in 28% of the cells at 48h post-exposure; under these conditions, control cells exposed to vehicle only did not show any features of apoptosis. The induction of apoptosis by DEB correlated to elevated p53 levels in the same cells in a concentration and time dependent manner. Although DEB-induced apoptosis was observed in the p53 proficient TK6 lymphoblasts, it failed to occur in the p53 deficient NH32 lymphoblasts under the same experimental conditions. Our results demonstrate the occurrence of DEB-induced apoptosis in human lymphoblasts for the first time. In addition, our results reveal that DEB-induced apoptosis is mediated by the tumor suppressor p53 protein. Collectively, this DEB-induced p53 mediated apoptosis may explain butadiene induced bone marrow depletion, as well as spleen and thymus atrophy in exposed animals(supported by grant# ES10018, funded by NIEHS).

1737 THE ROLE OF TNF-α RECEPTOR 2 IN BLEOMYCIN-INDUCED APOPTOSIS IN ALVEOLAR MACROPHAGES.

H. W. Zhao[1], S. Y. Hu[2], M. W. Barger[1], J. K. Ma[2], V. Castranova[1] and J. Y. Ma[1]. *[1]HELD, NIOSH, Morgantown, WV and [2]School of Pharmacy, WVU, Morgantown, WV.*

Activation of alveolar macrophages (AM) in the production of tumor necrosis factor (TNF)-αby bleomycin (BLM) is linked to AM apoptosis and the development of pulmonary fibrosis. AM may contribute to their own death through expression of TNF receptor (TNFR) 1 and 2. The present study was carried out to characterize the role of the TNFR in the mechanism(s) of BLM-induced apoptosis in AM. Sprague-Dawley rats were instilled intratracheally with saline or BLM at 1 mg/kg body weight. At 1, 3, or 7 days post-exposure, AM were isolated by bronchoalveolar lavage, and apoptosis was determined by ELISA. The activation of caspases 3, 8, and 9, the release of cytochrome c from mitochrondria, the cleavage of nuclear poly(ADP-ribose) polymerase (PARP), and the amount of TNFR1 and TNFR2 in AM were monitored by immunoblotting. The results showed that BLM treatment significantly induced AM apoptosis at all exposure time points, with peak apoptosis occurring at 1 day post BLM exposure. BLM treatment, at 1, 3 or 7 days post exposure, significantly increased active caspase 3 level with enhanced caspase 3 activity and increased PARP cleavage in AM in comparison to the control. The maximum activation of caspase 3 and PARP fragmentation occurred in AM from rats exposed to BLM for 3 days. The amount of cytochrome c released into cytosol was gradually increased with time after BLM treatment and peaked on day 7. This coincides with expression of TNFR2, which was significantly induced in BLM-exposed AM with a peak level at 7-day post exposure. In contrast, BLM treatment did not affect AM expression of TNFR1. BLM exposure also significantly activated caspase 9 but not caspase 8 in AM. These results show that BLM-induced apoptosis is TNFR1/caspase-8 independent, but involves over-expression of TNFR2, the release of cytochrome c from mitochondria, activation of caspases 9 and 3, and the cleavage of the DNA repair protein PARP.

1738 APOPTOTIC ALVEOLAR MACROPHAGES PLAY A ROLE IN THE DEVELOPMENT OF PULMONARY INFLAMMATORY DISEASE IN RATS.

L. Wang[1], J. Scabilloni[1], J. Antonini[1], Y. Rojanasakul[2], V. Castranova[1], B. Lu[2] and R. R. Mercer[1]. *[1]PPRB, NIOSH, Morgantown, WV and [2]West Virginia University Health Sciences Center, Morgantown, WV.*

Increasing evidence suggests that apoptosis plays an important role in pulmonary diseases; however, the molecular mechanism underlying this relationship is unclear. Previous studies from our laboratory have shown that pulmonary instillation of dimethyl sulfoxide (DMSO) induced apoptotic alveolar macrophage (AM) resulted in pulmonary inflammation and fibrosis. In this study, we further investigated the effect of intratracheal instillation of DMSO or UVB induced apoptotic AM and documented the time course of the resulting inflammatory and fibrotic responses in the Brown Norway rat lung. Morphological and immunohistochemical analyses of lung tissues showed an inflammatory response and increased connect tissue in the treatment groups, but not in control groups which were instilled with normal non-apoptotic AM. Instilled apoptotic cells were cleared at 4 weeks post-treatment, but the number of apoptotic lung cells was still higher than those in the control rats. Caspase 8 was elevated in the treatment group at 4 weeks post-treatment, suggesting a continuous induction of apoptosis *in vivo* via the death receptor-mediated pathway. Consistent with this finding, we observed a concomitant increase in TNF-α expression in treated lung tissues. *In vitro* studies also showed an increase in TNF-α production by naive AM incubated with apoptotic AM, but not when exposed to untreated AM. Together, our results indicate a role for AM apoptosis in the induction of inflammatory and fibrotic lung disorders, which may be mediated though cytokines, such as TNF-α.

