INTERSPECIES COMPARISON OF IMMUNOTOXICITY OF INHALED SULFURIC ACID: I. HUMAN SUBJECTS. M.W. Frampton, K.Z. Voter, P.E. Morrow, J.T. Zelikoff, R.B. Schlesinger, M.J. Utell. University of Rochester School of Medicine, Rochester, NY 14642 and NYU Medical Center, Institute of Environmental Medicine, 550 First Ave., NY, NY 10016, USA.

Particulate matter in the atmosphere has been associated with increased respiratory morbidity and mortality, and sulfuric acid aerosols comprise the predominant particulate species in many industrialized areas. Animal studies have demonstrated effects of H_2SO_4 aerosol exposure on alveolar macrophage function, but extrapolation of these findings to humans has remained problematic. As part of a collaborative study to compare the results of H_2SO_4 exposure in humans and animals under similar conditions, we exposed 7 healthy nonsmoking volunteers to aerosols of either H_2SO_4 or NaCl, 1 mg/m^3 for 3 hours, with intermittent exercise. Bronchoalveolar lavage (BAL) was performed 1 hour after exposure; total and differential cell counts were determined in both the first lavage aliquot (bronchial lavage) and the last three pooled lavage aliquots (alveolar lavage). Alveolar macrophages (AM) separated by adherence were examined for viability, phagocytosis of serum-opsonized latex particles, uptake and killing of *Staphylococcus aureus*, release of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), and expression of surface receptors important in host defense. Proportion of cells recovered in both bronchial and alveolar lavage samples were similar following NaCl and H_2SO_4 exposure, indicating the absence of an airway inflammatory response to the acidic aerosol. Release of O_2^- by AM stimulated with opsonized zymosan decreased following H_2SO_4 exposure (NaCl, 3.49±0.25 nmol; H_2SO_4, 3.01±0.28 nmol, $p<0.06$). Cell viability, release of H_2O_2, and phagocytosis were unaffected by the exposure. Preliminary results indicate no significant effects on bacterial uptake or killing, or on Fc receptor expression by AM. Concordance between findings in humans and New Zealand white rabbits with regard to release of superoxide anion by AM suggests exposure to H_2SO_4 aerosols may impair respiratory host defense against infection.

INTRATRACHEAL INSTILLATION OF SILICA UPREGULATES INDUCIBLE NITRIC OXIDE SYNTHASE GENE EXPRESSION AND INCREASES NITRIC OXIDE PRODUCTION IN ALVEOLAR MACROPHAGES. John A. Blackford Jr., James M. Antonini, Vincent Castranova, Richard D. Dey, Departments of Anatomy and Pharmacology and Toxicology, West Virginia University, Morgantown, WV 26506, and Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Morgantown WV, 26505.

Alveolar macrophages (AM) exposed to cytokines or bacterial lipopolysaccharide (LPS) produce the free radical nitric oxide (NO·) by an inducible nitric oxide synthase (iNOS) and release reactive oxygen free radicals following exposure to silica dust. The purpose of the present study was to determine if NO· is produced by rat AM following the intratracheal (IT) instillation of silica. Male Sprague-Dawley rats (175-225 gm) were IT instilled with either silica dust (10 mg/100 gbw) or LPS (0.25 mg/100 gbw). After 24 hr, bronchoalveolar lavage cells (BALC) and lavaged lung tissue were assayed for iNOS mRNA and BALC iNOS-dependent (L-NAME-inhibitable) chemiluminescence and cell counts were also determined. Northern blot analysis demonstrated that the steady-state levels of BALC iNOS mRNA was significantly increased by 3 fold following IT silica and by 7 fold following IT LPS. iNOS-dependent chemiluminescence was significantly increased in AM by 36 fold following IT silica and by 89 fold following IT LPS. Differential counts of BALC showed that AM numbers did not change in any of the treatments, RBC increased by 30 fold following IT silica and by 23 fold following IT LPS. Total leukocytes (polymorphonuclear leukocytes plus lymphocytes) increased by 58 fold following IT silica and by 274 fold following IT LPS. The results demonstrate that BALC iNOS steady-state mRNA levels, AM iNOS-dependent chemiluminescence and total numbers of RBC and leukocytes all increased following IT silica. These findings suggest that NO· production in rat AM is increased in response to silica. NO· has the ability to then combine with superoxide and form a highly reactive, toxic free radical, peroxynitrite that may be responsible for silica-induced lung damage.

PULMONARY INTRAVASCULAR MACROPHAGE CLEARANCE OF ALBUMIN COLLOIDAL GOLD DURING ENDOTOXEMIA: QUANTITATION BY NEUTRON ACTIVATION. B.J. Darien[1], K.T. Kruse-Elliott[2], P.A. Sims[3], R.M. Albrecht[3]. Departments of Medical[1] & Surgical[2] Sciences, School of Veterinary Medicine; Department of Animal Health & Biomedical Science[3], School of Agriculture and Life Science, University of Wisconsin, Madison WI 53707.

