Apoptosis in lung pathophysiology

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Fine, Alan, Yvonne Janssen-Heininger, Rebecca P. Soultanakis, Stephen G. Swisher, and Bruce D. Uhal. Apoptosis in lung pathophysiology. Am J Physiol Lung Cell Mol Physiol 279: L423–L427, 2000.—As recently as 1993, fewer than 10 manuscripts had been published on the topic of apoptosis specifically in the lung. Although that number is increasing, far fewer papers appear each year on apoptosis in the lung than in the other major organs. Therefore, our knowledge of this important aspect of lung cell physiology is relatively rudimentary. Recent literature is beginning to define important roles for apoptosis in normal lung cell turnover, lung development, and the pathogenesis of diseases such as interstitial pulmonary fibrosis, acute respiratory distress syndrome, and chronic obstructive pulmonary disease. Although the involvement of lung cell apoptosis in each of these examples seems clear, the many factors comprising the normal and abnormal regulation of cell death remain to be elucidated and are likely to be different in each situation. The definition of those factors will be an exciting and challenging field of research for many years to come. In that context, the goal of this symposium was to discuss, from a physiological perspective, some of the most recent and exciting advances in the definition of signaling mechanisms involved in the regulation of apoptosis specifically in lung cell populations.

APOPTOSIS IN THE LUNG is an exciting field in which to be working, in part because it is a relatively new field that is rapidly developing. Recent literature is beginning to define many roles for apoptosis in normal lung function as well as in pathophysiology unrelated to lung neoplasia. For example, in the lung as in other organs, apoptosis plays a critical role in postnatal development (40). The normal resolution of inflammation in the lung occurs through the regulated removal by apoptosis of unneeded cells such as granulocytes without the release of damaging histotoxins (17). Dexamethasone has been known for many years to induce apoptosis in some leukocyte subsets; although corticosteroids appear to induce apoptosis in lung eosinophil and lymphocyte subpopulations, recent evidence (30) suggests that dexamethasone may inhibit the apoptosis of lung neutrophils. Considerable literature now supports a role for apoptosis in the remodeling of lung tissue after acute lung injury for both the clearance of excess epithelial stem cells after repair (2) and the normal removal of excess mesenchymal cells from resolving lesions (36). Recent evidence (11) also suggests a role for apoptosis in the remodeling associated with chronic pulmonary hypertension.

A considerable body of recent literature (16, 25, 29, 42) describes roles for apoptosis in the pathogenesis of diseases such as interstitial pulmonary fibrosis, acute respiratory distress syndrome, and chronic obstructive pulmonary disease. Although the involvement of lung cell apoptosis in each of these examples seems clear, the many factors comprising the normal and abnormal regulation of cell death remain to be elucidated and are...
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FAS IN THE LUNG

Fas, the receptor signaling component of this system, is widely expressed in inflammatory, mesenchymal, and parenchymal cells of the skin, liver, small intestines, and ovary (14). The other essential component of the Fas killing pathway is the Fas ligand (FasL) (43). Identified as a type II membrane protein member of the tumor necrosis factor family of ligands, Fasl was originally characterized in T cells as the primary effector molecule of Th1 cytotoxicity (43). In previous work, Fine et al. (14) found that Fas is expressed on the luminal surface of a subset of alveolar type II cells (14). Consistent with this finding, injection of a Fas-activating antibody induced apoptosis of scattered type II cells. The relevance of Fas expression in a restricted type II cell subset is not clear at this time. Nevertheless, these findings are consistent with a role for this molecule in the turnover of the distal lung epithelium. Interestingly, bleomycin-induced lung injury, which is thought to be initiated by damage to epithelial cells, has been found to be resistant in lpr and gld mice (24).

In contrast, Gochuico et al. (15) found that Fasl is constitutively expressed in the Clara cell of the mouse airway. Because the airway is particularly susceptible to recurrent immune injury during continuous exposure to aerogenic antigens, we speculated that Clara cell-derived Fasl serves to monitor local immune activity. Consistent with this, we found that the airway of the adult gld mouse contains an infiltrating population of mononuclear immune cells. Furthermore, we found the inflammatory response in an allergen model of asthma coincides with the loss of Clara cell Fasl expression (15).

