Histol Histopathol (2004) 19: 867-881

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

The role of apoptosis in pulmonary fibrosis

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Summary. Pulmonary fibrosis is a common response to various injuries to the lung. The resolution of a fibroproliferative response after lung injury is key to survival. Although there are various initiating factors or causes, the terminal stages are characterized by proliferation and progressive accumulation of connective tissue replacing normal functional parenchyma. Conventional therapy consisting of glucocorticoids or immunosuppressive drugs is usually ineffective in preventing progression of fibrosis. Further understanding of the molecular mechanisms of endothelial and epithelial cell injury, inflammatory reaction, fibroblast proliferation, collagen deposition and tissue remodeling, should lead to the development of effective treatments against pulmonary fibrosis. Evidence that apoptosis plays an important role in the pathophysiology of pulmonary fibrosis has been accumulated. We overview the role of apoptosis in each of the pathogenic events which have emerged from animal models and human tissue studies.

Key words: Lung injury, Pulmonary fibrosis, Apoptosis, Epithelium, Fibroblast

Introduction

Apoptosis plays a major role in homeostasis to maintain a balance between cell proliferation and cell death. There are two principle signaling pathways of apoptosis. One is a direct pathway from death receptor ligation to caspase cascade activation and cell death. Death receptor ligation triggers recruitment of the precursor form of caspase-8 to a death-inducing complex, through the adaptor protein FADD, which leads to caspase-8 activation. The other pathway triggered by stimuli such as drugs, radiation, infectious agents and reactive oxygen species is initiated in mitochondria. After cytochrome c is released into the cytosol from the mitochondria, it binds to Apaf1 and ATP, which then activate caspase-9 (Kroemer and Reed, 2000). The activation of caspase-8 or caspase-9 leads to the activation of the caspase cascade and apoptosis. The vulnerability to apoptosis induced by death receptors or other apoptosis stimulators, and the ability to survive by inhibitors of apoptosis vary according to the cell type (Fig. 1).

Apoptosis may play important roles in lung diseases in two different ways. First, failure to clear unwanted cells by apoptosis will prolong the inflammation because of the release of their toxic contents. The resolution of a fibroproliferative response after lung injury is key to survival. If orderly reconstitution of the alveolar wall is to occur, the intraalveolar granulation tissue must regress without inducing an acute inflammatory response or disturbing the concomitant re-epithelialization. It is now increasingly recognized that the unwanted cells are cleared by apoptosis, which eliminates cells without inciting an inflammatory response, because apoptotic cells are quickly recognized and ingested by phagocytes before releasing their toxic contents, unlike accidental cell death or necrosis. Repair after an acute lung injury requires the elimination of proliferating mesenchymal and inflammatory cells from the alveolar airspace or alveolar wall (Polunovsky et al., 1996). Secondly, excessive apoptosis may cause diseases. It has been demonstrated that intratracheal instillation of agonistic anti-Fas antibody or recombinant Fas ligand (FasL) induces acute alveolar epithelial injury and lung inflammation (Matute-Bello et al., 2001a,b), and that repeated inhalations of agonistic anti-Fas antibody induce epithelial cell apoptosis and lung inflammation, which subsequently lead to pulmonary fibrosis in mice (Hagimoto et al., 1997).

Alveolar epithelial damage is an important initial event in pulmonary fibrosis. Epithelial cell damage and cell death during alveolitis induces the formation of gaps in the epithelial basement membranes. When the degree of lung injury is mild, damaged tissue will normally be repaired, whereas excess cell death may lead to unrepairable lung damage and pulmonary fibrosis. The

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migration of fibroblasts through these gaps into the alveolar space leads to intra-alveolar fibrosis (Fukuda et al., 1987). Interstitial fibrosis and the subsequent relining of intra-alveolar fibrosis by alveolar and bronchiolar epithelial cells result in structural remodeling after lung injury.

As well as death receptors/ligands, death signals such as reactive oxygen species, nitrogen species, proinflammatory cytokines, chemokines and others are involved in inflammatory lung diseases. In animal models of lung injury or human diseases such as acute respiratory distress syndrome (ARDS) and idiopathic pulmonary fibrosis (IPF), various inflammatory mediators and death factors induce epithelial cell damage and apoptosis (Fig. 2). The vulnerability to apoptosis induced by death receptors or other apoptosis stimulators, and the ability to survive by inhibitors of apoptosis varies according to the cell type. A clarification of the apoptosis regulatory mechanisms could lead to the development of novel strategies against inflammatory lung diseases, especially lung injury and fibrosis.

Epithelial cell apoptosis

Lung epithelium is not only the primary site of lung damage but it also participates in inflammatory reaction through a number of mechanisms, including the release of inflammatory mediators. Alterations in the structure and function of lung epithelial cells may affect the expression of these molecules. Epithelial cells in IPF can secrete a number of molecules, such as growth factors and their receptors, proteases, surfactant proteins, adhesion molecules and matrix component, which may regulate the inflammatory and fibrotic response within the lung. Prominent alveolar epithelial cell injury is the characteristic feature of IPF. Although type I pneumocytes comprise 40% of the alveolar epithelial cell population and over 90% of the alveolar surface in the normal lung (Mason et al., 1997), they are markedly decreased in the area of severe inflammation following extensive injury and cell death in the lung tissue from patients with IPF. The alveolar type II cell is a reparative cell and rapidly proliferates following epithelial cell injury. In areas most severely damaged, the basement membrane is covered by proliferating type II cells which are cuboidal, and death of both type I and type II cells is replaced by abundant fibroblasts and smooth muscle cells (Coalson et al., 1982) (Fig. 3).

Bleomycin rapidly produces extensive DNA damage in the lung (Harrison et al., 1989). *In vitro*, bleomycin can induce apoptosis (Tounekti et al., 1993). The persistence of DNA damage rather than the initial level of strand scission, is associated with pulmonary fibrosis (Harrison et al., 1989). Biphasic DNA fragmentation is observed by the gel electrophoretic results of DNA extracted from lung tissues in mice with bleomycin-induced pulmonary fibrosis. Electron microscopic findings show the characteristic features of apoptosis in bronchiolar and alveolar epithelial cells in this model (Hagimoto et al., 1997). Therefore, DNA damage and

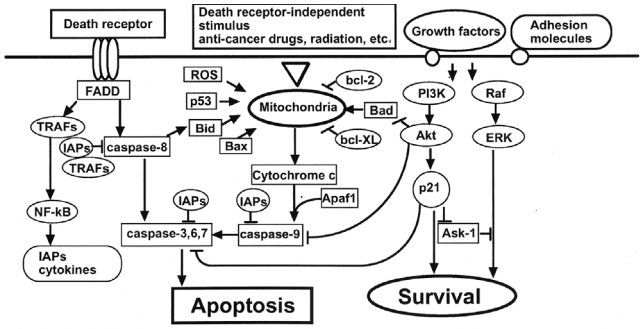

Fig. 1. Apoptosis-signaling pathways mediated by death receptor and mitochondria. Death receptor-mediated and mitochondria-mediated pathways are two principal signaling pathways of apoptosis. Activation of death receptors resulted in the recruitment of adaptor proteins through the interaction of the death domain (DD). Recruitment of FADD to Fas or to TNFR through TRADD activates initiator caspase-8. Other stimuli other than death-receptor activation, such as anti-cancer drugs, radiation, and reactive oxygen radicals etc., trigger apoptotic pathways initiating at the mitochondria.Cytochrome c is released into the cytosol from mitochondria and binds to Apaf1 with ATP, which results in the activation of caspase-9.The activation of caspase-8 or caspase-9 leads to the activation of the caspase cascade.The NF-κB signal transduction pathway is also initiated through the interaction of TRADD and TRAFs. The activated NF-κB promotes the transcription of IAPs as well as proinflammatory cytokines. IAPs block caspase-3, caspase-7, and caspase-9 directly, and also inhibit caspase-8 along with TRAFs. The precise functions of IAPs remain to be addressed in the future.

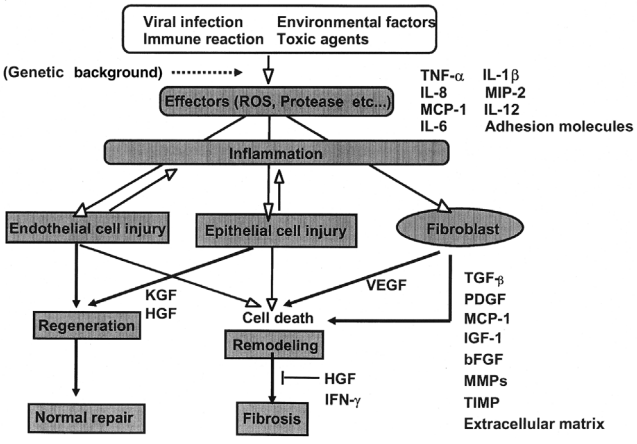

Fig. 2. Molecular mechanisms of pulmonary fibrosis. Recent advances concerning the molecular mechanisms of pulmonary fibrosis concerning parenchymal cell injury, inflammation, fibroblast proliferation and remodeling result in the increasing recognition of its complexity. Since various inflammatory mediators and death factors induce tissue damage, it is unlikely that a single treatment is effective in severe lung injury. In addition, to prevent tissue damage, and to accelerate repair and regeneration of damaged tissue could be an effective treatment to prevent irreversible fibrosis. The combination of effective treatments may overcome this devastating illness.

the apoptosis of epithelial cells may be associated with pulmonary fibrosis. There is DNA damage or apoptosis in bronchiolar and alveolar epithelial cells in IPF using an in situ DNA nick-end-labeling method and electron microscopy (Kuwano et al., 1996, 2002; Barbas-Filho et al., 2001). DNA damage and apoptosis in lung epithelial cells have been reported in acute lung injury (Bardales et al., 1996) and diffuse alveolar damage (Guinee et al., 1996), as well as IPF.

The evidence that apoptosis is involved in lung injury and fibrosis has also been demonstrated using caspase inhibitors. One of the intracellular events required for cell death in several systems, including the Fas-FasL pathway, is the activation of caspases. The activation of initiator caspase-8 is triggered by ligation of death receptors through the adapter molecule FADD. Active caspase-8 activates effector caspases such as caspase-3. Active effector caspases mediate the cleavage of protein substrates, resulting in morphological features of apoptosis. The tripeptide benzyloxycarbonyl-Val-Ala-Asp fluoromethylketone (Z-VAD.fmk), a broad-spectrum caspase inhibitor, inhibited the intracellular activation of caspase-like proteases in vivo, and protected mice against LPS-induced acute lung injury (Haimovitz-Friedman et al., 1997; Kawasaki et al., 2000). It also attenuated bleomycin-induced pulmonary fibrosis in mice (Uhal et al., 2000; Kuwano et al., 2001). Although the precise mechanisms of how epithelial cell apoptosis leads to pulmonary fibrosis remain to be examined, epithelial cell apoptosis probably has an important role in the pathogenesis of lung injury and fibrosis.

