HIGHLIGHTED TOPIC | Lung Growth and Repair

Aptoptosis in lung injury and remodeling

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Aptoptosis in lung injury and remodeling. J Appl Physiol 97: 1535–1542, 2004; 10.1152/japplphysiol.00519.2004.—The mode of cell death termed apoptosis, sometimes referred to as programmed cell death, is as critical a determinant of cell population size as is cell proliferation. Although the best characterized in cells of the immune system, apoptosis is now known to be a key factor in the maintenance of normal cell turnover within structural cells in the parenchyma of virtually every organ. Recent interest in apoptosis in the lung has sparked a surge of studies designed to determine the roles of apoptosis in lung development, injury, and remodeling. Of particular recent interest are the roles of apoptosis in disease pathogenesis and resolution, in which the concept of apoptosis as a “programmed” cell death, i.e., genetically determined, is often more accurately viewed as “inappropriate cell suicide” with regard to its extent and/or timing. Data accumulating over the past decade have made clear the complexity of the control of lung cell apoptosis; concepts of the regulation of apoptosis originally determined in classical cell culture models are often, but not always, applicable to structural cells. For this reason, each of the many cell types of the lung must be studied as a potentially new subject with its own idiosyncrasies yet to be discovered. In light of the large volume of literature now available, this article focuses on the roles of apoptosis in three pathophysiological contexts: acute respiratory distress syndrome, chronic obstructive pulmonary disease, and pulmonary fibrosis. Each section presents key data describing the evidence for apoptosis in the lung, its possible relevance to disease pathogenesis, and proposed mechanisms that might suggest potential avenues for therapeutic intervention.

programmed cell death; acute respiratory distress syndrome; chronic obstructive pulmonary disease; pulmonary fibrosis; caspases

APPTOSIS IN ACUTE RESPIRATORY DISTRESS SYNDROME

Acute respiratory distress syndrome (ARDS), the severe form of alveolar lung injury (ALI), is characterized by flooded alveolar air spaces and increased microvascular and epithelial permeability (112) due to disruption of the alveolar epithelium, damage to the alveolar capillary endothelium, and neutrophilic inflammation. Given that apoptosis plays an important role in the immune system and tissue repair after injury, there are several potential mechanisms by which apoptosis might play roles in ARDS; these include apoptosis of epithelial cells, neutrophils, and endothelial cells.

Epithelial cell apoptosis in ARDS. Apoptotic epithelial cells have been found in the damaged alveolar epithelium of patients with ARDS (93). In the resolution phase of acute lung injury, apoptosis of type II pneumocytes is believed to be largely responsible for the disappearance of excess epithelial cells (13, 105). Elevated concentrations of soluble Fas (Apo1, CD95) and Fas ligand (FasL) were detected in bronchoalveolar lavage (BAL) fluids from patients with ALI or ARDS (6, 39, 74, 112), suggesting that the Fas system might be involved in the apoptosis found in ALI or ARDS.

Type II alveolar epithelial cells (AECs) express both Fas and FasL protein (6, 27, 74), and the Fas/FasL system induces type II epithelial cell apoptosis both in vitro and in vivo (27, 75). Matute-Bello and colleagues (75) induced acute lung injury in mice by intratracheal administration of Fas-activating antibody and thereby demonstrated that apoptosis can lead directly to lung barrier collapse, at least in the mouse model. The notion that the Fas-FasL system might play a role in epithelial apoptosis in ARDS is supported by the finding that BAL fluids obtained from ARDS patients induced apoptosis in a distal lung epithelial cell line (74) and that apoptosis was inhibited by blocking the Fas-FasL system with Fas- or FasL-specific inhibitors (antibodies or Fas-Ig fusion proteins).

One of the possible consequences of epithelial (type II) cell apoptosis in ARDS or ALI is the reduction of lung surfactant pools. Surfactant protein A (SP-A) has inhibitory effects on type II cell apoptosis and injury of lung fibroblasts (114); therefore, surfactant replacement therapy might have therapeutic benefits for patients with ARDS through its inhibition of apoptosis in addition to other mechanisms. Although the pathways that signal apoptosis of epithelial cells in ARDS are not...
totally clear, studies of diffuse alveolar damage suggest the involvement of BAX, a homologue of Bcl-2 (41), and its likely proapoptotic effect on type II pneumocytes (32).