In skin keratinocytes, the Fas system is thought to play a role in deleting damaged cells. In this paradigm, Fas-mediated apoptosis removes cells that contain excess damaged DNA and, as a result, serves as a “brake” against the disregulated proliferation of mutant cells (18). Similarly, we have preliminary evidence that Fasl in the airway may serve a similar function. In these initial studies, we found that nonspecific DNA damage, such as that associated with gamma radiation, is associated with development of airway epithelial cell hyperplasia in gld animals. Overall, we speculate that impaired or inhibited Fas signaling in the airway could play a role in lung cancer pathogenesis. Of note, the DcR3 gene, which is commonly amplified in lung cancer, encodes for a soluble Fasl receptor that inhibits Fasl-induced apoptosis (35).

To summarize, accumulated data by us and others indicate that Fas signaling is involved in lung epithelial turnover and regulation of pulmonary inflammation and that disregulation of this system may be a key factor in the pathogenesis of lung disease syndromes. Future work will likely be directed at elucidating the specific role of Fas signaling in the evolution of these syndromes.

APOPTOTIC SIGNALING IN LUNG EPITHELIUM IN RESPONSE TO REACTIVE NITROGEN SPECIES

The lung can be exposed to a variety of reactive oxygen and nitrogen species by inhalation or by formation during inflammation. One oxidant that has received attention as an air pollutant is the highly reactive free radical gas nitrogen dioxide (NO2). NO2 is generated during inflammation, occurs as a pollutant of indoor and outdoor air, and has been associated with acute respiratory symptoms and the aggravation of asthma in children (7, 31). Mechanistically, NO2 is formed by the decomposition of peroxynitrite (ONOO−) or by myeloperoxidase- or eosinophil peroxidase-catalyzed reactions (5, 46). The formation of 3-nitrotyrosine is a footprint of NO2 exposure observed in models of pulmonary inflammation and infection.

However, it is unclear to date whether the NO2-induced protein nitration is causally linked to injury or is an epiphenomenon irrelevant to disease (46). Earlier studies (20) in our laboratory compared the ability of various oxidants to induce apoptosis in a rat alveolar type II epithelial cell line (RLE), which undergoes apoptosis after exposure to donors of nitric oxide or hydrogen peroxide. The apoptosis was preceded by upregulation of c-fos and c-jun protooncogenes and the transcription factor activator protein-1 (AP-1). In contrast, high concentrations of ONOO− or the ONOO− generator 3-morpholinosydnonimine (SIN-1) did not lead to c-fos, c-jun, or AP-1 activation and failed to induce apoptosis (20).

Recent experiments employing a gas-phase exposure system also demonstrated that exposure of quiescent RLE cells to 5 parts/million NO2 for 4 h does not induce apoptosis, suggesting a unique ability of different reactive nitrogen species to cause apoptosis. It is important to consider that under inflammatory conditions such as asthma, the epithelium is not quiescent but rather in a state of cell shedding and regeneration (4, 34, 37). Consequently, the cellular responses to nitrating species under conditions of injury and repair may also be different. Recent unpublished observations from our laboratory demonstrated a marked induction of apoptosis in log-phase cultures exposed to NO2 or ONOO−. Similarly, under conditions that mimic wound healing, NO2 causes apoptosis selectively in cells that are migrating into the wound. Therefore, the presence of a nitrating agent in the lung may interfere with epithelial repair and contribute to the continuous shedding of cells in pulmonary diseases such as asthma (3, 4, 34).

Apoptosis of lung epithelial cells can lead to pulmonary fibrosis (6, 16, 24). Understanding the signaling

1 Presented by Alan Fine.
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mechanisms that control apoptosis in response to NO₂ may therefore be critical to the prevention of fibrotic lung disease. One protein that is involved in stress-induced apoptosis is c-Jun NH₂-terminal kinase (JNK), a member of the family of mitogen-activated protein kinases. Oxidants have been demonstrated to activate JNK (1, 21, 26), which, in turn, leads to phosphorylation of its targets, c-Jun and activating transcription factor (ATF)-2 (19, 39). This JNK-dependent phosphorylation enhances the transcriptional activation of Jun and ATF-2 and activates gene expression (10).

