Apoptosis and Epithelial Injury in the Lungs

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Epithelial injury is a critical event in the development of acute lung injury, but the mechanisms that cause death of the alveolar epithelium are not completely understood. Epithelial death occurs by necrosis and apoptosis; more information is needed about the balance between these two types of cell death in the lungs. Direct epithelial necrosis probably occurs in response to bacterial exotoxins and overdistension of alveolar units by mechanical ventilation. Apoptosis is a regulated form of cell death that is mediated by membrane death receptors and direct mitochondrial injury. Apoptosis pathways are activated in the lungs of patients with acute lung injury, in part by activation of the membrane Fas death receptor by soluble Fas ligand (sFasL), which accumulates in biologically active form at the onset of lung injury. Accumulating evidence in humans and experimental models links sFasL and Fas pathway with lung epithelial injury and fibrosis. New strategies to inhibit Fas-mediated epithelial apoptosis need to be developed in order to develop new ways to preserve epithelial function in patients who develop acute lung injury.

Keywords: apoptosis; epithelial; lung injury

The acute respiratory distress syndrome (ARDS) causes approximately 80,000 deaths each year in the United States, and the mortality in unselected patients continues to exceed 40%. The ARDSnet studies of mechanical ventilation strategies in patients with ARDS established that a simple mechanical intervention, applying less stretch to the lungs, is consistently associated with lower mortality (1, 2). Yet overall mortality remains unacceptably high, and an improved understanding of the pathophysiology of acute lung injury (ALI) in humans is needed in order to develop treatments that will be additive or synergistic with mechanical ventilation strategies in reducing overall mortality. Soon after the initial description of ARDS in humans, it was recognized that alveolar inflammation was an important feature of ARDS (3), and many laboratories investigated the inflammatory events in the lungs of patients, and the role of inflammation in models of experimental lung injury (4). More recently, interest has focused on other mechanisms that cause alveolar injury, either instead of or in addition to inflammation. Apoptosis is a form of programmed cell death that is essential to tissue development and repair, and it also appears to be important in the pathophysiology of some diseases (5, 6). This brief review will summarize some of the potential links between apoptosis and alveolar injury in humans and experimental models of ALI.

ALVEOLAR EPITHELIAL INJURY IN PATIENTS WITH ALI

Epithelial injury in the lungs is one of the hallmarks of ALI in humans. The classical studies of Bachofen and Weibel identified ultrastructural injury to alveolar Type I epithelial cells in patients who had died of ARDS (7, 8). Although injury to the lung microvasculature was also detected, the vascular lesions were not as prominent as the epithelial destruction. Autopsy studies do not clarify how early epithelial injury occurs, or how it progresses in patients after onset of lung injury. One of the characteristics of acute inflammation is that endothelial permeability increases rapidly. In the lungs this results in the movement of plasma equivalents into the interstitial space, and this extravascular fluid moves into lymphatic spaces along bronchovascular bundles. If microvascular or interstitial pressures are high, alveolar flooding can result. The increase in extravascular fluid alters ventilation/perfusion relationships, and the resulting hypoxemia can be severe. Yet if endothelial permeability is the sole change in the lungs, extravascular fluid is eventually cleared from the interstitial and alveolar spaces, and the lungs heal without fibrosis because no major structural damage has occurred. In contrast, when the alveolar epithelium is damaged the change in epithelial permeability leads to major alveolar flooding with high-molecular-weight proteins, with prolonged changes in gas exchange and a much higher likelihood of disordered repair.

Endothelial permeability changes rapidly in response to local and systemic proinflammatory stimuli, and changes in endothelial permeability are rapidly reversible in the absence of severe endothelial damage. In contrast, the lung alveolar epithelium is an extremely tight barrier that restricts the movement of proteins and liquid from the interstitium into the alveolar spaces. In addition, the lung epithelium contains specialized transport systems that pump sodium chloride from the alveolar spaces into the interstitium (9). Protein is transported more slowly across the alveolar epithelium than electrolytes and water. As a result, when epithelial function is normal and plasma equivalents reach the alveolar spaces, the protein concentration in alveolar fluid rises with time, as water and electrolytes are reabsorbed faster than protein. Clinical studies show that patients in whom the alveolar protein concentration rises over a period of hours after the clinical onset of ALI have a better prognosis than those in whom the protein concentration remains constant or falls, consistent with the interpretation that impaired alveolar epithelial function in the lungs is a marker of poor outcome in humans (10, 11).

