Acute lung injury and cell death: how many ways can cells die?

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Invited Review

Tang PS, Mura M, Seth R, Liu M. Acute lung injury and cell death: how many ways can cells die? Am J Physiol Lung Cell Mol Physiol 294: L632–L641, 2008. First published January 18, 2008; doi:10.1152/ajplung.00262.2007.—Apoptosis has been considered as an underlying mechanism in acute lung injury/acute respiratory distress syndrome and multiorgan dysfunction syndrome. Recently, several alternative pathways for cell death (such as caspase-independent cell death, oncosis, and autophagy) have been discovered. Evidence of these pathways in the pathogenesis of acute lung injury has also come into light. In this article, we briefly introduce cell death pathways and then focus on studies related to lung injury. The different types of cell death that occur and the underlying mechanisms utilized depend on both experimental and clinical conditions. Lipopolysaccharide-induced acute lung injury is associated with apoptosis via Fas/Fas ligand mechanisms. Hyperoxia and ischemia-reperfusion injury generate reactive oxidative species, which induce complex cell death patterns composed of apoptosis, oncosis, and necrosis. Prolonged overexpression of inflammatory mediators results in increased production and activation of proteases, especially cathepsins. Activation and resistance to death of neutrophils also plays an important role in promoting parenchymal cell death. Knowledge of the coexisting multiple cell death pathways and awareness of the pharmacological inhibitors targeting different proteases critical to cell death may lead to the development of novel therapies for acute lung injury.

Apoptosis; necrosis; caspase-independent cell death; oncosis; acute respiratory distress syndrome

Acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS), represent a clinical syndrome that results from complex responses of the lung to a multitude of direct and indirect insults (57). Since ARDS was first described in 1967 (4), numerous studies have been conducted to better understand this complicated and serious syndrome that affects nearly 200,000 patients per year in the U.S. alone (94). Despite improvement in supportive care, ALI/ARDS still carries high mortality rates between 40 and 60% (94). Better understanding of the key mechanisms in the development of ALI/ARDS is absolutely crucial for developing novel therapeutic options for better management of these patients (70, 71).

Cell death has been demonstrated in the lung and other organs during the pathogenesis of ALI/ARDS (79, 80), of which apoptosis and necrosis remain prevalent in literature. Cell death in the absence of apoptotic markers is often assumed as necrosis. Extensive evidence is now emerging to suggest that the classical dichotomy of apoptosis and necrosis is an oversimplification of highly sophisticated processes that protect the organism against unwanted and potentially harmful cells (55, 104, 114). Various modes of alternative types of cell death, such as caspase-independent cell death (CICD), oncosis, and autophagy, have been the subject of recent excellent reviews (32, 46, 66). Awareness of these new concepts in ALI studies, however, awaits further development.

The present article reviews current knowledge of the role of cell death in ALI/ARDS and, in particular, provides information regarding alternative cell death mechanisms in ALI. We briefly introduce the concept of multiple modes of cell death and then focus on cell death in ALI/ARDS.

Multiple Pathways of Cell Death

It has been estimated that ~100,000 cells are produced every second through mitosis, and about a similar number of cells die per second via apoptosis in a human being (47). Apoptosis is therefore an essential mechanism to maintain homeostasis. Apoptosis and other types of cell death are also involved in a wide range of human diseases (22). In this section, we briefly introduce concepts related to different pathways of cell death.

Programmed cell death. Programmed cell death (PCD) is a process by which cells “commit suicide” through apoptosis or other alternative pathways. Cell death occurs at a specific point in the developmental process and is, therefore, considered as “programmed.” Because most examples of PCD are via apoptosis, these two terms have been interchangeably used in many studies. However, it is necessary to clarify that apoptosis is only one type of PCD (13).

