INVOLVEMENT OF VANADATE-MEDIATED REACTIVE OXYGEN SPECIES IN THE ACTIVATION OF TRANSCRIPTION FACTOR AP-1. V Vallyathan [1], M Ding [1], X Shi [1], JJ Li [1], S Leonard [1], JP Ye [1], NH Colburn [2] and V Castranova [1]. [1] NIOSH, Morgantown, WV and [2] NCI, Frederick, MD.

Previous studies from our laboratory have suggested a central role for reactive oxygen species (ROS) in the pathogenesis of occupational lung diseases. Environmental and occupational exposure to vanadium is common and is linked to an increased incidence of lung cancer. We hypothesized that the carcinogenicity of vanadium may be associated with its ROS-generating potential leading to activation of the transcription factor activator protein-1 (AP-1). In the present study, we investigated the activation of AP-1 with vanadate using mouse epidermal cells (JB6 P^+) stably transfected with AP-1 luciferase reporter plasmid. This resulted in a dose dependent transactivation of AP-1, which was inhibited by SOD and catalase. Sodium formate, a specific $^{\cdot}OH$ scavenger, did not inhibit vanadate-induced AP-1 activation, whereas NADPH enhanced AP-1activation. An antioxidant, *N*-acetyl-cysteine, decreased the activation, further showing that vanadate-induced AP-1 activation is involved in redox reactions. Calphostin C, a specific inhibitor of protein kinase C (PKC), inhibited the activation of AP-1, demonstrating that PKC is involved in the cell signal cascades leading to vanadate-induced AP-1 activation. Electron spin resonance (ESR) measurements showed that JB6 P^+ cells are able to reduce vanadate to vanadium(IV) in the presence of NADPH. Molecular oxygen was consumed during the vanadate reduction process to generate $O_2^{\cdot-}$. SOD inhibited the ESR spin adduct signal, further demonstrating the generation of $O_2^{\cdot-}$ in the cellular reduction of vanadate. These results provide support for a model in which vanadium, like other classes of tumor promoters (e.g., phorbol esters), transactivates AP-1-dependent gene expression. In the case of vanadium, the AP-1 transactivation is dependent on the generation of $O_2^{\cdot-}$ and H_2O_2, but not $^{\cdot}OH$.

THIOREDOXIN AS A MODULATOR FOR TRANSCRIPTION FACTOR ACTIVITY IN AIRWAY EPITHELIUM: Richart W. Harper, Reen Wu, Department of Internal Medicine and Center for Comparative Respiratory Biology and Medicine, University of California, Davis, Davis, CA

Our laboratory is interested in the role of thioredoxin in regulating cellular redox status and gene expression in airway epithelium. Specifically, we have examined how thioredoxin modulates transcription factor activity. Previously, we have elucidated that thioredoxin enhances binding of NF-κB, and human IL-8 promoter activity after treatment of cells with TNF-α. This increased binding was unique to thioredoxin. Other thiols such as N-acetyl-cysteine and glutathione were unable to increase binding of NF-κB. Using a similar approach, we observed thioredoxin also enhanced binding of transcription factor AP-1. Nuclear extracts were prepared from primary human/monkey tracheobronchial epithelial cell cultures and an immortalized human tracheobronchial cell line, HBE1, for gel mobility shift assays (GMSA). We observed that TNF-α, PMA, and vitamin A treatments enhanced the binding activity of AP-1 as measured by GMSA. This increased binding was further enhanced by the addition of thioredoxin to nuclear extracts. Antibodies to the c-Jun component of AP-1 were used to confirm AP-1 specificity. Other thiols had no effect on AP-1 binding activity. Using a promoter-reporter chimeric construct, we observed enhanced SPRR1 promoter activity by thioredoxin. This increased activity was abolished when TRE sites of the SPRR1 promoter were mutated. Other thiols again had no similar stimulatory effect. These results, together with our previous data, suggest that thioredoxin plays a unique role in the modulation of transcription factor activity in airway epithelial cells.