Mortality from adult respiratory distress syndrome (ARDS) approaches 50%, and is higher when ARDS is associated with gram-negative sepsis or endotoxemia (LPS). LPS is a potent activator of many cell types including monocytes/macrophages (Mø), neutrophils and endothelial cells (EC). LPS induces Mø and EC procoagulant activity and the release of tumor necrosis factor from Mø, which potentiate inflammatory lung injury and multiple organ failure. Mø play an important role in the clearance of blood-borne bacteria and LPS, thus attenuating their dissemination to other organs and the subsequent whole-body inflammatory response. Because of this intertwined relationship, we investigated the effects of LPS on pulmonary intravascular Mø (PMø) phagocytic function. Pigs were anesthetized and maintained with pentobarbital sodium, and instrumented for measurement of cardiopulmonary function. Colloidal gold particles with an average diameter of 16 nm were prepared by reducing $HAu_{16}Cl_4$ with tannic acid and sodium citrate. 180 μg of bovine serum albumin/ml Au_{16} ($AlbAu_{16}$) was required to stabilize the gold particles as determined by the salt flocculation test. The $AlbAu_{16}$ conjugate was concentrated by centrifugation to 60 ml. Blood (0.5 ml) and tissue (0.5 gm) were collected in pre-weighed standard irradiation vials and sealed by friction welding. Blank vials and control samples without gold were run as standards. Following sample collection and subsequent neutron activation, gold ($AlbAu_{16}^{198}$) samples were reported as counts per second (cps) per ml of fluid or gm of tissue. From 0-6 hours experimental and control pigs were administered LPS (*E. coli* 055:B5, 1.0 μg/kg/hr, IV) and physiologic saline, respectively. Blood samples were obtained at hours 5 & 6 for quantitating plasma $AlbAu_{16}$. At 5.25 hours $AlbAu_{16}$ was infused at 2 ml/min for 30 min. At 6 hours lung tissue and bronchoalveolar lavage (BAL) were collected from the left lobe for quantifying $AlbAu_{16}$ and the intermediated lobe was fixed for electron microscopy (EM). Plasma $AlbAu_{16}$ concentration in the LPS treated pig (4856.7 cps/ml) was markedly elevated in comparison to the control pig (3.8 cps/ml). Conversely, lung $AlbAu_{16}$ concentration in the LPS treated pig (2.6×10^5 cps/gm) was markedly reduced when compared to the control pig (5.46×10^5/gm). Additionally, no $AlbAu_{16}$ was measured in the BAL of the control pig vs 0.9 cps/ml in the LPS pig. This finding was supported by the EM data. On EM, $AlbAu_{16}$ was observed only in the PMø in the control pig; while in the LPS pig it was also found in the interstitium and airways. We conclude that at this dose, LPS affects PMø phagocytic function and may contribute to systemic gram-negative septicemia and associated endotoxemia. This study was supported by the University of Wisconsin-Madison.

ALVEOLAR MACROPHAGES IN PATIENTS WITH AIDS AND PULMONARY TUBERCULOSIS

Werneck-Barroso E.; Bonecini-de-Almeida, M.G.*; Carvalho, P.B.*; Vieira, M.A.M.S. ;Carvalho, C.E.**; Almada-Horta, R.**; Teixeira, A.K.***; Kritski, A.L.**** - Evandro Chagas Hospital, IOC-FioCruz; * AIDS e Molecular Imunology Lab-FioCruz; ** ITP-UFRJ *** LAGOA Hospital-INAMPS; **** CFF Hospital - UFRJ

Objective: To evaluate the changes in BAL fluid macrophage counts in patients with AIDS and infections that produces distinct lower respiratory tract inflammatory processes.

Methods: We performed an analysis of alveolar macrophages population in AIDS adults patients (CDC-1987) that were submitted to bronchoalveolar lavage during fiberoptic bronchoscopy for the evaluation of suspected pulmonary disease. Patients were classified into five groups based on microbiological studies, as follows: 1) TB Group: 20 patients with AIDS and pulmonary tuberculosis. 2) PCP Group: 21 patients with AIDS and *P. carinii* pneumonia. 3) BP Group: 5 patients with AIDS and bacterial pneumonia 4) AIDS Control Group: 5 patients with AIDS and without clinical pulmonary symptoms or radiological abnormalities to suggest respiratory infection; these patients underwent fiberoptic bronchoscopy because they had pyrexia of unknown origin. 5) Health Control Group: 3 healthy HIV-seronegative subjects underwent BAL for comparison of cellular recovery. Patients with dual infection were excluded. Data expressed as mean SEM.

Results: All AIDS patients showed lower percentage of macrophages than the healthy control group. The relative proportion of alveolar macrophages of BAL obtained from AIDS patients (78 5%) were lower than those obtained from healthy controls (89 3%), followed by TB (62 6%), PCP (38 5%) and BP (12 3%) patients. However, the absolute ($x10^5$ per ml) number of alveolar macrophages retrieved per aliquot volume was higher in tuberculous patients (3.21) than all other groups ($p < 0.05$), including healthy (1.76) or AIDS (1.66) controls and BP (1.32) or PCP (1.11) patients.

Conclusion: Although with an expected percentage of macrophages, our study show that tuberculous patients had comparatively the highest absolute number of alveolar macrophages. This finding is in contrast to the decrease of alveolar macrophage number in the lower respiratory tract of patients with miliary tuberculosis and in lesions of active pulmonary tuberculosis. These changes in the numbers of macrophages seem to be critical to the knowledge of tuberculous inflammatory process in AIDS patients and must be confirmed by others.