Apoptosis. Apoptosis is an active form of cell death, the timing of which is genetically determined during the course of development. Apoptosis can also be triggered by external...
stimuli, such as soluble cell death ligands, which are released during inflammatory responses, or intrinsic stimuli, resulting from alteration of cellular function and metabolism. Apoptosis is characterized by cell shrinkage and formation of apoptotic bodies. In general, the cellular membrane of apoptotic cells remains intact. Hence, this particular type of cell death is usually considered less inflammatory (66). Various biochemical features of apoptosis have been identified, which have been used frequently as an indication for apoptosis, such as caspase activation (measured by enzyme activity assay; cleavage of caspases and their substrates detected by Western blotting or immunostaining), DNA fragmentation [detected by DNA laddering or terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling (TUNEL) staining], and externalization of phosphatidylserine, a cell surface marker for phagocytosis (determined by immunostaining or flow cytometry) (39).

Caspases. Caspases (cysteine aspartyl-specific proteases) are the most extensively studied proteases that are activated during apoptosis. Thirteen distinct human caspase genes have been identified, of which caspase-12 is a pseudogene with unknown function in Caucasians but is functional in a subpopulation of African descendants (62). Seven of these caspases have been suggested to participate in apoptosis as their major roles and are grouped into initiators (caspase-2, -8, -9, and -10) and effectors (caspase-3, -6, and -7). Other caspases, such as human caspase-1, -4, and -5 and mouse caspase-11 and -12, are involved in cytokine processing and inflammation (62).

Caspase activation can be carried out by either the extrinsic or the intrinsic pathway (Fig. 1). These two pathways can be distinguished by the initiating events and the downstream molecules involved.

The extrinsic pathway of apoptosis. The extrinsic pathway of apoptosis is triggered by the ligation of cell surface death receptors and their ligands, such as tumor necrosis factor (TNF), Fas ligand (FasL), and TNF-related apoptosis-inducing ligand (15). Death receptors mediate apoptotic signals through death domains and death effector domain modular protein...
motifs (15). The activated death receptor induces formation of the DISC, which recruits and activates multiple procaspase-8 molecules through the adaptor molecule Fas-associated death domain protein (15, 62). The activated initiator caspase, caspase-8, induces activation of effector caspases, such as caspase-3 (Fig. 1). Activated caspase-3 further triggers enzymes responsible for apoptosis, resulting in phosphatidylserine externalization, nuclear condensation, and DNA fragmentation (15, 62).

The intrinsic pathway of apoptosis. Cell death can be triggered by intracellular stress, including ionizing radiation, cytokine deprivation, and chemotherapeutic agents (62). Under such conditions, apoptosis is propagated through the intrinsic pathway of PCD. This pathway is characterized by mitochondrial outer membrane permeabilization (MOMP) (51), which is regulated by the B cell lymphoma (Bcl)-2 family of proteins (15). About 20 members of this family have been identified in mammalian cells. These proteins contain at least one of four relatively conserved Bcl-2 homology (BH) domains, which categorize Bcl-2 family members into three groups (51). Members of the first group are antiapoptotic, including Bcl-2, Bcl-XL, A1, and Mcl-1. The other two groups are proapoptotic, including members of the “multi-BH domain protein” (Bax, Bak, and Bok) and members of the “BH3-only protein” (Bid, Bad, and Bim). Proapoptotic Bcl-2 proteins induce apoptosis by triggering MOMP, which is counteracted by the antiapoptotic Bcl-2 members. Therefore, the decision to undergo apoptosis depends on the balance of proapoptotic and antiapoptotic members of Bcl-2 proteins. MOMP leads to cell death either through the released mitochondrial molecules or as a result of abrogated mitochondrial functions that are indispensable for cell survival (31).

Among the released molecules, cytochrome c is the most critical factor in the intrinsic pathway. After MOMP, mitochondrial cytochrome c is released into cytosol, where it complexes with apoptosis protein activating factor-1 and caspase-9. Caspase-9 is then activated in this complex, the apoptosome (Fig. 1) (15). Caspase-3 is subsequently recruited to the apoptosome to mediate subsequent events for cell death (6).

