613 MODULATION OF OZONE ABSORPTION BY INTERFACIAL PHOSPHOLIPIDS.

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Reactions of ozone (O_3) and nitrogen dioxide (NO_2) with constituents of the lung surface lining layer mediate the net flux of inhaled oxidant into the aqueous phase (reactive absorption). Recent studies have shown that monolayers of dipalmitoyl phosphatidylcholine (DPPC), the major component of alveolar surfactant, modulate NO_2 uptake. When DPPC, deposited on an aqueous surface, was subjected to increased molecular packing (surface compression), NO_2 reactive absorption was significantly reduced. To investigate potential phospholipid (PL) induced effects on O_3 uptake, the rates of O_3 gas phase disappearance (25° C) were evaluated using a specialized exposure-compression apparatus. Determinations of O_3 mass balance across a defined surface area were conducted during graded PL surface compressions. DPPC, dilinoleoyl-PC (DLPC) or palmitoyloleoyl PG (POPG) monolayers were compressed from 121 → 49 Å^2/molecule, γ = 72 → 25 dyn/cm respectively. Apparatus constraints prevented further compression. **Results:** 1) At the initial area/molecule level, PL did not alter O_3 (0.6 ± 0.1 ppm) absorption rates. 2) Upon DPPC compression, uptake declined proportional to surface compression (3.5 → 1.2 μg O_3•min^{-1}•cm^{-2}). 3) DPPC-induced reductions occurred regardless of aqueous substrate concentration, composition, or [O_3] (0.3 to 1.0 ppm). 4) Compression of POPG or DLPC monolayers did not significantly decrease uptake. 5) DLPC, extracted from O_3-exposed liposomes, when compressed displayed similar surface tensions and O_3 absorption as previously unexposed. **Conclusions:** Compression of DPPC monolayers to surface tensions which are still greater than those occurring *in vivo* significantly limits O_3 flux into the aqueous phase. Under the exposure conditions employed in these studies, it is unlikely that O_3 reactions with interfacial phospholipids contribute significantly to absorption, nor do such reactions appear to alter PL surface active properties. (HL54696, ES05749, and HL24075)

614 ADHESION MOLECULES ON BLOOD NEUTROPHILS AND ALVEOLAR MACROPHAGES FROM RATS: MODULATION BY EXPOSURE TO OZONE.

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It is known that exposure of rats to the pulmonary irritant ozone results in an infiltration of polymorphonuclear leukocytes (PMNs) into the lungs. The purpose of this study was to investigate whether the ozone-induced inflammatory process is preceded by a change in the expression of integrins and selectins in peripheral blood PMNs and alveolar macrophages. Female Sprague Dawley rats were exposed to air or ozone (1 ppm, 2 or 4 hrs). Bronchoalveolar lavage (BAL) was carried out and blood was collected via intracardiac puncture at 0 or 18 hrs after the exposure. The percent of neutrophils in the BAL fluid was increased only in animals exposed to ozone for 4 hrs and not for 2 hrs, while the expression of CD18 on alveolar macrophages was lowered at all exposure times. The expression of CD62L on blood PMN was not affected by exposure to ozone, while the expression of CD11b was lowered after 2 hrs, and not after 4 hrs of exposure to ozone. This trend was also observed in experiments in which plasma of ozone-exposed animals was incubated with whole blood obtained from non-exposed animals. In these experiments, the expression of CD11b on PMNs of non-exposed animals was lower after incubation with plasma from 2 hours-ozone-exposed animals, but not after incubation with plasma from 4 hours-ozone-exposed animals. (This work was supported by a grant from the Committee on Prevention and Research in Occupational Health at the Ministry of Labour and Social Welfare.)

615 ACUTE INFLAMMATORY REACTION IN RATS AFTER INTRATRACHEAL INSTILLATION OF MATERIAL COLLECTED FROM A NYLON FLOCKING PLANT.

