1647 REDUCED RESPONSIVENESS OF CIRCULATING LEUKOCYTES FOLLOWING METAL-RICH PARTICULATE MATTER EXPOSURE.

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Exposure to metal-rich particulate matter generated from welding has been linked to cardiovascular dysfunction and reduced immune competence. The aim of this study was to evaluate the molecular changes and responsiveness of circulating leukocytes following welding fume exposure. Rats were exposed to manual metal arc stainless steel welding fume (MMAW-SS) at 2 mg/rat by intratracheal instillation and harvested 4 and 24 hr post-exposure. Blood was collected and analyzed for differential changes by flow cytometry and gene expression changes by microarray and subsequent pathway analysis. In addition, anticoagulated blood was incubated for 24 hr with and without LPS stimulation utilizing the TruCulture® whole blood collection system. After incubation, supernatants were collected for protein analysis and the cellular fraction was collected for gene expression changes. Analysis of microarray data from 4hr post-exposure showed 254 network eligible genes (137-up and 117-down). The top biological category "inflammatory response" had 70 molecules of which 75% were significantly reduced. By 24 hr there were 75% fewer network eligible and altered "inflammatory response" genes. These results indicate a rapid effect on the circulating blood cell population after pulmonary exposure that was less apparent with time. Ex vivo stimulation with LPS of circulating leukocytes showed reduced production of CCL4, CXCL2, CXCL10 and TNF alpha protein in MMAW-SS treated rats. Cellular gene expression changes from MMAW-SS and PBS rats were similar after ex vivo LPS stimulation indicating effects were not at the transcriptional level. These results showed a reduced capacity of circulating leukocytes to produce inflammatory proteins in response to a secondary stimulus following a metal-rich particulate matter pulmonary exposure and provide mechanistic insight into epidemiological and experimental evidence illustrating immunosuppression following welding fume exposure.

1648 OXIDATIVE STRESS, DNA DAMAGE, AND INFLAMMATION-INDUCED BY WOOD SMOKE PARTICULATE MATTER IN HUMAN A549 AND THP-1 CELLS.

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PURPOSE: Especially in wintertime, combustion of wood for residential heating and/or cooking substantially contributes to both outdoor air and indoor levels of particulate matter (PM). The purpose of this study was to investigate the pro-inflammatory effects of wood smoke PM in the human lung epithelial cell line A459 and in the human promyelocytic cell line THP-1 comparing wood smoke PM obtained from high or low oxygen combustion of beech and fir pellets and reference diesel exhaust particulates (DEP, NIES certified reference material NO.8 vehicle exhausted particles, Japan). In parallel, in A549 cells wood smoke PM-induced DNA damage and oxidative stress were also investigated. METHODS: Cells were treated with increasing concentrations of PM2.5 obtained from wood combustions and DEP (0-100 microg/ml) for different times (3-48 h). Cell viability was assessed by lactate dehydrogenase leakage, and the proinflammatory effect was evaluated by measuring the release of IL-8. Oxidative stress was evaluated by the DCFH-DA assay and DNA damage by the Comet assay. RESULTS: Both A549 and THP-1 cells responded in a dose and time related manner to PM2.5 obtained from incomplete wood combustion in term of release the proinflammatory cytokine IL-8. Similar responses were observed for the particulates obtained from both fir and beech woods as well as DEP. Part of the response observed was due to endotoxin contamination and a role of benzopyrene could be demonstrated for IL-8 release in A549 cells. Both fir and beech wood PM obtained from incomplete combustion also induced a dose related oxidative stress and DNA damage. These results suggest that the proper control of combustion condition strongly affects the characteristic of particulate and the consequent toxicity. This project was funded by Ministero dell'Istruzione, dell'Università e della Ricerca (PRIN2008).

