692 APOPTOSIS INDUCED BY INTERACTIONS BETWEEN MOLDY HOUSE MICROBES.

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Exposure to complex mixture of bacteria and fungi in moldy buildings is a potential cause of inflammatory related symptoms among the occupants. In this study, capability of these microbes to cause apoptotic cell death was investigated in more details. In addition, microbial interactions on apoptosis was identified in mouse macrophages, when the microbes were cultured separately and together on the same culture plate. The fungal strain *Stachybotrys chartarum* and the gram-positive bacterial strain *Streptomyces californicus* were selected in this study based on their characteristic occurrence in water damaged buildings. These microbes were co-cultivated on the same agar plate. The both strains were also cultured separately and the strains were combined at the same proportion as the co-cultivated combination of *S. chartarum* and *Str. californicus* (1:5). Mouse macrophages (RAW264.7) were exposed to combination of spores and spores individually. In the dose response study the macrophages were exposed to six doses (1×10^4-3×10^6 spores/ml) for 24 hours. For the time course study the macrophages were exposed to the dose of 3×10^5 spores/ml for 4, 8, 16 and 24 hours. Apoptosis was measured by flow cytometry using propidium iodide (PI) staining of permeabilized cells. Changes of caspase-3 activity in mouse macrophages were detected by fluorometric substrate cleavage assay. Flow cytometric analysis of DNA fragmentation showed, that there was 2.6±0.1 % apoptotic cells when exposed (3×10^5 spores/ml at 24 hr) to separately cultured combination, but the amount of apoptotic cells increased to 12.4±1.3 % when exposed to co-cultivated combination. Furthermore co-cultivation increased the caspase-3 activity by 2.4-fold compared to separately cultured combination. Altogether, this data suggests that co-culture of *S. chartarum* and *Str. californicus* leads to microbial interactions, which significantly affect on the ability of microbes' spores to cause apoptosis in mammalian cells.

693 DIFFERENCES IN THE ONSET OF APOPTOSIS IN OLFACTORY SENSORY CELLS OF MICE, RATS AND MONKEYS GIVEN AN INTRAVENOUS INJECTION OF MAXIMUM TOLERATED DOSE (MTD) OF VINCRISTINE, A VINCA-ALKALOID ANTITUMOR DRUG.

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We have previously reported that vincristine sulfate (VCR) causes apoptosis in the olfactory sensory cells of male mice following an intravenous injection. In the present study, we investigated differences in the onset of apoptosis in the olfactory epithelium of BALB/c mice and Crj:CD(SD)IGS rats given an intravenous injection of VCR at a MTD and estimated 10% lethal dose (LD10), and of common marmoset monkeys receiving MTD. Namely, VCR was intravenously administered once to mice (1.17 and 1.95 mg/kg), rats (0.21 and 0.35 mg/kg) and monkeys (0.35 mg/kg) of both sexes. The dosing day was designated as Day 1 in this study. The animals were serially necropsied on Days 2, 5 and 10, and the nasal tissue and sciatic nerve were examined light-microscopically. The olfactory sections of mice on Day 2 were subjected to the terminal deoxyribonucleotidyl transferase-mediated dUTP-digoxigenin nick-end labeling (TUNEL) assay and electron-microscopic observation. In mice, VCR elicited cell deaths with condensation and fragmentation of nuclei in the olfactory epithelia and vomeronasal organs in males given 1.95 mg/kg and in females receiving 1.17 mg/kg or more from Day 2. On Day 10, demyelination in the sciatic nerve was noted in both sexes at 1.17 mg/kg or more. TUNEL-positive cells were also recognized in the basolateral olfactory epithelium. Ultrastructurally, condensation and margination of chromatin were observed in sensory cells of the basolateral olfactory epithelium. However, no morphological changes related to VCR treatment were observed in rats and monkeys of either sex. In conclusion, these results demonstrate that MTD of VCR evokes severe olfactory apoptosis only in mice, especially in females.

694 EFFECTS OF STAINLESS STEEL MANUAL METAL ARC WELDING FUMES ON DNA DAMAGE AND APOPTOSIS INDUCTION *IN VITRO* AND *IN VIVO*.

