1368 EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD), 12-0-TETRADECANOYLPHORBOL-13-ACETATE (TPA), AND 17β-ESTRADIOL (E_2) ON ESTROGEN RECEPTOR REGULATION IN MCF-7 HUMAN BREAST CANCER CELLS

J F Gierthy, B C Spink, H F Figge [1], B T Pentecost, D C Spink. *Wadsworth Center, New York State Dept. of Health, Albany, NY; [1] Dept. of Pathology, Albany Medical Center, Albany, NY.* Sponsor: L Kaminsky

TCDD exhibits remarkably potent antiestrogenic activity. To further elucidate the role of estrogen receptor (ER) regulation in this response, we examined the effects in human MCF-7 breast cancer cells of TCDD treatment on ER mRNA levels using the RNase protection assay, ER accumulation by using an estrogen receptor immunocytological assay (ER-ICA), and function by competitive binding assays under conditions of saturating E_2. Comparative studies were conducted with E_2 or TPA treatment, both of which are known to suppress ER expression. The results indicate that 10^{-9} M E_2 and 10^{-7} M TPA both suppress ER mRNA levels as early as 4 hr after exposure and to 33.6% and 16.5% of control levels respectively after 72 hr. In contrast, no significant effect on ER mRNA levels was attributed to 10^{-8} M TCDD exposure. A greater than 50% reduction in percent positive staining was observed by ER-ICA assay after 72 hr exposure to 10^{-9} M E_2 and 10^{-7} M TPA, while no effect was observed with 10^{-8} M TCDD. Specific binding of [^{3}H]E_2 at saturating conditions (10^{-8} M) in whole cells was reduced by 50% in cultures exposed to 10^{-7} M TPA, with no effect using 10^{-8} M TCDD. In conclusion, while TPA and E_2 effectively down-regulate the ER, TCDD has little if any effect on total ER levels in MCF-7 cells, and thus ER modulation is probably not necessary for suppression of estrogenic activity by TCDD (Supported by NIEHS ES-03561).

1369 MOLECULAR MECHANISMS IN THE REGULATION OF IMMUNOLOGICAL RESPONSES BY 2,3,7,8-TETRACHLORO- DIBENZO-p-DIOXIN (TCDD)

D E Heck. *Dept. of Environmental and Community Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.* Sponsor: M A Gallo

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), a ubiquitous environmental pollutant, is known to act as a potent murine immunotoxin, inducing thymic atrophy, suppressing antibody responses, as well as profoundly inhibiting resistance to viral and parasitic infection. However, mechanisms by which TCDD induced these responses are not fully understood. Nitric oxide, a newly recognized reactive mediator, is produced by macrophages as well as epithelial cells in response to the inflammatory cytokine γ-interferon. Recent studies have demonstrated that nitric oxide is critical for cellular resistance to both viral and parasitic infection. We have found that the TCDD sensitive cell line Hepa 1c1c7 responds to γ-interferon by producing nitric oxide. Treatment with TCDD in the nanomolar concentration inhibited this response. Investigations into the mechanisms of the cellular alterations induced by this cytokine, have revealed that tyrosine kinases, activated by the interaction of γ-interferon with its receptor, mediate the induction of gene transcription. Tyrosine phosphorylation of unique proteins initiated by activation of the interferon receptor, results in formation of nuclear multimeric transcription complexes which bind cis-acting enhancer elements in the regulatory regions of interferon regulated genes, including nitric oxide synthase. We discovered that treatment of Hepa 1c1c7 cells with TCDD induces expression of a 48 kd protein antigenically related to this family of transcription factors. Although the cellular effects of alterations in the activity of these transcription factors are as yet undefined, TCDD-induced alterations in this pathway may disrupt cellular signaling initiated by γ-interferon leading to suppression of nitric oxide formation by Hepa 1c1c7 cells. These findings represent a potential mechanism in the immunosuppressive actions of TCDD. (Supported by ES 05022.)

1370 PULMONARY HOST DEFENSE ACTIVITIES IN RATS WITH AMIODARONE-INDUCED PULMONARY PHOSPHOLIPIDOSIS

M J Reasor, C M McCloud, M DiMatteo, R Shafer. *Robert C. Byrd Health Sciences Center, West Virginia University, Morgantown, WV*

Amiodarone (AD) is a cationic, amphiphilic drug with class III antiarrhythmic activity. In humans and animals, AD causes pulmonary phospholipidosis. The functional consequences of this disorder are unknown. The objective of this study was to compare pulmonary host defense activities of rats treated with AD (100 mg/kg/day for 7d) with those of control rats which received vehicle. AD treatment resulted in a 5-fold increase in total phospholipid in alveolar macrophages (AMs). Pulmonary antibacterial activity against intratracheally instilled *Listeria monocytogenes* was not significantly different between AD-treated and control rats at 3 and 7 days post instillation. The generation of zymosan-stimulated or PMA-stimulated chemiluminescence (a measure of oxidant production) by isolated AMs from AD-treated rats was not significantly different from control AMs, nor was phagocytosis of yeast particles. The results indicate that the presence of phospholipidosis in the lungs of rats does not have an adverse effect on *in vivo* or *in vitro* aspects of host defense in the context of the activities studied. (Supported by a grant from Procter & Gamble Pharmaceuticals).