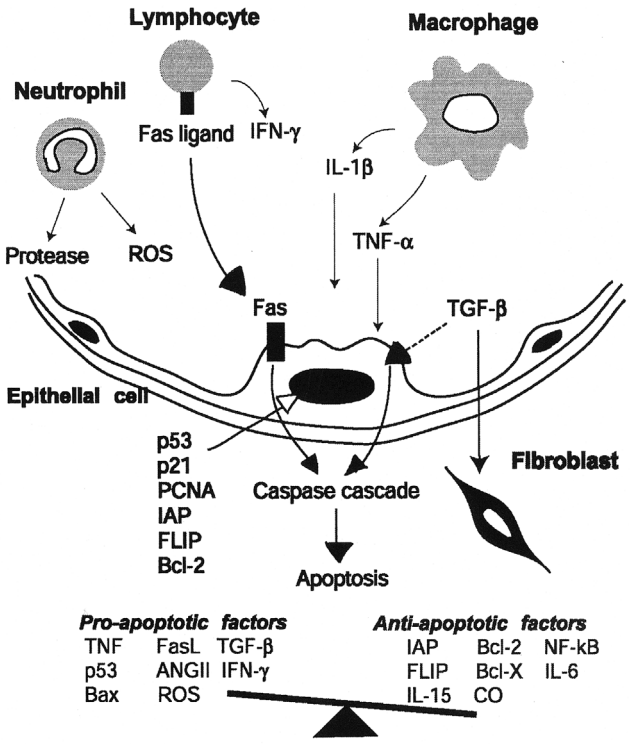

Fig. 3. Epithelial cell damage as an initial event in the pathogenesis of pulmonary fibrosis. Alveolitis is an initially important event in pulmonary fibrosis, regardless of whether this process is acute or chronic, or the etiology is known or unknown. When the damage on epithelial cells and basement membranes is too severe to be repaired, the migration of fibroblasts into the alveolar space leads to intraalveolar fibrosis as well as interstitial fibrosis.

Endothelial cell apoptosis

Pulmonary endothelial cells are involved in homeostasis and gas exchange in the lung. Damage to endothelial cells is associated with interstitial edema, leukocyte invasion and decreased gas exchange. The structural or functional deficiency of endothelial cells may lead to malfunction or cell death of type II epithelial cells and may promote pulmonary fibrosis (Pober and Cotran, 1990). Ultrastructural findings show that injured epithelial and endothelial cells are found in early fibrosing alveolitis (Harrison et al., 1991). Alveolar type II cells release a peptide(s) that protects endothelial cells from apoptosis induced by TNF-α (Wendt et al., 1994).

Pulmonary endothelial cells are activated in a variety of inflammatory lung diseases, and their dysfunction may be important in pulmonary fibrosis. Endothelial cells express inflammatory and fibrogenic cytokines, which have an important role in inflammatory infiltration, cellular growth and matrix synthesis (Wendt et al., 1994). Apoptosis occurs within the intra-alveolar granulation tissue during lung injury and BALF from these patients induces apoptosis in mesenchymal cells (Polunovsky et al., 1993). Endothelial cell proliferation and angiogenesis are essential in intra-alveolar granulation of injured lungs.

The existence of neovascularization in bleomycin-induced pulmonary fibrosis has been identified (Peao et al., 1994). Alveolar epithelial cells may regulate endothelial cell apoptosis during injury and repair. The CXC chemokine, IFN-γ-inducible protein-10 (IP-10) is inversely correlated to total lung collagen and a greater angiogenic response in mice with bleomycin-induced pulmonary fibrosis compared with control lung tissue (Keane et al., 1999). A significant increase in MIP-2 is correlated with a significant increase in angiogenesis in mice with bleomycin-induced pulmonary fibrosis compared with controls. Moreover, angiogenesis and pulmonary fibrosis are significantly attenuated with the anti-MIP-2 antibody (Keane et al., 1999). In addition to the fibroproliferative effect, the angiogenic effect of bFGF may play a role in pulmonary fibrosis (Peao et al., 1994).

Epithelium-fibroblast interaction

Severe injury and insufficient repair of lung epithelial cells disturb normal epithelial-fibroblast interaction, which leads to pulmonary fibrosis. If

epithelial cell repair does not proceed smoothly and completely, fibroblasts will proliferate, eventually leading to pulmonary fibrosis. Studies on the repopulation of denuded tracheal explants by epithelial cells show that the denuded tracheal implants are rapidly replaced by fibroblasts, unless enough epithelial cells are introduced into the lumen to control fibroblast proliferation (Terzaghi et al., 1978). Alternatively, epithelial cells may control fibroblasts by releasing cytokines that downregulate fibroblast activity. Mouse lung explants with severe epithelial damage induced by prior hyperoxic lung injury exhibit marked fibroblast proliferation and collagen deposition in culture, whereas less severely injured explants do not (Adamson et al., 1988). Normal repair of the epithelial layer occurs through the proliferation and differentiation of type II alveolar epithelial cells. This process is affected by factors produced by lung fibroblasts (Panos et al., 1993; Mason et al., 1994).

Abnormal fibroblast phenotypes isolated from the fibrotic human lung produce factors capable of inducing apoptosis and necrosis of alveolar epithelial cells in vitro (Uhal et al., 1995). The cuboidal epithelium of the fibrotic human lung is composed of both proliferating and dying cells, and apoptotic and necrotic epithelial cells are observed in proximity to a-actin-positive interstitial cells (Uhal et al., 1998). Neither inflammation nor fibrosis correlates with survival, and the only pathological data that showed a significant correlation with mortality are numbers of areas with fibroblastic foci (King et al., 2001). These abnormal epithelial-mesenchymal interactions contribute to the pathogenesis and exacerbation of fibrotic lung disease by preventing normal epithelial repair and progression of abnormal fibroblast proliferation.

Lovastatin is an HMG-CoA reductase inhibitor widely used in the treatment of hypercholesteremia. Lovastatin induces apoptosis in normal and fibrotic fibroblasts both in vitro and in vivo, and dramatically reduces granulation tissue formation in a guinea pig wound chamber model with ultrastructural evidence of fibroblast apoptosis (Tan et al., 1999). Soluble fibronectin peptides trigger non-transformed fibroblast apoptosis in routine culture and in fibrin gels by a mechanism that includes disruption of an integrin-mediated survival-signaling pathway (Hadden and Henke, 2000). CD44 on fibroblast is an adhesion molecule for extracellular matrix and mediates fibroblast invasion into fibrin matrices. Incubation of cultured fibroblasts with an anti-CD44 monoclonal antibody induces fibroblast detachment from the substratum and morphological changes compatible with apoptosis (Henke et al., 1996). To induce apoptosis of fibroblasts may be one possible approach in the attempt to block fibroblast proliferation and collagen synthesis.

p53 and p21$^{Waf1/Cip1/Sdi1}$

The wild-type p53 normally acts to suppress cell growth while the cell attempts DNA repair. It also promotes apoptosis in those cells, which have irreparably damaged DNA or continue to proliferate (Kasten et al., 1991; Ko and Prives, 1996). The expression level of wild-type p53 is kept very low in normal cells, and a half-life of wild-type p53 is as short as 20 minutes to 4 hours in normal cells (Yu et al., 2000). Expression of p53 is upregulated in response to a variety of stress such as DNA damage, heat shock, or reactive oxygen species (Maltzman and Czyzyk, 1984; Hubbert et al., 1992; Maki and Howley, 1997). Expression of p53 and p21 are detected in the hyperplastic bronchial and alveolar epithelial cells of lung tissue from patients with IPF by using immunohistochemistry (Kuwano et al., 1996).

Bleomycin-induced pulmonary fibrosis is an animal model of lung injury and fibrosis. Mishra et al. (2000) demonstrated that DNA damage to alveolar epithelial cells occurs in response to bleomycin and that p53 and p21 proteins were overexpressed within these cells. We have also demonstrated epithelial cell damage and apoptosis associated with p53 and p21 upregulation in this model (Kuwano et al., 1999). Accordingly, these results suggest that p53 may play an important role in epithelial cell damage and apoptosis in this model. In contrast, mice expressing dominant negative p53 in the lung epithelium have a decreased induction of p21 expression, and are impaired in the recovery from bleomycin-induced pneumopathy (Ghosh et al., 2002). p53 knockout mice present more severe inflammation and fibrosis after bleomycin instillation compared with wild-type mice (Davis et al., 2000). These results suggest that p53-inducible genes, such as p21, may protect epithelial cells and promote repair of epithelial cell injury. Whether p53 induces apoptosis or promotes repair in lung epithelial cells is likely to be tightly regulated by complex mechanisms within the cell.

p21$^{Waf1/Cip}$1/Sdi1 (p21) is induced in wild-type p53-containing cells following exposure to DNA-damaging agents. In normal human cells, p21 exists in a quaternary complex with a cyclin, a Cdk, and the proliferating-cell nuclear antigen (PCNA) (Waga et al., 1994). p21 inhibits cyclin-Cdk complex kinase activity and is a critical downstream effector in the p53-specific pathway of growth control in mammalian cells (El-Deily et al., 1993). On the other hand, PCNA functions in both DNA replication and repair. p21 directly inhibits PCNA-dependent DNA replication in the absence of a cyclin/Cdk, but does not inhibit DNA repair (Li et al., 1994). Forced p21 expression has been shown to have a protective effect against cell death caused by genotoxic stresses such as radiation or cytotoxic agents (Bissonnette and Hunting et al., 1998; Lu et al., 1998). p21 enhanced survival either by promoting DNA repair or by modifying cell death caused by exposure to hyperoxia (O'Reilly et al., 2001). The absence of p21 results in rapid necrotic alveolar cell death and mortality and also results in premature and extended proliferation of parenchymal cells, thereby creating hyperplastic

regions enriched in proliferating fibroblasts after oxidant injury (Sterversky et al., 2002). Interestingly, activation of caspase-3 is regulated by p21, and procaspase-3-p21 complex formation is an essential system for cell survival (Suzuki et al., 1998, 1999). These findings suggest that p21 may be a key regulator of DNA replication and repair after lung injury.

The Fas-Fas ligand pathway

The Fas-Fas ligand (FasL) pathway is a representative system of apoptosis- signaling receptor molecules. Fas antigen is a cell surface protein that mediates apoptosis. It is expressed in various cells and tissues including the thymus, liver, ovary, heart and lung. It has structural homology with a number of cell surface receptors, including tumor necrosis factor receptor and nerve growth factor (Itoh et al., 1991). Mice carrying the lymphoproliferative (lpr) mutation have defects in the Fas antigen gene (Watanabe-Fukunaga et al., 1992). The lpr mice develop lymphadenopathy and suffer from a systemic lupus erythematosus-like autoimmune disease, indicating an important role for Fas antigen in the negative selection of autoreactive T-cells in the thymus (Watanabe-Fukunaga et al., 1992). Fas ligand (FasL), a cell surface molecule belonging to the tumor necrosis factor family, binds to its receptor Fas, thus inducing the apoptosis of Fas-bearing cells (Suda et al., 1993). Generalized lymphoproliferative disease (gld) mice have a point mutation in the FasL and develop lymphadenopathy and suffer from autoimmune disease (Takahashi et al., 1994). FasL is expressed predominantly in activated T-lymphocytes and in tissues including the small intestines, kidney, testis and lung (Suda et al, 1993). In the immune system, Fas and FasL are involved in the downregulation of immune reactions (Brunner et al., 1995; Dhein et al, 1995; Ju et al., 1995).

Bleomycin-induced pulmonary fibrosis is an animal model for lung injury and fibrosis. In this model, FasL mRNA is upregulated in infiltrating lymphocytes, and Fas is upregulated in bronchiolar and alveolar epithelial cells, in which excessive apoptosis is detected (Hagimoto et al., 1997a). The repeated inhalation of anti-Fas antibody mimicking Fas-FasL cross-linking induces excessive apoptosis of epithelial cells and inflammation, which results in pulmonary fibrosis in mice (Hagimoto et al., 1997b). The neutralization of FasL by Fas-Ig fusion protein or neutralizing anti-FasL antibody could prevent the development of this model, and Fas- or FasL-deficient mice are resistant to the induction of this model (Kuwano et al., 1999). Furthermore, Fas ligation not only induces apoptosis but also induces IL-8 expression via NF-κB activation in bronchiolar epithelial cells in vitro (Hagimoto et al., 1998). The Fas-FasL system has also been demonstrated to be important in the pathogenesis of lipopolysaccharide-induced acute lung injury, and proper regulation of the Fas-FasL system might be important for potential treatment of ARDS (Kitamura et al., 2001). Although the Fas/FasL system does not have a major role in the clearance of aerosolized bacteria from the lungs, this system contributes to the development of permeability changes and tissue injury during gram-negative bacterial pneumonia (Matute-Bell et al., 2001). These results strongly support the hypothesis that the Fas-FasL pathway is involved in the pathogenesis of lung injury and fibrosis.