**Neutrophil apoptosis in ARDS.** Activated neutrophils cause lung injuries in patients with ARDS by releasing oxidants, proteases, leukotrienes, and other proinflammatory molecules, such as platelet-activating factor. The protein-rich exudates in the alveolar spaces then lead to the inactivation of surfactant. Clinical and experimental studies have shown the accumulation of neutrophils in the pulmonary edema fluid and BAL fluid obtained from patients with ARDS (82). Neutrophils, as well as other phagocytes, and endothelial and epithelial cells in the lung all express the receptor Fas. Knockout of toll-like receptor-4 in the mouse markedly reduced the influx of neutrophils to the lung, as indicated by a reduction in lung myeloperoxidase activity compared with fasL-gene-deficient or wild-type mice (11).

Neutrophils have a relatively short life span; therefore, the accumulation of neutrophils at inflammatory sites in the lung is due in part to delayed neutrophil apoptosis, although apoptosis of epithelial cells is enhanced at the same site. Both human and animal studies supported the inhibitory effects of BAL fluid on neutrophil apoptosis (30, 73, 80). BAL fluid from patients with early ARDS delays neutrophil apoptosis; this fluid also exhibits elevated levels of granulocyte colony-stimulating factor, granulocyte/macrophage colony-stimulating factor (GM-CSF; Ref. 73), and interleukin-8 (3). When incubated with human neutrophils, interleukin-8 and growth-related oncogene-α suppress neutrophil apoptosis and stimulate their own production (25). Klein et al. (54) identified the involvement of phosphoinositide 3-kinase and ERK pathway in the GM-CSF-dependent inhibition of neutrophil apoptosis. In vivo endotoxin administration to mice resulted in activation of phosphoinositide 3-kinase and Akt in neutrophils that accumulated in the lungs (122). Mcl-1, an antiapoptotic Bcl-2 family member, is present in normal neutrophils, but the expression of Mcl-1 is decreased in neutrophils undergoing apoptosis (62). After exposure of neutrophils to GM-CSF, the stability of Mcl-1 is enhanced (21). Evidence suggests that Mcl-1 is crucial for the delay of apoptosis initiated by antiapoptotic factors.

Other factors regulating the apoptosis of neutrophil include eicosapentaenoic acid and γ-linolenic acid. When used alone or in combination, these compounds significantly induced apoptosis and reduced cell viability in the HL-60 cell line (29). These data suggest that eicosapentaenoic acid and γ-linolenic acid might decrease pulmonary inflammation by reducing neutrophil counts and chemotactic factors in BAL fluid during ARDS.

Apoptosis of neutrophils is an important determinant of the consequences of lung injury. Sookhai et al. (94) showed that the induction of pulmonary neutrophil apoptosis by administration of aerosolized dead Escherichia coli before reperfusion injury resulted in a significant improvement in lung injury and in survival. In a rat model of oleic acid-induced lung injury, the onset of neutrophil apoptosis was shown to correlate with the resolution response and phagocytosis by macrophages (42). Thus neutrophil apoptosis likely plays an important role in attenuating neutrophil-mediated lung injury and may ultimately benefit the outcome of patients with ARDS. Although most studies support the notion that neutrophilic inflammation leads to ALI, there are some data that oppose this theory. Patients with acute neutropenia can develop ALI and ARDS (61), in agreement with neutrophil-independent animal models of ALI. In light of their prolonged life span, the removal of neutrophils without the release of their cytotoxic contents becomes very important in the resolution of inflammation. The apoptotic neutrophil is recognized by macrophage at inflammation sites (20, 86) via several cell surface molecules. Among them, the hyaluronan receptor CD44 is well studied and plays an important role in resolving lung inflammation. Using CD44-deficient mice, Teder et al. (98) showed unremitting inflammation accompanied by impaired clearance of apoptotic neutrophils. The introduction of CD44+ cells can partially reverse this phenotype (98). Thus both in vivo and in vitro data suggest a novel role for CD44 in inflammation and tissue repair.