MECHANISMS OF ALVEOLAR EPITHELIAL DEATH

Cell death occurs by two general mechanisms, necrosis and apoptosis, and we have an incomplete understanding about the mechanisms that determine which of these two processes occurs. Necrosis is likely when tissue becomes ischemic and tissue oxygen tension falls abruptly. This is probably less common in the lungs than in other tissues because of the dual pulmonary circulations and the ventilated alveolar spaces. Some bacterial exotoxins cause direct lysis of epithelial cells, including the type III toxins produced by *Pseudomonas* species, and exotoxins released by *Escherichia coli* and *Staphylococcus aureus* (12–14). Mechanisms

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Figure 1. Cellular pathways that mediate apoptosis. A family of death receptors can initiate apoptosis, and their relative importance depends on the cell type. Two major pathways are shown here: the Fas receptor pathway and the receptor-independent mitochondrial pathway. The various membrane receptors share some components of the signaling pathways shown. DcR3 is unique, however, as it is a truncated receptor that does not signal. The soluble form of DcR3 functions as a decoy receptor that blocks receptor/ligand-mediated apoptosis.

cal forces created by shear stress or overdistension may also cause direct disruption of epithelial membranes, particularly in injured lungs in which heterogeneous alveolar flooding leads to marked heterogeneity in regional compliance (15, 16).

Apoptosis is a form of regulated cell death in which activation of specific intracellular serine rich proteases (caspases) leads to DNA cleavage and cell death. Apoptosis is an essential feature of development, and provides a mechanism for tissue remodeling in specific regions such as the interdigital spaces of fingers and toes. In general, apoptosis occurs without the release of intracellular products, whereas necrosis is associated with cellular swelling, membrane rupture, and the escape of intracellular products into the local environment. *Apoptosis* is a Greek word meaning "falling away," like leaves falling from trees in the autumn. Apoptosis occurs in response to activation of specific cell membrane receptors, termed "death receptors," as well as in response to the release of mitochondrial products such as cytochrome C (17) (Figure 1). The death receptor family includes the TNF receptors I and II, and the Fas receptor (CD95), which is activated either by Fas ligand (FasL) on the surface of cytotoxic lymphocytes, or by a soluble form of FasL (sFasL), which can be cleaved from cell membranes by the action of specific serine proteinases such as matrix metalloproteinases 7 and 3 (MMP-7, MMP-3) (18, 19). Soluble FasL is also released from activated blood monocytes, but it does not appear to be released from activated alveolar macrophages (20). Activation of the mitochondrial and the receptor pathways of apoptosis have been reported in the lungs, but this review focuses primarily on the Fas receptor-mediated apoptosis pathway.

When membrane Fas is clustered by FasL, specialized docking proteins (including Fas-associated death domain [FADD]) aggregate around the intracellular tails of clustered Fas molecules. This death-initiating complex (DISC) recruits procaspase 8 molecules, which undergo activation by autocatalytic cleavage, resulting in the activation of a cascade of downstream intracellu-

lar caspases. This eventually causes activation of endonucleases, with cleavage of nuclear DNA and cell death. The DNA cleavage is detectable as a "laddering" effect of DNA fragments of different molecular weights when cellular DNA is analyzed by electrophoresis in agarose gels. DNA cleavage is also detectable by assays which identify nucleotide cleavage sites using a terminal deoxynucleotidyl transferase (TUNEL assay) (21, 22). Activated caspase 3, a distal enzyme in the caspase cascade, can be detected in cells and tissues using antibodies specific for the cleaved (activated) form of caspase 3. In addition to the Fas receptor-mediated pathway, mitochondrial damage caused by ultraviolet light and other toxins initiates apoptosis via the release of cytochrome C, which binds to Apaf-1 and activates caspase 9, and subsequently caspase 3, followed by DNA fragmentation and apoptotic cell death (Figure 1).