The involvement of the Fas-FasL pathway in lung injury and fibrosis of human diseases has also been demonstrated. The Fas-FasL pathway has been demonstrated to contribute to severe epithelial damage that occurs in ARDS. FasL can be released as a biologically active, death–inducing mediator capable of inducing apoptosis of epithelial cells during acute lung injury (Matute-Bello et al., 1999). Alveolar epithelial damage in human with acute lung injury or ARDS is in part associated with local upregulation of the Fas-FasL pathway and activation of the apoptotic cascade in epithelial cells (Albertine et al., 2002). Fas protein expression is upregulated in lung epithelial cells, and FasL mRNA and protein expression are upregulated in infiltrating inflammatory cells in lung tissues from patients with IPF (Kuwano et al., 1999). Soluble FasL levels in BALF are significantly increased in the active phase of IPF (Kuwano et al., 2000). BALF from patients with ARDS or IPF could induce apoptosis on small airway epithelial cells which are dependent on the Fas-FasL pathway (Matute-Bello et al., 1999; Hagimoto et al., 2002). Inhibiting this pathway may be one of novel treatment strategies against lung injury and fibrosis.

TNF-α

Tumor necrosis factor-α (TNF) causes inflammation by damaging tissues and by inducing the expression of adhesion molecules and cytokines in epithelial and endothelial cells as well as in inflammatory cells. TNF induces fibroblasts to produce PDGF, prostaglandin E2, collagenase, gelatinase, chemokines, GM-CSF, IL-1 and IL-6. The cellular effects of TNF are mediated by two distinct cell surface receptors, termed TNF-receptor 1 (TNFR1) and TNF-receptor 2 (TNFR2) (Tartaglia and Goeddel, 1992). Most of the cytotoxic effects of TNF are mediated by TNFR1 through interaction of its death domain with the TNFR-associated death domain (TRADD) protein (Hsu et al., 1995). TRADD interacts with Fas-associated death domain protein (FADD) (Chinnaiyan et al., 1995) to activate caspase-8, thereby initiating the apoptosis pathway. Since the Fas-mediated apoptosis-signaling pathway is relatively short and straight compared with that of TNFR, Fas-ligation takes hours to kill target cells, while TNF takes a day or more. Furthermore, TNF does not usually kill most types of cells without metabolic inhibitors, which is different from Fas-ligation.

Although TNF and its mRNA have been identified in alveolar macrophages in normal lung tissue, they are predominantly detected in type II epithelial cells lining

the thickened alveolar septa, as well as in macrophages in fibrotic lung tissue (Piguet et al., 1993). Transgenic mice, in which TNF gene expression has been driven from the lung-specific SP-C promoter, go on to develop an early lymphocytic alveolitis, followed by a fibrogenic response (Miyazaki et al., 1995). Mice exposed to silica upregulate their expression of TNF mRNA and protein in lung tissue and in BAL cells. Enhanced TNF expression precedes the onset of fibroblast proliferation and collagen deposition in the lung (Piguet et al., 1990; Ohtsuka et al., 1995). Anti-TNF antibodies or soluble TNFR can prevent the development of pulmonary fibrosis induced by silica or bleomycin exposure in mice (Piguet et al., 1990; Piguet and Vesin, 1994). Mice deficient in two different cell surface receptors for TNF (TNFR1 and TNFR2), p55 and p75, are protected from the fibroproliferative effects of inhaled asbestos fibers (Liu et al., 1998) and silica, or bleomycin instillation (Ortiz et al., 1999). These results suggest that TNF is one of the important cytokines associated with the pathophysiology of pulmonary fibrosis.

Although TNFR mediates apoptotic signal transduction, it can transduce intracellular signals that activate transcription factor NF-κB by proteolytic breakdown of IκB. TNFR-associated factor-2 (TRAF2) and receptor interacting protein (RIP) (Hsu et al., 1996) indirectly bind to TNFR1 through TRADD or directly bind to TNFR2, and activate the NF-κB-inducing kinase (NIK) (Malinin et al., 1997), which in turn activates the IκB kinase (IKK) complex (Mercurio et al., 1997; Regnier et al., 1997; Woronicz et al., 1997; Zandi et al., 1997). IKK phosphorylates IκB, which leads to IkB degradation, and allows NF-B to translocate to the nucleus to activate transcription (Fig. 1). TNF or agonistic anti-Fas antibody administration can lead to production of IL-8 by colon epithelial cells (Abreu-Martin et al., 1995) or bronchial epithelial cells in addition to inducing apoptosis in vitro (Hagimoto et al., 1999). Disruption of the NF-κB pathway with the dominant-negative TRAF2 enhances the cytolytic effects of TNF (Hsu et al., 1996). Cellular proteins homologous to baculovirus inhibitors of apoptosis (IAPs) block cell death. TRAF1, TRAF2, XIAP, c-IAP1 and c-IAP2 were identified as gene targets of NF-κB transcriptional activity (Stehlik et al., 1998). The recruitment of c-IAP1 and c-IAP2 to the TNF receptor complex through interactions with TRAF1 or TRAF2 inhibits the activation of the initiator caspase, caspase-8 (Wang et al., 1998). Therefore, death receptor activation induces NF-κB activation, which triggers inflammation and also plays an important role in regulating apoptosis.

TGF-ß1

TGF-ßs are multifunctional cytokines that exist in three isoforms designated as TGF-ß1, TGF-ß2 and TGF-ß3. Although the biological activity of these isoforms overlaps, TGF-ß1 appears to be predominant among them in being expressed in pulmonary fibrosis (Coker et al., 1997). There are three TGF-ß receptors, type I (TGFR1), type II (TGFRII) and type III (TGFRIII). All three receptors bind to all three TGF-ßs with a high affinity. TGF-ß is the most potent promoter of extra cellular matrix (ECM) production, and also a strong chemotactic factor for monocytes and macrophages. TGF-ß activates them to release a number of cytokines such as PDGF, IL-1ß, bFGF, TNF-α, and TGF-ß itself. There is a consistent increase in TGF-ß production in epithelial cells and macrophages in lung tissue from patients with IPF (Khalil et al., 1991) and in bleomycin-induced pulmonary fibrosis in rodents (Raghow et al., 1989).

Transient overexpression of active TGF-ß1 through the transfection of porcine TGF-ß1 cDNA to the rat lung, results in prolonged and severe interstitial and pleural fibrosis (Sime et al., 1997). The increase in lung collagen accumulation in bleomycin-induced lung fibrosis is reduced by treatment with either TGF-ß2 , TGF-ß1 antibody, or the recombinant TGFRII (Giri et al., 1993; Wang et al., 1999). Decorin, a naturally occurring biological molecule that antagonizes TGF bioactivity, may ameliorate excessive TGF signaling in injured lungs. Adenovirus-mediated decorin gene transfer reduces fibrotic response to bleomycin (Kolb et al., 2001).

Smad proteins regulate intracellular signals from the membrane to the nucleus of TGF-ß (Helden et al., 1997). The activated TGF-ß receptors induce phosphorylation of Smad2 and Smad3, which form complexes with Smad4. The complexes translocate to the nucleus and regulate transcriptional responses. Smad3 deficiency attenuates bleomycin-induced pulmonary fibrosis in mice (Zhao et al., 2002). Smad7 prevents the phosphorylation of Smad2 and Smad3 by association with activated TGF-ß receptors (Hayashi et al., 1997; Nakao et al., 1997). Transient gene transfer and the expression of exogenous Smad7 into the lung by adenoviral vectors prevent bleomycin-induced lung fibrosis (Nakao et al., 1999).

In addition to multiple effects on the process of fibrogenesis, TGF-ß1 can induce apoptosis in gastric carcinoma cells (Yanagihara et al., 1992), primary hepatocytes, hepatoma cells (Gressner et al., 1997) and human lung epithelial cell lines (Yanagisawa et al., 1998). The mechanism of TGF-ß1-mediated apoptosis probably varies among cell types, although caspase activation (Chen et al., 1997; Hung et al., 1998), upregulation of p21 (Kim et al., 1998), and downregulation of Bcl-XL expression (Saltzman et al., 1998) are commonly observed. However, TGF-ß1 induces apoptosis of retinal endothelial cells with decreased expression of p21 (Yan et al., 1998). p21 regulates the activation of caspase-3 through the procaspase-3-p21 complex formation and protects human hepatoma cells from Fas-mediated apoptosis (Suzuki et al., 1998, 1999). TGF-ß also augments Fas-mediated apoptosis on lung epithelial cells in vitro and in vivo (Hagimoto et al., 2002). BALF obtained from

patients with IPF induces apoptosis on lung epithelial cells, which is blocked by anti-Fas or by anti-TGF-ß antibody in vitro. TGF-ß1 is a potent inducer of apoptosis through the caspase-3 activation and the downregulation of p21 and is also an enhancer of Fas-mediated apoptosis of lung epithelial cells (Hagimoto et al., 2002). This novel function of TGF-ß1 in apoptosis of lung epithelial cells should be considered in the treatment of lung injury and fibrosis (Fig. 4).

Reactive oxygen and nitrogen species

Lung epithelial cells are always exposed to a variety of stresses, and are a primary target for reactive oxygen species (ROS). High intracellular and extracellular levels of antioxidants protect lung epithelial cells. The generation of ROS is increased in conditions such as inflammation, or exposure to air pollutants and cigarette smoke. ROS and their reactions with lung epithelial cells participate in the pathophysiology of several lung diseases, including IPF, ARDS, and lung cancer (Gaston et al., 1994; Shi et al., 2001). Pulmonary lesions seen in patients dying from ARDS are not only caused by the initial disease, but also by the toxic effects of oxygen therapy. It has been reported that a damaged lung is more susceptible to oxygen than an undamaged one (Witschi et al., 1981). There have been a number of studies demonstrating the increased oxidative stress in IPF. The spontaneous production of oxidants by lung inflammatory cells and the myeloperoxidase concentration are both increased in the alveolar epithelial lining fluid of patients with IPF (Cantin et al., 1987). Nitrotyrosine, a byproduct of protein nitration by peroxynitrite, is increased in the lungs of patients with IPF (Saleh et al., 1997). F2-isoprostanes, products of the radical-catalyzed lipid peroxidation, are increased in BALF in patients with interstitial lung diseases (Monttuschi et al., 1998). Products of lipid peroxidation, measured as thiobarbituric acid-malondialdehyde adducts in plasma and BALF are increased in IPF. In contrast, there is a marked reduction in antioxidant capacity, measured as Trolox equivalent antioxidant capacity, in the plasma and BALF from patients with IPF (Rahman et al., 1999). These results demonstrate the evidence of increased oxidative stress and of oxidant / antioxidant imbalance in patients with IPF.

