products. The present study investigated the mechanism of this inflammatory response. Cultures of human nasal epithelial (HNE) cells were initiated from excised nasal turbinates and exposed to 120, 240, or 500 ppb ozone for 3 hours. Basal media was then collected for tumor necrosis factor alpha (TNF-α) quantification by enzyme-linked immunosorbent assay. Mean TNF-α concentration in cultures exposed to 120 ppb ozone (10.34 ± 1.32 pg/ml) was not significantly different than in those exposed to air (11.01 ± 1.10 pg/ml). Exposure to 240 and 500 ppb ozone significantly increased mean TNF-α expression relative to controls by 75% (from 34.25 ± 4.39 pg/ml to 59.77 ± 8.72 pg/ml) and 28% (from 30.54 ± 4.61 pg/ml to 39.25 ± 7.42 pg/ml), respectively. These findings support the active role of HNE in ozone-induced airway inflammation and warrant further studies that delineate the interaction between ozone and inflammatory signal transduction molecules. Examples include possible ozone-induced activation of NF-κB, a transcription factor known to upregulate TNF-α expression. (Supported by NIH grant ES07033.)

244 AP-1 BINDING ACTIVITY IS ALTERED IN LPS-TREATED TNF ALPHA (p55/p75) DOUBLE RECEPTOR KNOCK-OUT MICE.

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It is well established that pro-inflammatory mediators of LPS induction of the acute phase response (APR) include interleukins (IL), interferons, and tumor necrosis factor alpha (TNF). Using TNF p55/p75 double receptor knockout mice (DKO), the role of TNF on transcription factor activation during the APR was examined. DKO and C57BL6 mice were administered LPS (2 mg/kg, i.p.) or saline and euthanatized 2 or 24 hours following LPS administration. Sera and liver tissue were then collected and analyzed for cytokine levels (sera) and transcription factor activation (liver). Serum cytokine levels were determined using direct ELISA. Serum levels of IL-1, IL-6, and TNF supported induction of the APR. Hepatic nuclear extracts were isolated and DNA binding to STAT3, $NF_κB$, and AP-1 elements was analyzed using EMSA. The binding activity for STAT3 and $NF_κB$, was increased with LPS treatment, but did not differ between DKO and C57BL6 mice at either time point. However, AP-1 binding activity was decreased in the DKO mice at 2 and 24 hours. The dampened AP-1 binding activity in the DKO mice may be explained by disruption of the TNF/SAPK/MAPK signalling pathway. The ultimate step in this pathway is the phosporylation of c-Jun; part of the dimeric complex of AP-1. These results support the role of TNF in the signalling pathways necessary for AP-1 activation following LPS administration.

245 JP-8 JET FUEL INDUCED RELEASE OF PRO-INFLAMMATORY CYTOKINES IL-1β AND IL-6 FROM RAT ALVEOLAR TYPE II CELLS IN CO-CULUTRE WITH RAT ALVEOLAR MACROPHAGES.

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Jet propulsion-8 (JP-8) jet fuel has been shown to induce lung injury after inhalation, however the mechanism of injury still remains unknown. Previous work conducted in our laboratory shows that aerosolized JP-8 jet fuel causes increased lung permeability and changes in lung compliance and resistance in an animal model. The purpose of this study focused on the interaction of the alveolar type II cell and the resident immune cell in the lung, the pulmonary alveolar macrophage (PAM). RLE-6TN cells (RLE cells), a rat alveolar type II epithelial cell line, were grown to confluence, dosed with JP-8 jet fuel at concentrations of 0.25, 0.3, 0.4, 0.5, 0.6, and 0.8 ug/ml, alone or in co-cultures with PAM isolated from F344 rats. At time points of 1, 2, 6, 12, and 24 hours cell culture supernatant was removed and assayed, by ELISA, for the presence of IL-β and IL-6. Results from these experiments show that JP-8 induces the release of IL-β and IL-6 in a time dependent manner, however the levels of cytokines released were not dose dependent in the cultures of RLE-6TN cells alone. In co-cultures of RLE cells and PAM the levels of cytokines released were increased significantly ($p<0.05$) versus the RLE cultures alone. The pattern of release of cytokines was similar to that seen in the cultured RLE cells. From this study it appears that injury to the alveolar epithelium may be caused by immune activation of the alveolar type II cells and this injury may be exacerbated by the presence and activation of PAM in the alveolar space. The release of these pro-inflammatory and chemotactic cytokines may lead to increased airway inflammation and recruitment of various immune cells to the lung which could exacerbate injury. (This work supported by AFOSR 49260-97-0217 and DoD AASERT program.)