The extrinsic pathway and intrinsic pathway are interlinked by caspase-8 in certain situations. Activated caspase-8 truncates the proapoptotic Bcl-2 protein Bid. The truncated Bid translocates onto the mitochondrial outer membrane, where it induces MOMP and causes subsequent cytochrome c release (15) (Fig. 1). The released cytochrome c initiates formation of the apoptosome, an important mediator in the intrinsic pathway of cell death.

Caspase-independent cell death. For many years, the focus of apoptosis research has been on caspases and their possible roles as the executioners of PCD (32). Caspase activation was considered an indispensable hallmark of apoptosis (105). Recently, increasing evidence has pointed to the existence of alternative pathways of cell death that are independent of caspases. It should be mentioned that caspase-coding sequences are absent from the genomes of many nonanimal species (3), suggesting that PCD in lower species is conducted in a caspase-independent fashion.

CICD is mediated by noncaspase proteases and switched on by either death receptors or mitochondrial pathways (66). It is known that lysosomes are involved in necrotic and autophagic cell death; however, recent studies have demonstrated that lysosomes can also be involved in apoptosis or apoptosis-like PCD, depending on the magnitude of lysosomal permeability and the amount of proteolytic enzymes released (Fig. 2) (32). Among the proteases released from lysosomes, cathepsins have gained the most attention for their roles in cell death. Cathepsins are the largest group of proteolytic enzymes in lysosomes, with 11 members in human, known as cathepin B, H, L, S, F, K, C, W, X, V, and O. Of these members, cathepin B and L are ubiquitously expressed and most abundant in lysosomes (32). The role of cathepin B has been shown in many cases of apoptosis, including TNF-induced cell death in WEHI-S cell line (29) and cell death of non-small cell lung cancer induced by microtubule-stabilizing agents (7, 32). Other cathepsins are increased in response to inflammation, and some are related to cell death as well (67, 118).

Lysosomal membrane permeabilization is a critical step in CICD (Fig. 2). Release of lysosomal enzymes can be triggered by reactive oxygen species (ROS), DNA damage, calcium, microtubule stabilization, sphingosine, and proapoptotic Bcl-2 family proteins; activation of death receptors may also induce lysosomal release indirectly (61). Some modes of cell death appear to be exclusively mediated by cathepsins, whereas others may require both cathepsins and caspases for the initiation and execution (32).

Mitochondria are the regulators for both extrinsic and intrinsic apoptosis. Multiple cell death signals converge on the mitochondria to regulate their membrane integrity. As a result, multiple death-promoting factors can be released from the mitochondrial intermembrane space to the cytosol (96). As discussed above, apoptosis is mainly mediated through cytochrome c, whereas CICD is mediated through other mitochondrial proteins, such as apoptosis-inducing factor (AIF), endonuclease G, and/or high-temperature requirement protein A2 (HTRA2/OMI) (Fig. 1) (13, 96). The role of these factors in the
regulation of cell death has been reviewed in detail elsewhere (14, 51, 62, 91, 96).

Oncosis. Oncosis is derived from a Greek word “onkos,” meaning swelling. Oncosis, therefore, refers to the cell death program that is characterized by cell swelling, organelle swelling, membrane blebbing, and increased membrane permeability. The morphological features of oncosis include nuclear chromatin clumping in the absence of evident dense chromatin bodies, cytoplasmic swelling and vacuolation, and lysosomal disruption and leakage, as well as mitochondrial swelling and disruption. At the biochemical level, oncosis is usually induced by pathological stimuli such as ischemia and/or reperfusion. Oncosis often leads to nonspecific DNA fragmentation with impaired ATP generation and early mitochondrial damage, increased plasma membrane permeability, and ionic pump dysfunction resulting in leakage of lysosomal enzymes and intracellular structural proteins. As the number of cells undergoing oncosis increases, physiological function diminishes, eventually leading to organ dysfunction (21, 36, 48, 53, 59, 74). Oncosis occurs when ATP generation is attenuated or when cellular energy consumption becomes unregulated (74). During the repair process of massive DNA destruction, cellular energy depletion by poly(ADP-ribose) polymerase results in oncosis (25). The inhibition of poly(ADP-ribose) polymerase in oxidant-stressed endothelial cells attenuates oncotic cell death (25).

