in vitro exposure to asphalt fume condensate (AFC) on AM functions, such as cell viability, resting release of cytokines and oxidants, and the ability of microbial products to stimulate cytokine or oxidant production. Rat AM cultures were incubated with various condensations of AFC for 24 hours at 37°C. AM-conditioned media were collected and assayed for lactate dehydrogenase (LDH), interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α). AFC (≤ 200 μg/ml for 24 hours) did not affect cell viability (measured as LDH release). Such AFC exposure failed to induce IL-1 release while causing a relatively small but significant increase in TNF-α production. In addition, AFC exposure (≤ 200 μg/ml for 24 hours) did not alter TNF-α release from AM exposed to LPS (a bacterial product). A 15-minute exposure to AFC (≤ 200 μg/ml) did not increase the production of oxidants by AM (measured as resting chemiluminescence). However, AFC resulted in a small but significant decline in chemiluminescence generated in response to zymosan (a fungal product). These data suggest that exposure of AM in vitro to reasonably high levels of AFC exposure did not adversely affect cell viability, failed to induce the release of high levels of inflammatory cytokines or oxidants, and did not have major effects on the ability of AM to produce cytokines or antimicrobial oxidants in response to bacterial LPS or zymosan.

1701 OXIDATIVE STRESS INDUCED BY MARIJUANA SMOKE.

T A Sarafian, J Marques, H Shau, M D Roth and D P Tashkin. *Depts. of Pathology, Medicine and Surgery, UCLA, Los Angeles, CA.*

While cigarette smoke has been the subject of extensive study with respect to toxicity associated with various constituents, far less research has been conducted on marijuana (MJ) smoke. We have examined oxidative toxicity of marijuana smoke in the cultured human endothelial cell line ECV304 using fluorescent probes dichlorofluorescein (DCF) and Monochlorobimane. Marijuana smoke produced a 1.8-fold increase in accumulation of reactive oxygen species (ROS) over a 30 min period, accompanied by a 85 % decrease in cellular glutathione (GSH) levels. Smoke from marijuana cigarettes lacking Δ^9-tetrahydrocannabinol (-THC) produced little or no ROS above air-exposed control cells, despite 78% depletion of GSH, suggesting that THC combustion in marijauna cigarettes plays a major role in the generation of ROS. Ascorbate-inhibitable ROS production was 4.6-fold greater in MJ smoke-exposed cells and 1.7-fold greater in -THC smoke-exposed cells compared with control cells. Cytotoxicity studies revealed similar rates of necrotic cell death 24 hr after exposure with 49 % cell death from MJ smoke and 41 % cell death from -THC smoke. The gaseous component of MJ smoke passed through a Cambridge filter produced 4-fold higher levels of ROS compared with unfiltered MJ smoke, suggesting that the particulate phase of MJ smoke suppresses DCF oxidation. These results indicate that marijauna smoke is very potent in the production of oxidative stress and that THC significantly contributes to the production of ROS.

1702 GLYCINE BLUNTS ALVEOLAR MACROPHAGE ACTIVATION BY A MECHANISM INVOLVING A GLYCINE-GATED CHLORIDE CHANNEL.

M D Wheeler[1], R F Stachlewitz[1], and R G Thurman[1]. [1]*Department of Pharmacology, University of North Carolina, Chapel Hill, NC.*

It has been shown that glycine blunts lipopolysaccharide (LPS)-induced increases in intracellular calcium ($[Ca^{2+}]_i$) as well as TNFα production in Kupffer cells by activating glycine-gated chloride channels. Because Kupffer cells and alveolar macrophages are derived from the same monocyte lineage, studies were designed to test the hypothesis that alveolar macrophages also contain glycine-gated chloride channels. The ability of glycine to prevent LPS-induced increases in intracellular calcium and subsequent production of superoxide anion as well as the ability of glycine to promote the influx of radiolabelled extracellular chloride in alveolar macrophages was investigated. LPS (1μg/mL + 5% rat serum) caused transient increases in intracellular calcium with maximal values reaching nearly 160 nM. Glycine blunted the increase in $[Ca^{2+}]_i$ in a dose-dependent manner with an IC_{50} value of <10 μM. The effects of glycine on increases in $[Ca^{2+}]_i$ were both strychnine sensitive and chloride dependent. Also glycine stimulated the influx of radiolabelled extracellular chloride into primary isolated alveolar macrophages in a dose-dependent manner. LPS-stimulated superoxide production was also blunted in a dose-dependent manner by glycine with an IC_{50} value of 10 μM. Taken together, these data support the hypothesis that alveolar macrophages contain glycine-gated chloride channels which are protective against the increase in $[Ca^{2+}]_i$ and subsequent production of toxic oxygen radicals. Since Adult Respiratory Distress Syndrome and asthma are mediated by alveolar macrophage activation, glycine may be therapeutically useful in these diseases (AA-03624).