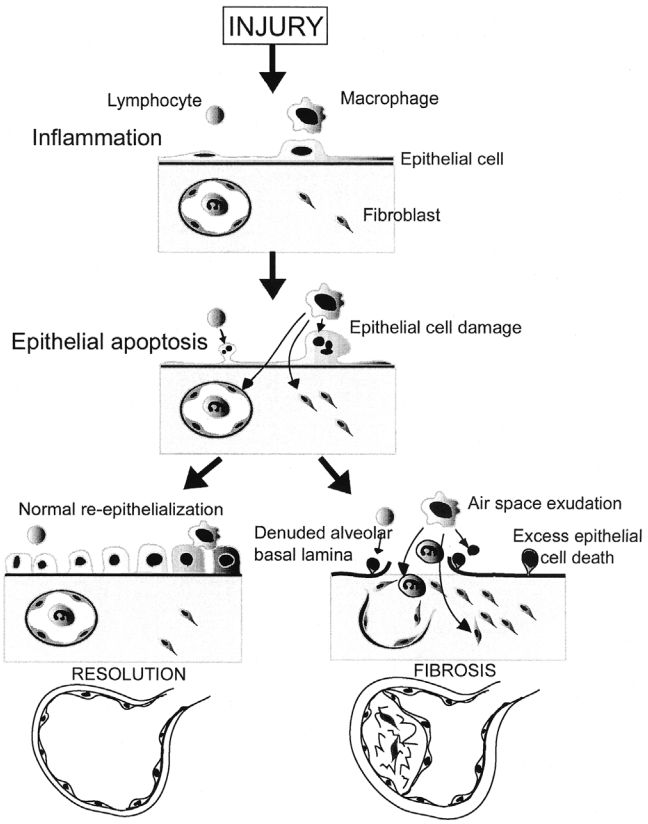

Fig. 4. Pro- and anti-apoptotic molecules affecting epithelial cells in pulmonary fibrosis. Apoptosis plays a major role in homeostasis to maintain a balance between cell proliferation and cell death. Increasing attention has been paid to the importance of epithelial cell death during the process of pulmonary fibrosis. The imbalance between pro- and anti-apoptotic factors may participate in the pathogenesis of pulmonary fibrosis.

Apoptosis plays a central role in DNA damage during the pathogenesis of hyperoxic lung injury (Albertine and Plopper, 2002). Hyperoxic injured cells in neonatal lungs undergo both apoptotic and nonapoptotic cell death (McGrath-Morrow and Stahl, 2001). Hydrogen peroxide induces Fas upregulation by promoting cytoplasmic transport of Fas to the cell surface in human airway epithelial cells, and the activation of the poly (ADP-ribose) polymerase-p53 pathway may be involved in this mechanism (Fujita et al., 2002). Exaggerated apoptosis through Fas-mediated signaling may accelerate hyperoxia-induced acute lung injury in Legionella pneumonia (Tateda et al., 2003). Hyperoxia, by virtue of activating NAPDH oxidase, generates ROS, which mediates cell death of lung epithelium via ERK1/2 MAPK activation in lung epithelial cells (Zhang et al., 2003). Administration of hydroxycortisone during hyperoxia aggravates lung injury. The extent of cell death by ROS correlates with the degree of NF-κß suppression (Barazzone-Argiroffo et al., 2003). The adrenal response aggravates alveolar epithelial cell damage and is likely to be mediated by the decrease of NF-κß function involved in cell survival (Franek et al., 2003).

Glutathione (GSH) is one of the major antioxidant molecules present in normal epithelial lining fluid and it plays a role in protecting epithelial cells against oxidant-mediated injury. Therefore, epithelial cell damage in IPF may be augmented by a deficiency of GSH in the epithelial lining fluid (Cantin et al., 1989). In this regard, strategies to reduce oxidants may be beneficial in decreasing alveolar epithelial cell injury and may consequently reduce the progressive deterioration of

patients with IPF. A significant increase of GSH in BAL fluid was found after a period of treatment with high-dose oral N-acetylcysteine (NAC), the GSH precursor, in patients with IPF (Meyer et al., 1994; Behr et al., 1997). The decreased levels of GSH and increased levels of ceramide correlate with the induction of apoptosis in these lung epithelial cells. GSH and NAC inhibit hydrogen peroxide-mediated induction of ceramide and apoptosis (Lavrentiadou et al., 2001). NAC ameliorates the acute pulmonary inflammation induced by bleomycin injection via the repression of chemokines and lipid hydroperoxide production, resulting in the attenuation of pulmonary fibrosis in mice (Hagiwara et al., 2000). NAC inhibited MPO activity and lipid peroxidation, which resulted in the reduction of apoptosis in lung in cecal ligation and puncture-induced sepsis model (Ozdulger et al., 2003).

Heme oxygenase-1 (HO-1) confers protection against a variety of oxidant-induced cell death and tissue injuries. HO-1 overexpression using adenovirus exhibited attenuation of hyperoxia-induced neutrophil inflammation and apoptosis (Otterbein et al., 1999), or prevented bleomycin-induced pulmonary fibrosis by attenuating apoptotic cell death (Tsuburai et al., 2002). CO, a major by-product of heme catalysis by HO-1, exhibited a marked attenuation of hyperoxia-induced neutrophil infiltration into the airways and total lung apoptotic index (Otterbein et al., 1999). CO utilizes p38 MAPK and caspase-3 in exerting its anti-apoptotic effect both in vitro and in vivo during ischemia-reperfusion injury (Zhang et al., 2003).

Nitric oxide (NO) is produced by three isoforms of NO synthases. Constitutive NOS (cNOS) including neuronal NOS (nNOS) and endothelial NOS (eNOS) are expressed constitutively, and expression of inducible NOS (iNOS) is induced by some stimuli, such as inflammatory cytokines, in a variety of cell types. NO is a potent vasodilator that has various functions including antimicrobial, antiproliferative, and anticoagulant effects. Peroxynitrite, which is produced by the rapid reaction of NO and superoxide, mediates the cytotoxic effect of nitric NO. Peroxynitrite is increased in acute lung injury and ARDS (Haddad et al., 1994; Kooy et al., 1995), and the source of both NO and peroxynitrite is activated macrophages (Ischiropoulos et al., 1992). Normal epithelial cells express high levels of cNOS and only limited amounts of iNOS. However, there is downregulation of cNOS and upregulation of iNOS in lung tissue from patients with IPF and the active stage of IPF is associated with increased formation of NO and peroxynitrite (Saleh et al., 1997). Increased peroxynitrite may have an important role in IPF, either through oxidation of glutathione, inhibition of surfactant function, or direct epithelial cell damage (Haddad et al., 1993; Behr et al., 1995). In contrast, iNOS plays anti-apoptotic and protective roles on lung epithelial cells in bleomycin-induced pulmonary fibrosis in mice (Davis et al., 2000). The role of NO in lung injury and fibrosis remains to be clarified.

Angiotensin II

Angiotensin-converting enzyme (ACE) levels in BALF and serum are increased in fibrosing lung diseases, including sarcoidosis, IPF, asbestosis, silicosis and ARDS. Angiotensin II concentrations increase during radiation-induced pulmonary fibrosis (Ward et al., 1983). Angiotensin II induces human lung fibroblast proliferation in vitro via activation of the angiotensin type I receptor and the autocrine action of TGF-ß(Marshall et al., 2000). The ACE inhibitor, captopril, ameliorates pulmonary fibrosis induced by monocrotaline in rats (Molteni et al., 1985). Captopril inhibits the accumulation of collagens and mast cells in the irradiated rat lung, and also inhibits fibroblast proliferation in the presence of bFGF in vitro (Ward et al., 1990; Uhal et al., 1998). Fas-induced apoptosis of human lung epithelial cells in culture is potently inhibited by captopril at concentrations readily attained in vivo (Uhal et al., 1998). Fibloblasts isolated from human lung secrete a soluble inducer of apoptosis in alveolar epithelial cells in vitro. The protein inducers of alveolar epithelial cell apoptosis produced by fibroblasts from patients with IPF were identified as angiotensinogen and its derivative angiotensin II (Wang et al., 1999). Induction of alveolar epithelial cell apoptosis and lung fibrosis are induced in Wister rats to which amiodarone was administered orally over six months, and concurrent administration of captopril or losartan attenuates both alveolar epithelial cell apoptosis and collagen accumulation (Uhal et al., 2003). Bleomycin, like FasL or TNF-α, induces transactivation of angiotensin II synthesis de novo that is required for alveolar epithelial cell apoptosis (Li et al., 2003). The inhibitory actions of captopril on pulmonary fibrosis may be due to the prevention of lung epithelial cell apoptosis.

Cytokines and growth factors

Alveolar epithelial cells are damaged and induced to cell death in association with the accumulation of inflammatory cells and the presence of their mediators. Cytokines and chemokines, which are chemotactic for macrophages and lymphocytes, are closely associated with the survival of these cells and the remodeling process. Anti-apoptotic effects on monocyte and alveolar macrophages of MCP-1 may be associated with their profibrotic capacity. IL-8 recruits and activates neutrophils in vitro and in vivo to release proteases and LTB4 and can prolong the survival of neutrophils and endothelial cells. Apoptotic cells produce significantly more TGF-ß and IL-4 than viable cells. An increased production of TGF-ß and IL-4 by epithelial cells undergoing apoptosis may contribute to the inhibition of proliferation, squamous metaplasia, and to the reduction of the inflammatory response in acute lung injury (Hodge et al., 2002). IL-6 enhances Fas-mediated apoptosis and expression of Bax in normal fibroblasts,

but inhibits apoptosis and induces expression of Bcl-2 in fibroblasts obtained from patients with IPF (Moodley et al., 2003). IL-6 diminishes hyperoxic lung injury and this protection is associated with a marked diminution in hyperoxic cell death, probably through the induction of bcl-2 and TIMP-1 (Ward et al., 2000). Overexpression of IL-11 in the lung enhances murine tolerance of 100% oxygen and diminishes hyperoxia-induced apoptosis (Waxman et al., 1998). IL-15 overexpression can prevent TNF-α-induced apoptosis and protect against *E. coli*-induced shock, indicating a possible therapeutic application of IL-15 for septic shock (Hiromatsu et al., 2003).

PDGF is one of the most potent mitogens and chemoattractants for mesenchymal cells and induces the proliferation of fibroblasts and the synthesis of extracellular matrix. IGF-1 acts synergistically with PGDF to promote fibroblast proliferation (Rom et al., 1988). Basic FGF is a potent stimulator of both fibroblast and endothelial cell proliferation and is associated with the fibroproliferative response, similar to PDGF. Basic FGF expression has been found to be upregulated in healing wounds, recombinant bFGF has been shown to accelerate wound healing, and anti-bFGF antibody inhibits granulation tissue formation and normal wound repair. Alveolar macrophages are a predominant source of bFGF in intra-alveolar fibrotic lesions following acute lung injury (Henke et al., 1993). Mast cells are predominantly bFGF-producing cells in IPF, and bFGF levels are correlated with bronchoalveolar lavage cellularity and with the severity of gas exchange abnormality (Inoue et al., 1996). TGF-α induces the proliferation of endothelial and epithelial cells and fibroblasts. TGF-α expression is upregulated in alveolar epithelial cells and macrophages in fibroproliferative lesions in rats with asbestos- or bleomycin-induced pulmonary fibrosis (Madtes et al., 1994). Transgenic mice, in which human TGF-α is expressed in the lung in an epithelial cell-specific manner, develop a fibroproliferative response in the interstitium and pleural surface (Korfhagen et al., 1994).

KGF has been demonstrated to enhance the functional differentiation of rat alveolar type II cells, to increase DNA synthesis in these cells in vitro, and to stimulate the proliferation of these cells in vivo. KGF is produced in the mesenchymal cells and the KGF receptor is expressed in the epithelium of the developing lung. Intratracheal instillation of KGF significantly attenuates bleomycin-induced pulmonary fibrosis in rats (Sugahara et al., 1998). KGF may participate in maintaining and repairing the alveolar epithelium and has the potential to become an effective agent against lung injury and pulmonary fibrosis. Oxygen-induced damage of alveolar epithelium and of endothelium was prevented by KGF treatment, as seen by electron microscopy. The induction of p53, Bax, and Bcl-x mRNAs during hyperoxia is to a large extent prevented by KGF, most probably by suppressing the expression of plasminogen- activator inhibitor 1 expression (Barazzone et al., 1999). KGF attenuates hydrogen peroxide-induced DNA strand break formation in cultured alveolar epithelial cells by mechanisms that involve tyrosine kinase, PKC, and DNA polymerases (Wu et al., 1998). Lung epithelial cell death can be inhibited in transgenic mice when the animals are subjected to hyperoxia. KGF is able to activate antiapoptotic Akt signaling (Ray et al., 2003).