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Epidemiological studies examining the potential association of occupational inhalation of welding fumes and increased incidence of lung cancer have not reached definite conclusions. Stainless steel (SS) welding fumes are of particular interest because they contain potential human carcinogens, such as chromium and nickel. Animal studies addressing toxicological responses related to carcinogenicity are currently lacking, although SS welding fumes have been shown to be mutagenic *in vitro*. The goals of this study were to examine the potential for SS welding fumes to damage DNA and induce apoptosis, events that are associated with possible carcinogenic activity. In a previous study using electron spin resonance, the generation of hydroxyl radicals from Cr(VI) in the SS fume (1.0 mg/ml) was observed. In the current study, SS fume at the same concentration caused plasmid DNA strand breakage *in vitro* under similar conditions. To examine the effects of SS fumes on apoptosis *in vivo*, male Sprague-Dawley rats were intratracheally instilled with SS fumes from manual metal arc welding (1.0 mg/100 g bw), Cr(VI) (0.2 mg/100 g bw) as a positive control, or the saline vehicle. On days 1, 3, 6, 8, and 10, the rats were euthanized and their left lungs were excised and cryo-sectioned. Apoptosis was examined in the tissue sections by TUNEL assay. Increased numbers of apoptotic cells were found in lung tissue treated with either the SS fume or the positive control, Cr(VI), as compared to lungs from saline-treated animals. These preliminary studies indicate that SS welding fumes can damage DNA *in vitro* and induce apoptosis *in vivo*. Studies are ongoing to determine if the apoptotic event is associated with an increase in oxidative DNA damage in lung tissue.

695 INHALED OZONE INDUCES DNA-DNA CROSS-LINKING IN EXPOSED RAT LUNG.

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Ozone (03) exposure has been shown to cause a variety of debilitating respiratory disturbances including possibly cancer. The prevalence of 03 as the dominant oxidant of world-wide photochemical smog, its occupational utilization and its application as a direct or adjunct therapy for treatment of various diseases make it a global human health concern. As the major target of 03 inhalation, lung tissue has been reported to sustain both cytotoxic and genotoxic damage as well as cause neoplastic transformation *in vivo* and *in vitro*. However, whether exposure to O3 can cause DNA-DNA cross-linking is as of yet unknown. Thus, this study examined whether long-term /chronic 03 exposure could induce pulmonary genomic DNA-DNA cross-linking. Adult male rats were exposed nose-only, 5h/d, 5d/wk for 4, 8, 12, 24 and 48 wks to filtered air or one of three different O3 concentrations (i.e., 0.1, 0.3 and 0.6 ppm). Rats were sacrificed 3d after the cessation of exposure lungs were removed and flash frozen and DNA-DNA cross-links determined by gel electrophoresis. Effects of 03 appeared to be time- and dose- dependent. Animals exposed to 0.3 ppm 03 for 8 wks demonstrated significantly elevated levels of cross-linking compared to controls and 0.1 and 0.6 ppm 03 exposed rats; exposure for twelve wks to 0.3 and 0.6 ppm 03 resulted in greater DNA cross-linking than controls or the 0.1 ppm 03 group; inhalation exposure of rats for twenty four wks to all three concentrations caused a significant dose-related increase in cross-linking; at 48 wks rats exposed to 0.3 ppm O3 demonstrated significantly elevated levels of cross-linking (compared to air controls). These findings suggest that long-term inhalation of 03 can produce genotoxic events that could be associated with neoplastic changes. NIOSH # OH03607.

696 ROLE OF INDUCIBLE NITRIC OXIDE-DERIVED NITRIC OXIDE IN SILICA-INDUCED PULMONARY INFLAMMATION AND INJURY.

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Our lab has demonstrated previously that exposure of rats to silica resulted in induction of inducible nitric oxide synthase (iNOS) in alveolar macrophages (AM) and alveolar type II epithelial cells (TII). The production of nitric oxide (NO) by these pneumocytes and resultant NO-dependent damage (nitrotyrosine residues) have been related temporally and anatomically with silica-induced inflammation and granuloma formation (Am J Physiol Lung Cell Mol Physiol 283:L485, 2002). The objective of the present study was to determine if these associations represent a causal role for NO in silica-induced lung disease. To address this question, the subchronic response of C57BL/6J wild type (WT) mice and iNOS knockout (KO) mice to aspiration of silica (40 mg/kg) was compared 42 days post-exposure. Exposure of WT mice to silica was marked by the following sub-chronic responses: pulmonary damage (increased LDH and albumin levels in lung lavage fluid, and increased lung weight), inflammation (increased lung lavage polymorphonuclear leukocytes, TNF-alpha, MIP-2 and TGF-beta, and histological evidence of alveolitis and lipoproteinosis), oxidant stress (increased zymosan-stimulated chemiluminescence from AM, and decreased total antioxidant levels in lung lavage fluid), and

fibrosis (increased lung hydroxyproline levels). Absence of the iNOS gene in KO mice resulted in a significant decrease in markers of pulmonary damage, inflammation, oxidant stress and fibrosis 42 days after silica exposure. These data indicate that NO production plays a causal role in the progression of silica-induced lung disease.

