underlying mechanisms in polymicrobial sepsis-induced lung dysfunction are far from clear. Although various researchers have studied the lung inflammation/injury in a lethal endotoxemia animal model (106, 107), the amount of research using

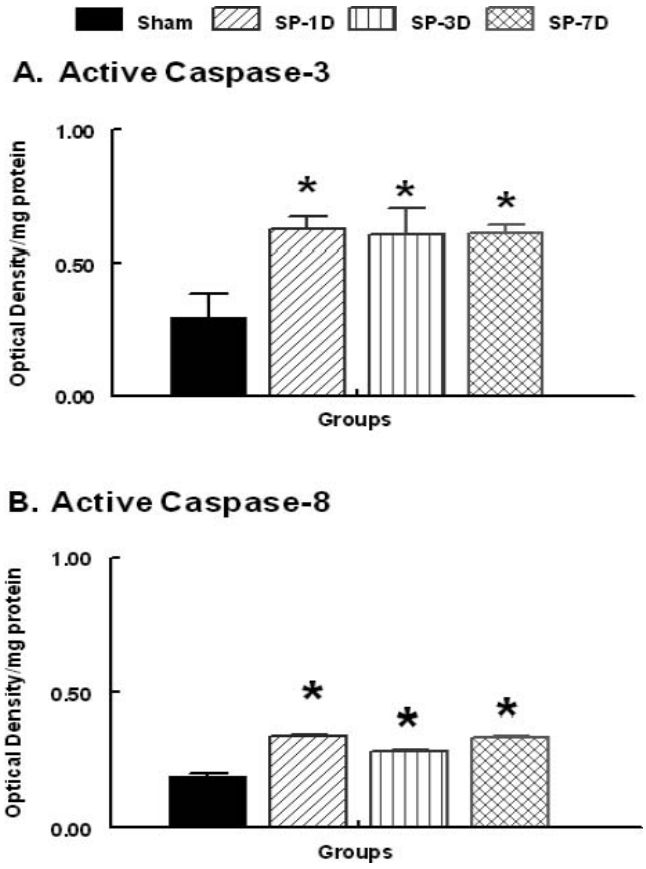

Figure 2. Effect of duration of sepsis on the concentration of (A) active caspase-3 and (B) caspase-8 in lung tissue supernatants (optical density/mg protein) in sham, and 1-, 3-, 7-day post-sepsis groups ($n = 5$ in each case). Data are expressed as mean ± standard error to mean (SEM). The biochemical data were analyzed with a one-way analysis of variance (ANOVA) using *SPSS* software. After obtaining a significant *F* value, a post-hoc Student-Newman-Keul's test was performed for inter- and intra-group comparisons. * $P \leq 0.05$ compared to the sham group.

clinically relevant models of sepsis is sparse. However, a recent study provides evidence that there is a time-dependent increase in lung injury in the CLP model of bacterial sepsis (108). This lung injury is initially characterized by moderate edema at 24 h post-CLP, followed by diffuse alveolar hemorrhage, alveolar wall thickening and increased cellularity at 48 h post-CLP. This study agrees with similar findings in which alveolar hemorrhage, septal wall thickening and capillary congestion occurred in the mouse model at a similar time-point (24).

Using a cecal inoculum method in a septic male Sprague-Dawley rat model, we have tested the hypothesis that increased duration of sepsis stimulates apoptosis in lung tissue (109–111). In the cecal inoculum (CI) model, animals were made septic using cecal material obtained from a healthy donor rat and suspended in 5 mL sterile 5% dextrose water (D_5W). The sham-operated animals receive only 5 mL/kg dose of sterile D_5W, which is a vehicle used for preparing cecal inoculum. The main advantage of CI model over CLP model is the use of a quantifiable dose of cecal material for sepsis induction. Unlike CI model, in the CLP model the dose of cecal material remains unquantifiable that produces an inconsistent response and is laboratory- and personnel-dependent. Post-mortem analysis of these septic animals in CI model revealed a severely inflamed peritoneum with the presence of pus, which was directly proportional to the duration of sepsis (111). We observed that animals at 3 and 7 days post-sepsis had a significant progressive increase in wet lung weight and body weight ratio, suggesting signs of pulmonary edema (111). The histological examination using hematoxylin-eosin staining revealed an extensive inflammatory damage in the lung following induction of sepsis (Fig. 1A). The lung tissue obtained from a sham animal showed a normal histology. However, with the progression of sepsis from 1 day to 7 days, the lung tissue showed an increased accumulation of inflammatory cells, along with deformed alveoli filled with proteinaceous material, granulocytes, necrotic debris, and inflammatory cells (Fig. 1A). In paraffinized lung tissue sections of sham and septic animals, DNA breaks were determined using the APO-BrdU^{TM} TUNEL Assay Kit (Invitrogen). This TUNEL assay detects the DNA fragmentation of apoptotic cells by labeling the 3′-hydroxyl ends of the DNA breaks (111). The TUNEL assay revealed the presence of DNA breaks expressed by the green fluorescence at 488 nm in the nuclei (exhibiting blue fluorescence at 633 nm) in the infiltrating cells in the alveolar spaces and endothelial cells during sepsis (Fig. 1B). On further analyses, we observed that 10–38% infiltrating cells expressed TUNEL positive nuclei as opposed to alveolar septal cells. These findings are in agreement with the observation of Masaki *et al*., who first reported *in situ* TUNEL DNA strand breaks in endothelial, bronchial and alveolar epithelial cells as well as inflammatory cells in the interstitium (112). In this study, we also performed caspase-3 and caspase-8 colorimetric assay (BioVision, Inc.) using homogenized lung tissues harvested from sham and septic animals, which is based on the principle of spectrophotometric detection of chromophore p-nitroaniline (pNA) after its cleavage from the labeled substrate IETD-pNA. The absorbance values of caspases 3 and 8 were measured at 450 nm. We observed that the induction of sepsis produced increased concentrations of both caspase-3 and caspase-8 in the lung tissue supernatants compared to the sham group (Fig. 2). Overall, these findings further emphasize that the induction of apoptosis-mediated DNA damage is an important molecular characteristic in the development of ALI during sepsis.