Of potential importance in epithelial apoptosis are recent observations that FasL contains a mitogen-activated protein kinase kinase response element in its promoter that binds c-Jun and ATF-2 and is activated in a JNK-dependent manner (12, 13, 23). The JNK-dependent transcriptional activation of FasL may therefore be an important pathway for apoptosis in bronchiolar epithelium. The generation of cell lines or mice that express dominant negative JNK constructs will be required to determine a causal role for this signaling mechanism in oxidant-induced epithelial apoptosis in vivo.

LOCAL RENIN-ANGIOTENSIN SYSTEMS AND APOPTOSIS IN THE LUNG³

Many cell types outside the vasculature have recently been shown to synthesize and respond to angiotensin (ANG) II, among them adipocytes (41), neurons and astrocytes (52), and testicular epithelial cells (27), all of which can now be described as members of local “intrinsic” renin-angiotensin systems (RASs). By definition, these systems are microenvironments in which all of the components required for the synthesis of ANG II as well as of ANG II receptor(s) are expressed locally and function as a unit independent of the circulation (8). Intrinsic local RASs are in contrast to “extrinsic” RASs, which are defined as microenvironments in which circulating renin of renal origin (or other RAS components) is sequestered from the circulation locally and contributes significantly to local ANG II production, as is believed to occur in the heart (9).

Recent evidence indicated that a local intrinsic RAS is expressed in the distal lung parenchyma and also plays a central role in the signaling of apoptosis in at least one cell type, alveolar epithelial cells (AECs) (48–50). Angiotensinogen (ANGEN) was recently shown to be synthesized and secreted by human lung myofibroblasts isolated from patients suffering from interstitial pulmonary fibrosis or chronic hypersensitivity pneumonitis (48), in agreement with simultaneous work by Katwa et al. (22) demonstrating ANGEN expression by rat heart myofibroblasts. Normal human lung fibroblasts did not express ANGEN. Although human lung myofibroblasts in culture have a limited capacity to convert ANGEN to ANG II (48), primary cultures of AECs were earlier found to be capable of proteolytically processing ANGEN and to undergo dose-dependent apoptosis in response to the ANG II produced from it (50). Together, these data support the hypothesis that the production of ANGEN by myofibroblasts and its conversion to ANG II by the epithelium provide a mechanism to explain AEC death adjacent to underlying myofibroblasts within the fibrotic human lung (45).

The local intrinsic RAS also functions in apoptosis of AECs in the absence of myofibroblasts. A recent study (49) has shown that activation of the receptor Fas in AECs causes a significant increase in the abundance of ANGEN mRNA and protein, which, in turn, results in the generation and secretion of ANG II (49). Moreover, apoptosis in response to Fas activation can be abrogated by antisense oligonucleotides against ANGEN, by ANG-converting enzyme (ACE) inhibitors, or by ANG receptor antagonists, indicating that the de novo synthesis of ANG II and receptor interaction are required for the induction of apoptosis by Fas.

Autocrine production of ANG II is required for chemically induced apoptosis of AECs as well. Apoptosis of cultured AECs in response to the antiarrhythmic benzofuran amidarone (3) or the fibrinogenic agent bleomycin (47) could also be blocked by either an ACE inhibitor or an ANG receptor antagonist. The blockade of bleomycin-induced AEC apoptosis was demonstrated both in vitro and in vivo, suggesting that the requirement for ANG II as a “second messenger” for apoptosis exists in the intact lung as well as in cultured cells. Taken together, these studies suggest that the local intrinsic RAS plays a critical role in the signaling of apoptosis in response to a variety of stimuli, at least in AECs. Whether or not ACE inhibitors and/or ANG receptor antagonists prove useful in human lung disease will depend, at least in part, on the cell-type specificity of ANG II as a second messenger system for apoptosis.

INDUCTION OF APOPTOSIS IN LUNG CANCER CELLS⁴

The identification of specific genes critical to the development of carcinogenesis offers the opportunity to target these genes or their products for treatment. One possible gene therapy strategy would be to replace nonfunctional tumor suppressor genes such as mutated or deleted p53 genes with wild-type (wt) p53 genes. Despite the presence of multiple genetic defects, the restoration of wt p53 gene expression with retroviral or adeno viral p53 (Adp53) vectors has been shown in an orthotopic nushi mouse model to induce apoptosis and inhibit cell growth in lung cancer cell lines.