697 ROLE OF NITRIC OXIDE IN MEDIATING ALVEOLAR MACROPHAGE RESPONSES TO DIESEL EXHAUST PARTICLES.

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Diesel exhaust particles (DEP) are known to suppress the innate immune responses of alveolar macrophages (AM) against bacterial infection. In particular, although DEP induce AM production of nitric oxide (NO) through particle stimulation, the organic component of DEP strongly inhibits pathogen-induced NO production by AM. The present study examined whether NO may affect the secretion of pro- and anti-inflammatory cytokines by AM resulting in suppression of the host defense in response to DEP exposure. Male Sprague-Dawley rats were intratracheally (IT) instilled with saline or DEP (35 mg/kg body weight). To inhibit DEP-induced inducible nitric oxide synthase (iNOS), another group of rats were treated with an iNOS inhibitor, aminoguanidine (AG), by i.p. injection of 100 mg/kg AG, 30 min prior to and 3, 6 and 9 h after IT instillation of DEP or saline. Rats were sacrificed at 1 day post-DEP exposure, and AM were harvested. The results show that DEP induced iNOS expression in AM, which was not affected by AG. AG did not affect DEP-induced inflammatory markers in the lung (neutrophil infiltration and protein content), but significantly lowered the DEP-induced iNOS activity in AM. The production of nitrite by DEP-exposed AM was reduced from 52 ± 16 to 18 ± 4 μM by AG treatment. AG treatment inhibited the secretion of TNF-α, pro-inflammatory cytokine, by AM in response to DEP exposure (from 3424 ± 676 to 1511 ± 428 pg/ml). DEP exposure induced AM production of IL-10, an anti-inflammatory cytokine that is often induced by intracellular pathogens to dampen the host defense mechanism. Inhibition of iNOS activity by AG further enhanced this DEP-induced IL-10 production (from 38 ± 20 to 199 ± 86 pg/ml), suggesting that NO, by inhibiting IL-10, plays an important anti-bacterial role. In summary, this study shows that inhibition of NO by AG significantly decreases TNF-α secretion, while further enhancing IL-10 production by DEP-exposed AM. This change in pro-/anti-inflammatory cytokine balance may make the lung more susceptible to bacterial infection.

698 *IN-VITRO* INFLAMMATORY AND CYTOTOXIC RESPONSES TO AMBIENT AIR PARTICULATE SAMPLES COLLECTED DURING LONG-RANGE TRANSPORT (LRT) OF FOREST FIRE SMOKE TO HELSINKI, FINLAND.

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Ambient air particulate (PM) samples from three different air pollution situations in autumn 2002 were studied: normal seasonal sample and two episodes of LRT forest fire smoke. The samples were collected with a high volume cascade impactor in three size fractions: Fine2 (F2, PM1-0.2), Fine1 (F1, PM2.5-1) and Coarse (CO, PM10-2.5). Episodes affected mostly the F2 fraction: the total mass concentration increased 5-10 times and the $PM_{2.5}$ mass concentrations for K+, oxalate and malonate increased also strongly. Cultures of mouse macrophage cell line (RAW 264.7) were exposed in a dose-related manner (15, 50, 150 and 300 μg/ml) to the PM samples for 8 or 24h. We measured nitric oxide (NO) production by the Griess method, production of cytokines (TNFα, IL-1, IL-6, IL-10 and MIP-2) immunochemically (ELISA) and cytotoxicity with the MTT test. Both the size fraction and the air pollution situation affected the PM induced cytotoxic and proinflammatory responses. The CO and F1 fractions were roughly equipotent but at the same time more potent inducers of cytotoxicity and cytokine production than the F2 fractions. The potency difference in TNFa production was 100-fold. The first forest fire episode increased TNFa production most within F2 fractions, whereas within F1 fractions, the second forest fire episode and the normal seasonal sample gave the larger responses. In NO and MIP-2 production, and cytotoxicity, the normal seasonal F2 fraction was more potent than the forest fire episodes. Thus, the PM chemistry changes caused by the LRT-episodes of forest fire smoke were differently reflected in the toxicity of the F2 and F1 fractions.