(Supported by grants from the American Lung Association and the NIH)
This abstract is funded by:

METAL-INDUCED GENE EXPRESSION IN BRONCHIAL EPITHELIAL CELLS: ROLE OF ROI-DEPENDENT ACTIVATION OF NF-κB. I. Jaspers, J.M. Samet, W. Reed, R.B. Devlin; CEMLB, UNC-CH, Chapel Hill, NC; U.S. EPA, NHEERL, Research Triangle Park, NC USA 27711

Metals associated with particulate matter (PM) have been suggested to mediate PM-induced adverse health effects, including inflammation of the lower respiratory tract. Expression of many inflammatory genes, such as the chemokine IL-8, is regulated at the transcriptional level by NF-κB. Many studies suggest that reactive oxygen intermediates (ROI) are involved in NF-κB activation and subsequent gene expression. The purpose of this study is to investigate the potential of transition metals to induce oxidative stress and the role of ROI in metal-induced NF-κB activation and gene expression in normal human bronchial epithelial (NHBE) cells. NHBE cells were treated with copper, vanadium, or ferrous iron, transition metals often associated with PM, and were examined for formation of ROI, activation of NF-κB, and gene expression. Stimulation with any of the three transition metals resulted in a dose-dependent generation of oxidative stress. Both vanadium and ferrous iron induced NF-κB DNA-binding as well as IL-8 gene transcription. The role of ROI in metal-induced activation of NF-κB and gene expression was tested by infecting NHBE cells with adenoviral expression vectors for antioxidant enzymes. Overexpressing catalase, blocked vanadium-induced activation of NF-κB and IL-8 gene transcription. These data demonstrate that transition metals, which are often associated with PM, generate oxidative stress in NHBE cells and that metal-induced formation of ROI mediates IL-8 gene expression. (This abstract does not reflect U.S. EPA policy)
This abstract is funded by:

NITRIC OXIDE INCREASES GENE TRANSCRIPTION IN PORCINE PULMONARY ARTERY ENDOTHELIAL CELLS. J.L. Zhang, J.M. Patel, and E.R. Block. Department of Medicine, University of Florida and VA Medical Center, Gainesville, FL 32610.

Nitric oxide (NO) is known to suppress gene expression and catalytic activity of a number of proteins, including the redox regulatory protein thioredoxin, in pulmonary artery endothelial cells (PAEC) (Am. J. Physiol. 275: L288-L293, 1998). We sought to determine whether exposure to NO results in increased gene transcription in PAEC. Cell monolayers in RPMI 1640 were exposed to 8 ppm NO gas or air (control) for 24 hr at 37° C. After exposure, total mRNA was extracted and cDNA was synthesized. The subtraction suppression hybridization was performed between control and NO-exposed samples using a PCR-Select cDNA Subtraction kit. Using suppressor PCR, only differentially expressed sequences were amplified exponentially for further identification. Three novel cDNAs up-regulated by NO exposure were sequenced and identified by data base analysis with 75-85% homology. NO-exposure significantly increased expression of mitochondrial 16s ribosome RNA, tumor suppressor, and cyclic nucleotide-activated channel genes compared to controls ($p < 0.01$ for all). These results suggest that exposure to NO can increase expression of genes that may be associated with modulation of mitochondrial and other regulatory processes in response to oxidative stress in PAEC.

Funded by NIH grant HL58679
This abstract is funded by:

Antibody-Directed Targeting of Catalase to the Endothelial Antigen PECAM Augments Pulmonary Antioxidant Defense in Vivo. M.Christofidou-Solomidou, K.Ng, S.Kennel, S.Albelda, and V.Muzykantov. U.Penn., Phila., PA, Oak Ridge Natl. Lab., TN

