suppresses bleomycin-induced apoptosis in the lung (Davis, et. al.). Consistent with a role for apoptosis in chronic lung injury, levels of bleomycin-induced inflammation were substantially higher in iNOS$^{-/-}$ and p53$^{-/-}$ mice compared to wild-type controls. Here we report the chronic effects of bleomycin in the lung implicating downstream targets of p53. TUNEL analysis demonstrated cell death in the lung several days following bleomycin exposure. Laser Scanning Cytometry(LSC) analysis demonstrated that bleomycin induced an increase in p53 expression in wild-type mice. p53 activation was followed by an induction in Fas expression which increased during the course of inflammation. Bleomycin did not induce Fas expression in p53$^{-/-}$ mice. However, constitutive levels of Fas were higher in p53$^{-/-}$ mice than wild-type control mice. LSC analysis revealed no significant differences in Fas Ligand expression in wild-type or p53$^{-/-}$ mice. Furthermore, bleomycin induced an increase in p21 expression in wild-type mice. The effects of bleomycin on these targets will be confirmed using Fas deficient mice (lpr$^{-/-}$) mice and p21$^{-/-}$ mice.

389 INDUCTION OF APOPTOSIS BY CHRONICALLY INHALED SILICA.

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Whether apoptotis is induced and/or plays a significant role in lung pathology from inhaled silica is unknown. We examined apoptosis in rat lungs following exposure to 15 mg/m^3 silica (average particle size 1.6um) or filtered air for 6 hours/day, 5 days/week for up to 116 days. Lungs from filtered air and exposed animals were preserved by intra-tracheal instillation of fixative. Additional groups were sacrificed for isolation of genomic DNA. Sections were processed for TdT labeling of apoptotic nuclei and counter-stained with propidium iodide to label non-apoptotic nuclei. Morphometric methods were used to determine the number of apoptotic cells per lung. Additional sections were stained with Sirius Red to detect areas of lung fibrosis. Ladder patterns demonstrating DNA breakdown products of apoptotic nuclei were present in agarose gels of genomic DNA in 79 and 116 day exposure groups. Significant increases in the number of apoptotic nuclei were observed at 40, 79 and 116 days of exposure. At 40 days of exposure, apoptotic nuclei were observed in focal accumulations of lung surfactant and alveolar macrophages. At 79 and 116 days of exposure these masses of lung surfactant and inflammatory cells were more diffusely distributed throughout the airspaces. Fibrosis of the alveolar walls and isolated fibrotic nodules, similar to those in human silicosis, were present at 79 and 116 days of exposure. Apoptotic cells were limited to the airspaces and were not found within areas of fibrosis. These results demonstrate that silica inhalation produces significant increases in apoptosis before and during the development of fibrotic lesions. It is proposed that apoptosis of alveolar macrophages rather than interstitial cells is associated with the development of fibrotic foci in the lungs.

390 BOTH ORGANIC AND METALLIC FRACTIONS OF PARTICULATE MATTER INDUCE APOPTOSIS OF ALVEOLAR MACROPHAGES.

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Recent epidemiology studies have linked mortality, increased asthma morbidity and other respiratory disorders in urban areas to increases in fine particulate matter (PM). Neither the components of PM nor the biological mechanisms that explain the reported effects are known, but fine PM is known to target the pulmonary epithelium and resident alveolar macrophages (AM). This study tested the hypothesis that both organic and metallic fractions of PM contribute to AM apoptosis by interacting with scavenger receptors. Freshly isolated AM from Balb/c mice were incubated with different fractions of PM 1648. Fractions were generated by solvent, acid, and temperature treatments and analyzed by GC/MS for semi-volatile compounds. Surface analysis of each residual fraction was done by X-Ray Photoelectron Spectroscopy to determine the metallic contents. Cell viability was assessed by trypan blue exclusion and apoptosis was demonstrated by examination of cell morphology and by Cell Death ELISA. All post-treatment PM fractions except MilliQ water and 100°C showed significant reduction in apoptosis compared to untreated PM. The acid-digested fraction, confirmed by surface analysis, had the least amount of metals and induced the least apoptosis and loss of cell viability. The cyclohexane-treated fraction induced less apoptosis than the 500°C heat-treated fraction; while acetone-treated fraction induced greater apoptosis than 500°C treated PM. Inhibition of Type I and Type II scavenger receptors using polyinosinic acid and antibody 2F8 significantly reduced the level of apoptosis of untreated and generated fractions. Taken together, these results suggest that both the metal and organic compound constituents on the surface of PM can induce comparable toxicity through mechanisms that involve macrophage scavenger receptors. Supported by EPA Grant G8K104427

