

mation is inflammasome activation in lung macrophages and secretion of IL-1 β . Allergen-challenged mice are susceptible to MWCNT-induced airway inflammation and have elevated levels of the Th2 cytokines IL-13 and IL-4. We postulate that Th2 cytokines modulate MWCNT-induced inflammasome activation and IL-1 β release by human monocytes (THP-1 cells) *in vitro* and in the lungs of mice *in vivo* following house dust mite (HDM) allergen challenge. Methods: THP-1 cells were differentiated with vitamin D3 and TPA, primed with LPS, and dosed with MWCNT alone or with IL-13 or IL-4. IL-1 β in cell supernatants was measured by ELISA. C57Bl/6 mice were exposed to HDM allergen intranasally for two weeks, dosed with MWCNT (2mg/kg via oropharyngeal aspiration), and necropsied 1 day post-MWCNT exposure. Bronchoalveolar lavage fluid (BALF) and serum were analyzed for IL-1 β and IgE, respectively, via ELISA. BALF was also used to determine inflammatory cell differential counts. The left lung was formalin-fixed for histopathology. Results: THP-1 cells treated with MWCNT showed a dose-dependent increase in IL-1 β , while IL-13 or IL-4 significantly decreased MWCNT-induced IL-1 β . HDM sensitization significantly decreased MWCNT-induced IL-1 β levels in BALF from the lungs of mice. MWCNT suppressed HDM-induced serum IgE, but enhanced HDM-induced eosinophil counts in BALF. Conclusions: The allergen-inducible cytokines IL-13 and IL-4 negatively regulate MWCNT-induced production of IL-1 β by macrophages *in vitro*. HDM allergen also decreases MWCNT-induced IL-1 β production in the lungs of mice *in vivo*. However, MWCNT enhance HDM-induced eosinophilic lung inflammation. Further study will address the mechanism through which Th2 cytokines modulate MWCNT-induced inflammasome activation and how MWCNT exacerbate allergic airway inflammation in asthma.

Funding: Supported by NIEHS grants RO1-ESO20897 and RC2- ESO18772.

PS 583 Atomic Layer Deposition Coating of Multiwalled Carbon Nanotubes with Aluminum Oxide Alters Innate Immune Responses *In Vitro* and *In Vivo*

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Multi-walled carbon nanotubes (MWCNTs) pose a possible human health risk for lung disease as a result of occupational or environmental exposure. Macrophages are the first line of defense that engulf and remove inhaled MWCNTs from the lungs. MWCNTs stimulate macrophage inflammasome activation and interleukin (IL)-1 β release, key cellular components of the innate immune response that mediate the initiation and resolution of acute inflammation. Alternatively, macrophages are a source of secreted osteopontin (OPN), which promotes tissue matrix remodeling and fibrosis. Atomic layer deposition (ALD) is a novel process used to enhance surface and functional properties of MWCNTs. The purpose of this study was to determine whether ALD coating with Al₂O₃ would alter toxicity, phagocytosis, or the production of soluble mediators (IL-1 β or OPN) in human macrophages or monocytes exposed to MWCNTs as well as in mice *in vivo*. IL-1 β and OPN protein secretion was measured via ELISA at 24hr post nanotube exposure. ALD surface coating of MWCNTs with Al₂O₃ enhanced IL-1 β secretion, yet inhibited OPN production by THP-1 cells. Experiments conducted with primary peripheral blood monocytes obtained from normal human donors also showed that the Al₂O₃-coating on MWCNTs enhanced IL-1 β secretion and yet suppressed OPN production. C57Bl/6 mice exposed to uncoated MWCNTs for 1 day had increased levels of IL-1 β and OPN in the bronchoalveolar lavage fluid. ALD coated MWCNTs increased IL-1 β in the BAL fluid as well, yet had a suppressive effect on OPN levels. These findings indicate that thin film surface coating of MWCNTs with Al₂O₃ by ALD enhances the innate immune response of mononuclear phagocytes but decreases pro-fibrogenic activity *in vitro* and *in vivo*.