Calpains, a family of Ca\(^{2+}\)-activated neutral cysteine proteases, have been shown to play a role in oncotic cell death (73). The involvement of this particular cysteine protease family suggests that, unlike necrosis, oncosis is carefully regulated. On the basis of their tissue expression patterns, calpains are classified as either ubiquitous or tissue specific (103). Two ubiquitous isoforms, \(\mu\) (calpain I) and m-calpain (calpain II), have been identified. Physiologically, calpains play critical roles in embryogenesis, cell cycle progression, proliferation, differentiation, and migration (73). Endoplasmic reticulum actin as a sensor of cellular stress that regulates cell metabolism and differentiation, and mitochondrial damage, increased plasma membrane permeability, and ionic pump dysfunction resulting in leakage of lysosomal enzymes and intracellular structural proteins. As the number of cells undergoing oncosis increases, physiological function diminishes, eventually leading to organ dysfunction (21, 36, 48, 53, 59, 74). Oncosis occurs when ATP generation is attenuated or when cellular energy consumption becomes unregulated (74). During the repair process of massive DNA destruction, cellular energy depletion by poly(ADP-ribose) polymerase results in oncosis (25). The inhibition of poly(ADP-ribose) polymerase in oxidant-stressed endothelial cells attenuates oncotic cell death (25).

**Cell Death and Acute Lung Injury**

Apoptosis and alternative cell deaths have been found in experimental settings where lung injury has been proposed to be a mechanism for the pathogenesis of ALI/ARDS and other lung diseases. The types of cell death and underlying mechanisms vary among different experimental conditions and clinical situations.

*LPS-induced caspase-dependent apoptosis in ALI.* LPS is an immunogenic component of the outer membrane of gram-negative bacteria, which can trigger innate immune and inflammatory responses via Toll-like receptors (2). One of the common mediators of Toll-like receptors, MyD88, binds Fas-associated death domain protein and caspase-8, which is sufficient to induce apoptosis (1). LPS has been commonly used as a tool to study the mechanisms of sepsis in ALI, both in animals (30, 52, 54, 95, 110) and in cultured cells (90, 107). LPS induced apoptosis of vascular endothelial cells, bronchial and alveolar epithelial cells, and inflammatory cells in the pulmonary interstitium of mice (30). Evidence for apoptosis was sought using TUNEL staining, DNA ladder, caspase activation, and, most importantly, electron microscopy (30, 52). Caspase inhibitor (z-VAD.fmk) suppressed LPS-induced caspase activation, significantly decreased the number of TUNEL-positive cells, and improved survival of mice (52). Pretreatment of mice with a specific inhibitor for inducible nitric oxide synthase (iNOS) significantly enhanced LPS-induced pulmonary apoptosis and increased activities of caspase-3 and caspase-7. In support, iNOS-deficient mice developed a greater degree of pulmonary apoptosis compared with wild-type mice, suggesting that NO derived from iNOS has a protective role against LPS-induced apoptosis in the lung (95).