1703 ALPHA PARTICLES LIKE THOSE EMITTED BY RADON INITIATE BIOLOGICAL PRODUCTION OF IL-8 IN HUMAN CELLS.

P K Narayanan, K E LaRue, Y E Valdez, E H Goodwin, and B E Lehnert. *Los Alamos National Laboratory, Los Alamos, NM.*

The pulmonary microenvironment is a primary target that alpha (α) particles like those emitted by inhaled radon and its progeny will encounter, but their role in generating oxidative stress-related mediators in the airways is not well understood. Interleukin-8 (IL-8) is a cytokine recognized for its potent role as a chemoattractant and activator of neutrophils. Oxidative stress is known to regulate IL-8 gene expression in a variety of cell types. We have recently found that oxidative stress is among the many mechanisms that low doses of α particles can initiate in fibroblasts (*Cancer Res.* 57:3963-3971, 1997). We therefore sought to examine potential linkage between the generation of reactive oxygen species (ROS) and IL-8 in α-irradiated normal human lung fibroblasts. ELISA revealed that exposure of the fibroblasts to α particles (3.6 – 19 cGy) caused significant increases in IL-8 protein generation as early as 30 min post-irradiation. Cells exposed to α particles in the presence of antioxidants and dexamethasone resulted in a significant down-regulation of IL-8 protein. Cellular IL-8 measured by flow cytometry also showed similar increases after exposure to α particles, as was IL-8 mRNA. In addition, immunofluorescence assays revealed that the transcription factor nuclear factor kappa-B (NF-κB), which regulates IL-8 gene expression, showed increased nuclear translocation after α particle exposure. Our results indicate that α particle-induced increases in IL-8 production temporally in parallel with elevated ROS production. [Funded by the U.S. DOE].

1704 MODULATION OF NF-KappaB AND CYTOKINE RESPONSES IN LUNG CELLS BY PRO-OXIDANT AND ANTIOXIDANT AGENTS.

D Ufferfilge[1], G Cosma[1], A Martinez[1], H Gardner[2], and V Vallyathan[3]. [1]*Dept. Environ Health, Colorado State Univ, Ft. Collins, CO;* [2] *US Army Ctr Environ Health Research;* [3] *NIOSH/HELD, Morgantown, WV.*

Previous studies from our laboratory have indicated a central role for oxygen free radicals in lung pathogenesis following exposures to particulates. Current studies are investigating the modulation of DNA binding of transcription factor NF-kappaB by pro-oxidant and antioxidant agents, and the activities of proinflammatory cytokines interleukin-1/6, and TNF-alpha. Stimulation of rat alveolar macrophage NR8383 and human monocytic THP-1 cell lines with organic particulates resulted in a ten-fold increase in NF-kappaB activity, as determined by electrophoretic mobility shift assay, by 45 min post-treatment, which coincided with the production of hydrogen peroxide. IL-1 and TNF-alpha levels, as detected by ELISA, increased by 6 hr, and IL-6 levels rose by 24 hr. Pre-treatments of cells with 20 mM n-acetylcysteine, previously shown to significantly inhibit hydrogen peroxide formation and cytokine production in macrophages, did not result in inhibition of NF-kappaB activity in stimulated THP-1 cells. The pineal hormone melatonin also did not appear to modulate DNA binding of NF-kappaB protein, despite a modest inhibition of hydrogen peroxide formation in cells. Interestingly, melatonin treated cells displayed a biphasic dose-response of synthesis of pro-inflammatory cytokines. Ongoing studies are further investigating the effects of melatonin on transcription factor activities and stress protein responses in lung cells, and its relationship to quenching of reactive oxygen species as detected by electron spin resonance. [Support by NIOSH and USACEHR]

1705 DIFFERENTIAL REGULATION OF MACROPHAGE INFLAMMATORY PROTEIN-2 GENE EXPRESSION BY OXIDATIVE STRESS IN RAT ALVEOLAR MACROPHAGES.