HGF, composed of a α-subunit (35 kDa) and a ß-subunit (64 kDa), is produced by mesenchymal cells and has been identified as a potent mitogen for mature hepatocytes. HGF is known to act not only as a mitogen but also as a motogen or a morphogen for many kinds of epithelial cells. The receptor for HGF is the c-Met proto-oncogene product, which is predominantly expressed in various types of epithelial cells. HGF levels in BALF and the sera of patients with IPF are higher than those of healthy control subjects (Maeda et al., 1995; Sakai et al., 1997). As well as other epithelial cells, HGF promotes DNA synthesis in alveolar type II cells in vitro (Shiratori et al., 1995). A simultaneous or delayed administration of HGF equally represses apoptosis and fibrotic changes in murine lung injury induced by bleomycin (Yaekashiwa et al., 1997). The combination of HGF and IFN-γ enhances the migratory activity of A549 cells through the upregulation of the c-Met/HGF receptor (Nagahori et al., 1999). HGF administration may be a novel strategy in the effort to inhibit apptosis and to promote repairing and healing of inflammatory lung damage in cases of pulmonary fibrosis.

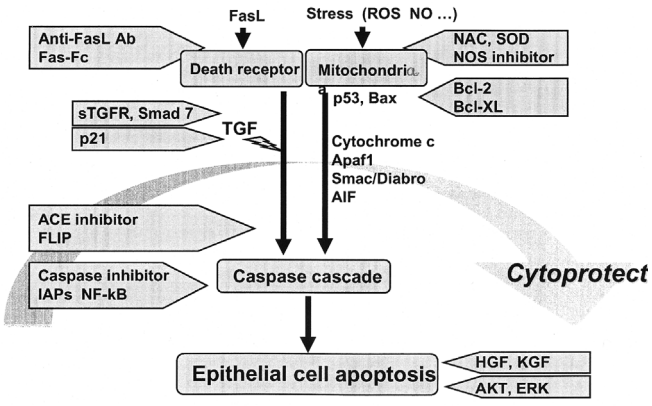

Fig. 5. Targeting apoptosis as a treatment of lung injury and fibrosis. The survival and recovery of epithelial cells, and the prevention of fibroblast proliferation and ECM deposition appear to be the key to the prognosis of patients. Protecting parenchymal cells from injury and maintaining their function may be an effective therapeutic strategy against pulmonary fibrosis.

Conclusions

Recent advances with regard to the molecular mechanisms in pulmonary fibrosis concerning endothelial and epithelial cell injury, inflammatory

reactions, fibroblast proliferation, collagen deposition and lung repair have resulted in the increasing recognition of its complexity. The major targets of therapy have focused on inflammatory cells, and this has led to the use of anti-inflammatory agents. However, conventional therapies, such as corticosteroids and cytotoxic agents, have been reported to be only minimally effective. In animal models of pulmonary fibrosis or human diseases such as IPF, various inflammatory mediators and death factors induce epithelial cell damage. Therefore, it is unlikely that any single treatment would be sufficiently effective in cases of severe lung injury. The survival and recovery of epithelial cells, and the prevention of fibroblast proliferation and ECM deposition appear to be the key to the prognosis of patients. Protecting parenchymal cells from injury and maintaining their function may be an effective therapeutic strategy against pulmonary fibrosis (Fig. 5).

References

Abreu-Martin M.T., Vidrich A., Lynch D.H. and Targan S.R. (1995). Divergent induction of apoptosis and IL-8 secretion in HT-29 cells in response to TNF-alfa and ligation of Fas antigen. J. Immunol. 155, 4147-4154.

Adamson I.Y., Young L. and Bowden D.H. (1988). Relationship of alveolar epithelial injury and repair to the induction of pulmonary fibrosis. Am. J. Pathol. 130, 377-383.

Albertine K.H., Soulier M.F., Wang Z., Ishizaka A., Hashimoto S., Zimmerman G.A., Matthay M.A. and Ware L.B. (2002). Fas and fas ligand are up-regulated in pulmonary edema fluid and lung tissue of patients with acute lung injury and the acute respiratory distress syndrome. Am. J. Pathol. 161, 1783-1796.

Albertine K.H. and Plopper C.G. (2002). DNA oxidation or apoptosis: will the real culprit of dna damage in hyperoxic lung injury please stand up? Am. J. Respir. Cell Mol. Biol. 26, 381-383.

Barazzone-Argiroffo C., Pagano A., Juge C., Metrailler I., Rochat A., Vesin C. and Donati Y. (2003). Glucocorticoids aggravate hyperoxia-induced lung injury through decreased nuclear factor-kappa B activity. Am. J. Physiol. Lung Cell. Mol. Physiol. 284, L197-204.

Barazzone C., Donati Y.R., Rochat A.F., Vesin C., Kan C.D., Pache J.C. and Piguet P.F. (1999). Keratinocyte growth factor protects alveolar epithelium and endothelium from oxygen-induced injury in mice. Am. J. Pathol. 154, 1479-1487.

Barbas-Filho J.V., Ferreira M.A., Sesso A., Kairalla R.A., Carvalho C.R. and Capelozzi V.L. (2001). Evidence of type II pneumocyte apoptosis in the pathogenesis of idiopathic pulmonary fibrosis (IFP)/usual interstitial pneumonia (UIP). J. Clin. Pathol. 54, 132-138.

Bardales R.H., Xie S.S., Schaefer R.F. and Hsu S.M. (1996). Apoptosis is a major pathway responsible for the resolution of type II pneumocytes in acute lung injury. Am. J. Pathol. 149, 845-852.

Behr J., Degenkolb B., Maier K., Braun B., Beinert T., Krombach F., Vogelmeier C. and Fruhmann G. (1995). Increased oxidation of extracellular glutathione by bronchoalveolar inflammatory cells in diffuse fibrosing alveolitis. Eur. Respir. J. 8, 1286-1292.

Behr J., Maier K., Degenkolb B., Krombach F. and Vogelmeier C. (1997). Antioxidative and clinical effects of high-dose N-acetylcysteine in fibrosing alveolitis. Adjunctive therapy to maintenance immunosuppression. Am. J. Respir. Crit. Care Med. 156, 1897-1901.

Bissonnette N. and Hunting D.J. (1998). p21-induced cycle arrest in G1 protects cells from apoptosis induced by UV-irradiation or RNA polymerase II blockage. Oncogene 16, 3461-3469.

Brunner T., Mogil R.J., LaFace D., Yoo N.J., Mahboubi A., Echeverri F., Martin S.J., Force W.R., Lynch D.H., Ware C.F. and Green D.R. (1995). Cell-autonomous Fas(CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. Nature 373, 441-444.

Cantin A.M., North S.L., Fells G.A., Hubbard R.C. and Crystal R.G. (1987). Oxidant-mediated epithelial cell injury in idiopathic pulmonary fibrosis. J. Clin. Invest. 79, 1665-1673.

Cantin A.M., Hubbard R.C. and Crystal R.G. (1989). Glutathione deficiency in the epithelial lining fluid of the lower respiratory tract in idiopathic pulmonary fibrosis. Am. Rev. Respir. Dis. 139, 370-372.

Chen R.H. and Chang T.Y. (1997). Involvement of caspase family proteases in transforming growth factor- beta-induced apoptosis. Cell Growth Differ. 8, 821-827.

Chinnaiyan A.M., O'Rourke K., Tewari M. and Dixit V.M. (1995). FADD, a novel death domain-containing protein, interacts with the death domainof Fas and initiate apoptosis. Cell 81, 505-512.

Coalson J.J. (1982). The ultrastructure of human fibrosing alveolitis. Virchows Arch. Pathol. Anat. Histol. 395, 181-199.

Coker R.K., Laurent G.J., Shahzeidi S., Shahzeidi S., Lympany P.A., du Bois R.M., Jeffery P.K. and McAnulty R.J. (1997). Transforming growth factors-beta 1, -beta 2, and -beta 3 stimulate fibroblast procollagen production in vitro but are differentially expressed during bleomycin-induced lung fibrosis. Am. J. Pathol. 150, 981-991.

Davis D.W., Weidner D.A., Holian A. and McConkey D.J. (2000). Nitric Oxide–dependent Activation of p53 Suppresses Bleomycin-induced Apoptosis in the Lung. J. Exp. Med. 192, 857-870.

Dhein J., Walczak H., Bäumler C., Debatin K.M., and Krammer P.H. (1995). Autocrine T-cell suicide mediated by APO-1/(Fas/CD95). Nature 373, 438-441.

El-Deily W.S., Takashi T., Veluculescu V.E., Levy D.B., Parsons R., Trent J.M., Lin D., Mercer W.E., Kintzler K.W. and Vogelstein B. (1993). WAF1, a potential mediator of p53 tumor suppression. Cell 75, 817-825.

Franek W.R., Chowdary Y.C., Lin X., Hu M., Miller E.J., Kazzaz J.A., Razzano P., Romashko J. 3rd, Davis J.M., Narula P., Horowitz S., Scott W. and Mantell L.L. (2002). Suppression of nuclear factor-kappa B activity by nitric oxide and hyperoxia in oxygen-resistant cells. J. Biol. Chem. 277, 42694-42700.

Fujita T., Maruyama M., Araya J., Sassa K., Kawagishi Y., Hayashi R., Matsui S., Kashii T., Yamashita N., Sugiyama E. and Kobayashi M. (2002). Hydrogen peroxide induces upregulation of Fas in human airway epithelial cells via the activation of PARP-p53 pathway. Am. J. Respir. Cell Mol. Biol. 27, 542-552.

Fukuda Y., Ishizaki M., Masuda Y., Kimura G., Kawanami O. and Masugi Y. (1987). The role of intraalveolar fibrosis in the process of pulmonary structural remodeling in patients with diffuse alveolar damage. Am. J. Pathol. 126, 171-182.

Gaston B., Drazen J.M., Loscalzo J. and Stamler J.S. (1994). The biology of nitrogen oxides in the airways. Am. J. Respir. Crit. Care Med. 149(2 Pt 1), 538-551.

Ghosh S., Mendoza T., Ortiz L.A., Hoyle G.W., Fermin C.D., Brody A.R., Friedman M. and Morris G.F. (2002). Bleomycin sensitivity of mice expressing dominant-negative p53 in the lung epithelium. Am. J.

Respir. Crit. Care Med. 166, 890-897.

Giri S.N., Hyde D.M. and Hollinger M.A. (1993). Effect of antibody to transforming growth factor beta on bleomycin induced accumulation of lung collagen in mice. Thorax 48, 959-966.

Gressner A.M., Lahme B., Mannherz H.G. and Polzar B. (1997). TGF-beta-mediated hepatocellular apoptosis by rat and human hepatoma cells and primary rat hepatocytes. J. Hepatol. 26, 1079-1092.

Guinee D. Jr., Fleming M., Hayashi T., Woodward M., Zhang J., Walls J., Koss M., Ferrans V. and Travis W. (1996). Association of p53 and WAF1 expression with apoptosis in diffuse alveolar damage. Am. J. Pathol. 149, 531-538.

Haddad I.Y., Pataki G., Hu P., Galliani C., Beckman J.S. and Matalon S. (1994). Quantitation of nitrotyrosine levels in lung sections of patients and animals with acute lung injury. J. Clin. Invest. 94, 2407-2413.

Haddad I.Y., Ischiropoulos H., Holm B.A., Beckman J.S., Baker J.R. and Matalon S. (1993). Mechanisms of peroxynitrite-induced injury to pulmonary surfactants. Am. J. Physiol. 265, L555-564.