Cellular apoptosis is tightly regulated by several different inhibitory proteins, so that cell death can be controlled at the appropriate times. A family of proteins, inhibitors of apoptosis (IAP), directly bind and inhibit caspases 3, 6, 7, and 9 (23). The mitochondrial pathway is inhibited by the Bcl-2 family of proteins, which block activation of caspases by cytochrome C (24, 25). An additional family, FLICE/caspase-8 inhibitory proteins (FLIP), includes mammalian and viral proteins, which interfere directly with the activation of caspase-8 recruited to the Fas/FADD membrane complex (17, 26, 27). Viral FLIPs provide a unique mechanism to prolong the lives of infected cells by blocking apoptosis pathways (28).

Although the membrane receptor pathway and the mitochondrial pathways for apoptosis differ initially, the terminal events leading to DNA damage are similar, and available assays cannot differentiate the initial stimuli which cause apoptosis. Apoptosis leads to the loss of polarization of the cell membrane, which is detectable as an increase in the expression of phosphatidylserine on the outer leaflet of the lipid bilayer using the binding of the fluorescent indicator annexin V (29, 30). Apoptotic leukocytes

Figure 2. Soluble Fas ligand concentrations (sFasL) measured by specific immunoassay in the bronchoalveolar lavage fluid of normal volunteers (Normal), patients at risk for ARDS because of either clinical sepsis or trauma (At risk), and patients with established ARDS (ARDS). The horizontal axis shows the day on which bronchoalveolar lavage was performed (from Reference 39).

are recognized and ingested by tissue macrophages via specific surface receptors on the cell membrane (31). Because this process is extremely rapid, the number of visible apoptotic leukocytes at sites of tissue inflammation is usually low, and probably underestimates the extent to which apoptosis is occurring (32).

One of the consequences of apoptosis is loss of cellular attachment to the underlying basement membrane. This could result in the exposure of the underlying alveolar epithelial basement membrane to inflammatory products in the alveolar spaces, such as oxidants, proteinases, and other elements of the inflammatory milieu. Destruction of the alveolar walls and activation of fibroblast proliferation and collagen production, which is known to occur at the onset of ALI in humans, could lead to fibrosis during the repair process (33). Identifying the mechanisms that link apoptosis with acute and chronic fibrosis in the lungs is an important objective of ongoing research.

APOPTOSIS IN ALI

The importance of apoptosis in the resolution of inflammation led us to investigate the number of apoptotic PMN recovered from the lungs of patients at the onset and during the course of ALI. Apoptotic PMN are recognizable using hematoxylin eosin staining by their characteristic pattern of nuclear condensation. Examination of bronchoalveolar lavage cells prepared by cyto-centrifugation revealed few apoptotic PMN, as less than 10% of recovered PMN had morphologic features of apoptosis (34). Studies of freshly isolated PMN showed that the bronchoalveolar lavage fluid from patients with lung injury contained factor(s) that delayed PMN apoptosis, principally granulocyte-macrophage colony stimulating factor (GM-CSF) (34). This cytokine provides a mechanism for prolonging PMN survival in tissue and its role is not limited to inflammation in the lungs. Interestingly, electron microscopic analysis of alveolar macrophages in the same specimens showed that more than 30% of the macrophages contained particles suggesting PMN cellular debris such as myeloperoxidase granules. This is consistent with the view that apoptotic PMN are taken up rapidly by activated lung macrophages. Thus two processes are likely to be occurring simultaneously: PMN apoptosis is delayed in the airspaces, and apoptotic PMN are taken up very rapidly by activated macrophages.

Because bronchoalveolar lavage fluid contained factors that modified PMN apoptosis, we also investigated the effect of lung lavage fluid on epithelial cell apoptosis and the results were strikingly different. Earlier studies of human lung tissue had shown that Fas is present on proliferating Type II pneumocytes, as well as airway epithelial cells, and Fas-mediated apoptosis

had been proposed as a mechanism to clear proliferating Type II pneumocytes during lung repair (35–38). We found that the bronchoalveolar lavage fluid of patients with ARDS induced apoptosis in airway epithelial cells derived from the distal lung airways of normal human lungs (39). These distal lung epithelial cells contain lamellar bodies and surfactant protein A (SP-A), but they are not true Type II pneumocytes because they are obtained by microdissection of 1- to 2-mm airways of excised human lungs. The bronchoalveolar lavage fluid was found to contain sFasL (Figure 2) before and after the onset of ARDS, but the sFasL was biologically active only at the onset of ARDS (39). The decoy receptor, DcR3 (40), was also detectable in the lung lavage fluid of these same patients, but the ratio of DcR3 to sFasL provided an incomplete explanation for the bioactivity of the sFasL at the onset of ARDS.