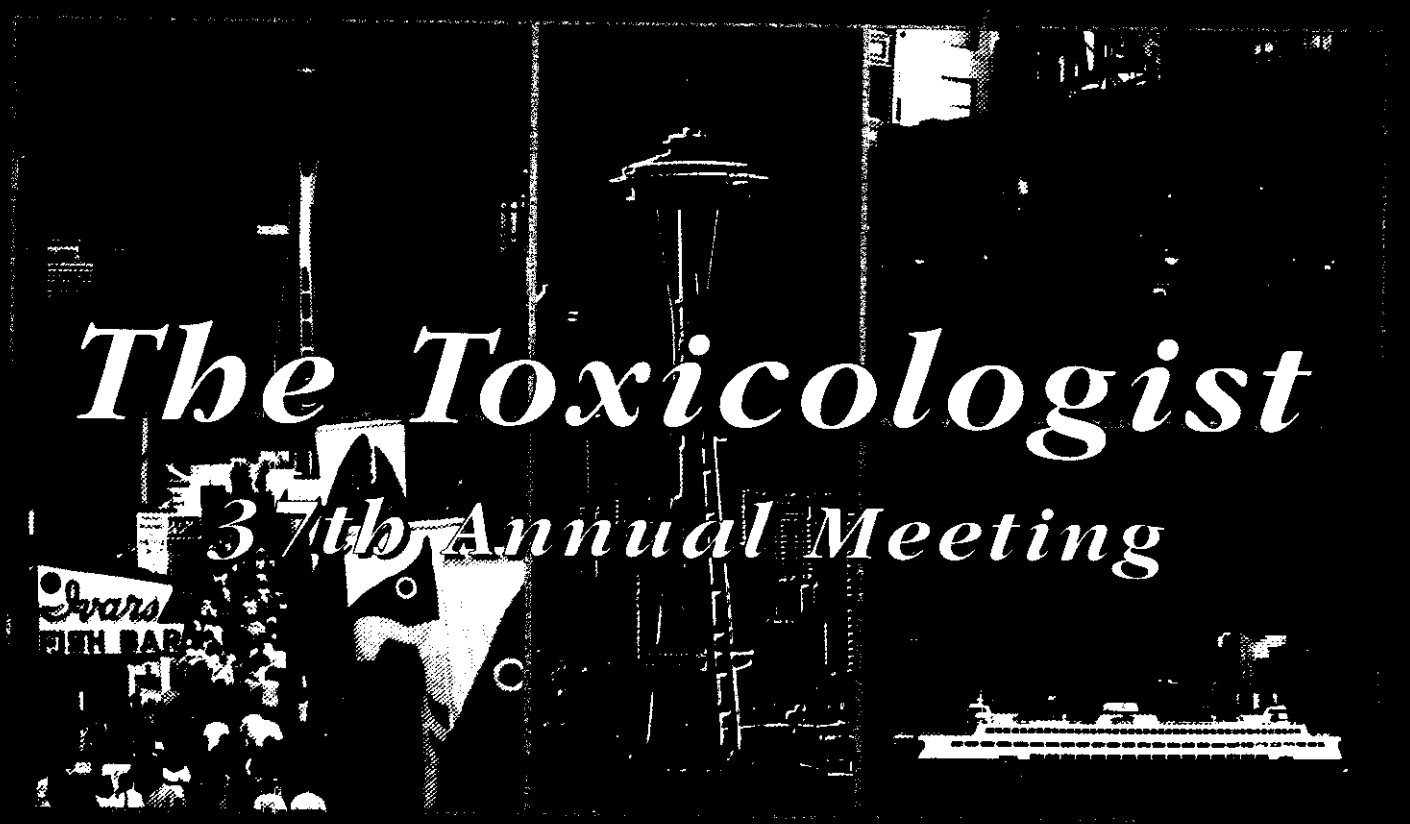
M M Shi[1], I -W Chong[2], J J Godleski[2] and J D Paulauskis[2]. [1]*Pathology and Experimental Toxicology, Parke-Davis Pharmaceutical Research, Ann Arbor, MI;* [2]*Department of Environmental Health, Harvard School of Public Health, Boston, MA.*

The chemokine macrophage inflammatory protein-2 (MIP-2) is one of the primary inflammatory mediators inducing neutrophil chemotaxis and activation. Our previous work demonstrated that oxidative stress can up-regulate mRNA expression of this chemokine. In the present study, we further investigate the molecular mechanisms controlling this chemokine's gene expression by oxidative stress. A rat alveolar macrophage cell line (NR8383) was exposed

An Official Journal of the
Society of Toxicology
Supplement

TOXICOLOGICAL SCIENCES

Formerly Fundamental and Applied Toxicology



The Toxicologist

37th Annual Meeting

AP

Academic Press

Volume 42, Number 1-s, March 1998

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 37th Annual Meeting of the Society of Toxicology, held at the Washington State Convention Center, Seattle, Washington, March 1-5, 1998.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 407.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 433.

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

Copies of *The Toxicologist* are available at $45 each plus $5 postage and handling (U.S. funds) from:

Society of Toxicology
1767 Business Center Drive, Suite 302
Reston, VA 20190-5332