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

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