SYNTHETIC COPPER-CONTAINING PARTICLES ENHANCE ALLERGIC AIRWAY RESPONSES IN MICE. SH Gavett, MI Gilmour, and N Haykal-Coates. National Health and Environ Effects Research Lab, USEPA, Res Triangle Park, NC USA

Respiratory morbidity and mortality associated with increases in ambient levels of particulate matter (PM) may be dependent on particle elemental composition. Particle-associated metals such as copper may catalyze formation of reactive oxygen species leading to inflammation and lung injury. We studied the ability of chemically defined synthetic particles to enhance allergic inflammation and airway hyperresponsiveness in ovalbumin (OVA)-allergic mice. Particles (< 2 μm) were synthesized with a base of 50% carbon, 15% NH_4Cl, and 30% Na_2SO_4. Control carbon (C) particles contained an extra 5% carbon, while carbon-zinc (CZn) or carbon-copper (CCu) particles contained 5% zinc sulfate or copper sulfate [2.0% Zn(II) or Cu(II) metal; 0.31 mmol/gm particle]. Female BALB/cJ mice were sensitized i.p. with OVA in aluminum hydroxide adjuvant or were administered adjuvant only; all mice were challenged with OVA aerosol 14 or 15 d later. One hour after challenge mice were intratracheally instilled with saline, C, CZn, or CCu in saline vehicle (2 mg/kg; ~40 μg). Both nonallergic and allergic CCu-exposed mice were hyperresponsive to methacholine aerosol challenge (Buxco; 4-32 mg/ml) 1 day (~4-fold increase) and 8 days (~2-fold increase) after exposure compared with all other groups. Bronchoalveolar lavage (BAL) fluid eosinophils, neutrophils, lactate dehydrogenase, protein, albumin, N-acetyl-β-D-glucosaminidase, interleukins-4, -5, -13, interferon-γ, and tumor necrosis factor-α were all significantly increased in CCu-exposed mice compared with saline-, C-, or CZn-exposed groups. These data show that PM-associated copper enhances allergic inflammation and airway hyperresponsiveness in mice, and are consistent with recent studies (Dye, Env Health Perspect 2001; Ghio, AJRCCM 2001) relating toxicological effects with epidemiological findings of health effects of PM containing high concentrations of copper.

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QUANTITATIVE TRAIT LOCUS ANALYSIS OF THE DEVELOPMMENT OF PULMONARY TOLERANCE TO INHALED ZINC OXIDE. SC Wesselkamper[1], LC Chen[1], SR Kleeberger[2], M-S Tang[1], and T Gordon[1]. [1]Dept. of Env. Med., NYU School of Med., Tuxedo, NY; [2]Dept. of Env. Health Sci., Johns Hopkins Univ., Baltimore MD

As a result of repeated exposure to inhaled toxic agents in different occupations and environmental settings, individuals may develop tolerance to adverse health effects. Thus, the identification of innate and extrinsic factors that influence the ability to develop tolerance is a significant issue. Among inbred strains of mice, the BALB/cByJ (CBy) strain exhibits greater pulmonary tolerance to PMN and protein levels in lavage fluid than DBA/2J (D2) following 5 successive exposures (5X) to 1.0 mg/m^3 ZnO. A genome-wide screen using 298 F2 mice (CByD2F1xCByD2F1) that were phenotyped for the development of pulmonary tolerance following 5X ZnO exposure identified a significant quantitative trait locus (QTL) for the BAL protein phenotype on chromosome 1. An excellent candidate gene within this QTL is the toll-like receptor 5 gene (*Tlr5*) which is part of a family of toll-like receptor genes that are important in the induction of the innate immune response. Affymetrix GeneChip oligonucleotide microarray analyses were conducted to examine ZnO-induced expression of genes up- and downstream in the toll-like receptor signaling pathway and identify candidate genes present in other chromosomal regions of interest. Interestingly, the toll-like receptor 4 gene (*Tlr4*) has been shown to be a strong candidate susceptibility gene for ozone-induced increases in the BAL protein phenotype in inbred mice. Taken together, these studies suggest that the toll-like receptor pathway may play a role in genetic regulation of the pulmonary response to inhaled particles and gases.

EPA, NIOSH

This abstract is funded by:

MECHANISMS OF Zn-INDUCED SIGNAL INITIATION THROUGH THE EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)

James M. Samet*, Lee M. Graves§ and Weidong Wu§. *Human Studies Division, NHEERL, ORD, Research Triangle Park, NC 27711, and § Center for Environmental Medicine, University of North Carolina, Chapel Hill, NC 27599.