Cytosolic caspase-3 activation is regulated by both TNF-α receptor-mediated extrinsic and intrinsic (mitochondrial-dependent) apoptosis cascades. It is now established that the majority of the cytotoxic effects of TNF-α are mediated by TNF-receptor-1 (TNFR1) through the interaction of its death domain protein, TRADD (113). In our polymicrobial sepsis model, we followed a standard immunoblot procedure as described in our previous

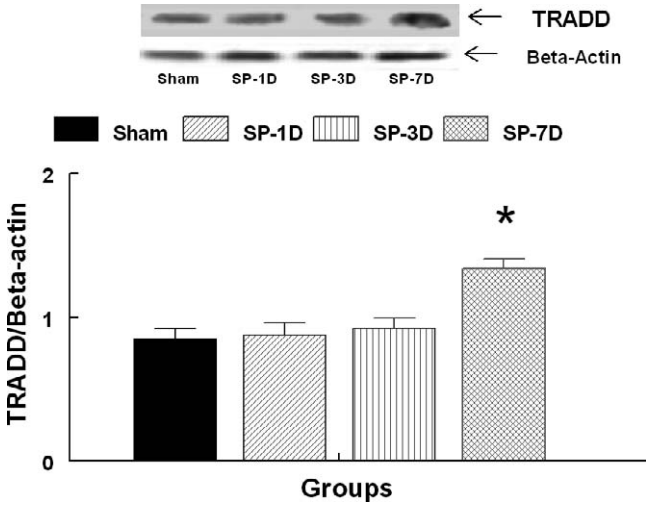

Figure 3. Expression of extrinsic apoptosis marker, TRADD, in sham, 1-, 3-, and 7-day post-sepsis groups. The immunoblot indicates the expression of TRADD normalized to beta-actin in the lung tissue samples. Each well was loaded with 15 μg protein determined by Bradford's detection method. The data were analyzed as explained in the legend of Figure 2. * $P \leq 0.05$ compared to respective sham group. The blot is a representation of five experiments in each group.

publication and observed an increase in the TRADD protein expression (Fig. 3) at 7 days post sepsis induction (109, 111). Thus, it is apparent that TRADD-mediated extrinsic apoptosis plays a critical regulatory role in the upregulation of caspase-3 during the development of ALI in sepsis. In addition to mediating signal transduction, TNFR also

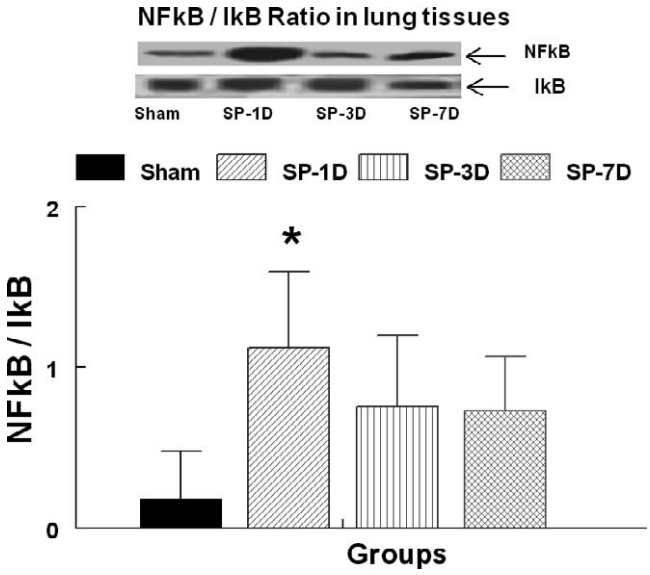

Figure 4. Expression of other apoptosis markers, NFκB and IκB ratio in sham, 1-, 3-, and 7-day post-sepsis group. The immunoblots indicate the expressions of NFκB and IκB in the lung tissue samples. Each well was loaded with 15 μg protein determined by Bradford's detection method. The data were analyzed as explained in the legend of Figure 2. * $P \leq 0.05$ compared to respective sham group; # $P \leq 0.05$ compared to the 1-day sepsis groups. The blot is a representation of five experiments in each group.