We have developed a strategy of vascular immunotargeting by coupling enzymes to monoclonal antibodies recognizing endothelial cells. For example, our data showed that iv injection of the H_2O_2-producing enzyme glucose oxidase coupled to thrombomodulin antibody (anti-TM/GOX) in mice caused oxidative edematous injury in the lungs (wet-to-dry ratio 7.6±0.2 vs 4.7±0.1 in control) and led to 100% lethality within 7 h. In this study we evaluated the protective effect of the H_2O_2-degrading enzyme catalase conjugated via streptavidin-biotin with PECAM antibody. Anti-PECAM/^{125}I-catalase, but not IgG/^{125}I-catalase, bound specifically to endothelial cells in culture (67.2±2.9 vs 1.3±0.1 ng/well), entered the cells (91.2% internalization) with modest intracellular degradation (<5%) and protected cells against H_2O_2 (14.5±1.3 vs 56.1±5.7% of ^{51}Cr release). Anti-PECAM/^{125}I-catalase, but not IgG/^{125}I-catalase, accumulated in the perfused rat lungs (37.7±4.4 vs 2.1±0.2 %ID/g) and protected against pulmonary edema caused by infusion of 5 mM H_2O_2 (lung wet-to-dry ratio 5.5±0.1 vs 8.1±0.7 for IgG/catalase). Anti-PECAM/^{125}I-catalase, but not IgG/^{125}I-catalase, accumulated in the lungs after iv injection in intact animals: lung/blood ratio of ^{125}I was 39.8±4.1 vs 1.1±0.2 in rats and 7.5±1.1 vs 0.6±0.1 in mice. Co-injection of PEG-catalase with anti-TM/GOX only slightly attenuated lung injury (wet-to-dry ratio 6.9±0.2) and lethality (80% within 10 h). In contrast, co-injection of anti-PECAM/catalase with anti-TM/GOX markedly attenuated lung injury (wet-to-dry ratio 5.7±0.4) and lethality (20% within 12 h). Therefore, anti-PECAM carrier can deliver active catalase to endothelium in intact animals and markedly augment antioxidant defense in the lung. Our results validate the general strategy of vascular immunotargeting as a novel approach for specific and effective antioxidant therapy.

This abstract is funded by: AHA EIG and NIH SCOR in Acute Lung Injury (VRM)

GLUTATHIONE REDUCTASE ACTIVITY DIRECTED TO THE MITOCHONDRIA. X. Xu, R. Husser, T. Tamura, C.V. Smith, S.E. Welty, J.P. Katkin. Department of Pediatrics, Baylor College of Medicine, Houston, TX USA