Albertine and coworkers also found sFasL in the lung edema fluids of patients with ALI, and confirmed that soluble Fas is detectable by immunoassay (41). Histologic studies of the lungs of different patients with ALI showed increased expression of Fas on the alveolar epithelium of patients who died, as compared with patients who died of other causes. The studies by Matute-Bello and colleagues and by Albertine and coworkers suggested that activation of the Fas pathway is an important mechanism of alveolar epithelial injury in the lungs of patients with ALI, in addition to direct epithelial necrosis caused by mechanical factors, local ischemia, or bacterial products in the airspaces (39, 40).

Clearly, other pathways in addition to the Fas receptor pathway also have the potential to induce epithelial apoptosis in ALI. Importantly, hyperoxia and hypoxia each can induce apoptosis of pulmonary epithelial cells in experimental systems, and both are relevant in patients with ALI who are ventilated with a high FI_{O_2} , yet at the same time may have regions of tissue hypoxia where alveolar collapse has occurred. Buccellato and associates found that hyperoxia induced apoptosis of primary rat alveolar epithelial cells *in vitro* by activating the pro-apoptotic protein “Bax” at the mitochondrial membrane (42). This leads to the release of mitochondrial cytochrome C, which activates caspase 9, leading to cellular apoptosis. In contrast, Krick and coworkers found that exposure of rat type II cells to graded hypoxia suppressed proliferation and caused apoptosis by a mechanism that involves activation of hypoxia inducible factor (HIF-1 α), and the hypoxia response element (HRE) in nuclear DNA (43). These authors speculated that targeting the HIF-1 α /HRE pathway, perhaps with a low-molecular-weight inhibitor, might be an additional strategy to reduce epithelial apoptosis in ALI.

APOPTOSIS IN HUMAN AIRWAY EPITHELIAL CELLS

The consequences of Fas activation differ at different locations along the airway and alveolar epithelium. We compared the responses of proximal and distal nontransformed human airway epithelial cells and found that proximal airway epithelial cells are insensitive to Fas-induced apoptosis, whereas distal epithelial cells are much more sensitive (44). Rabbit and mouse alveolar type II cells are sensitive to Fas activation either by human sFasL (in rabbit pneumocytes) or a specific monoclonal antibody which clusters membrane Fas (in murine pneumocytes) (45). The mechanism for the differential sensitivity of proximal and distal pneumocytes to sFasL remains unclear, as both types of cells express membrane Fas and contain similar patterns of steady-state mRNAs for the Fas pathway intermediate proteins. These studies raise the possibility that there is a gradient of Fas sensitivity in the lungs, with the proximal airways relatively insensitive to sFasL in airspace fluids and increasing sensitivity moving distally in the airways and into the alveolar space.

APOPTOSIS IN MODELS OF ACUTE AND CHRONIC LUNG INJURY

The importance of apoptosis pathways in acute and chronic lung injury has been investigated in a number of different animal models. When recombinant human sFasL was instilled into the lungs of rabbits, localized alveolar hemorrhage was observed. This was not present in the contralateral lungs of the same animals that had been treated with human sFasL together with an inhibitory immunoglobulin construct containing a human Fas peptide sequence in the variable region. Interestingly, the sFasL activated inflammatory pathways in the lungs, as reflected by immunohistochemical evidence of IL-8 expression in alveolar macrophages where sFasL had deposited in the airspaces. Activation of Fas in the lungs of mice using a single intratracheal dose of the activating monoclonal antibody, Jo2, causes acute inflammation and evidence of apoptosis in alveolar walls within 24 h after instillation of the Jo2 antibody (46). Recently, Wang and associates showed that the caspase-8/Bid pathway is important in signaling associated with hyperoxic lung injury and cell death *in vivo* and *in vitro* (47). In addition to epithelial apoptosis, endothelial cell apoptosis also been found to occur in the lungs in a murine model of hemorrhagic shock (48).