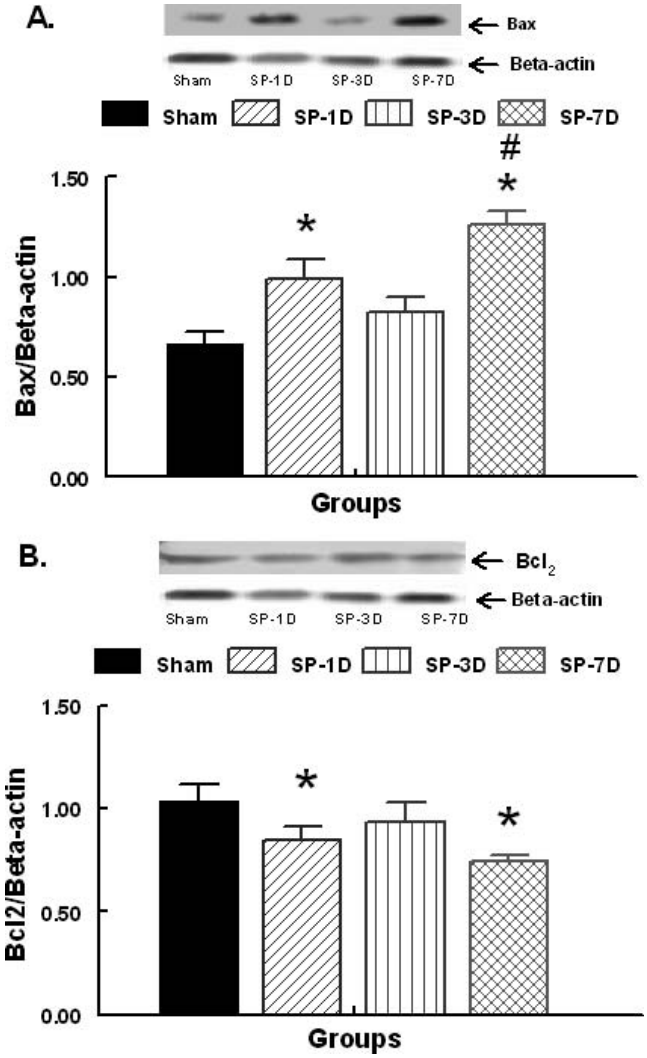

Figure 5. Expression of intrinsic apoptosis markers (A) Bax and (B) Bcl_2 in sham, 1-, 3-, and 7-day post-sepsis group. The immunoblots indicate the expressions of Bax and Bcl_2 normalized to beta-actin in the lung tissue samples. Each well was loaded with 15 μg protein determined by Bradford's detection method. The data were analyzed as explained in the legend of Figure 2. * $P \leq 0.05$ compared to respective sham group; # $P \leq 0.05$ compared to the 1-day sepsis groups. The blot is a representation of five experiments in each group.

activates NFκB by proteolytic breakdown of its inhibitor, IκB. The Iκκ phosphorylates IκB, which results in IκB degradation and translocation of NFκB to the nucleus to activate transcription. In addition, cytosolic caspase-3 activation also causes the upregulation of IκB and the activation of cytosolic NFκB (60, 78).

In the present study, we observed that the NFkB expression was upregulated at day 1 and remained elevated at day 7, although the levels were lower than 1-day post sepsis (Fig. 4). It is not clear from these results whether the decreased expression of NFκB on days 3 and 7 compared to day 1 was due to an increased translocation of NFκB in the nucleus. Guinee *et al.* reported that an elevated Bax expression was linked to diffuse alveolar damage (114).

The activation of the intrinsic apoptosis cascade was supported by the observation of Bax protein upregulation on day 1 post-sepsis and day 7, suggesting a biphasic response in our sepsis model (Fig. 5). In contrast, the Bcl_2 protein expression was down-regulated by day 1 and remained lower than sham levels up to 7 days post-sepsis. These data obtained in our polymicrobial septic rat model support the contention that the activation of extrinsic and intrinsic apoptotic cascades correlates with an increase in pulmonary edema, as seen in the lung tissues during the progression of sepsis. However, additional studies are needed to determine the apoptosome formation and to profile other mitochondrial target apoptotic cascade proteins, such as cytochrome c, in order to explore their association with lung injury during sepsis.

Future Prospects

Despite the reduction in the rate of mortality in the last 10–15 years, ALI/ARDS still remains an important cause of pulmonary and non-pulmonary morbidity (30–40%) in discharged patients (115). A rise in the incidence of morbidity has been predicted because of the increased frequency of several predisposing conditions, such as sepsis, that precipitate ALI/ARDS (116). At an NHLBI workshop (2003), an unequivocal consensus was reached regarding future endeavors in this field: that research leading to increased comprehension of the mechanisms involved in the development of ALI must take place at all levels (basic, translational and cellular). Investigational and collaborative efforts directed toward understanding key cellular and molecular events in both animal and clinical studies will provide the necessary insight for improving the detection and treatment of ALI (117).

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