1371 EFFECTS OF SINGLE AND REPEATED EXPOSURE TO PHOSGENE ON PULMONARY BACTERIAL INFECTION IN RATS

M J Selgrade [1], M I Gilmour [2]. [1] *U.S. EPA, HERL, Research Triangle Park, NC; [2] Center for Environmental Medicine and Lung Biology, UNC, Chapel Hill, NC*

Acute phosgene exposure impairs resistance to streptococcal infection in mice. This study assessed effects of acute (0.1 or 0.2 ppm, 6 hr) and subchronic (4 or 12 wk, 0.1 or 0.2 ppm, 6 hr/day, 5 day/wk or 0.5 ppm, 2 day/wk) phosgene exposure on resistance to streptococcal infection in rats. Bronchoalveolar lavage (BAL) was assessed at intervals postinfection for bacteria and inflammatory cells. All exposures impaired bacterial clearance from the lungs and caused an increase in neutrophils in BAL. Effects of repeated exposure were similar to those observed after a single exposure. Effects in the 0.5 ppm repeated exposure group were greatest, and significantly different from those in the 0.2 ppm group although the concentration X time was equal. All parameters were normal in rats assessed 4 wks after the 12 wk exposure. The data indicate that streptococcal infection is the most sensitive endpoint for phosgene toxicity in rats. Effects of repetitive exposure were not additive nor was there attenuation of the response; however, recovery after exposure was complete. (This abstract does not reflect EPA policy.)

1372 DIFFERENTIAL MODULATION OF IMMUNITY TO HOUSE DUST MITE ANTIGEN BY EXPOSING RATS TO O_3 BEFORE OR AFTER IMMUNIZATION

M I Gilmour [1], P A Park, M J Selgrade. [1] *UNC Chapel Hill, RTP, NC; U.S. EPA, HERL, RTP, NC*

We have previously demonstrated that exposure to NO_2 after immunization and challenge with house dust mite antigen causes upregulation of immune responses and increased allergen-mediated lung disease in rats. This present study was designed to establish whether similar effects would occur with O_3 exposure, and to compare the effects of O_3 before or after administration of antigen. Exposure to 0.8 ppm O_3 for 3 hrs immediately after immunization and intratracheal challenge with antigen caused an increase in antigen specific IgG, IgA and IgE antibody, lymphocyte proliferation, and inflammatory cells in the lung 7 days post challenge compared to air-exposed controls. When animals were exposed to O_3 just prior to immunization, immune function and lung inflammation was significantly less than air controls. The data suggest that the timing of pollutant exposure in relation to immunization plays a critical role in determining whether immune function and subsequent lung disease is increased or decreased. (This abstract does not reflect EPA policy.)

1373 WOODSMOKE EMISSIONS: EFFECTS ON PULMONARY IMMUNE DEFENSE

J T Zelikoff, K Baker, M D Cohen, L C Chen. *New York University Medical Center, Inst. Environ. Med., 550 First Ave., NY, NY*

Epidemiological evidence suggests that inhalation of woodsmoke enhances the incidence, duration and, possibly, severity of respiratory illness in children. The hypothesis of this toxicological study is that woodsmoke exposure compromises pulmonary host resistance to infectious bacteria by altering immune defense mechanisms, particularly at the level of the macrophage. Rats were exposed nose-only to woodsmoke emissions generated from the burning of Red Oak (RO) and Douglas Fir (DF) in a furnace developed in this laboratory. Generated smoke was characterized in terms of respirable suspended particulate (RSP) matter, carbon monoxide (CO) levels, NO_x (NO and NO_2, respectively) concentration, and total PAH [measured as benzo(a)pyrene]. Under the present burning conditions, RSP concentration was maintained at a level of 750 $\mu g/m_3$ for 1 hr for RO; mass median diameter of the emitted particles was 0.15 μm ($\sigma g = 1.8$). CO levels emitted during combustion of DF and RO were 1 ppm, and all (100%) of the gaseous material produced was volatile. Levels of NO_x were 0.062 ppm for RO and 0.020 for DF. Following a single 1 hr exposure, rats were either anesthetized and intratracheally instilled with *Staphylococcus aureus* to assess effects on pulmonary clearance, or sacrificed

and their lungs lavaged to provide pulmonary macrophages. Inhalation of RO emissions reduced bacterial killing *in vivo* and diminished *in vitro* phagocytosis by macrophages recovered 1 and 2 h post-exposure. Results demonstrate that acute inhalation of woodsmoke generated from a model system that produces emissions comparable to those produced in homes using woodburning devices, compromises important immune defense mechanisms of the lung. Supported by Center Indoor Air Research. Contract No. CIAR 94-03.