Fas activation has also been linked to chronic lung injury, because repeated exposure to daily aerosols of the Jo-2 antibody for 14 d caused acute inflammation and delayed evidence of collagen accumulation, consistent with a fibrotic response in the lungs (49). Mice lacking the Fas receptor (*lpr* mice) had a blunted response to intrapulmonary bleomycin, with reduced hydroxyproline accumulation following bleomycin exposure, suggesting a role for the Fas pathway in pulmonary fibrosis due to other agents (50). Stimulation of Fas in the lungs of mice is associated with delayed expression of mRNA for TGF- β s, and TGF- β was found to potentiate the effects of sFasL in human lung epithelial cells *in vitro* (51). Because a relatively small increase in the rate of epithelial cell apoptosis can result in considerable cell loss over time, a relatively minor upregulation of epithelial apoptosis, particularly of alveolar Type II cells, could account for the excessive epithelial loss and failure of reepithelialization that is characteristic of pulmonary fibrosis. Epithelial apoptosis is found in the lungs of patients with idiopathic pulmonary fibrosis and in the lungs of mice and rats with bleomycin-induced pulmonary fibrosis (52–55). Interestingly, fibroblast activation in acute or chronic lung injury may compound epithelial cell apoptosis, as Uhal and coworkers and Wang and colleagues found that fibroblasts from fibrotic lungs induced apoptosis of alveolar epithelial

cells *in vitro*, and that angiotensin peptides appeared to have a causal role in this process (56, 57).

The release of sFasL in the systemic circulation may connect lung injury with distant organ injury. Studies of mechanically ventilated rabbits showed more apoptosis in the renal tubular epithelial cells than in the lung epithelium, and serum from patients with ALI contained detectable sFasL in concentrations that were directly related to the serum creatinine concentration (58). Soluble FasL could not be measured in the rabbit serum because of the lack of an immunologic assay at the time of the study. Similarly, a caspase inhibitor (ZVAD) protected mice from lung inflammation and death after systemic treatment with LPS, suggesting that apoptosis pathways are involved in LPS-dependent inflammatory events in mice (59). More information is needed about the relationships between lung injury, activation of Fas pathways in the lungs and systemic circulation, and apoptosis in distant organs.

Recent evidence indicates that phagocytic clearance of apoptotic cells primes the immune system against autoantigens, and plays an immunoregulatory role (60). Apoptotic cells have been increasingly recognized as targets of autoantibodies that arise in a broad spectrum of autoimmune diseases. The evidence that apoptotic cells are targeted by autoantibodies was provided by studies showing that autoantibodies from humans and mice with systemic lupus erythematosus (SLE) recognize autoantigens that are prominently clustered in the surface blebs of apoptotic cells (61, 62). In addition, immunization of mice with syngeneic apoptotic cells has been reported to induce a humoral immune response to apoptotic cells (63). Thus, cells undergoing apoptosis provide “proinflammatory” signals, mediating autoimmune and inflammatory responses. This could be relevant for the pathogenesis of collagen-related lung diseases, which have high frequencies of associated lung fibrosis.

APOPTOSIS AND INFECTION

A report by Grassme and coworkers indicating that *Pseudomonas* organisms disseminated more readily from the lungs of mice deficient in Fas ligand suggested a role for the Fas system in host defenses in the lungs, even though *Pseudomonas* caused apoptosis in the lungs of normal mice (64). The implication was that strategies to block Fas-dependent responses in the lungs might increase host susceptibility to bacterial infection. In this study, the bacteria were delivered by intratracheal inoculation, and the bacterial inoculum was relatively high. We used a bacterial aerosol model to compare bacterial clearance from the lungs of normal and Fas-deficient mice (*lpr* mice). The clearance of *S. aureus*, *Streptococcus pneumoniae*, and *E. coli* was similar in the normal and the *lpr* mice, and there were no major differences in neutrophil recruitment to the lungs (65). However, the normal mice had more severe tissue injury than the *lpr* mice in response to *E. coli*, with evidence of alveolar wall thickening, vascular congestion, and neutrophilic inflammation. Thus, at the bacterial inocula that are achieved by the aerosol method (about 10^6 cfu/lung), Fas dependent signaling is not critical for the effective clearance of these gram-positive and gram-negative bacteria, but the Fas system appears to have a role in tissue injury caused by gram-negative bacteria.