Multiple studies point out the role of Fas/FasL system in the pathogenesis of epithelial apoptosis in LPS-induced ALI. Increased concentrations of soluble Fas and FasL were found in the bronchoalveolar lavage (BAL) fluid from ARDS patients (83). BAL fluid from ARDS patients induced apoptosis of distal lung epithelial cells, which was inhibited by anti-FasL monoclonal antibody (mAb), anti-Fas mAb, and a Fas-ligand fusion protein (83). In LPS-induced lung injury, a dose-dependent overexpression of Fas was accompanied by lung edema, neutrophil infiltration, and epithelial cell death (54). These detrimental effects were attenuated by the intratracheal administration of F2 antibody, which impeded the Fas-FasL signaling transduction (54). In support, expression of Fas receptor has been demonstrated on pulmonary endothelial and epithelial cells, as well as on neutrophils and other phagocytes (68). Intranasal instillation of the Fas-activating antibody (Jo2) significantly increases proteins and neutrophils in the BAL (85). Lung histology of Jo2-treated mice showed neutrophilic infiltrates, alveolar septa thickening, hemorrhage, and TUNEL-positive cells in alveolar septae and air space. Electron micros-
copy confirmed apoptosis of type II pneumocytes (85). These effects were attenuated in mice deficient in Fas (85). Sustained LPS-induced neutrophilic inflammation in the lungs was attenuated in mutant mice deficient in either Fas (lrp mice) or FasL (gld mice) (86). On the other hand, it should be pointed out that during the resolution phase of ALI, apoptosis of type II pneumocytes mediated through the Fas/FasL pathway has been suggested as a mechanism for clearance of these cells (112). Together, these studies support a specific mechanism of Fas/FasL system in LPS-induced apoptosis in the lung.

TNF-α and its receptors represent another major extrinsic cell death pathway (15). It is also involved in cell death during ALI/ARDS. BAL fluid from patients at early and late stages of ARDS has been shown to be cytotoxic to human lung microvascular endothelial cells (33). Concentrations of TNF-α and angiotatin, a natural inhibitor of angiogenesis, were increased in the BAL fluid. Neutralization of TNF-α or angiotatin attenuated the cytotoxicity on cultured endothelial cells (33). However, in vivo studies demonstrated that TNF-α appears to be less important in the LPS-induced pulmonary apoptosis, since anti-TNF-α treatment, intratracheal TNF-α instillation, or the use of TNF-α knockout mice did not dramatically affect LPS-induced cell death (110). On the other hand, exogenous TNF-α improved the survival of Legionella pneumophila-infected mice kept under hyperoxic condition. TNF-α effects were associated with restoration of total lung weight and histone DNA and glutathione levels (89). The roles of Fas/FasL, TNF-α/receptors, and other extrinsic apoptotic signals in mediating apoptosis resulting from other insults needs to be studied further.

Hyperoxia-induced cell death: apoptosis and nonapoptotic cell death. Hyperoxia could induce necrosis as well as both apoptotic and nonapoptotic PCD (75, 77). Mantell and colleagues (75–77) exposed mice to hyperoxia and identified apoptosis as a prominent component of the acute inflammatory responses in the lung. By using strains of mice that are differentially sensitive to hyperoxic lung injury, they observed that the percentage of apoptotic cells was well correlated with the severity of lung injury (76). Hyperoxia induced Fas/FasL expression and apoptosis in lung epithelial cells (16). Fas-deficient mice showed partial resistance to the lethal effects of Legionella pneumonia in the presence of hyperoxia (108).

Angiopoietin 2 (Ang2) is an angiogenic growth factor that destabilizes blood vessels, enhances vascular leak, and induces vascular regression and endothelial cell apoptosis. When mice were exposed to hyperoxia, Ang2 expression was induced in lung epithelial cells. Hyperoxia-induced oxidant injury, cell death, inflammation, increased permeability, and mortality were ameliorated in Ang2−/− mice or in animals treated with small interference RNA to reduce Ang2 expression. Hyperoxia activated both extrinsic and intrinsic mitochondrial cell death pathways and activated both initiator and effector caspases through Ang2-dependent pathways. The levels of Ang2 in plasma and alveolar edema fluid were increased in patients with ALI and in tracheal fluid of neonates that developed bronchopulmonary dysplasia (5). This study demonstrated not only the importance of Ang2 in ALI but also the interaction between the extrinsic and intrinsic pathways of cell death in ALI.

In these studies, the “nonapoptotic cell death” is based on TUNEL staining, caspase activation, or annexin V/propidium iodide double staining followed by flow cytometry. Other techniques, such as electron microscopy and calpain activity assays, should be considered to determine whether a subpopulation of cells have undergone oncosis.