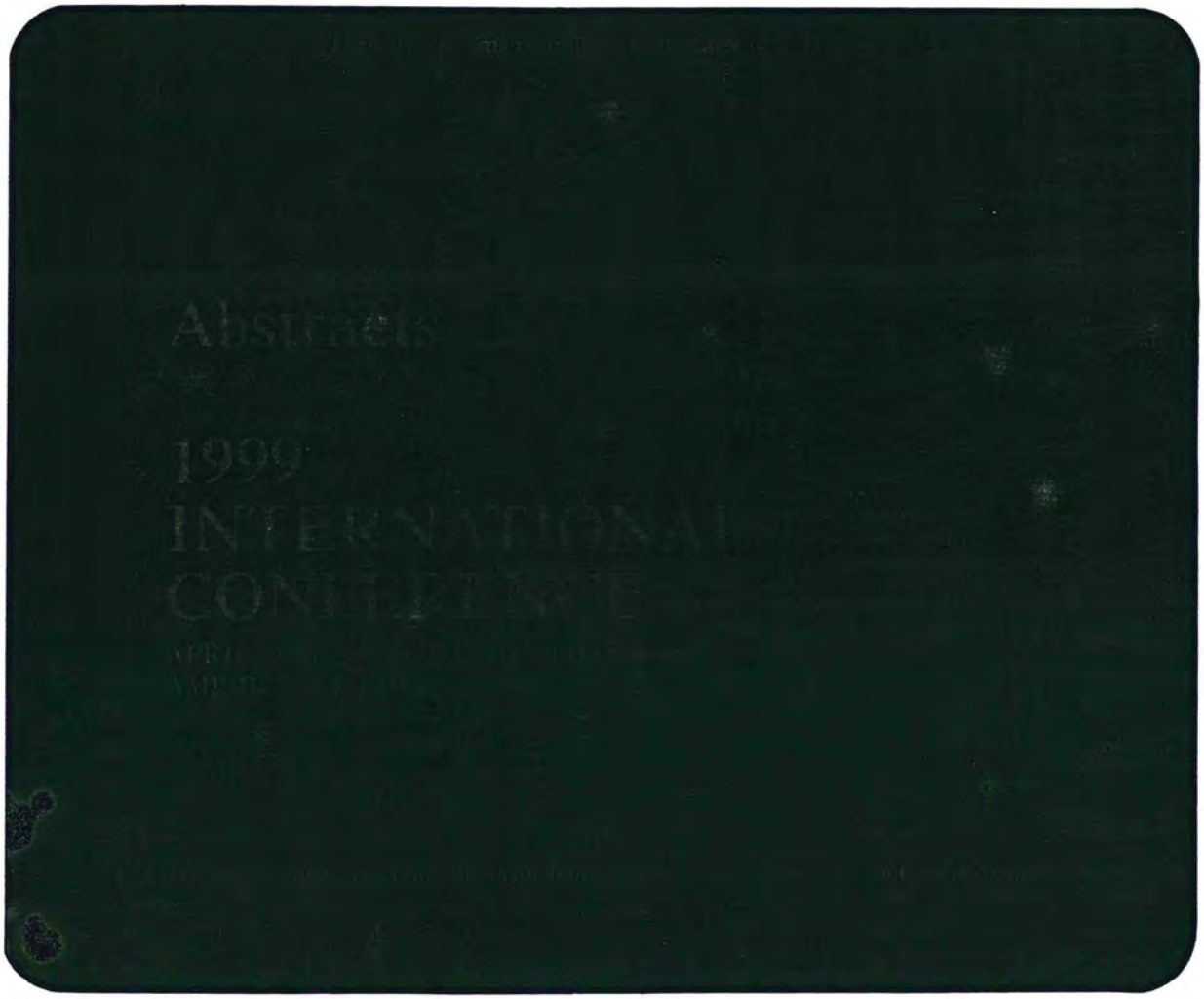
Acute lung injury is a frequent consequence of therapy with hyperoxic gas mixtures, to which premature infants may be particularly susceptible. Glutathione (GSH) plays a pivotal role in protecting cells from oxidative stress. Glutathione reductase (GR), which is normally present in the cytosol and mitochondria, maintains intracellular GSH levels by catalyzing the reduction of glutathione disulfide (GSSG). Chinese hamster ovary (CHO) cells transfected with the (human) hGR cDNA had increased resistance to oxidant-mediated cytotoxicity, coincident with increased cytosolic GR activities. Modification of the hGR plasmid to include the mitochondrial targeting signal (MTS) for human MnSOD, imparted even greater resistance to oxidant stresses. The majority of increased GR activities in cells transfected with MTS-hGR were observed in the mitochondrial compartment, although cytosolic GR activities were significantly greater than those seen in non-transfected control cells. As the MTS-hGR construct retains the original hGR start codon 3' to the MTS, we tested the hypothesis that initiation of translation at the ATG in the hGR sequence could be responsible for the increase in cytosolic GR activities observed in cells transfected with the MTS-GR. We mutated the initial ATG of the hGR cDNA and transfected CHO cells with the modified construct. Although cells transfected with the mutated MTS-hGR construct tended to have lower elevations of cytosolic GR activities than did cells transfected with MTS-hGR, the overall distributions of GR activities in the two groups were not different. These results suggest that translation of MTS-hGR begins preferentially at the MTS start site and that the resultant protein is directed to the mitochondria efficiently. The greater resistance to oxidant injury imparted to cells by increased mitochondrial expression of hGR is even more striking in view of this data indicating that these cells have minimal increases in cytosolic GR activities.

This abstract is funded by: NIH U10 HL52637 and GM44263

AMERICAN JOURNAL OF

Respiratory and Critical Care Medicine

Volume 159 • Number 3 • March 1999
(*part 2 of 2 parts*)



AN OFFICIAL JOURNAL OF THE AMERICAN THORACIC SOCIETY
MEDICAL SECTION OF THE AMERICAN LUNG ASSOCIATION