Aptosis of epithelial cells and neutrophils are interrelated events. Serrao et al. (92) showed that neutrophil-induced epithelial apoptosis can be blocked by inhibitory anti-Fas and anti-FasL monoclonal antibodies, suggesting that neutrophils secrete soluble Fas ligand. In response to Fas ligation or TNF-α, bronchiolar epithelial cells undergo apoptosis and secrete interleukin-8 and NF-κB (33), which in turn suppresses the apoptosis of neutrophils. Thus the Fas-FasL pathway mediates epithelial apoptosis and also plays a proinflammatory role in lung injury. In ALI and ARDS, as well as in pulmonary fibrosis, circulating neutrophils release hepatocyte growth factor (HGF) (95). Because HGF and keratinocyte growth factor (KGF) are potent growth factors for type II pneumocytes (71), these data suggest a positive role for neutrophils in promoting alveolar epithelial repair after acute or chronic lung injury.

**Endothelial cell apoptosis in ARDS.** Hamacher et al. (36) demonstrated that BAL fluid from patients at risk of developing ARDS or from patients with early- and late-phase ARDS is cytotoxic to human lung microvascular endothelial cells. Subsequently, increased levels of TNF-α and angiostatin, an inhibitor of angiogenesis in vivo, were determined in BAL fluid from those patients or from volunteers treated with endotoxin (36, 67). Neutralization of TNF-α or angiostatin inhibited the cytotoxic activity of BAL fluid on cultured endothelial cells. These results indicate that TNF-α and angiostatin may contribute to the endothelial injury observed in ARDS. Apoptosis signal-regulating kinase (ASK)-1 is a ubiquitously expressed mitogen-activated protein kinase kinase kinase that activates the MKK3/MKK6-p38 MAPK and the SEK1-JNK signaling cascade. Machino et al. (69) identified the involvement of ASK-1 in apoptosis of pulmonary vascular endothelial cells challenged with hydrogen peroxide (H₂O₂). Using parental porcine artery endothelial cells that were stably transfected with the dominant-negative form of ASK-1, they found that p38 MAPK and JNK phosphorylation, caspase-3 activation, and apoptosis were all decreased by gene knockdown of ASK-1. Given the known roles of H₂O₂ generated by activated neutrophils and other phagocytes, these data suggest the possibility that inhibitors of ASK-1 and its signaling pathway might have potential benefits in the management of ARDS or ALI.

**APOPTOSIS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE.**

The notion that cell death by apoptosis is a likely contributor to the pathogenesis of some forms of chronic obstructive pulmonary disease (COPD), particularly in diseases involving
irreversible tissue destruction such as emphysema, might seem obvious to some readers. However, actual data demonstrating the presence of apoptotic cells in the lungs of patients with COPD did not begin to appear until relatively recently. The first report of apoptosis within parenchymal cells in biopsy tissues from COPD patients was that by Segura-Valdez et al. (88), who showed increased in situ end labeling of DNA within endothelial, alveolar epithelial, and interstitial cells accompanied by increased gelatinase and collagenase activities. These authors also noted labeling of some inflammatory cells, a finding that agreed with the earlier demonstration of Hamzaoui et al. (37) of increased lymphocyte apoptosis in patients with acute exacerbation of asthma.

More recently, Hodge et al. (39) found an association of increased apoptosis of T lymphocytes in COPD patients with elevated transforming growth factor-β. The detection of apoptotic cells in both inflammatory cell and alveolar wall cell populations, specifically in emphysema, was later confirmed by several independent research groups (49, 52, 70, 120). Efforts to more carefully define the specific cell types undergoing apoptosis, and potential mechanisms that might signal their demise, have identified apoptotic vascular endothelial cells in COPD (104) and specifically in emphysema (48). Effort is also being directed toward understanding the roles and consequences of apoptosis in the skeletal muscles of patients with COPD (4, 5, 31, 63). Regardless, the significant implications of increased apoptosis in parenchymal cells of the lungs toward the pathogenesis and treatment of emphysema and other forms of COPD are now being realized (100). The notion that apoptosis of parenchymal wall cells can, at least theoretically, play a causative role in the genesis of COPD is supported by the demonstrations of Aoshiba et al. (10) that experimental induction of apoptosis by instillation of the active form of caspase-3 could cause architectural changes in the lungs of mice similar to those observed in human emphysema.