Roles of neutrophilic apoptosis in ALI. Recruitment and activation of neutrophils are considered essential to the pathogenesis of ALI/ARDS. When activated, neutrophils contribute to lung injury by releasing ROS, proteolytic enzymes, leukotrienes, and other proinflammatory mediators (64). Neutrophils are short-lived phagocytic cells, and apoptosis of neutrophils appears to be inhibited in ALI/ARDS. In patients with sepsis, apoptosis of peripheral blood neutrophils is inversely proportional to the severity of sepsis (24). In a clinical study, it was found that delayed neutrophil apoptosis in sepsis patients is associated with maintenance of mitochondrial transmembrane potential and reduced caspase-9 activity (106). Intravascular injection of oleic acid is a model to simulate pulmonary fat embolism-induced ALI (38). At the peak of lung injury, 1 and 4 h following oleic acid injection, a massive neutrophil response was found in the lung, without any evidence of apoptosis. At 24 h, when the lung injury is in the early resolution stage, intense neutrophil apoptosis was found (41). Intestinal ischemia-reperfusion-induced ALI was also associated with reduced neutrophilic apoptosis in the BAL (88).

BAL fluid from patients with early ARDS attenuated apoptosis development in normal neutrophils (81). The inhibitory effect of BAL fluid on neutrophil apoptosis is mediated by soluble factors, primarily the proinflammatory cytokines, granulocyte colony-stimulating factor (G-CSF), and granulocyte/macrophage colony-stimulating factor (GM-CSF) (82, 84). Concentrations of G-CSF and GM-CSF in BAL fluid from ARDS patients parallel the antiapoptotic effect of ARDS BAL fluid on neutrophils (81). Pre-B cell colony-enhancing factor has been found as a novel inflammatory cytokine that plays a requisite role in the delayed neutrophil apoptosis of clinical and experimental sepsis (50). Another proinflammatory cytokine, IL-1β, may also possess an antiapoptotic effect on neutrophils (78). Moreover, neutrophils have been demonstrated to induce epithelial apoptosis by releasing soluble FasL, since epithelial apoptosis was attenuated in the presence of inhibitory anti-Fas or anti-FasL mAb (102).

On the other hand, neutrophils can modify the inflammatory response via modulating the production of proinflammatory cytokines by alveolar macrophages (84). Phagocytosis of apoptotic neutrophils by macrophages inhibits macrophages to produce proinflammatory cytokines (TNF-α, GM-CSF, IL-1β, IL-8) and to release anti-inflammatory mediators [i.e., transforming growth factor (TGF)-β1] (23, 42). It was shown that instillation of apoptotic cells into LPS-stimulated lung reduced proinflammatory chemokine levels in BAL fluid and enhanced the resolution of acute inflammation, which required phosphatidylserine on the apoptotic cells and local induction of TGF-β1 (42). Ingestion of apoptotic cells by macrophages may induce TGF-β1 secretion, which may have an anti-inflammatory effect and suppress proinflammatory mediators. Neutrophils can release hepatocyte growth factor during ALI/ARDS and pulmonary fibrosis. Hepatocyte growth factor and keratinocyte growth factor are potent growth factors for type II pneumocytes, suggesting that neutrophils may also promote alveolar epithelial repair after acute or chronic lung injury (68).
**Cell death during lung transplantation.** Ischemia-reperfusion (IR) is an unavoidable step in organ transplantation, and IR-induced pulmonary dysfunction is a significant clinical problem in lung transplantation (40), characterized by nonspecific alveolar damage, lung edema, and hypoxemia that occurs within 72 h posttransplantation (18). Many TUNEL-positive cells have been found in the lungs within 2 h of reperfusion during human lung transplantation; evidence of apoptosis was confirmed with electron microscopy (26). It should be pointed out that although many cells in these lungs underwent apoptosis, the lung function and clinical outcome were very good. This raises the question of the clinical significance of apoptosis. To address this question, a rat lung transplantation model with a triple staining technique was used. After permeabilization of the plasma membrane, propidium iodide was used to stain nuclei, representing the total number of cells in the lung. TUNEL staining was used for "apoptotic cells," and trypan blue staining was used for total dead cells. The "necrotic cells" were calculated by subtracting TUNEL-positive cells from the total number of dead (trypan blue positive) cells (28). Many TUNEL-positive cells were found after 2 h of reperfusion with rat lungs preserved for either 6 or 12 h, which were associated with good lung function. In contrast, a preponderance of necrotic cells in lung tissue was present in the lung after prolonged (18 or 24 h) hypothermic preservation, which was associated with deterioration of lung function (28). When the donor lung was pretreated with prostaglandin E1 (17) or adenosine-mediated gene delivery of an anti-inflammatory cytokine, IL-10 (27), improved lung function was associated with a shift from a pro- to an anti-inflammatory cytokine profile. In these studies, the deterioration of lung function (arterial partial pressure of O2, wet/dry lung weight ratio, and peak airway pressure) was correlated only with the degree of "necrosis" but not with "apoptosis" (17, 27).