PS 584 Activation and Interplay of the Tumor Suppressor Genes p53 and STAT-1 in Response to Multiwalled Carbon Nanotubes

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Multi-walled carbon nanotubes (MWCNTs) are engineered nanomaterials that possess unique physical properties, making them ideal candidates for use in electronics, engineering and medicine. As they are used in increasing quantities, the potential for occupational and/or environmental exposures is inevitable. There is increasing evidence that MWCNTs may produce toxic effects both in cultured cells and in the lungs of rodents. Studies in human epithelial cells and rats *in vivo*

have shown MWCNTs to cause DNA damage. The tumor suppressor gene p53 is a DNA damage inducible transcription factor involved in cell cycle arrest and apoptosis. Likewise, the transcription factor, STAT-1 serves many of the same functions as p53 and STAT-1 has been found to interact with p53 in mouse embryonic fibroblasts and human fibrosarcoma cells *in vitro*. We explored a possible relationship between p53 and STAT-1 in human mesothelial cells, the target cells of mesothelioma, as well as in the mouse lung *in vivo*, following exposure to MWCNTs. Human mesothelial cells (Met5A cells) were dosed with 10 μ g/cm² MWCNTs, and using western blot analysis, we observed that the phosphorylated or "active" forms of both p53 and STAT-1 were increased 4 hours post-exposure. To determine whether the induction in p-p53 was affected by the presence of STAT-1, we utilized both STAT-1 siRNA to knockdown STAT-1 message in the Met5A cells, as well as a STAT-1 knockout mouse model. In both models, decreased or absent STAT-1 protein, resulted in decreased p53 protein levels. Our results suggest that both p-p53 and p-STAT-1 can be induced as a result of MWCNT exposure and that the induction in p-p53 is dependent on the presence of STAT-1. The findings are relevant to our understanding of the potential for carbon nanotubes to cause pleural disease after inhalation exposure.

Funding: Supported by NIEHS grant RO1-ESO20897.

PS 585 Different Functionalizations of MWCNT Influence Transformation Potential in Primary Human Lung Epithelial Cells

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MWCNT have received increased scrutiny for potential human health impacts based on their fibrogenicity and promotion of pulmonary carcinogenesis. Functionalized CNT (fCNT) development has intensified to improve surface activity in technological applications, and potentially reduce toxicity. Subchronic *in vitro* CNT exposure causes neoplastic-like transformation; however, tumorigenic risk associated with fMWCNT exposure in human lung epithelium is presently unknown. To identify early steps in fMWCNT-induced cell transformation, this study hypothesized that different functional groups of MWCNT determine their neoplastic transformation potential in primary human small airway epithelial cells (SAECs). Cells were continuously exposed (0.06 μ g/cm²) to dispersed pure (pMWCNT), carboxylated (cMWCNT), and aminated MWCNT (nMWCNT) for 8 and 12 weeks. Dispersed ultrafine carbon black (UFCB) and crocidolite asbestos served as controls. Exposed cells were assessed for several established cancer hallmarks and morphological transformation. UFCB and pMWCNT cells at 48h and all MWCNT cells at 6d post-treatment exhibited significant increased proliferation compared to controls. UFCB exposure stimulated significant invasion and migration behavior while pMWCNT and cMWCNT showed moderate significant increases; however, these trends disappeared at 6d post-exposure. Conversely, nMWCNT displayed the largest significant increase in colony formation potential while UFCB showed a moderate significant increase. All other treatments did not differ from controls. UFCB and nMWCNT cells exhibited increased foci frequency, indicative of neoplastic transformation, compared to all other treatments. In summary, surface charge characteristics of carbon nanoparticles can impact transient, early neoplastic transformation events *in vitro* following occupationally relevant exposures.

Disclaimer: Present findings and conclusions are the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

PS 586 Lactoperoxidase-Mediated Degradation of Oxidized Single-Walled Carbon Nanotubes and Its Modulatory Effects on Airway Antibacterial Activity

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Studies in recent years have shown that carbon nanotubes (CNTs) may elicit inflammatory responses. On the other hand, enzymatic biodegradation of CNTs by inflammatory cells has also been reported thus illustrating the reciprocal interactions between CNTs and the immune system. Here we analyzed the biodegradation of oxidized SWCNTs by recombinant lactoperoxidase (LPO), in the presence or absence of lung surfactant (Curosurf®). UV/Vis-NIR and Raman spectroscopy techniques were employed to monitor the biodegradation. There was effective degradation of SWCNTs by LPO and no interference by Curosurf®. AFM and

The Toxicologist

Supplement to *Toxicological Sciences*

53rd Annual Meeting and ToxExpo™

March 23-27, 2014 • Phoenix, Arizona



OXFORD
UNIVERSITY PRESS

ISSN 1096-6080
Volume 138, Issue 1
March 2014

www.toxsci.oxfordjournals.org

An Official Journal of
the Society of Toxicology

SOT | Society of
Toxicology

Creating a Safer and Healthier World
by Advancing the Science of Toxicology

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