1739 C2-CERAMIDE *VS.* TNF-α INDUCED CYTOTOXICITY AND APOPTOSIS IN A RAT HEPATOMA (H4IIE) CELL LINE.

J. F. Pregenzer, P. C. Wilga, D. K. Petrella, R. K. Patel and J. M. McKim, Jr.. *Investigative Toxicology, Pharmacia, Kalamazoo, MI.*

Understanding the various mechanisms of cellular toxicity and cell death can aid in drug design. Previously, we noted that TNF-α and C2-ceramide produced different toxicity and apoptosis profiles in rat hepatoma (H4IIE) cells. Tumor necrosis factor alpha (TNF-α) is a cytokine involved in certain apoptotic pathways and can stimulate the hydrolysis of sphingomyelin to ceramides. Ceramides are putative lipid second messengers implicated in apoptotic responses. In this study, the effects of TNF-α and ceramide on toxicity and apoptosis were investigated in H4IIE cells using multiple biochemical markers. H4IIE cells were exposed to ceramide (0-300 μM) or TNF-α (0-150 ng/mL, specific activity 1000 University/ng) for 24 hours. Changes in general cell health were determined by monitoring mitochondrial function, membrane leakage, and cell proliferation. Oxidative stress was assessed by measuring changes in reduced glutathione (GSH) and membrane lipid peroxidation. Apoptosis was evaluated by caspase (3, 8, and 9) activity, DNA fragmentation, and using a Cellomics™ ArrayScan®: mitochondrial mass potential, f-actin levels, and nuclear condensation/fragmentation. Cellular calcium changes were measured for up to 2 hours after treatment using Fluo3-AM dye and fluorescence microscopy or a Molecular Devices FLIPR system. As expected, TNF-α caused a marked increase in caspase 3, 8, and 9 activities as well as DNA fragmentation and f-actin content. TNF-α was cytotoxic above 5ng/mL, however, no significant change was observed in GSH or in membrane lipid peroxidation prior to toxicity. In contrast, ceramide caused a significant increase in membrane lipid peroxidation and DNA fragmentation but had no effect on caspase 3, 8, and 9 activities. Ceramide increased intracellular calcium levels while TNF-α did not produce significant calcium influx. These data suggest that apoptosis induced by TNF-α occurs through caspase dependent pathways whereas exogenous C2-ceramide induced cell death involves calcium influx, oxidative stress, or other caspase independent pathways.

1740 MECHANISMS OF OCHRATOXIN A-INDUCED INHIBITION OF HUMAN LYMPHOCYTE PROLIFERATION.

H. Assaf[1,2], H. Azouri[2] and M. Pallardy[1]. *[1]Faculty of Pharmacy, INSERM U461, Châtenay-Malabry, France and [2]Faculty of Pharmacy, Laboratory of Toxicology, Beyrouth, Lebanon.*

Ochratoxin A (OTA) is a fungal secondary metabolite produced in agricultural commodities by Aspergillus and Penicillium fungi species. Human exposure to OTA is both due to consumption of directly contaminated food or of food products derived from animals consuming contaminated feedstuffs. OTA was implicated in the pathogenesis of Balkan endemic nephropathy due to its nephrotoxicity ; in addition, it is hepatotoxic, genotoxic, carcinogenic and immunosuppressive. This latter toxicity has been widely studied in experimental animals such as mice and chickens. In this study, we investigated the effects of OTA on human peripheral blood mononuclear cells (PBMC). PBMC stimulated with phytohaemagglutinin (PHA) and incubated with OTA at different concentrations for 48 h showed an inhibition of proliferation at $5x10^{-6}$ M and 10^{-5} M of OTA as measured by ^{3}H-thymidine uptake. However, IL-2 secretion was not affected for all concentrations tested as determined by ELISA. In addition, exogenous IL-2 added to cell culture at 1 and 10 ng/ml did not overcome the inhibition of lymphocyte proliferation. A slight decrease in CD25 (IL-2R alpha) surface expression at $5x10^{-6}$ M of OTA was observed compared to control cells (60 vs 73 % and 47 vs 72 % at 24 h and 48 h respectively). Moreover, stimulated and unstimulated PBMC incubated with OTA entered apoptosis in a time and concentration-dependent manner. The mechanism is likely to be mediated by caspases since the pan-caspase inhibitor VAD reduced apoptosis induced by OTA. Our results suggest that downregulation of CD25 and induction of apoptosis are involved in OTA inhibition of lymphocyte proliferation. The role of cell cycle proteins such as cdk2 and cyclin E, as well as Bcl-2 family proteins, are currently under investigation.

1741 CHANGES IN LYMPHOCYTE SUBSETS AND APOPTOSIS IN LYMPHOID TISSUES OF NIVALENOL-TREATED MICE.

A. Poapolathep[1], T. Nagata[1], Y. Sugita-Konishi[3], S. Kumagai[2] and K. Doi[1]. *[1]Department of Vet. Pathol., The University of Tokyo, Tokyo, Japan, [2]Division of Microbiol., National Inst. of Health Sciences., Tokyo, Japan and [3]Department of Vet. Public Health, The University of Tokyo, Tokyo, Japan.* Sponsor: K. Ebino.

Nivalenol (NIV) is the major group B trichothecene mycotoxin and NIV widely contaminates agricultural commodities. In this study, ICR mice were orally administered with 15 mg/kg of NIV to elucidate the development of apoptosis and