To further test the role of apoptosis in IR-induced lung injury, caspase inhibitors were administered after hypothermic preservation and prior to reperfusion, which blocked apoptosis (TUNEL positive) and reduced the total number of dead cells with improved lung function (93). Therefore, apoptosis alone may not be responsible for the deterioration of lung function, but apoptosis may lead to necrosis. Apoptosis and necrosis may coexist, and alteration of cell death modes may be an important mechanism responsible for organ damage induced by the IR process during lung transplantation.

IR can activate JNK and p38 MAP kinases in the kidney and heart. In contrast, during the IR process of lung transplantation, ERK is the major signaling molecule activated (97, 98). Activation of ERK is attributable to hyperoxia-induced cell death in lung epithelia (117) and IL-13-induced inflammation and remodeling in the lung (65). This coincidence indicates that the ERK activation observed in lung transplantation could be a major signaling pathway for cell death as well. This hypothesis merits further investigation.

**Roles of cathepsins in cell death and lung injury.** Evidence of cathepsins in lung injury is mainly from cell culture and studies related to pulmonary fibrosis and emphysema. It was demonstrated that LPS increased death of human lung epithelial cells in a caspase-independent but cathepsin B-dependent manner (107). Lung epithelial cells are not only the target of inflammation but also a source of inflammatory mediators (19, 34, 37, 69, 116). LPS induced cytokine production in primary cultured rat pneumocytes, including TNF-α (45, 87) and macrophage inflammatory protein-2 (43, 44, 115). LPS also induced production of IL-8 and GM-CSF (60) and expression of ICAM-1 (63) in human lung epithelial A549 cells. When A549 cells were challenged with LPS, cell death with apoptotic features was demonstrated by TUNEL and annexin V staining and by DNA laddering. However, two commonly used pan-caspase inhibitors, z-VAD.fmk and BOC-D.fmk, did not block cell death. In contrast, cathepsin B inhibitors, Ca074-Me and N-1845, reduced cell death significantly. LPS increased expression and translocation of AIF from mitochondria to the nucleus, a feature of CICD. LPS-induced cell death was significantly attenuated by ROS scavengers (107). Caspase-independent apoptosis has also been found in human pulmonary artery endothelial cells treated with wood smoke extract (72). These cell biology studies demonstrated the presence of CICD in lung cells.

It was shown that the level and activity of cathepsins increased transiently in lung tissue during regeneration processes in bleomycin-induced lung injury (58). Bleomycin induced activation and release of cathepsin D in primary cultured rat type II pneumocytes. Pepstatin A, an aspartyl protease inhibitor, completely blocked cathepsin D enzymatic activity and inhibited bleomycin-induced nuclear fragmentation and caspase-3 activation. Antisense oligonucleotides against cathepsin D also inhibited bleomycin-induced nuclear fragmentation in these cells (67).