1374 IMMUNOTOXIC EFFECTS IN THE RAT LUNG FROM INHALATION OF VANADIUM

T P McManus, J T Zelikoff, R B Schlesinger, M D Cohen. *New York University Department of Environmental Medicine, Tuxedo, NY*

Male Fisher 344 rats (10 wk old, 200–250 g) were exposed to atmospheres containing 2 mg vanadium (V)/m^3 (as ammonium metavanadate NH_4VO_3, 0.32 μm diameter particles) for 8 hr/d for 4 d in a nose-only exposure system. In exposed rats, lung burdens of V increased in a time-dependent fashion. Nearly all (88%) V-exposed rats displayed markedly increased levels of bronchus-associated lymphoid tissue (BALT) after each exposure, though the effect was only transitory. Analysis of lung cells and lavage fluid 24 hr after the final exposure suggested that a strong inflammatory response was elicited; levels of free neutrophils and immature monocytes, as well as of lavage protein and lactate dehydrogenase, were greatly elevated as compared with levels observed in air-exposed controls. Vanadium also affected the capacity of pulmonary macrophages (PAM) to both produce and respond to important immunoregulatory cytokines. PAM production of tumor necrosis factor-α in response to lipopolysaccharide was significantly inhibited, as was their ability to synthesize/express MHC Class II/Ia molecules in response to interferon-γ (IFNγ). The PAM from V-exposed hosts were also inhibited in their ability to be primed by IFNγ to produce superoxide anion and hydrogen peroxide in response to opsonized zymosan stimulation. These studies indicate that subchronic exposure of rats to workplace levels of atmospheric V can cause strong immunomodulatory effects in the lungs, with a major effect occurring at the level of cytokine-related functions. These alterations may be underlaying mechanisms for the well-documented increases in bronchopulmonary infections and cancers in workers chronically exposed to V-containing atmospheres. This study was supported by NIOSH (Grant No. OH03064-01).

1375 VANADIUM ALTERS MACROPHAGE INTERFERON-γ-INTERACTIONS AND -INDUCIBLE RESPONSES

M D Cohen, T P McManus, Z Yang, Q Qu, J T Zelikoff, R B Schlesinger. *New York University Department of Environmental Medicine, Tuxedo, NY*

Vanadium (V) impairs host resistance overall, and the antimicrobial activity and function of several intracellular enzymes of macrophages (Mø) in particular. Mouse WEHI-3 Mø-like cells were exposed overnight to subcytotoxic concentrations of ammonium metavanadate (NH_4VO_3) to determine whether this observed immunomodulation might be due, at least in part, to altered Mø interactions with interferon-γ (IFNγ) or IFNγ-inducible responses, i.e., increased reactive oxygen intermediate (ROI) production, Ca^{2+} ion influx, and surface Class II/Ia antigen expression. Binding studies performed at 22°C indicated that V-treated cells had ≅50% fewer actively-binding receptors, but binding affinities 450-times greater than that of control receptors. At 4°C, V-treated cells had 98% fewer functional receptors, but again higher (145-fold) affinities. IFNγ-receptor complex internalization was unaffected by V, although significantly higher in cells incubated at temperatures which minimize uptake. ROI production in IFNγ-stimulated V-treated (either NH_4VO_3 or V_2O_5 [vanadium pentoxide]) cells was decreased relative to spontaneous production, while control cells showed consistent increases due to IFNγ priming. Vanadium also reduced the Ca^{2+} ion influx rate into stimulated cells without affecting final cell Ca^{2+} burdens. Although V did not affect IFNγ-induced Ia expression, exposures resulted in increased numbers of Ia-bearing cells with lower maximal antigen densities than control cells. The results of this study show that V exposure may alter Mø-mediated functions by modifying cell interactions with IFNγ and subsequent IFNγ-dependent parameters. This study was supported by NIOSH (OH03064-01) and by EPRI (RP2155-1).