699 NRF2 PLAYS A CRITICAL ROLE IN CONFERRING PROTECTION AGAINST INFLAMMATION IN A MOUSE MODEL OF ASTHMA.

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The critical role of Nrf2, a b-zip transcription factor that positively regulates several antioxidant enzymes and cytoprotective proteins, against inflammatory response in lung was investigated in a murine model of asthma. Both Nrf2 +/+ and -/- mice were sensitized (on day 0 and 14) and challenged (on day 24th, 26th and 31 st) with ovalbumin (OVA). In response to OVA challenge, there was a progressive increase in the infiltration of inflammatory cells, particulary eosinophils (80 %) into the lungs of Nrf2 -/- than Nrf2 +/+ mice from 1st to 3rd OVA challenge. Staining of the lung sections with PAS and H&E also showed increased mucus cell metaplasia and infiltration of eosinophils into the lungs of the OVA treated Nrf2 -/- mice. There was an elevated level of of TH-2 cytokines (IL-4 and IL-13) in the BAL fluid of OVA treated Nrf2 -/- mice. In response to acetylcholine, OVA treated Nrf2 -/- mice showed increased airway hyperresponsiveness than Nrf2 +/+ mice. Activation of Nrf2 in the lungs of Nrf2 +/+ mice in response to OVA was confirmed by EMSA. Real Time PCR analysis showed the increased transcriptional induction of an array of antioxidant genes in the lungs of OVA treated Nrf2 +/+ mice. Increased eosinophilia, mucus cell metaplasia, airway hyperresponsiveness and elevated level of TH-2 cytokines in Nrf2-deficient mice in response to allergen suggests the pivotal role of Nrf2 in the protection of lungs against ovalbumin induced asthmatic response. (This work was supported by grants: P50 CA058184-09 and NIEHS center grant P30 ES 03819)

700 LACK OF NRF2 AUGMENTS LIPOPOLYSACCHARIDE INDUCED INFLAMMATION IN MICE LUNGS.

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Lung inflammatory diseases such as acute lung injury and chronic obstructive pulmonary disease are predominantly associated with oxidative stress. Recent findings on the transcriptional targets of Nrf2 revealed its regulatory role on several detoxifying enzymes, antioxidants and NADPH-regenerating enzymes suggest its protective function against a wide spectrum of toxicants. To evaluate the protective role of Nrf2 against inflammation in lungs, we employed the murine model of lipopolysaccharide (LPS) induced acute lung injury. LPS caused greater increase in wet/dry lung weight, inflammatory cells, TNF-a level in bronchoalveolar lavage fluid of Nrf2 -/- mice than those of Nrf2 +/+ mice. Electrophoretic mobility shift assay showed greater increase of NF-κB activity in the Nrf2 -/- lungs than Nrf2 +/+ lungs in response to LPS, which may be responsible for differences in inflammation. LPS treatment also resulted in higher oxidative stress in the lungs of Nrf2 -/- than Nrf2 +/+ mice as determined by the ratio of GSH/GSSG. mRNA levels of antioxidant genes such as g-glutamylcysteine synthetase (regulatory subunit), glutathione reductase, heme oxygenase and the specific activity of glutathione peroxidase, gluathione reductase, and glucose-6-phosphate dehydrogenase were significantly higher in the lungs from Nrf2 +/+ mice compared to Nrf2 -/- mice after LPS challenge. Results demonstrated that Nrf2 plays a significant role in protection against endotoxin-induced lung inflammation and resulting lung injury by elevating antioxidant defense systems (supported by P50 CA058184-09 and NIEHS Center grant-P30-ES03819).

701 ROLE OF TOLL LIKE RECEPTOR-4 IN OZONE-INDUCED PRODUCTION OF INFLAMMATORY MEDIATORS AND TOXICITY.

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Studies from our laboratory have demonstrated that the toxicity of inhaled ozone is due, in part, to cytotoxic inflammatory mediators including nitric oxide, generated from inducible nitric oxide synthase (NOSII), tumor necrosis factor alpha (TNFα), and PGE2 produced *via* cyclooxygenase-2 (COX-2) released from activated alveolar macrophages. Toll like receptor-4 (TLR-4) is a transmembrane protein important in macrophage-mediated inflammatory responses initiated by lipopolysaccharide. Engagement of TLR-4 leads to activation of the transcription factor NF-κB which regulates NOSII, COX-2 and TNFα. In the present studies we analyzed the role of TLR-4 in ozone-induced macrophage activation, inflammatory mediator production, and toxicity. Treatment of control C3H/OuJ mice with ozone (0.8 ppm, 3 hr) resulted in alveolar epithelial injury as measured by protein accumulation in bronchoalveolar lavage fluid (BAL). In contrast, BAL fluid protein

Society of Toxicology
43rd Annual Meeting
Baltimore, Maryland

THE TOXICOLOGIST

a supplement to
TOXICOLOGICAL SCIENCES

An Official Journal of the Society of Toxicology

Volume 78, Number S-1, March 2004