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The etiologic agent responsible for several cases of interstitial lung disease among workers at a nylon flock plant was unknown. Airborne dust collected at the plant was examined for its inflammatory potential in rat lungs. The endpoints measured were: (1) breathing rates, (2) differential cell counts of bronchoalveolar lavage cells, (3) alveolar macrophage (AM) chemiluminescence, (4) albumin in the acellular bronchoalveolar lavage, and (5) pulmonary histopathology. In the first study, rats received a single dose of the airborne dust sample (10 mg/kg body weight) by intratracheal (IT) instillation. At 1 day post-IT, all inflammatory endpoints were significantly increased ($p<0.05$) versus controls but by 29 days post-IT they did not differ significantly ($p>0.05$) from controls. Histopathology demonstrated mild to moderate, multifocal, suppurative pneumonia usually centered around bronchioles at 1 day post-IT. At 29 days post-IT, pulmonary inflammation was minimal to mild and characterized by alveolar histocytosis usually restricted to the immediate area of retained birefringent fibers. In subsequent experiments, airborne dust was extracted with water and the dust (washed airborne dust) and water extract (soluble fraction) were separated by centrifugation. Nylon dust was prepared in the laboratory by milling uncut nylon strands that had not been treated with finish or dyes which are commonly used in nylon flock plants. Rats were administered a single dose of a dust sample (10 mg/kg body weight) or the soluble fraction (1.3 ml/kg body weight) by IT administration and the same endpoints were measured at 1 day post-IT. The dust samples caused significant increases in all of the inflammatory end points; however the soluble fraction was by far the least active. Histological analysis of the lungs 1 day post-IT confirmed lung inflammation was occurring only after dust instillation and tended to center around bronchioles. The results suggest: (1) nylon flocking generates particles of respirable size which can interact with AM and can be detected in the lung 29 days after exposure, (2) the dusts examined cause an inflammatory response, (3) water extractable agent(s) from airborne dust contribute minimally to the inflammatory response, and (4) the acute inflammatory response to these dusts is substantial when compared to other pathologic occupational dusts previously examined.

616 INTRATRACHEAL INSTILLATION OF WELDING FUMES ALTERS THE PULMONARY CLEARANCE OF *LISTERIA MONOCYTOGENES* IN THE RAT.

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Epidemiological studies have shown an increased incidence of respiratory illness in welders. Stainless steel (SS) welding fumes have been shown to have a greater effect on alveolar macrophage (AM) function than mild steel (MS) fumes. The objective of this study was to evaluate the effects of different welding fumes on the clearance of a bacterial pathogen from the lungs. Fumes were collected during flux-cored manual metal arc (MMA) and gas metal arc (GMA) welding using either SS or MS consumable electrodes. The fume composition was: **1) GMA-SS**: 52.3% Fe, 22.2% Cr, 18.3% Mn, 4.9% Ni, 2.3% Si; **2) GMA-MS**: 89.2% Fe, 8.2% Mn, 2.6% Si; **3) MMA-SS**: 22.3% K, 19.4% Fe, 13.1% Cr, 12.6% Si, 8.2% Ca, 8.0% Mn, 17.4% other. CD/VAF rats were dosed intratracheally with saline (control) or the welding samples at a dose of 1.0 mg/100 g b wt. At 1 and 35 days post-instillation, 5000 *Listeria monocytogenes* were intratracheally instilled into the treated animals. Five days after intratracheal exposure to *L. monocytogenes*, the lungs and spleen were removed, homogenized, and cultured quantitatively on Brain Heart Infusion agar at 37° C. Colony forming units (CFUs) were counted after an overnight incubation. After a 1 day fume treatment, all three welding samples caused a significant decrease ($p<0.05$) in the 5-day clearance of *L. monocytogenes* from the lungs as compared to control. At 35 days post-instillation, the MMA-SS and GMA-SS samples significantly increased ($p<0.05$) *L. monocytogenes* clearance from the lungs as compared to the GMA-MS and control groups. No significant differences were seen among the treatment groups in the number of bacteria cultured from the spleen. We have demonstrated that subchronic exposure to SS fumes may have a greater effect on the pulmonary clearance of *L. monocytogenes* as compared to MS fumes. This is most likely due to differences in fume composition and the greater effect of SS fumes on AM function.