246 INSTILLED SOLUBLE VANADIUM EVOKES PULMONARY INFLAMMATION BUT DOES NOT ELICIT PRO-INFLAMMATORY CHEMOKINE mRNA IN THE HEART.

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Inhalation of vanadium-rich fly-ash (~ 500 μ g/m³) results in increases in pulmonary inflammation and increased expression of pro-inflammatory chemokine levels in cardiac macrophages (Killingsworth et al, *Inhal Tox* 9:541). Intratracheal instillation of vanadium compounds causes significant pulmonary inflammation and enhanced pulmonary chemokine mRNA levels (Pierce et al, *Tox Appl Pharm* **138**:1). HYPOTHESIS: Cardiac chemokine induction is due to soluble metal ions transported from the pulmonary region. METHODS: Rats were lightly anesthetized and intratracheally instilled with 210 μ g/kg sodium metavanadate or PBS. Following sacrifice at 24, 48, and 168 hr post-instillation (PI), bronchoalveolar lavage (BAL) was performed and cardiac tissue prepared for RNA extraction and Northern analysis. RESULTS: As previously observed, significant metavanadate-induced pulmonary inflammation was observed in BAL samples at 24 and 48 hr PI marked by significantly increased neutrophil and eosinophil levels ($p < 0.05$) and significant changes in inflammatory biochemical parameters in acellular BAL fluid; inflammation was resolved by 168 hr. Northern analysis for the pro-inflammatory chemokines macrophage inflammatory protein (MIP)-1α and MIP-2 showed only trace amounts of mRNA in heart tissue from vanadium-exposed animals at 24 and 48 hr PI. CONCLUSION: These results indicate that soluble vanadium induces pulmonary, but not cardiac, inflammation following instillation. This suggests that inflammation-related cardiac dysfunction is not caused by soluble vanadium transported following pulmonary exposure. (Supported by ES00002, ES08129, HL05947, and HL54958.)

247 DEVELOPMENT OF A TAQMAN® BASED QUANTITATIVE RT-PCR ASSAY FOR CANINE CYTOKINE INDUCTION.

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The inflammatory response consists of multiple components, including induction of several cytokine mRNA's. Accurate quantitation of cytokine levels in specific tissues can aid in understanding the extent and source of inflammatory mediators. Using a fluorescence-based ABI Prism® 7700 Sequence Detection System (TaqMan®), a quantitative RT-PCR assay has been developed to measure specific cytokine gene induction in canine tissues and cultured cells. Using the Primer Express software developed by Perkin-Elmer, primer/probe sets were designed for canine IL6, IL8, IL10, TNFα, and GMCSF. Each primer/probe set was specifically designed to span intron-exon junctions to eliminate detection of genomic DNA. GAPDH and 18s primer/probes were used as internal standards in co-amplification reactions. RNA expression vectors containing cloned RT-PCR product from canine RNA were used to generate RNA and DNA standards for absolute quantitation. LPS treatment was used to validate the assays. In canine liver, all the cytokines tested showed a dramatic induction relative to control 1 hour after oral gavage with LPS with the exception of GMCSF and IL 10. LPS also induced cytokines in canine large vessel endothelial cells. The TaqMan RT-PCR method of detecting and quantifying RNA has multiple advantages over endpoint RT-PCR, Northern blot and Rnase protection analysis. The use of specific primers and fluorescent probe results in an assay that is extremely sensitive and specific, does not require gel-based analysis, is very reproducible, and gives a linear response over 6 orders of magnitude without the need for dilutions.

248 A MODEL OF SUBCUTANEOUS ABSCESS FORMATION IN NON-HUMAN PRIMATES.

M A Gallo and C J Horvath. *Primedica Corporation, Dept. Experimental Medicine, Worcester, MA, USA.* Sponsor: F J Mecler.

Many anti-inflammatory agents affect the trafficking, adhesion and activation of cells of the immune system. Treatment with anti-inflammatory drugs

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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 38th Annual Meeting of the Society of Toxicology, held at the Ernest N. Morial Convention Center, New Orleans, Louisiana, March 14-18, 1999.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 419.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 444.

The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology and appear in numerical sequence.

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