Hadden H.L. and Henke C.A. (2000). Induction of lung fibroblast apoptosis by soluble fibronectin peptides. Am. J. Respir. Crit. Care Med. 162, 1553-1560.

Hagimoto N., Kuwano K., Nomoto Y., Kunitake R. and Hara N. (1997a). Apoptosis and expression of Fas/Fas ligand mRNA in bleomycin-induced pulmonary fibrosis in mice. Am. J. Respir. Cell Mol. Biol. 16, 91-101.

Hagimoto N., Kuwano K., Miyazaki H., Kunitake R., Fujita M., Kawasaki M., Kaneko Y. and Hara N. (1997b). Induction of apoptosis and pulmonary fibrosis in mice in response to ligation of Fas antigen. Am. J. Respir. Cell Mol. Biol. 17, 272-278.

Hagimoto N., Kuwano K., Kawasaki M., Yoshimi M., Kaneko Y., Kunitake R., Maeyama T., Tanaka T. and Hara N. (1999). Induction of interleukin-8 secretion and apoptosis in bronchiolar epithelial cells by Fas ligation. Am. J. Respir. Cell Mol. Biol. 21, 436-445.

Hagimoto N., Kuwano K., Inoshima I., Yoshimi M., Nakamura N., Fujita M., Maeyama T. and Hara N. (2002). TGF-beta 1 as an enhancer of Fas-mediated apoptosis of lung epithelial cells. J. Immunol. 168, 6470-6478.

Hagiwara S.I., Ishii Y. and Kitamura S. (2000). Aerosolized administration of N-acetylcysteine attenuates lung fibrosis induced by bleomycin in mice. Am. J. Respir. Crit. Care Med. 162, 225-231.

Haimovitz-Friedman A., Cordon-Cardo C., Bayoumy S., Garzotto M., McLoughlin M., Gallily R., Edwards C.K. 3rd., Schuchman E.H., Fuks Z. and Kolesnick R. (1997). Lipopolysaccharide induces disseminated endothelial apoptosis requiring ceramide generation. J. Exp. Med. 186, 1831-1841.

Harrison J.H. Jr., Hoyt D.G. and Lazo J.S. (1989). Acute pulmonary toxicity of bleomycin: DNA scission and matrix protein mRNA levels in bleomycin-sensitive and -resistant strains of mice. Mol. Pharmacol. 36, 231-238.

Harrison N.K., Myers A.R., Corrin B., Soosay G., Dewar A., Black C.M., Du Bois R.M. and Turner-Warwick M. (1991). Structural features of interstitial lung disease in systemic sclerosis. Am. Rev. Respir. Dis. 144, 706-713.

Hayashi H. Abdollah S., Qiu Y., Cai J., Xu Y.Y., Grimmell B.W., Richardson M.A., Topper J.N., Gimbrone M.A., Wrana J.L. and Falb D. (1997). The MAD-related protein Smad 7 associates with the TGFbeta receptor and functions as an antagonist of TGFbeta signaling. Cell 89, 1165-1173.

Heldin C.H., Miyazono K. and Dijke P. (1997). TGF-beta signalling from cell membrane to nucleus through SMAD proteins. Nature 390, 465-471.

Henke C., Bitterman P.B., Roongta U., Ingbar D. and Polunovsky V. (1996). Induction of fibroblast apoptosis by anti-CD44 antibody: implications for the treatment of fibroproliferative lung disease. Am. J. Pathol. 149, 1639-1650.

Henke C., Marineili W., Jessurun J., Fox J., Harms D., Peterson M., Chiang L. and Doran P. (1993). Macrophage production of basic fibroblast growth factor in the fibroproliferative disorder of alveolar fibrosis after lung injury. Am. J. Pathol. 143, 1189-1199.

Hiromatsu T., Yajima T., Matsuguchi T., Nishimura H., Wajjwalku W., Arai T., Nimura Y. and Yoshikai Y. (2003). Overexpression of interleukin-15 protects against Escherichia coli-induced shock accompanied by inhibition of tumor necrosis factor-alpha-induced apoptosis. J. Infect. Dis. 187, 1442-1451.

Hodge S., Hodge G., Flower R., Reynolds P.N., Scicchitano R. and Holmes M. (2002). Up-regulation of production of TGF-beta and IL-4 and down-regulation of IL-6 by apoptotic human bronchial epithelial cells. Immunol. Cell Biol. 80, 537-543.

Hsu H., Huang J., Shu H.B., Baichwal V. and Goeddel D.V. (1996). TNF-dependent recruitment of the protein kinase RIP to the TNF receptor-1 signaling complex. Immunity 4, 387-396.

Hsu H., Xiong J. and Goeddel D.V. (1996). TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. Cell 84, 299-308.

Hsu H., Xiong J. and Goeddel D.V. (1995). The TNF receptor 1-associated protein TRADD signals cell death and NF-kB activation. Cell 81, 495-504.

Hubbert N.L., Sedman S.A. and Schiller J.T. (1992). Human papillomavirus type 16 E6 increases the degradation rate of p53 in human keratinocytes. J. Virol. 66, 6237-6241.

Hung W.C., Chang H.C. and Chuang L.Y. (1998). Transforming growth factor beta 1 potently activates CPP32-like proteases in human hepatoma cells. Cell Signal. 10, 511-515.

Inoue Y., King T.E. Jr., Tinkle S.S., Dockstader K. and Newman L.S. (1996). Human mast cell basic fibroblast growth factor in pulmonary fibrotic disorders. Am. J. Pathol. 149, 2037-2054.

Ischiropoulos H., Zhu L. and Beckman J.S. (1992). Peroxynitrite formation from macrophage-derived nitric oxide. Arch. Biochem. Biophys. 298, 446-451.

Itoh N., Yonehara S., Ishii A., Yonehara M., Mizushima S., Sameshima M., Hase A., Seto Y. and Nagata S. (1991). The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell 66, 233-243.

Ju S.T., Panka D.J., Cui H., Ettinger R., El-Khatib M., Sherr D.H., Stanger B.Z. and Rothstein A.M. (1995). Fas(CD95)/FasL interactions required for programmed cell death after T-cell activation. Nature 373, 444-448.

Kastan M.B., Onyekwere O., Sidransky D., Vogelstein B. and Craig R.W. (1991). Participation of p53 protein in the cellular response to DNA damage. Cancer Res. 51, 6304-6311.

Kawasaki M., Kuwano K., Hagimoto N., Matsuba T., Kunitake R., Tanaka T., Maeyama T. and Hara N. (2000). Protection from lethal apoptosis in lipopolysaccharide-induced acute lung injury in mice by a caspase inhibitor. Am. J. Pathol. 157, 597-603.

Keane M.P., Belperio J.A., Arenberg D.A., Burdick M.D., Xu Z.J., Xue Y.Y. and Strieter R.M. (1999). IFN-gamma-inducible protein-10 attenuates bleomycin-induced pulmonary fibrosis via inhibition of angiogenesis. J. Immunol. 163, 5686-5692.

Keane M.P., Belperio J.A., Moore T.A., Moore B.B., Arenberg D.A., Smith R.E., Burdick M.D., Kunkel S.L. and Strieter R.M. (1999). Neutralization of the CXC chemokine, macrophage inflammatory protein-2, attenuates bleomycin-induced pulmonary fibrosis. J. Immunol. 162, 5511-5518.

Khalil N., O'Connor R.N., Unruh H.W., Warren P.W., Flanders K.C., Kemp A., Bereznay O.H. and Greenberg A.H. (1991). Increased production and immunohistochemical localization of transforming growth factor-beta in idiopathic pulmonary fibrosis. Am. J. Respir. Cell Mol. Biol. 5, 155-162.

Kim J.W., Kim H.S., Kim I.K., Kim M.R., Cho E.Y., Kim H.K., Lee J.M. and Namkoong S.E. (1998). Transforming growth factor-beta 1 induces apoptosis through down- regulation of c-myc gene and overexpression of p27Kip1 protein in cervical carcinoma. Gynecol. Oncol. 69, 230-236.

King T.E. Jr., Schwarz M.I., Brown K., Tooze J.A., Colby T.V., Waldron J.A., Flint A., Thurlbeck W. and Cherniack R.M. (2001). Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality. Am. J. Respir. Crit. Care Med. 164, 1025-1032.

Kitamura Y., Hashimoto S., Mizuta N., Kobayashi A., Kooguchi K., Fujiwara I. and Nakajima H. (2001). Fas/FasL-dependent apoptosis of alveolar cells after lipopolysaccharide-induced lung injury in mice. Am. J. Respir. Crit. Care Med. 163(3 Pt 1), 762-769.

Ko L.J. and Prives C. (1996). p53: puzzle and paradigm. Genes Dev. 10:,∫π 1054-1072.

Kolb M., Margetts P.J., Galt T., Sime P.J., Xing Z., Schmidt M. and Gauldie J. (2001). Transient transgene expression of decorin in the lung reduces the fibrotic response to bleomycin. Am. J. Respir. Crit. Care Med. 163, 770-777.

Kooy N.W., Royall J.A., Ye Y.Z., Kelly D.R. and Beckman J.S. (1995). Evidence for in vivo peroxynitrite production in human acute lung injury. Am. J. Respir. Crit. Care Med. 151, 1250-1254.

Korfhagen T.R., Swantz R.J., Wert S.E., McCarty JM., Kerlakian CB., Glasser S.W. and Whitsett J.A. (1994). Respiratory epithelial cell expression of human transforming growth factor-alpha induces lung fibrosis in transgenic mice. J. Clin. Invest. 93, 1691-1699.

Kroemer G. and Reed J.C. (2000). Mitochondrial control of cell death. Nat. Med. 6, 513-519.

Kuwano K., Kunitake R., Kawasaki M., Nomoto Y., Hagimoto N., Nakanishi Y. and Hara N. (1996). P21Waf1/Cip1/Sdi1 and p53 expression in association with DNA strand breaks in idiopathic pulmonary fibrosis. Am. J. Respir. Crit. Care Med. 154, 477-483.

Kuwano K., Miyazaki H., Hagimoto N., Kawasaki M., Fujita M., Kunitake R., Kaneko Y. and Hara N. (1999a). The involvement of Fas-Fas ligand pathway in fibrosing lung diseases. Am. J. Respir. Cell Mol. Biol. 20, 53-60.

Kuwano K., Hagimoto N., Kawasaki M., Yatomi T., Nakamura N., Nagata S., Suda T., Kunitake R., Maeyama T. and Hara N. (1999b). Essential roles of the Fas-Fas ligand pathway in the development of pulmonary fibrosis. J. Clin. Invest. 104, 13-19.

Kuwano K., Kawasaki M., Maeyama T., Hagimoto N., Nakamura N., Shirakawa K. and Hara N. (2000a). Soluble form of fas and fas ligand in BAL fluid from patients with pulmonary fibrosis and bronchiolitis obliterans organizing pneumonia. Chest 118, 451-458.

Kuwano K., Kunitake R., Maeyama T., Hagimoto N., Kawasaki M., Matsuba T., Yoshimi M., Inoshima I., Yoshida K. and Hara N. (2001). Attenuation of bleomycin-induced pneumopathy in mice by a caspase inhibitor. Am. J. Physiol. Lung Cell. Mol. Physiol. 280, L316-325.

Kuwano K., Hagimoto N., Maeyama T., Fujita M., Yoshimi M., Inoshima I., Nakashima N., Hamada N., Watanabe K. and Hara N. (2002). Mitochondria-mediated apoptosis of lung epithelial cells in idiopathic interstitial pneumonias. Lab. Invest. 82, 1695-1706.

Lavrentiadou S.N., Chan C., Kawcak T., Ravid T., Tsaba A., van der Vliet A., Rasooly R. and Goldkorn T. (2001). Ceramide-mediated apoptosis in lung epithelial cells is regulated by glutathione. Am. J. Respir. Cell Mol. Biol. 25, 676-684.