391 ROLE OF MACROPHAGE APOPTOSIS IN PULMONARY INFLAMMATION.

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Apoptosis is a gene-controlled process of cellular self-destruction implicated in pathogenesis of inflammatory and fibrotic lung disorders. Macrophages are key regulatory cells in the lung; however, the role of apoptotic macrophages in pulmonary disorders is not known. We instilled apoptotic macrophages into the lungs of Fischer 344 rats to study lung response to apoptotic cells and products. The effects on apoptosis induction, lung histopathology, and fibrosis-related gene expression of matrix metalloproteinase (MMP) and TGF-β were examined. Apoptosis was determined by DNA ladder and TUNEL TdT assays, while lung pathology and protein expression were determined by histological analysis and immunohistochemistry of lung sections. To induce macrophage apoptosis, several known apoptosis inducers including salicylates, TGF-β, ethanol, silica, and DMSO were evaluated. Maximal apoptosis (~30%) was obtained using either DMSO or silica. DMSO was chosen in all subsequent studies. Instillation of apoptotic macrophages (1-5 million cells/rat) with or without Survanta (a bovine surfactant) resulted in increased accumulation of macrophages and apoptotic lung cells after 4 weeks of treatment. In contrast, PBS- and normal macrophage-instilled controls showed no stimulatory effects. Increased expressions of MMP-9 and TGF-β were also observed in the apoptotic treatment group, but not in control groups. No significant effects were seen in rats treated with surfactant alone. Because MMP and TGF-β have been demonstrated to play crucial roles in the pathogenesis of inflammatory and fibrotic lung disorders, our results suggest a role for macrophage apoptosis in lung pathologies. In summary, we demonstrated that apoptotic macrophages can induce increased expression of MMP and TGF-β and that this induction may be associated with the development of lung pathology.

392 ENVIRONMENTAL TOBACCO SMOKE INDUCES INFLAMMATION AND APOPTOSIS IN THE LUNG OF SPONTANEOUSLY HYPERTENSIVE RATS.

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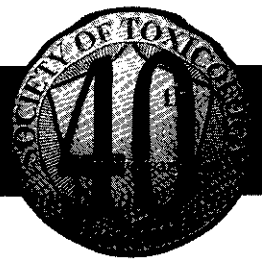
Apoptosis is a form of cell death believed to be involved in the resolution of inflammation. Environmental tobacco smoke (ETS) is believed to be a risk factor in individuals with cardiovascular disease. However, the role of apoptosis in ETS-related cardiovascular and pulmonary diseases remains unknown. We hypothesize that rats with hereditary systemic hypertension are more susceptible than healthy normotensive rats to acute ETS exposure. Ten spontaneously hypertensive (SH) and ten normotensive Wistar-Kyoto (WKY) rats (12 weeks old) were exposed to ETS or filtered air (FA). ETS exposure was at a total particulate concentration of 70-80 mg/m^3, 6 hours/day for 2 days. There were more neutrophils in bronchoalveolar lavage (BAL) from ETS exposed SH and WKY rats compared to FA controls ($p<0.0005$). TUNEL assay was used for *in situ* detection of characteristic fragmented DNA in apoptotic cells in BAL and lung tissue sections. TUNEL analysis revealed that 1.8 and 4.5% of the total BAL cells were apoptotic in SH rats exposed to FA and ETS, respectively ($p=0.037$). The number of apoptotic BAL cells from FA exposed WKY rats was not significantly different from ETS exposed WKY rats (3.6 and 5.8% of total BAL cells, respectively, $p=0.077$). Further, apoptotic cells in lung tissue sections were primarily neutrophils, located near airways, with potentially more cells in ETS exposed SH and WKY rats compared to FA controls. Acute ETS exposure increased, though not significantly, the level of oxidized glutathione in BAL fluid from SH and WKY rats, suggesting that an oxidative stress may have played a role in the inflammatory and apoptotic responses. We conclude that (1) acute ETS exposure increases pulmonary inflammation in both SH and WKY rats and (2) the greater apoptotic response to acute ETS exposure in the lung of SH rats compared to WKY rats suggests that hypertensive rats are more susceptible than normotensive rats.



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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, roundtable, and poster sessions of the 40th Annual Meeting of the Society of Toxicology, held at the Moscone Convention Center, San Francisco, California, March 25–29, 2001.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 451.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 479.

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