Zn is a ubiquitous component of ambient air particulate matter and an occupational toxicant. We have shown that Zn ions induce EGFR activation and signaling. To elucidate the mechanism involved in Zn-induced activation of this receptor kinase, we studied EGFR dimerization, phosphorylation and kinase activity in A431 cells treated with $ZnSO_4$ and compared the effects to those induced by its ligand EGF. A431 extracts were incubated with 500 uM $ZnSO_4$ or 200 ng/ml EGF for 30 min, followed by treatment with a cross-linking agent (BS^3) and Western blotting. As expected, EGF induced pronounced dimerization of EGFR molecules. However, Zn treatment failed to induce detectable EGFR dimerization. Analyses of intact A431 cells treated in a similar manner confirmed that Zn does not induce dimerization of EGFR. Western blotting using phospho-specific antibodies showed that Zn induced phosphorylation of EGFR at tyrosines 845, 1068 and 1173, a pattern identical to that produced by EGF treatment. The EGFR kinase inhibitor PD153035 effectively ablated all phosphorylation induced by EGF but none caused by Zn treatment of A431 cells. Similarly, PD153035 abolished EGF-induced phosphorylation of the EGFR substrate cbl, while it had no effect on levels of phospho-cbl that resulted from Zn treatment. Together, these results confirm that Zn ions effectively activate EGFR and show that the mechanism includes EGFR phosphorylation but does not involve EGFR dimerization or EGFR kinase activation, suggesting that Zn induces a transactivation of EGFR in A431 cells. These findings may provide a mechanistic basis for the adverse effects of Zn inhalation. **This abstract of a proposed presentation does not necessarily reflect EPA policy.**

This abstract is funded by:

VANADYL SULFATE INDUCES PULMONARY VASOCONSTRICTION BY DECREASING NITRIC OXIDE (NO) BIOAVAILABILITY. Y.C. Huang, L. Dailey, I. Demchenko, J.D. Carter. ORD, US EPA, RTP, NC and Duke University Medical Center, Durham, NC.

Exposure to particulate matter (PM) has been associated with acute cardiovascular adverse events, but biologically relevant mechanisms are unclear. PM of combustion sources contain redox-active metals such as vanadium (V) and copper, which may alter vasoreactivity. We showed previously that intratracheal instillation of a V-rich PM dust, residual oil fly ash, produced pulmonary vasoconstriction that could be attributed in part to V permeating into the pulmonary circulation. The present study investigated if the V-induced pulmonary vasoconstriction was related to decreased NO bioavailability in isolated perfused lungs (IPL) and pulmonary artery (PA) rings. Vanadyl sulfate (0.5-50 μM) produced a dose-dependent increase in PA pressure in IPL, and inhibited acetylcholine-mediated vasodilation in pre-constricted PA rings. V inhibited accumulation of nitrite/nitrate and free nitrotyrosine in the perfusate, and decreased nitrotyrosinated α-tubulin in the lung. V-induced pulmonary vasoconstriction was attenuated by SOD, PAPANONOate (an NO donor) and DPI (an NADH oxidoreductase inhibitor), but not by catalase, genistein or PD153035 (an epidermal growth factor receptor antagonist). Copper sulfate, but not zinc sulfate, causes similar pulmonary vasoconstriction. These results indicate that V-induced pulmonary vasoconstriction may be mediated in part by NADH oxidoreductase-derived superoxide, which may act as a vasoconstrictor or react with NO resulting in the loss of NO-mediated vasodilation. The results raise the hypothesis that exposure to PM containing high levels of certain metals may produce adverse pulmonary vasoconstriction causing acute decompensation of the heart in patients with pre-existing cardiopulmonary diseases. (This abstract does not reflect EPA policy).

This abstract is funded by:

THE EFFECTS OF SINGLE AND SERIAL ZINC OXIDE EXPOSURES ON AIRWAY INFLAMATION IN HEALTHY HUMANS K Reinties, A Witten, M Arjomandi, J Balmes, P Blanc[1], C Solomon Lung Biology Center; [1]Department of Medicine, University of California, San Francisco, CA., U.S.A.