Potential mechanisms by which apoptosis contributes to COPD. Given the important roles of inflammation in the pathogenesis of COPD (78), much interest was generated by the findings of reduced apoptosis in neutrophils of COPD patients (83) and a reduced capacity of macrophages toward phagocytosis of apoptotic epithelial cells (40). Although little is yet known about the mechanisms underlying these changes, experiments to define them will be interesting and compelling studies for the future. In the meantime, inferences can be drawn on the basis of in vitro studies and the possible roles of key molecules known to affect cell survival and/or known to be prominent in the inflamed lung.

Key among these are the various reactive species generated in the oxidant burst of activated phagocytes (15, 91) or inhaled as part of the toxic repertoire of cigarette smoke (16, 17). As discussed elsewhere in this manuscript, oxidant stress contributes to both apoptosis and necrosis of a variety of lung cell types studied in vitro, and a growing number of research groups are studying the mechanisms by which cigarette smoke kills lung cells by apoptosis and other mechanisms (16). For example, Ishii et al. (46) showed that glutathione S-transferase P1 has a protective effect on tobacco smoke-induced apoptosis in human lung fibroblasts and thereafter found that depletion of glutathione S-transferase P1 by itself induces apoptosis in the same cells (45). Rahman et al. (84) found that 4-hydroxy-2-nonenal, a product of lipid peroxidation capable of inducing apoptosis in vitro, is elevated in lungs of patients with COPD. Studies such as these support the notion that pharmacological manipulation of oxidant stress, and in particular manipulation of the glutathione system, may hold therapeutic potential for the treatment of some forms of COPD.

Other investigations have begun to implicate a variety of endogenous growth factors and cytokines in the apoptosis associated with COPD. For example, overexpression of placental growth factor in mice caused apoptosis of type II pneumocytes, reduced endothelial cell number and the expression of vascular endothelial growth factor (VEGF), and caused emphysematous changes in lung architecture (99). It has been hypothesized that these changes in lung architecture may be an indirect effect of the decrease in VEGF expression, on the basis of demonstrations that chronic blockade of VEGF receptors with the receptor blocker SU-5416 caused alveolar septal cell apoptosis and air space enlargement (50). Later studies found that the effect of VEGF was enhanced by oxidative stress, a conclusion formed on the basis that the superoxide dismutase mimetic M-40419 prevented the apoptosis and alveolar enlargement in response to VEGF receptor blockade (101). From these and related findings, Sakao et al. (85) theorized that a polymorphism yielding significantly lower plasma levels of circulating VEGF might affect the frequency of COPD, but such a relationship was not found.

Another group of endogenous mediators believed to play an important role in apoptosis of parenchymal cells of the lung is TNF-α, FasL, and its receptor Fas. A role of TNF-α was suggested by the finding that TNF-knockout mice have reduced severity of elastase-induced emphysema (68). The studies of Takabatake et al. (97) were based on the theory that circulating levels of these molecules might be increased in patients with COPD, but this study showed that serum and plasma levels of soluble Fas or soluble FasL were unchanged. On the other hand, Yuzuka et al. (117) found that increases of soluble Fas, which can act as an inhibitor of apoptosis, are associated with the progression of COPD. Clearly, more work is needed to clarify the role(s) of TNF-α, Fas, FasL, and VEGF in apoptosis and COPD in the human lung.