Li R., Waga S., Hannon G.J., Beach D. and Stillman B. (1994). Differential effects by the p21 CDK inhibitor on PCNA-dependent DNA replication and repair. Nature 371, 534-537.

Li X., Zhang H., Soledad-Conrad V., Zhuang J. and Uhal B.D. (2003). Bleomycin-induced apoptosis of alveolar epithelial cells requires angiotensin synthesis de novo. Am. J. Physiol. Lung Cell. Mol. Physiol. 284, L501-507.

Liu J.Y., Brass D.M., Hoyle G.W. and Brody A.R. (1998). TNF-alpha receptor knockout mice are protected from the fibroproliferative effects of inhaled asbestos fibers. Am. J. Pathol. 153, 1839-1847.

Lu Y., Yamagishi N., Yagi T. and Takebe H. (1998). Mutated p21(WAF1/CIP1/SDI1) lacking CDK-inhibitory activity fails to prevent apoptosis in human colorectal carcinoma cells. Oncogene 16, 705-712.

Madtes D.K., Busby H.K., Strandjord T.P. and Clark J.G. (1994). Expression of transforming growth factor-alpha and epidermal growth factor receptor is increased following bleomycin-induced lung injury in rats. Am. J. Respir. Cell Mol. Biol. 11, 540-551.

Maeda J., Ueki N., Hada T. and Higashino K. (1995). Elevated serum hepatocyte growth factor/scatter factor levels in inflammatory lung disease. Am. J. Respir. Crit. Care Med. 152, 1587-1591.

Maki C.G. and Howley P.M. (1997). Ubiquitination of p53 and p21 is differentially affected by ionizing and UV radiation. Mol. Cell Biol. 17, 355-363.

Malinin N.L., Boldin M.P., Kovalenko A.V. and Wallach D. (1997). MAP3K-related kinase involved in NF-κB induction by TNF, CD95, and IL-1. Nature 385, 540-544.

Maltzman W. and Czyzyk L. (1984). UV irradiation stimulates levels of p53 cellular tumor antigen in nontransformed mouse cells. Mol. Cell Biol. 4, 1689-1694.

Marshall R.P., McAnulty R.J. and Laurent G.J. (2000). Angiotensin II is mitogenic for human lung fibroblasts via activation of the type 1 receptor. Am. J. Respir. Crit. Care Med. 160, 1999-2004.

Mason R.J. and Shannon J.M. (1997). Alveolar type II cells. In: The lung: scientific foundations. Crystal R.G. (ed). Lippincot-Raven Publishers. Philadelphia.

Mason R.J., Leslie C.C., McCormick-Shannon K., Deterding R.R., Nakamura T., Rubin J.S. and Shannon J.M. (1994). Hepatocyte growth factor is a growth factor for rat alveolar type II cells. Am. J. Respir. Cell Mol. Biol. 11, 561-567.

Matute-Bello G., Liles W.C., Frevert C.W., Nakamura M., Ballman K., Vathanaprida C., Kiener P.A. and Martin T.R. (2001). Recombinant human Fas ligand induces alveolar epithelial cell apoptosis and lung injury in rabbits. Am. J. Physiol. Lung Cell. Mol. Physiol. 281, L328-335.

Matute-Bello G., Winn R.K., Jonas M., Chi E.Y., Martin T.R. and Liles W.C. (2001a). Fas (CD95) induces alveolar epithelial cell apoptosis in vivo: implications for acute pulmonary inflammation. Am. J. Pathol. 158, 153-161.

Matute-Bello G., Frevert C.W., Liles W.C., Nakamura M., Ruzinski J.T., Ballman K., Wong V.A., Vathanaprida C. and Martin T.R. (2001b).

Fas/Fas ligand system mediates epithelial injury, but not pulmonary host defenses, in response to inhaled bacteria. Infect. Immun. 69, 5768-5776.

Matute-Bello G., Liles W.C., Steinberg K.P., Kiener P.A., Mongovin S., Chi E.Y., Jonas M. and Martin T.R. (1999). Soluble Fas ligand induces epithelial cell apoptosis in humans with acute lung injury (ARDS). J. Immunol. 163, 2217-2225.

McGrath-Morrow S.A. and Stahl J. (2001). Apoptosis in neonatal murine lung exposed to hyperoxia. Am. J. Respir. Cell Mol. Biol. 25, 150-155.

Mercurio F., Zhu H., Murray B.W., Shevchenko A., Bennett B.L., Li J., Young D.B., Barbosa M., Mann M., Manning A. and Rao A. (1997). IKK-1 and IKK-2: cytokine-activated IkB kinases essential for NFκB activation. Science 278, 860-866.

Meyer A., Buhl R. and Magnussen H. (1994). The effect of oral N-acetylcysteine on lung glutathione levels in idiopathic pulmonary fibrosis. Eur. Respir. J. 7, 431-436.

Mishra A., Doyle N.A. and Martin W.J. (2000). Bleomycin-mediated pulmonary toxicity: evidence for a p53-mediated response. Am. J. Respir. Cell Mol. Biol. 22, 543-549.

Miyazaki Y., Araki K., Vesin C., Garcia I., Kapanci Y., Whitsett J.A., Piguet P.F. and Vassalli P. (1995). Expression of a tumor necrosis factor-alpha transgene in murine lung causes lymphocytic and fibrosing alveolitis. A mouse model of progressive pulmonary fibrosis. J. Clin. Invest. 96, 250-259.

Molteni A., Ward W.F., Ts'ao C.H., Solliday N.H. and Dunne M. (1985). Monocrotaline-induced pulmonary fibrosis in rats: amelioration by captopril and penicillamine. Proc. Soc. Exp. Biol. Med. 180, 112-120..

Montuschi P., Ciabattoni G., Paredi P., Pantelidis P., du Bois R.M., Kharitonov S.A. and Barnes P.J. (1998). 8-Isoprostane as a biomarker of oxidative stress in interstitial lung diseases. Am. J. Respir. Crit. Care Med. 158, 1524-1527.

Moodley Y.P., Scaffidi A.K., Misso N.L., Keerthisingam C., McAnulty R.J., Laurent G.J., Mutsaers S.E., Thompson P.J. and Knight D.A. (2003). Fibroblasts isolated from normal lungs and those with Idiopathic pulmonary fibrosis differ in literleukin-6/gp130-mediated cell signaling and proliferation. Am. J. Pathol. 163, 345-354.

Nagahori T., Dohi M., Matsumoto K., Saitoh K., Honda Z., Nakamura T. and Yamamoto K. (1999). Interferon-gamma upregulates the c-Met/hepatocyte growth factor receptor expression in alveolar epithelial cells. Am. J. Respir. Cell Mol. Biol. 21, 490-497.

Nakao A., Afrakhte M., Moren A., Nakayama T., Christian JL., Heuchel R., Itoh S., Kawabata M., Heldin N.E., Heldin C.H. and ten Dijke P. (1997). Identification of Smad7, a TGF beta-inducible antagonist of TGF-beta signalling. Nature 1997, 389, 631-635.

Nakao A., Fujii M., Matsumura R., Kumano K., Saito Y., Miyazono K. and Iwamoto I. (1999). Transient gene transfer and expression of Smad 7 prevents bleomycin-induced lung fibrosis in mice. J. Clin. Invest. 104, 5-11.

Ohtsuka Y., Munakata M., Ukita H., Takahashi T., Satoh A., Homma Y. and Kawakami Y. (1995). Increased susceptibility to silicosis and TNF-alpha production in C57BL/6J mice. Am. J. Respir. Crit. Care Med. 152, 2144-2149.

O'Reilly M.A, Staversky R.J, Watkins R.H., Reed C.K., de Mesy Jensen K.L., Finkelstein J.N. and Keng P.C. (2001). The cyclin-dependent kinase inhibitor p21 protects the lung from oxidative stress. Am. J. Respir. Cell Mol. Biol. 24, 703-710.

Ortiz L.A., Lasky J., Lungarella G., Cavarra E., Martorana P., Banks W.A., Peschon J.J., Schmidts H.L., Brody A.R. and Friedman M. (1999). Upregulation of the p75 but not the p55 TNF-alpha receptor mRNA after silica and bleomycin exposure and protection from lung injury in double receptor knockout mice. Am. J. Respir. Cell Mol. Biol. 20, 825-833.

Otterbein L.E., Kolls J.K., Mantell L.L., Cook J.L., Alam J. and Choi A.M. (1999). Exogenous administration of heme oxygenase-1 by gene transfer provides protection against hyperoxia-induced lung injury. J. Clin. Invest. 103, 1047-1054.

Otterbein L.E., Mantell L.L. and Choi A.M. (1999). Carbon monoxide provides protection against hyperoxic lung injury. Am. J. Physiol. 276, L688-694.

Ozdulger A., Cinel I., Koksel O., Cinel L., Avlan D., Unlu A., Okcu H., Dikmengil M. and Oral U. (2003). The protective effect of n-acetylcysteine on apoptotic lung injury in cecal ligation and puncture-induced sepsis model. Shock 19, 366-372.

Panos R.J., Rubin J.S., Csaky K.G., Aaronson S.A. and Mason R.J. (1993). Keratinocyte growth factor and hepatocyte growth factor/scatter factor are heparin-binding growth factors for alveolar type II cells in fibroblast-conditioned medium. J. Clin. Invest. 92, 969-977.

Peao M.N., Aguas A.P., de Sa C.M. and Grande N.R. (1994). Neoformation of blood vessels in association with rat lung fibrosis induced by bleomycin. Anat. Rec. 238, 57-67.

Piguet P.F. and Vesin C. (1994). Treatment by human recombinant soluble TNF receptor of pulmonary fibrosis induced by bleomycin or silica in mice. Eur. Respir. J. 7, 515-518.

Piguet P.F., Collart M.A., Grau G.E., Sappino A.P. and Vassalli P. (1990). Requirement of tumour necrosis factor for development of silica-induced pulmonary fibrosis. Nature 344, 245-247.000

Piguet P.F., Ribaux C., Karpuz V., Grau G.E. and Kapanci Y. (1993). Expression and localization of tumor necrosis factor-alpha and its mRNA in idiopathic pulmonary fibrosis. Am. J. Pathol. 143, 651-655.

Pober J.S and Cotran R.S. (1990). The role of endothelial cells in inflammation. Transplantation 50, 537-544.

Polunovsky V.A., Chen B., Henke C., Snover D., Wendt C., Ingbar D.H. and Bitterman P.B. (1993). Role of mesenchymal cell death in lung remodeling after injury. J. Clin. Invest. 92, 388-397.

Raghow B., Irish P. and Kang A.H. (1989). Coordinate regulation of transforming growth factor beta gene expression and cell proliferation in hamster lungs undergoing bleomycin-induced pulmonary fibrosis. J. Clin. Invest. 84, 1836-1842.

Rahman I., Skwarska E., Henry M., Davis M., O'Connor C.M., FitzGerald M.X., Greening A. and MacNee W. (1999). Systemic and pulmonary oxidative stress in idiopathic pulmonary fibrosis. Free Radic. Biol. Med. 27, 60-68.

Ray P., Devaux Y., Stolz D.B., Yarlagadda M., Watkins S.C., Lu Y., Chen L., Yang X.F. and Ray A. (2003). Inducible expression of keratinocyte growth factor (KGF) in mice inhibits lung epithelial cell death induced by hyperoxia. Proc. Natl. Acad. Sci. USA 100, 6098-6103.

Regnier C.H., Song H.Y., Gao X., Rothe M. and Goeddel D. (1997). Identification and characterization of an IkB kinase. Cell 90, 373-383.