617 APOPTOSIS INDUCTION AFTER SILICA INHALATION IN RATS.

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Occupational exposure to crystalline silica (quartz) has been associated with lung damage and fibrosis. In vitro studies have shown that silica induces apoptosis in human alveolar macrophages and in vivo after intratracheal

instillation in rats. These studies suggested that silica inhalation may stimulate apoptosis and that apoptosis may play a role in the silicotic process. A first step in investigating these questions would be to establish the temporal relationship between silica exposure, apoptosis and lung inflammation and damage. Thus, rats were exposed to filtered air (control) or silica aerosol of 15 mg/m^3 (6 hr/day, 5 days/week) and apoptosis in bronchoalveolar lavage cells (BALC) or lung tissue were determined during a 116 day exposure. The lung inflammatory response to inhaled silica was monitored by determining polymorphonuclear leukocyte and alveolar macrophages (AM) in BALC while lung damage was assessed by measuring acellular bronchoalveolar lavage fluid albumin. In silica-exposed rats, all three parameters were elevated versus controls from 5-41 days of exposure, then dramatically increased thereafter versus controls. Apoptosis in BALC was measured with a commercial ELISA assay kit, and values from silica-exposed rats were normalized to controls. The BALC data indicate that apoptosis was evident after only 5 days of exposure, increased steadily until 20 days of exposure, remained relatively constant through 79 days of exposure and then decreased. Lungs, removed from both control and silica-exposed rats, were fixed with 10% neutral buffered formalin and embedded in paraffin for further examination. Apoptosis was assessed in lung tissue slices using the TdT-mediated dUTP biotin nick end-labeling (TUNEL) assay. The TUNEL assay results were consistent with the observed trends in apoptosis measured in BALC and also indicated that the apoptotic cells were AM. These data indicate that the inhalation of silica stimulates apoptosis as predicted, and the magnitude of the apoptotic response changed during the silica exposure. Finally, the observed acceleration of lung inflammation and damage was associated with a decline in apoptosis, suggesting that apoptosis may play a role in the silicotic process.

618 TEMPORAL RELATIONSHIPS BETWEEN BIOCHEMICAL MEDIATORS OF LUNG DAMAGE AND FIBROSIS AFTER SILICA INHALATION IN RATS.

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Crystalline silica (quartz) is a well established inflammatory and fibrogenic occupational dust. Past studies have established numerous biochemical mediators of these processes, but the temporal relationships between them have not been determined. To investigate these temporal relationships, rats were exposed to filtered air (control) or silica aerosol of 15 mg/m^3 (6 hr/day, 5 days/week) and assays were conducted after 5, 10, 16, 20, 30, 41, 79 and 116 days of exposure. Rat lungs were lavaged to isolate bronchoalveolar lavage cells (BALC) and acellular bronchoalveolar lavage fluid (BALF). Pulmonary inflammation was monitored by measuring BALC polymorphonuclear leukocytes (PMN) and alveolar macrophages (AM) differential cell counts. Compared to control, PMN in BALC isolated from silica-exposed rats were significantly increased after 5 days exposure, remained elevated until 41 days, then increased further. BALC AM also were increased in silica-exposed rats, but only after 41 days exposure. Silica cytotoxicity was monitored by analysis of BALF for lactate dehydrogenase (LDH) and albumin. In silica-exposed rats, both LDH and albumin levels were increased versus control after 5 days exposure, remained relatively constant until day 41, then increased further. AM chemiluminesence, a measure of AM activation and reactive oxygen species production, was higher in silica-exposed rats when compared to control. After 41 days of exposure, lung lipid peroxidation was also higher in silica-exposed rats. BALC secretion of TNF-α and IL-1, when considered on a per cell basis, was higher in silica-exposed versus control rats by 116 days exposure. Lung fibrosis was confirmed by increased hydroxyproline levels in the lungs of silica-exposed but not control rats after 116 days exposure. These data indicate that a progressively severe inflammatory reaction occurs in response to inhaled silica and begins to establish the temporal relationships between the various components of the inflammatory response and the development of pulmonary fibrosis.