There is an important degree of cross-talk between Fas-dependent apoptosis pathways and proinflammatory pathways, despite the fact that apoptosis was originally thought to be a relatively “silent” mode of cell death. In human airway epithelial cells, activation of Fas with an activating monoclonal antibody (CH-11) has been reported to cause NF- κ B activation and IL-8 secretion (66). In human monocyte-derived macrophages, Fas stimulation does not lead to apoptosis, but rather to NF- κ B activation and the

Figure 3. Interactions between the Fas-dependent apoptosis pathway and the TLR4 pathway that mediates cellular activation in response to bacterial lipopolysaccharide. FADD, Fas-associated death domain protein. The FADD protein shuttles back and forth between the Fas intracellular tail, where it promotes apoptosis, and the TLR4 adaptor protein, MyD88, where it antagonizes TLR4-dependent signaling.

production of $\text{TNF-}\alpha$, IL-8, and other proinflammatory cytokines (67). Earlier, we had found that instillation of recombinant human sFasL into the lungs of rabbits caused IL-8 expression in lung macrophages *in vivo*, but the mechanism for this was unclear at the time (45). Mice lacking Fas have reduced intrapulmonary inflammatory responses to LPS *in vivo*, showing that the interaction between the Fas pathway and the LPS recognition pathways occur *in vivo* (68). Bannerman and associates showed that the key intracellular docking protein which mediates Fas-dependent cellular activation, FADD, negatively regulates LPS-dependent NF- κ B activation (69). Expression of a dominant-negative FADD construct blocked NF- κ B activation by LPS, and the absence of FADD was associated with increased NF- κ B activation as well as increased cytokine production. Ma and associates expanded these findings when they found that Fas-deficient mice had reduced joint inflammation in a model of collagen-induced arthritis, despite having more IL-1 β in their joints (70). These studies showed that FADD regulates signaling through TLR4 via binding to MyD88, a proximal signaling molecule common to both IL-1R and TLR4 intracellular pathways. The current paradigm is that when Fas is clustered and FADD is bound to the intracellular tail of Fas, MyD88 is active and signaling through IL-1R and TLR4 is enhanced (Figure 3) (71). In contrast, when Fas is blocked, or inactive as in *lpr* mice, FADD is more likely to be bound to MyD88, reducing IL-1R- and TLR4-dependent signaling. This model is consistent with our prior observations that LPS-dependent intrapulmonary inflammation is reduced in the Fas-defective *lpr* mice, and that tissue inflammatory responses after inhaled *E. coli* are reduced in *lpr* mice (65, 68).

SHOULD APOPTOSIS BE INHIBITED IN THE LUNGS?

The hypothesis that apoptosis pathways should be inhibited in the lungs of patients with ALI had not been tested rigorously, but several experimental clues make this approach attractive. An important concept is that the role of apoptosis in the patho-

genesis of lung disease is likely to depend on the specific cells that are becoming apoptotic. For example, apoptosis of neutrophils may benefit the host by helping the resolution of inflammation, but apoptosis of epithelial cells may lead to disruption of the epithelial barrier and contribute to alveolar flooding. Thus, it may be important to develop cell-specific anti-apoptotic strategies, rather than strategies aimed at blocking apoptosis of all types of cells within the lungs.

We have found that mechanical ventilation and LPS have synergistic effects on the release of sFasL in the lungs of ventilated mice, which may promote apoptosis in the lungs of ventilated mice when bacterial LPS reaches the distal airspaces (unpublished observations). Blockade of caspase activation with ZVAD protected mice from death after systemic LPS administration (59). The studies of Imai and colleagues suggest that inhibiting apoptosis in the systemic circulation could prevent secondary apoptosis to renal tubular epithelial cells and concomitant acute renal insufficiency (58). This observation links lung injury with injury to distant organs, consistent with the lungs as a driver of the multiple organ failure syndrome, and raises hope that inhibitors of apoptosis will be useful in preventing localized injury in the lungs, as well as multiple organ failure. More experimental information is needed to identify the rate-limiting steps in the apoptosis pathways that might be inhibited by new drugs, and the consequences of such inhibition on lung repair, in addition to injury. Nevertheless, new strategies to modulate apoptosis in the lungs could be very useful in reducing ALI in humans. If early apoptosis responses in the alveolar walls are mediated by excess concentrations of agonists like sFasL, then blocking apoptosis early after lung injury might be beneficial. However, if apoptosis is important in the later repair phase, it might be worthwhile to allow apoptosis to proceed later in the course of the illness. Additional experimental data in animals and humans will be required to clarify these important possibilities.

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