With the use of a transgenic approach, it has been shown that IFN-γ (a TH1 cytokine) is a prominent stimulator of cathepsin B, D, H, and S (113). Selective cathepsin S inhibition or null mutation of cathepsin S decreased IFN-γ-induced apoptosis, inflammation, protease accumulation, and alveolar remodeling. IFN-γ-induced cathepsin expression and apoptosis are mediated through multiple inflammatory mediators. In the absence of CCR5 (null mutant), cathepsin-dependent apoptosis and emphysema-like changes were reduced (20). Interestingly, a TH2 cytokine, IL-13, when induced in the lung, also augmented the production of cathepsins (B, H, D, and S) (119); treatment with caspase inhibitor (z-VAD.fmk) or null mutation of cathepsin S also reduced IL-13-induced apoptosis (20). Therefore, cathepsins can be induced by multiple inflammatory mediators. Whether cathepsins also mediate cell death in ALI induced by infection, mechanical ventilation, or other insults is unknown and should be further explored.

**Oncosis and acute lung injury.** Oncosis is an emerging focus of cell death studies. Recent work by a number of groups has suggested a role of oncosis in inflammatory diseases. However, the evidence for oncosis in ALI is limited, perhaps, mainly due to the lack of awareness.

Apoptotic and necrotic cells in the lung were quantified after hemorrhagic shock in Mongrel pigs. Apoptosis (cell shrinkage, membrane blebbing, apoptotic bodies) and necrosis (cellular swelling, membrane lysis) in neutrophils and macrophages, as well as in alveolar cells, were found by hematoxylin and eosin staining (49). It is plausible that some of the claimed necrotic cells could be oncosic.

In a recent study, a murine intestinal ischemia-reperfusion (IIR) model of extrapulmonary ARDS was used to investigate mechanisms related to multiorgan dysfunction syndrome (MODS). Cell death was found in the lung, heart, and kidney, as determined by TUNEL staining and DNA fragmentation;
however, the activities of caspase-3 were not increased in the lung, kidney, and liver and were barely detectable in the heart (88). Activated caspase-3 and cathepsin B activities were also not increased in these tissues. Electron microscopy revealed features of oncotic cell death in the lung airway epithelial cells, type II pneumocytes, and microvascular endothelial cells, such as cytoplasmic swelling, mitochondrial swelling, loss of mitochondrial cristae, reduced number of lamellar bodies, and cytoplasmic disorganization (88). The IIR group also showed evidence of oncotic cell death in the heart and kidney (88). The animals in this study were subjected to mechanical ventilation with oxygen, and the IR process in the intestine may result in oxidative stress. These factors may lead to oncosis in different organs.

Necrosis: the End of the Story?

As reviewed in this article, apoptosis, nonapoptotic cell death, CICD, oncosis, and necrosis coexist in the lung during ALI. What is the relationship between these pathways? Two paradigms have been proposed. Each has its own experimental evidence and scientific merits.

Four deaths and one funeral: a continuous spectrum from apoptosis to necrosis. In the first paradigm, apoptosis and necrosis are viewed as two far ends of a continuum of cell death programs. Other types of cell death within the spectrum include apoptosis-like PCD and necrosis-like PCD (66) (Fig. 3A). Nuclear change is a major criterion to distinguish these four cell death patterns. Kroemer and Martin (61) used a similar paradigm but called it autophagic cell death, instead of necrosis-like PCD. In this concept, necrosis is regarded as accidental; it occurs when cells are exposed to high concentra-

![Diagram](attachment:diagram.png)

**Fig. 3.** Relationship among different types of cell death: two proposed models. A: it has been proposed that there are 4 pathways of cell death, of which apoptosis and necrosis are the 2 extremes. Blocking caspase activities may switch some of cells from apoptosis to CICD, which may be more detrimental (62). The definitions of cell death in brackets were defined by Leist and Jaatela (66). B: in the second model system, necrosis is considered the common destiny of cell death. The routes of cell death could be interrelated and switched based on underlying mechanisms and conditions (25, 74). Both paradigms should be considered, especially in the investigation of mechanisms of diseases in vivo.

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REFERENCES