619 PULMONARY RESPONSES TO SINGLE VERSUS MULTIPLE INTRATRACHEAL INSTILLATIONS OF SILICA IN RATS.

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The pulmonary toxicity of particles is often studied using a single intratracheal (I.T.) instillation of the material. Criticism has been levied that this procedure is not representative of inhalation exposure. In this study, we compared the pulmonary responses in male F344 rats to a single I.T. instillation of crystalline silica (5 mg/100 gm BWt) given on day 0 with those resulting from 5 consecutive daily I.T. instillations of the dust (1 mg/100 gm BWt per day) with the initial dose given on day 0. Controls received the sterile saline vehicle I.T.. The multiple instillation protocol was used as an experimental surrogate for inhalation exposure. The total amount of silica instilled was the same in the two protocols. Responses were assessed on day 14. The indices of response were cellular differentials recovered by bronchoalveolar lavage (BAL) and the level of albumin in BAL fluid (BALF). With both instillation protocols, the total cells, PMNs, and lymphocytes recovered and albumin levels in BALF were increased significantly in the silica-treated rats versus the respective controls. There were no significant differences between the single and multiple instillation protocols for any of these values for either controls or silica-treated rats. Therefore, at this high dose of silica, where particle overload may be occurring, the single and multiple instillation protocols gave similar pulmonary responses using these endpoints. Responses at other doses of silica and the use of additional endpoints are being investigated.

620 HUMAN BRONCHOEPITHELIAL CELLS CAN DIRECTLY INDUCE LUNG FIBROBLAST GENE EXPRESSION OF EXTRACELLULAR MATRIX PROTEINS AND FIBROGENIC CYTOKINES FOLLOWING ASBESTOS EXPOSURE IN VITRO.

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Direct interactions between human pulmonary epithelial cells and lung fibroblasts, representing two cell types of central regulatory potential in (chronic) lung disease, were studied in a coculture system in vitro as a model for chemical-induced pulmonary lesions such as fibrosis. Membrane cultures of human bronchoepithelial cells (BEAS-2B) were exposed to crocidolite asbestos (2-100μg/cm^2) for 24 h or 96 h and subsequently cocultivated with human lung fibroblast (WISTAR-38) cultures in collagen gels for 48 h or 70 h, respectively. Gene expression of procollagens type I and III, fibronectin (FN) and of the fibrogenic cytokines IL-6 and GM-CSF was determined by RT-PCR. Gene expression of procollagens and FN showed slight but consistent increases less than twofold above control levels after 48 h or 70 h of cocultivation with bronchoepithelial cells pretreated with 50 and 100 μg/cm^2 crocidolite, which was most evident for procollagen type III and FN. Cytokine mRNA levels were already enhanced at the lowest fibre concentration and dose-dependently increased more than twofold above control values after 48 h cocultivation. Constitutive expression of steady-state mRNA levels of both cytokines appeared almost maximally induced in fibroblasts when cocultivated with epithelial cells for 70 h and therefore further enhancement by increasing asbestos concentrations remained less than twofold above controls. 24 h pretreatment of bronchoepithelial cells with nonfibrogenic titanium dioxide could only induce mRNA expression of the cytokines in fibroblasts which occured to a considerably lower extent and only at concentrations above 50 μg/cm^2 TiO_2. Our preliminary results suggest that bronchoepithelial cells can mediate enhanced fibroblast activity in vitro by interacting with fibrogenic agents and thus directly contribute to the development of long-term lung lesions such as fibrosis.

621 CELLULAR INJURY AND REPAIR: REVERSIBILITY OF PULMONARY FIBROTIC LESIONS IN RATS INHALING p-ARAMID RFP.

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Inhalation of asbestos and other fiber-types can induce lung fibrosis. This is considered to be a permanent cellular effect characterized by a progressive tissue thickening response. This study was conducted to assess injury and repair at sites of fiber deposition following high dose inhalation exposures to p-aramid RFP in rats. Rats were exposed to 419 and 772 p-aramid respirable-sized fiber-shaped particulates (RFP) per cubic centimeter (f/cc) for two weeks. Animals were sacrificed and tissue effects analyzed immediately after a 2-week exposure as well as 1, 4, 12, 26, and 52 weeks postexposure. Bronchiole alveolar duct junctions (BADJ) were identified, magnified, and morphometrically analyzed. The volume to surface area ratio (i.e., tissue density or thickness) of BADJs from both exposure groups were compared with sham control rats exposed to filtered air. The results showed that exposures to 419 and 772 p-aramid RFP/cc induced initial increases in tissue density at BADJs for the early postexposure time points. These increases in alveolar tissue volume were most prominent at the 1-4 week postexposure

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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 38th Annual Meeting of the Society of Toxicology, held at the Ernest N. Morial Convention Center, New Orleans, Louisiana, March 14-18, 1999.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 419.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 444.

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