1376 EFFECTS OF INHALED NITRIC OXIDE (NO) ON RAT LUNG

K J Pendino, D L Laskin. *Rutgers University, Piscataway, NJ*

Inhaled NO has been shown to be a potent pulmonary vasodilator clinically useful in adult respiratory distress syndrome and persistent pulmonary hypertension of the newborn. However, little is known about the potential toxicity of this agent. In the present studies, we analyzed the effects of inhaled NO on the lungs. Exposure of female SD rats to 80 ppm NO for 3 hr resulted in a small increase in polymorphonuclear leukocytes in lung lavage fluid 24 hr after exposure. Superoxide anion production by lung lavage cells was also elevated, an effect which persisted for 48 hr. In contrast, although exposure of rats to 80 ppm NO for 3 days (6 hr/day) had no major effect on superoxide anion production, cells isolated from these animals produced significantly increased levels of NO *in vitro* in response to lipopolysaccharide. Interestingly, inhalation of NO prior to ozone resulted in partial reversal of ozone-induced increases in lavage protein levels and decreases in superoxide anion release. Taken together, these data suggest that inhalation of NO activates lung lavage cells to release increased quantities of reactive intermediates. Furthermore, NO may modify the response of the lung to pulmonary irritants. (ES04738) and a fellowship from the American Lung Association (ALA).

1377 MODIFICATION OF AIRWAY EPITHELIAL PERMEABILITY AND NITRIC OXIDE RELEASE UPON OZONE EXPOSURE

I Jaspers, L C Chen. *NYU Medical Center, Nelson Institute of Environmental Medicine, Tuxedo, NY*

Results from epidemiological and laboratory animal studies have demonstrated that ozone (O_3) is an inducer of lower airway inflammation and nonspecific airway hyperreactivity. The airway epithelium is the first cell type inhaled O_3 will encounter. The production and release of mediators such as reactive oxygen intermediates (ROI) and nitric oxide (NO) as well as of chemotactic factors (i.e. IL-8) by epithelia in response to ozone might be contributory to these two observed responses. The present study was designed to investigate the effects of O_3 exposure on epithelial NO release, IL-8 production, as well as disruption of the epithelial permeability barrier. A Human type II-like cell line (A549) was grown to a monolayer in Costar Transwells (Costar, Cambridge, MA) and exposed to 0.4 ppm O_3 for 1 hour. Epithelial resistance was measured using a "chopstick" voltohmmeter (World Precision Instruments, Sarasota, FL). Resistance was decreased 72 hours after exposure (91.4 Ω cm^{2-1}) as compared to before the O_3 exposure (193.2 Ω cm^{2-1}) in unstimulated cells. No change was observed in non-exposed cell. Additionally, the effect of O_3 on NO release by epithelial cells was assessed. Immediately after exposure, A549 cells were stimulated with cytomix (IL-1β, TNFα, IFNγ, all at 10 ng/ml) for 72 hours and the nitrite content was determined in the basolateral media. O_3 exposed A549 cells showed an increased nitrite content (1.338 μM) as compared to non-exposed cells (0.846 μM). These results indicate the airway epithelium as a target as well as an effector cell of ozone toxicity in the lower airways. (Sponsored by EPA R819342).

1378 AIRWAY INFLAMMATION AFTER EXPOSURE TO OZONE — CYTOKINE mRNA PRODUCTION IN LUNG CELLS

J Wijnands, R Vandebriel, H van Loveren, L van Bree. *Laboratories for Toxicology and Pathology, RIVM, Bilthoven, The Netherlands*

Animal and human studies have shown that acute inhalation of ozone causes airway inflammation and tissue damage, lung function decline, and airway hyperreactivity. Although these responses are also considered as important components in the pathogenesis of asthma, spirometric data following ozone exposure do not show that asthmatics are more susceptible than healthy people. Cytokines are thought to play a role in ozone-induced airway inflammation and structural tissue damage. Therefore, studies were initiated to investigate the airway inflammatory cell and mediator response to ozone and to develop (early) markers for (asthmatic) susceptibility.

In cells from lung lavages as well as in lung tissue from ozone exposed rats (6 hours, 2400 μg/m^3 O_3), changes in cytokine mRNA levels (IL-1α, IL-6, IL-12p40, TNF-α, TGF-β, and the chemokine KC) were assessed upon exposure by a semiquantitative RT/PCR using GAPDH as internal control. In cells from lung lavages increased levels were found for IL-6, IL-12p40, and KC mRNA, whereas decreased levels were found for IL-1α mRNA. No changes were found in TNF-α and TGF-β mRNA levels. In lung tissue increased levels were found for IL-1α and IL-6 mRNA, whereas decreased levels were found for IL-12p40 mRNA. No changes were found in TNF-α, TGF-β, and KC mRNA levels.

Data show that acute ozone exposure is able to induce pulmonary inflammatory mediator signals involved in cellular and immunological defense.

Future studies will include mRNA production of additional cytokines, in situ hybridization, and protein measurements.

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