1649 EFFECTS OF GLUTAMATE ON B1A B CELL FUNCTION.

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Inhalation of asbestos fibers can lead to multiple disease states including asbestosis, pleural plaques, and mesothelioma. Additional studies have linked asbestos exposure with systemic autoimmune disease. Macrophages exposed to asbestos demonstrate an increase in reactive oxygen species. To help neutralize reactive oxygen species the xCT system imports cystine to be used for synthesis of antioxidants. xCT also exports glutamate as part of this process, yet the role of glutamate in activating B cells is unknown. B1a B cells are implicated in autoimmunity and are potentially impacted in the pleural cavity following asbestos exposure. Our study focuses on the effects of exported glutamate on B1a cells and its possible connection with the development of autoimmune disease.
The expression of NMDA and mGluR5 glutamate receptors on CH12.LX cells, a B1a cell line, was demonstrated using flow cytometry. Agonists to these glutamate receptors were used to determine the physiological effects of activation of glutamate receptors on B1a cells including proliferation, cytokine production, and antibody production. Additionally, RNA was extracted from CH12.LX cells after exposure to glutamate and expression of genes involved B and T cell activation was quantified using a qPCR array. Results show that glutamate and glutamate receptor agonists exert their effect on proliferation of Ch12.LX cells. This indicates that exported glutamate does have an effect on B1a cells and thereby may contribute to immune activation leading to autoimmunity.

1650 PULMONARY HYPERTENSION-INDUCED BY EXPOSURE TO ANTIGEN AND URBAN PARTICULATE MATTER, ROLE OF IL-33.

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Previous studies in our lab have shown that a prolonged T helper 2 (Th2) response to inhaled antigen induces severe pulmonary arterial remodeling. Urban particulate matter (PM) from air pollution is known to exacerbate lung and cardiovascular conditions. The first goal of our study was to test the hypothesis that PM would also exacerbate pulmonary arterial remodeling and would cause pulmonary hypertension. The second goal was to identify the molecular mechanisms. We focused on IL-33, which can induce Th2 responses, lung inflammation and remodeling. On the other hand, IL-33 signaling via its receptor, ST2, is cardio-protective for the left heart. Urban PM2.5 was collected in New York City. Th2 primed wild type or IL-33 deficient (KO) mice were intranasally challenged with soluble antigen combined with urban PM2.5. Scores for pulmonary arterial remodeling were determined as well as right ventricular systolic pressure by heart catheterization of anaesthetized, spontaneously breathing mice; IL-33 and ST2 gene expression by qPCR. Results and Conclusions: 1) Urban PM2.5 exacerbated antigen-induced pulmonary arterial remodeling.
2) Combined exposure with urban PM2.5 and antigen induced pulmonary arterial hypertension as shown by increased right ventricular systolic pressure and right ventricular hypertrophy. 3) IL-33KO mice were no different from wild type with respect to pulmonary hypertension or right heart hypertrophy induced by combined exposure to antigen and urban PM2.5. IL-33KO mice had similar airway inflammation but reduced dendritic cells in the bronchoalveolar lavage relative to wild type in response to antigen and urban PM.
4) Initial data suggest that IL-33 is down- (not up-) regulated in the right hearts of animals exposed to an exaggerated Th2 response. This finding could explain the lack of a difference between IL-33KO and wild type mice in our experiments. In the future, we plan to test the idea that expressing IL-33 at control levels is beneficial for the right heart during a Th2 response.

1651 CHEMICAL ALLERGEN-INDUCED INTERLEUKIN-17 PRODUCTION: ROLE OF THE INNATE AND ADAPTIVE IMMUNE SYSTEMS.

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T helper (Th)17 cells and innate immune cells, including γδ T cells, express IL-17 cytokines and play important roles in the pathogenesis of autoimmunity. However, little is known of their involvement in immune responses to contact and respiratory allergens. The kinetics of allergen-induced IL-17 expression has now been examined following acute exposure and following immune priming. Using different topical treatment regimens, mice (BALB/c) were exposed to the contact allergen 2,4-dinitrochlorobenzene (DNCB) or to the respiratory sensitiser trimellitic anhydride (TMA). Controls received vehicle alone. At selected time points draining (auricular) lymph nodes were excised and pooled per animal (n=3 per group). Single cell suspensions were prepared, cultured for 120h and supernatants analysed by cytokine ELISA. A single topical (acute) exposure to both allergens on the dorsum of the ears resulted in transient up-regulation of IL-17 cytokines. Maximal levels were observed at 6h and 48h following DNCB and TMA treatment, respectively. Subsequently, mice were primed on both flanks on days 0 and 5, followed by challenge on the dorsum of both ears on days 10, 11 and 12, with lymph nodes excised on day 13. This "chronic" exposure regimen results in the induction of preferential

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