The first clinical trial involving transfer of wt p53 into lung cancer used a retroviral vector containing a wt p53 cDNA-driven by an actin promoter (38). Retroviral wt p53 supernatant was injected directly into endobronchial tumors under bronchoscopic guidance in four patients and CT guidance in five patients. Antitumor activity was demonstrated in six of seven evalua
tive posttreatment tumor biopsies by terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling (TUNEL) staining for apoptotic cells. In addition, three of seven evaluable patients showed

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³ Presented by Bruce Uhal.

⁴ Presented by Stephen G. Swisher.
evidence of tumor regression. This preliminary trial demonstrated the feasibility and safety of gene therapy strategies in advanced non-small cell lung cancer based on the restoration of wt p53 gene function.

Preclinical studies showed that apoptosis with Adp53 gene transfer could be enhanced if administered with cis-platinum. Accordingly, a phase I trial was performed in which Adp53 with and without cis-platinum (80 mg/m²) was injected directly into the tumors of patients with non-small cell lung cancer. Eleven of twenty-four evalative patients treated with Adp53 alone showed increases in apoptotic indexes in post-treatment tumor biopsy samples by TUNEL. Clinical tumor regression was also demonstrated (44).

There is a clinical need to identify other candidate genes for use in treating cancer patients with adenoviral vectors. One promising group is the proapoptotic members of the Bcl-2 family (Bax and Bak), which have been shown to induce apoptosis after gene transfer via plasmid vectors in vivo (33). Initial attempts to develop an adenoviral vector with the proapoptotic Bak gene were complicated by the high level of toxicity induced in the 293 packaging cell line. This problem was overcome with a binary adenoviral vector system in which Bak gene expression was transcriptionally controlled by the GT promoter and the Gal4/GV16 fusion protein allowing production in 293 cells (32). With this binary system (Ad/GT-Bak+Ad/GV16), high levels of apoptosis were induced in lung cancer cells that had been resistant to Adp53. In a subcutaneous tumor model, Ad/GT-Bak-injected tumors were smaller and demonstrated increased TUNEL. Importantly, intratumoral injection of Ad/GT-Bak caused no significant systemic toxicity, suggesting a clinical potential.

Enthusiasm for gene therapy strategies to induce apoptosis in lung cancers with Adp53 or Ad/GT-Bak must be tempered, however, by the limitations of the vector, which include the need for direct injection by bronchoscopy or CT scan and difficulty in targeting multiple metastatic sites. Nevertheless, these preliminary results offer encouragement that strategies designed to induce apoptosis in lung cancer may ultimately provide a novel strategy to complement conventional therapies (51).

CLOSING

In this symposium, one of the most interesting and important unanswered questions is the specificity, with respect to both cell type and organ, of each of the signaling paradigms discussed above. Clearly, in some situations, the intentional induction or inhibition of apoptosis could be beneficial but only if achieved in a cell-specific manner. With respect to lung tumors, it is interesting to note that several studies (28, 49) have suggested that systemic administration of antagonists of the RAS do not promote and may, in fact, inhibit tumorigenesis in long-term therapy, contrary to what might be predicted for pharmacological agents capable of potent inhibition of apoptosis in lung epithelial cells.

That example demonstrates the difficulty of predicting the outcome of a theoretical strategy designed on the basis of cell culture studies but applied to the whole animal. It also reaffirms the key role of the physiologist in “bridging the gap” between the molecular basis of cell function and the whole organism. Fortunately, the anatomy and physiology of the lung provide unique opportunities for drug delivery strategies to manipulate apoptosis that could, in theory, limit the systemic effects of agents delivered by aerosol. Taking these opportunities, however, will first require more knowledge about the many factors regulating apoptosis of individual lung cell types as well as the mechanisms of removal of the apoptotic cells under the circumstances involved. On behalf of all the presenters, we hope that this session has sparked the interest of physiologists and other scientists alike, who might contribute to this interesting and important aspect of respiratory physiology.

REFERENCES