A single exposure to zinc oxide (ZnO) results in an increase in inflammatory cells and cytokines. Serial exposures to ZnO can decrease this inflammatory response. This experiment was designed to test the hypothesis that exposure to ZnO on two consecutive occasions would decrease both the cellular and cytokine responses compared to one exposure. Utilizing a repeated measure, randomized, single-blind design, 6 healthy subjects (4 females; age range 26-53 yr; methacholine PC_{20} > 10 mg/ml) were exposed for 30 min, at rest, to: 1) filtered-air (FA); 2) ZnO on one occasion (ZnO-1: conc: 10 mg/m^3); 3) ZnO on two consecutive occasions (ZnO-2: conc: 10 mg/m^3). Sputum induction (3% saline, t=20 min) was performed 65 h pre- and 6 h post-exposure, Conditions were compared using the post minus pre delta value. In ZnO-1, compared to FA, there was a significant (P<0.05) increase in the total leukocyte concentration (median; 25-75% range: 16.6; 7.5-23.0 vs -0.3; 0.3--0.8 x 10^4 cells/ml), and TNFα (1.4; 0.6-6.5 vs 0.6; -0.1-1.8 pg/ml). In ZnO-2, compared to FA, there was a significant increase in TNFα (2.8; 1.0-7.0 vs 0.6; -0.1-1.8 pg/ml). For all three conditions there were no other differences in total leukocyte concentration, or macrophage, neutrophil, lymphocyte, or eosinophil differential percent, or in total protein. TNFα, or IL-8. These results indicate that serial exposure to ZnO does not result in the increase in leukocytes observed for a single exposure, but that serial exposure does not change the TNFα response.

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RESIDUAL OIL FLY ASH (ROFA)-INDUCED LUNG INJURY IN MICE: ROLE OF TOLL-LIKE RECEPTOR 4 (TLR4) SIGNALING. Hye-Youn Cho[1], Anne E. Jedlicka[1], Liu-Yi Zhang[1], Robert Clarke[2], and Steven R. Kleeberger[1]. [1]Dept. of Environmental Health Sciences, Johns Hopkins University, Baltimore, MD and [2]Advanced Inhalation Research, Inc., Cambridge MA.

We previously demonstrated significant inter-strain (genetic) variation in ROFA-induced lung inflammation and hyperpermeability. A significant difference in the hyperpermeability response between susceptible C3H/HeOuJ (OuJ) and resistant C3H/HeJ (HeJ) mice was particularly interesting as they differ only at a polymorphism in the coding region of the gene for Toll-like receptor 4 (*Tlr4*). *Tlr4* has an important role in innate immunity and responsiveness to bacterial endotoxin and the oxidant ozone. The objective of this study was to characterize the *Tlr4*-mediated signaling pathway in HeJ and OuJ mice following ROFA challenge and to identify candidate effector genes that mediate differential responses in these strains. Marked upregulation of lung *Tlr4* message level was observed in OuJ mice but not in HeJ mice following ROFA challenge. Basal and ROFA-induced lung TLR4 protein levels were higher in OuJ mice than in HeJ mice. Greater activation of the TLR4 downstream signal molecules MyD88, TRAF6, IRAK-1, IκB-α, and NF-κB in OuJ mice compared to HeJ mice supported a role for *Tlr4* in hyperresponsiveness to ROFA in murine lungs. Microarray cytokine profile analysis of ROFA-exposed lungs indicated several inflammatory cytokines, including interleukin (IL)-1β, were differentially regulated in HeJ and OuJ mice. Results suggest an important role of genetic background in response to ROFA exposure, and indicate innate immune defense mechanisms through TLR4 are important determinants of differential susceptibility.

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ABSTRACTS

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This special supplement of the *American Journal of Respiratory and Critical Care Medicine* contains abstracts of the scientific papers to be presented at the 2002 International Conference. The abstracts appear in order of presentation, from Sunday, May 19 through Wednesday, May 22 and are identified by session code numbers. To assist in planning a personal schedule at the Conference, the time and place of each presentation is also provided.

Bennett, William D. (Bill)

From: Dickerson, Richie
Sent: Thursday, June 19, 2003 2:15 PM
To: Bennett, William D. (Bill)
Subject: RE: Unknown Grant/Cooperative Agreement

Sorry to report, I couldn't locate anything under the names or that title.

-----Original Message-----
From: Bennett, William D. (Bill)
Sent: Wednesday, June 18, 2003 4:04 PM
To: Dickerson, Richie
Subject: Unknown Grant/Cooperative Agreement

You know the best way to determine if you are doing a good job is by the number of repeat customers. Well, here I am again.

The attached file contains an image of the document that we are entering into NIOSHTIC-2. Unfortunately, the authors only indicate that NIOSH funded the research, and do not identify the funding mechanism or number. I do not even know if the funding went to the NYU School of Medicine, or Johns Hopkins. Can you help? We will need all of the usual information.

<< File: Doc1.doc.zip >>