Several recent lines of investigation have suggested novel strategies for potential therapeutic interventions in COPD based, at least in part, on studies of apoptosis. For example, activation of peroxisome proliferator-activated receptors in human airway smooth muscle cells imparts potent anti-inflammatory properties (81); peroxisome proliferator-activated receptors affect apoptosis and/or differentiation of many cell types, including endothelial cells of the lungs (7). In mice developing emphysema, SP-D reduces alveolar macrophage apoptosis and proinflammatory cytokines (19), suggesting that modulation of SP-D or other members of the collectin family might offer therapeutic potential. On the other hand, blocking apoptosis may not prove to be beneficial, as both nicotine and crude cigarette smoke have been shown to inhibit apoptosis of neutrophils and Jurkat cells (8, 114). Before the practical value of these and related observations can be fully realized, further study is required for a better understanding of the role(s) of apoptosis within the various parenchymal and infiltrating cell types believed to be key in the pathogenesis of COPD.
APOPTOSIS IN LUNG FIBROSIS

Pulmonary fibrosis results from injury to the lung and an ensuing fibrotic response that leads to thickening of the alveolar walls and the obliteration of alveolar air spaces. If the etiology is unknown, the condition is designated as idiopathic pulmonary fibrosis (IPF; Ref. 28). The main histological features of the fibrotic lung are persistent and unrepaird epithelial damage, proliferation and accumulation of fibroblasts and myofibroblasts, and increased collagen deposition (89, 90). Recent studies support the notion that apoptosis contributes to the pathogenesis of lung fibrosis as well as to its resolution. This section will discuss the roles of apoptosis of AECs in fibrotic lung diseases.

Apoptosis in the lung could be envisioned as detrimental or beneficial, depending on the cell type, the circumstances, and the timing. For example, the stimulation of apoptosis in myofibroblasts and fibroblasts in the fibrotic lung could be beneficial because they are the major source of excess extracellular matrix. Apoptosis of inflammatory cells in fibrotic lung might also be beneficial (64), but excess epithelial cell apoptosis could lead to barrier collapse and a fibrotic response through the mechanisms to be discussed below. Therefore, in future attempts to manipulate apoptosis therapeutically, the cell-type specificity, timing, and location of apoptosis must all be considered.

Epithelial cell apoptosis in fibrotic lungs. A relatively small increase in the incidence of apoptosis within a given cell population can result in considerable cell loss over time. Therefore, a seemingly minor upregulation of apoptosis is theoretically capable of accounting for the excessive loss of AECs and the failure of the reepithelization characteristic of pulmonary fibrosis. Recent studies strongly suggested a role for epithelial apoptosis as a key profibrotic event in lung fibrogenesis. Evidence in support of this viewpoint is summarized below.

First, apoptosis of AECs is found both in the lungs of patients with IPF and in animal models of the disease. Fragmented DNA, a hallmark of apoptosis, was found in bronchioalveolar cells and AECs within lung biopsies from patients with IPF (57) and in the lungs of mice and rats with bleomycin-induced lung fibrosis (35, 107). This finding was confirmed by simultaneous double labeling of fragmented DNA and α-smooth muscle actin, a marker for myofibroblasts, in biopsies from patients with IPF (102). Fragmented DNA in the alveolar epithelium was found frequently and immediately adjacent to α-smooth muscle actin-positive interstitial cells and foci of collagen. Thus epithelial apoptosis colocalizes with myofibroblasts where collagen deposition is severe, at least in patients with IPF. Very recently, apoptosis within AECs of fibrotic human lungs was reconfirmed by Barbas-Filho et al. (12), also through the detection of fragmented DNA.

Consistent with those findings, the “death receptor” Fas was found to be expressed in AECs within the lungs of IPF patients by several independent research groups (24, 51). Thereafter, increased circulating levels of soluble FasL were shown to correlate with disease activity in patients with IPF (59). In animal models, similar observations were obtained; Hagimoto et al. (34) showed epithelial apoptosis and upregulation of Fas on the epithelium in bleomycin-induced pulmonary fibrosis in mice (35). Another study demonstrated that Fas is expressed on the luminal surface of a subset of alveolar type II cells in the mouse model (27).