Rom W.N., Basset P., Fells G.A., Nukiwa T., Trapnell B.C. and Crysal R.G. (1988). Alveolar macrophages release an insulin-like growth factor I-type molecule. J. Clin. Invest. 82,1685-1693.

Sakai T., Satoh K., Matsushima K., Shindo S., Abe S., Abe T., Motomiya M., Kawamoto T., Kawabata Y., Nakamura T. and Nukiwa

T. (1997). Hepatocyte growth factor in bronchoalveolar lavage fluids and cells in patients with inflammatory chest diseases of the lower respiratory tract: detection by RIA and in situ hybridization. Am. J. Respir. Cell Mol. Biol.16, 388-397.

Saleh D., Barnes P.J. and Giaid A. (1997). Increased production of the potent oxidant peroxynitrite in the lungs of patients with idiopathic pulmonary fibrosis. Am. J. Respir. Crit. Care Med. 155, 1763-1769.

Saltzman A., Munro R., Searfoss G., Franks C., Jaye M. and Ivashchenko Y. (1998). Transforming growth factor-beta-mediated apoptosis in the Ramos B- lymphoma cell line is accompanied by caspase activation and Bcl-XL downregulation. Exp. Cell Res. 242, 244-254.

Shi X., Ding M., Chen F., Wang L., Rojanasakul Y., Vallyathan V. and Castranova V. (2001). Reactive oxygen species and molecular mechanism of silica-induced lung injury. J. Environ. Pathol. Toxicol. Oncol. 20 (Suppl 1), 85-93.

Shiratori M., Michalopoulos G., Shinozuka H., Singh G., Ogasawara H. and Katyal S.L. (1995). Hepatocyte growth factor stimulates DNA synthesis in alveolar epithelial type II cells in vitro. Am. J. Respir. Cell Mol. Biol. 12, 171-180.

Sime P.J., Xing Z., Graham F.L., Csaky K.G. and Gauldie J. (1997). Adenovector-mediated gene transfer of active transforming growth factor-beta1 induces prolonged severe fibrosis in rat lung. J. Clin. Invest. 100, 768-776.

Staversky R.J., Watkins R.H., Wright T.W., Hernady E., LoMonaco M.B., D'Angio C.T., Williams J.P., Maniscalco W.M. and O'Reilly M.A. (2002). Normal remodeling of the oxygen-injured lung requires the cyclin-dependent kinase inhibitor p21(Cip1/WAF1/Sdi1). Am. J. Pathol. 161, 1383-1393.

Stehlik C., de Martin R., Kumabashiri I., Schmid J.A., Binder B.R. and Lipp J. (1998). Nuclear factor (NF) -κB-regulated X-chromosome-linked iap gene expression protects endothelial cells from tumor necrosis factor α -induced apoptosis. J. Exp. Med. 188, 211-216.

Suda T., Takahashi T., Golstein T. and Nagata S. (1993). Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. Cell 75, 1169-1178.

Sugahara K., Iyama K., Kuroda M.J. and Sano K. (1998). Double intratracheal instillation of keratinocyte growth factor prevents bleomycin-induced lung fibrosis in rats. J. Pathol. 186, 90-98.

Suzuki A., Tsutomi Y., Akahane K., Araki T. and Miura M. (1998). Resistance to Fas-mediated apoptosis: activation of caspase-3 is regulated by cell cycle regulator p21WAF1 and IAP gene family ILP. Oncogene 17, 931-939.

Suzuki A., Tsutomi Y., Miura M. and Akahane K. (1999). Caspase-3 inactivation to suppress Fas-mediated apoptosis: identification of binding domain with p21 and ILP and inactivation machinery by p21. Oncogene 18, 1239-1244.

Takahashi T., Tanaka M., Brannan C.I., Jenkins N.A., Copeland N.G., Suda T. and Nagata S. (1994). Generalized lymphoproliferative disease in mice, caused by a point mutation in the Fas ligand. Cell 76, 969-976.

Tan A., Levrey H., Dahm C., Polunovsky V.A., Rubins J. and Bitterman P.B. (1999). Lovastatin induces fibroblast apoptosis in vitro and in vivo. A possible therapy for fibroproliferative disorders. Am. J. Respir. Crit. Care Med. 159, 220-227.

Tartaglia L.A. and Goeddel D.V. (1992). Two TNF receptors. Immunol. Today 13, 151-153.

Tateda K., Deng J.C., Moore T.A., Newstead M.W., Paine R. 3rd, Kobayashi N., Yamaguchi K. and Standiford T.J. (2003). Hyperoxia mediates acute lung injury and increased lethality in murine legionella pneumonia: the role of apoptosis. J. Immunol. 170, 4209-4216.

Terzaghi M., Nettesheim P. and Williams M.L. (1978). Repopulation of denuded tracheal grafts with normal, preneoplastic, and neoplastic epithelial cell populations. Cancer Res. 38, 4546-4553.

Tounekti O., Pron G., Belehradek J. Jr. and Mir L.M. (1993). Bleomycin, an apoptosis-mimetic drug that induces two types of cell death depending on the number of molecules internalized. Cancer Res. 53, 5462-5469.

Tsuburai T., Suzuki M., Nagashima Y., Suzuki S., Inoue S., Hasiba T., Ueda A., Ikehara K., Matsuse T. and Ishigatsubo Y. (2002). Adenovirus-mediated transfer and overexpression of heme oxygenase 1 cDNA in lung prevents bleomycin-induced pulmonary fibrosis via a Fas-Fas ligand-independent pathway. Hum. Gene Ther. 13, 1945-1960.

Uhal B.D., Joshi I., True A.L., Mundle S., Raza A., Pardo A. and Selman M. (1995). Fibroblasts isolated after fibrotic lung injury induce apoptosis of alveolar epithelial cells in vitro. Am. J. Physiol. 269, L819-828.

Uhal B.D., Joshi I., Hughes W.F., Ramos C., Pardo A. and Selman M.(1998a). Alveolar epithelial cell death adjacent to underlying myofibroblasts in advanced fibrotic human lung. Am. J. Physiol. 275, L1192-1199.

Uhal B.D., Gidea C., Bargout R., Bifero A., Ibarra-Sunga O., Papp M., Flynn K. and Filippatos G. (1998b). Captopril inhibits apoptosis in human lung epithelial cells: a potential antifibrotic mechanism. Am. J. Physiol. 275, L1013-1017.

Uhal B.D., Wang R., Laukka J., Zhuang J., Soledad-Conrad V. and Filippatos G. (2003). Inhibition of amiodarone-induced lung fibrosis but not alveolitis by angiotensin system antagonists. Pharmacol. Toxicol. 92, 81-87.

Waga S., Hannon G.J., Beach D. and Stillman B. (1994). The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA. Nature 369, 574-578.

Wang C.Y., Mayo M.W., Korneluk R.G., Goeddel D.V. and Baldwin A.S. (1998). NF-κB antiapoptosis: Induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. Science 281, 1680-1683.

Wang R., Ramos C., Joshi I., Zagariya A., Pardo A., Selman M. and Uhal B.D. (1999). Human lung myofibroblast-derived inducers of alveolar epithelial apoptosis identified as angiotensin peptides. Am. J. Physiol. 277, L1158-1164.

Wang Q., Wang Y., Hyde DM., Gotwals P.J., Koteliansky V.E., Ryan S.T. and Giri S.N. (1999). Reduction of bleomycin induced lung fibrosis by transforming growth factor beta soluble receptor in hamsters. Thorax. 54, 805-812.

Ward W.F., Molteni A., Ts'ao C.H. and Hinz J.M. (1990). Captopril reduces collagen and mast cell accumulation in irradiated rat lung. Int. J. Radiat. Oncol. Biol. Phys. 19, 1405-1409.

Ward N.S., Waxman A.B., Homer R.J., Mantell L.L., Einarsson O., Du Y. and Elias J.A. (2000). Interleukin-6-induced protection in hyperoxic acute lung injury. Am. J. Respir. Cell Mol. Biol. 22, 535-542.

Ward W.F., Solliday N.H., Molteni A. and Port C.D. (1983). Radiation injury in rat lung. II. Angiotensin-converting enzyme activity. Radiat. Res. 96, 294-300..

Watanabe-Fukunaga R., Brannan C.I., Copeland N.G., Jenkins N.A., and Nagata S. (1992). Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature

356, 314-317.

Waxman A.B., Einarsson O., Seres T., Knickelbein R.G., Warshaw J.B., Johnston R., Homer R.J. and Elias J.A. (1998). Targeted lung expression of interleukin-11 enhances murine tolerance of 100% oxygen and diminishes hyperoxia-induced DNA fragmentation. J. Clin. Invest. 101, 1970-1982.

Wendt C.H., Polunovsky V.A., Peterson M.S., Bitterman P.B. and Ingbar D.H. (1994). Alveolar epithelial cells regulate the induction of endothelial cell apoptosis. Am. J. Physiol. 267, C893-900.

Witschi H.R., Haschek W.M., Klein-Szanto A.J. and Hakkinen P.J. (1981). Potentiation of diffuse lung damage by oxygen: determining variables. Am. Rev. Respir. Dis. 123, 98-103..

Woronicz J.D., Gao X., Cao Z., Rothe M. and Goeddel D. (1997). IκB kinase-b: NFκB activation and complex formation with IB kinase-α and NIK. Science 278, 866-869.

Wu K.I., Pollack N., Panos R.J., Sporn P.H. and Kamp D.W. (1998). Keratinocyte growth factor promotes alveolar epithelial cell DNA repair after H_2O_2 exposure. Am. J. Physiol. 275, L780-787.

Yaekashiwa M., Nakayama S., Ohnuma K., Sakai T., Abe T., Satoh K., Matsumoto K., Nakamura T., Takahashi T. and Nukiwa T. (1997). Simultaneous or delayed administration of hepatocyte growth factor equally represses the fibrotic changes in murine lung injury induced by bleomycin. A morphologic study. Am. J. Respir. Crit. Care Med. 156, 1937-1944.

Yanagisawa K., Osada H., Masuda A., Kondo M., Saito T., Yatabe Y., Takagi K. and Takahashi T. (1998). Induction of apoptosis by Smad3 and down-regulation of Smad3 expression in response to TGF-beta in human normal lung epithelial cells. Oncogene 17, 1743-1747.

Yanagihara K. and Tsumuraya M. (1992). Transforming growth factor beta 1 induces apoptotic cell death in cultured human gastric carcinoma cells. Cancer Res. 52, 4042-4045.

Yan Q. and Sage E.H.. (1998). Transforming growth factor-beta1 induces apoptotic cell death in cultured retinal endothelial cells but not pericytes: association with decreased expression of p21waf1/cip1. J. Cell Biochem. 70, 70-83.

Yu Z.K., Geyer R.K. and Maki C.G. (2000). MDM2-dependent ubiquitination of nuclear and cytoplasmic P53. Oncogene. 19, 5892-5897.

Zandi E., Rothwarf D.M., Delhase M., Hayakawa M. and Karin M. (1997). The IκB kinase complex (IKK) contains two kinase subunits, IKKa and IKKb, necessary for IkB phosphorylation and NFκB activation. Cell 91, 243-252.

Zhang X., Shan P., Otterbein L.E., Alam J., Flavell R.A., Davis R.J., Choi A.M. and Lee P.J. (2003). Carbon monoxide inhibition of apoptosis during ischemia-reperfusion lung injury is dependent on the p38 mitogen-activated protein kinase pathway and involves caspase 3. J. Biol. Chem. 278, 1248-1258.

Zhao J., Shi W., Wang Y.L., Chen H., Bringas P. Jr., Datto M.B., Frederick J.P., Wang X.F. and Warburton D. (2002). Smad3 deficiency attenuates bleomycin-induced pulmonary fibrosis in mice. Am. J. Physiol. Lung Cell. Mol. Physiol. 282, L585-593.

Accepted January 14, 2004