Second, induction of apoptosis in the epithelium is sufficient to initiate a fibrotic response in animal models. Hagimoto et al. (34) showed that instillation of an antibody that activates Fas-induced apoptosis of bronchial and AECs (both of which express Fas constitutively) initiated a fibrotic response detectable 1 wk later. Moreover, knockout mice deficient in the receptor Fas were found to be resistant to the profibrotic effect of bleomycin (55). However, another study found that the development of bronchial and alveolar epithelial apoptosis and fibrosis after bleomycin instillation in the lungs in Fas-null lpr mice and gld mice was similar to development shown in wild-type mice (9). Thus the role of Fas-induced apoptosis in the development of the pulmonary fibrotic response is still controversial; in addition, pathways other than Fas can initiate epithelial apoptosis and facilitate fibrogenesis.

Third, pharmacological blockade of apoptosis can prevent the fibrotic response. Wang et al. (107) first showed that bleomycin-induced accumulation of lung collagens could be blocked by the angiotensin-converting enzyme (ACE) inhibitor captopril or by daily intraperitoneal injections of N-benzylcarboxy-Val-Ala-Asp-fluoromethylketone (ZVADfmk), a broad-spectrum inhibitor of caspases (cysteine proteases) required for the induction of apoptosis. Soon thereafter, Kuwano et al. (58) confirmed the blockade by using the same caspase inhibitor (ZVADfmk) administered by aerosol to mice. Another strategy to interrupt AEC apoptosis proved effective in blocking bleomycin-induced pulmonary fibrosis; Inoshima et al. (43) showed that the forced expression of p21, predominantly in lung epithelial cells, exerted both antiapoptotic and antifibrotic effects. Thus the blockade of collagen deposition in vivo by inhibitors of apoptosis suggests that the fibrotic response is secondary to the apoptotic death of certain lung cell types. This premise in turn is consistent with the theories put forth by Witschi (115) and Adamson and Bowden (1) that alveolar reepithelialization is necessary to prevent subsequent fibrogenesis after lung injury. Other data consistent with this theory include studies of KGF, a potent proliferation and differentiation factor for alveolar type II cells known to promote alveolar epithelial repair (95). Both KGF and the related HGF prevented bleomycin-induced lung fibrosis in rats and mice (22, 23, 96, 118, 119).

Mechanism and signaling of epithelial apoptosis in fibrotic lung. Alterations in the expression of various antiapoptotic and proapoptotic factors likely regulate apoptosis of AECs. The proapoptotic factors p53 and p21 are upregulated in bronchiolar and AECs within lung biopsy specimens from patients with IPF and in bleomycin-induced pulmonary fibrosis animal models (56, 57). Upregulation of the proapoptotic factor BAX in patients with diffuse alveolar damage and in bleomycin-induced pulmonary fibrosis may enhance the susceptibility of AECs to apoptosis (32, 56).

In vitro studies of epithelial cell lines or primary cells showed that JNK, a member of the MAPK family is involved in stress-induced apoptosis. Activation of JNK (2, 47, 60) leads to phosphorylation of its targets, c-Jun and activating transcription factor (ATF)-2 (44, 87), and activation of downstream gene expression when epithelial cells are exposed to oxidative stress, which is a very important contributor to pulmonary injury and fibrosis. An in vivo study of p38 MAPK in bleo-
mycin-induced pulmonary fibrosis showed that p38 MAPK and its substrate, ATF-2, were phosphorylated in BAL fluid cells after intratracheal instillation of bleomycin. The phosphorylation of ATF-2 was inhibited by subcutaneous administration of a specific inhibitor of p38 MAPK, FR-167653. FR-167653 also prevented the apoptosis of lung cells and fibrosis induced by bleomycin administration (72). Signaling pathways that can contribute to the apoptosis of AECs in IPF patients were investigated by Yoshida et al. (121), who found that active ERK was decreased and active JNK was increased in epithelial cells and was accompanied by the progression of fibrosis. Activated p38 MAPK in epithelial cells was increased at the intermediate stage of fibrosis, in which the TUNEL-positive cells were predominantly detected (121).

The angiotensin system and lung cell apoptosis. Purified ANG II induces apoptosis of AECs in primary culture (110). This effect was mediated by the AT1 receptor subtype through protein kinase C (PKC) and could be prevented by the specific PKC inhibitor chelerythrin (79).

Subsequently, it was shown that apoptosis of AECs in response to FasL (109), TNF-α (106), amiodarone (14), and bleomycin (66) requires the de novo synthesis of ANG II and its binding to receptor AT1. In all cases, AEC apoptosis could be blocked by antisense oligonucleotides against angiotensinogen mRNA, by ACE inhibitors, or by AT1 receptor antagonists in vitro. Furthermore, human lung myofibroblast-derived inducers of alveolar epithelial apoptosis, originally identified in myofibroblast-conditioned media (102), were eventually identified as angiotensin peptides (108). These data suggest that ANG II is produced by at least two sources outside the lung, i.e., by myofibroblasts and by AECs undergoing apoptosis in response to FasL, TNF-α, amiodarone, or bleomycin. Because ANG II itself can also induce apoptosis of otherwise normal AECs, these effects of angiotensin on AEC apoptosis could account, at least partially, for the inhibitory effects of angiotensin system antagonists on lung fibrogenesis (to be discussed below).

Blockade of experimental lung fibrosis by angiotensin system antagonists. ACE inhibitors have been shown to attenuate experimental pulmonary fibrosis induced by various agents in animal models. The ACE inhibitor captopril inhibited monocrotaline-induced fibrosis (77) as well as irradiation-induced lung fibrosis in rats (111). More recently, the AT1 receptor-selective antagonist L158809 and the nonthiol ACE inhibitor enalapril were shown to have similar inhibitory effects on radiation-induced pulmonary fibrosis in rats (76). Wang et al. (107) demonstrated that captopril prevented both AEC apoptosis and collagen deposition in bleomycin-treated rats. A more recent study showed essential roles for angiotensin receptor AT1α by demonstrating that the AT1α-selective receptor antagonist losartan, or targeted deletion of the AT1α gene, can block bleomycin-induced epithelial apoptosis and pulmonary fibrosis in mice (65).

The role of apoptosis in lung fibrosis has also been evaluated in amiodarone-induced pulmonary toxicity. Amiodarone is an effective antiarrhythmic agent that induces pulmonary toxicity, including fibrosis in long-term therapy, in 6% of patients with 5–10% mortality in affected patients. In vitro studies have shown that amiodarone induces apoptosis or necrosis in primary AECs isolated from adult Wistar rats and in the human AEC-derived A549 cell line (14, 26). This effect was present at drug concentrations below the therapeutic serum concentration for amiodarone in patients receiving the drug for 9 mo (14). In addition, amiodarone increased the mRNA levels of BAX and caspase-3 in L-132 human lung epithelial cells (18). Both the apoptosis and net cell loss in vitro were blocked by the caspase inhibitor ZVADfmk, by the ACE inhibitor captopril, and by the ANG II-receptor antagonists saralasin or losartan (14, 103). Moreover, 6-mo oral administration of amiodarone to Wistar rats induced AEC apoptosis and lung fibrosis, both of which were inhibited by the ACE inhibitor captopril or by the angiotensin receptor AT1 antagonist losartan (103). Together, these results indicate that the angiotensin system plays an important role in AEC apoptosis and in lung fibrogenesis via the AT1 receptor.

SUMMARY

The induction of apoptosis within each of the many cell types of the lung could theoretically be detrimental or beneficial to lung homeostasis. Which of these outcomes is realized depends on the cell type, the timing, and the location of the cell at the time apoptosis is induced. Adding to this complexity is the growing evidence that individual cell types do not necessarily respond identically to a given stimulus. Understanding the many ways in which apoptosis contributes to lung injury and remodeling will require continued efforts to define the influence of key cytokines, extracellular matrix components, and cell–cell interactions on cell death in each of the many lung cell types critical to its function. Although isolated cell types are very useful and necessary tools to this end, in vitro studies are most meaningful when balanced against in vivo studies, which test the roles of specific gene products in the whole organism through transgenic models. The continued use of each of these experimental tools may ultimately provide sufficient understanding of the roles and regulation of lung cell apoptosis to offer novel future strategies for therapeutic control of lung injury and remodeling through pharmacological or genetic manipulation of apoptosis.

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Invited Review

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