4142 Proteinase-activated receptor-2 regulates the production of fibrotic mediators by *ex vivo* murine macrophages and mouse lung fibroblasts in response to multiwalled carbon nanotubes in an asthma-like microenvironment

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Background and Purpose: Multiwalled carbon nanotubes (MWCNTs) are increasingly implicated in the exacerbation of allergic asthma. We previously reported that MWCNTs synergistically enhanced allergic lung disease that featured eosinophilic inflammation, mucous cell metaplasia, and airway fibrosis. Moreover, mice deficient in the proteinase-activated receptor 2 (*Par2*$^{-/-}$) had reduced airway fibrosis and expression of the pro-fibrotic mediator arginase-1 (Arg-1) in lung macrophages *in situ* compared to wildtype (WT) mice. PAR2 is a G protein-coupled receptor that is activated by serine proteases such as those found in house dust mite (HDM) extract and has been implicated in the pathogenesis of asthma and pulmonary fibrosis. In the current study, we utilized bone marrow-derived macrophages (BMDMs), murine *ex vivo* alveolar macrophages (mexAMs), and mouse lung fibroblasts (MLFs) isolated from WT or *Par2*$^{-/-}$ mice to investigate the dynamic phenotype and functional changes of these cells exposed to MWCNTs in an asthma-like microenvironment consisting of the T_H2 cytokines IL-4 and IL-13. Additionally, we expose MLFs to BMDM-conditioned media to determine functional changes induced by macrophage-derived mediators. We hypothesized that PAR2 regulates the MWCNT-induced enhancement of allergic lung disease by regulating macrophage expression of arginase-1 and, in turn, fibroblast phenotype. **Methods:** Wildtype (WT) C57BL/6 mice and *Par2*$^{-/-}$ mice were used to isolate murine ex vivo alveolar macrophages (mexAM), bone marrow-derived macrophages (BMDM), and mouse lung fibroblasts (MLF). MexAMs were generated by obtaining alveolar macrophages from mice by bronchoalveolar lavage and then cultured in complete culture media supplemented with recombinant human TGF-β1, murine GM-CSF, and rosiglitazone. The remaining lung tissue was proteolytically digested, strained, and used to isolate MLFs. BMDMs were obtained from the bone marrow of the hind legs of mice. Bone marrow was flushed from the femur and tibia and filtered. The resulting cell mixture was cultured in L929-conditioned media for 7 days. Mature cells were pre-treated with IL-4 and IL-13 for 24 hours, followed by MWCNT treatment for an additional 24 hours. Recombinant murine IL-4 and IL-13 were used to simulate an asthma-like microenvironment. Macrophage-conditioned supernatants were used to expose MLFs for 24 hours. After cell treatments, samples were collected for immunoblotting, qRT-PCR, cytokine analysis, and flow cytometry. **Results:** Flow cytometry analysis revealed that MWCNT treatment increased the expression of the M2 polarization marker CD206 in WT and *Par2*$^{-/-}$ BMDMs compared to media control groups. Furthermore, MWCNTs increased Arg-1 production in WT and *PAR2*$^{-/-}$ BMDMs and MexAMs. Additionally, MWCNTs increased IL-4/13-induced Arg-1 expression in WT BMDMs and MexAMs. Western blot analysis corroborated these findings, showing exacerbated IL-4/13-induced Arg-1 and phosphorylated STAT6 (pSTAT6) protein expression in WT BMDMs and MexAMs exposed to MWCNTs. Collectively, these results indicate that MWCNT exposure promotes macrophage differentiation towards an M2 or profibrotic phenotype, evidenced by increased CD206 and Arg-1 expression. Additionally, the expression of collagen mRNAs (*Col1a1* and *Col1a2*) was upregulated in *Par2*$^{-/-}$ MLFs compared to WT MLFs stimulated with macrophage-conditioned media. This further suggests that PAR2 plays a role in collagen production and pulmonary fibrosis. **Conclusions:** These findings indicate that PAR2 regulates the production of fibrotic mediators by MWCNTs in macrophages and fibroblasts in a cell-type-specific manner. The data suggests that PAR2 suppresses the production of profibrotic mediators and collagen production in mouse lung fibroblasts. However, PAR2 appears to function differently in macrophages. In BMDMs and mexAMs, PAR2 regulates the production of ARG-1, which suggests that PAR2 helps regulate macrophage differentiation in addition to fibrotic mediators. Furthermore, MWCNTs exacerbate the production of profibrotic mediators as well as macrophage polarization in an asthma-like microenvironment containing T_H2 cytokines IL-4 and IL-13. Taken together, our data show that PAR2 regulates the profibrotic effects of MWCNTs, in part by modulating macrophage polarization. These findings have potentially important implications for understanding the mechanisms involved in MWCNT-induced exacerbation of allergic lung disease.

4143 4,4'-Methylene Diphenyl Diisocyanate-Glutathione Conjugate Exposure Downregulates Endogenous *hsa-miR-381-3p* Through Induction of Circular RNA *hsa_circ_0099188* in Macrophages

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Background and Purpose: Exposure to 4,4'-methylene diphenyl diisocyanate (MDI), the most widely used monomeric diisocyanate, in the occupational setting may lead to the development of occupational asthma (OA). Currently, the underlying molecular mechanism(s) by which MDI induces OA have yet to be elucidated. Alveolar macrophage (MØ) dysfunction plays an important role in asthma pathogenesis. Previously, our laboratory showed that MDI exposure downregulates endogenous *microRNA(miR)-206-3p/-381-3p,* activating *miR-206-3p/-381-3p*-regulated signaling, including PPP3CA/calcineurin/NFAT and KLF4 signaling activation in MØs, to increase chemokine production and promote chemotaxis activities of immune cells. Our previous report showed that MDI exposure in the form of MDI-glutathione (GSH) conjugate may influence the expression of endogenous circular RNA (circRNA) *hsa_circ_0008726* to modulate endogenous *hsa-miR-206-3p* and *hsa-miR-206-3p*-mediated downstream regulatory pathways; however, the MDI-mediated circRNA response(s) that target endogenous *hsa-miR-381-3p* and *hsa-miR-381-3p*-mediated downstream regulatory pathways is currently unknown. Several endogenous circRNAs have been reported to regulate endogenous *hsa-miR-381-3p* levels through potential competitive endogenous RNA (ceRNA) mechanisms. We hypothesize that MDI-GSH conjugate exposure induces endogenous circRNA(s) to regulate *hsa-miR-381-3p* in MØs. **Methods:** We determined the expression of candidate *hsa-miR-381-3p* binding circRNAs including *hsa_circ_0021593, hsa_circ_0084003,* and *hsa_circ_0099188* from MDI-GSH conjugate-treated differentiated THP-1 macrophages using RT-qPCR. **Results:** *In vitro* MDI-GSH conjugate exposures result in the upregulation of endogenous *hsa_circ_0099188* and its host gene transcript thyrotropin releasing hormone degrading enzyme (*TRHDE*);whereas other circRNA(s) examined were neither detected nor changed in MDI-GSH conjugate exposed MØs. RNA-induced silencing complex-immunoprecipitation (RISC-IP) experiments indicated that *hsa-miR-381-3p* binds to *hsa_circ_0099188* in MØs. The expression of endogenous *hsa-miR-381-3p* was either up- or down-regulated by transfection of either *hsa_circ_0099188* siRNAs or *hsa_circ_0099188* overexpression plasmid in MØs, respectively. **Conclusions:** These results suggest MDI exposure may downregulate endogenous *hsa-miR-381-3p* through induction of *hsa_circ_0099188/TRHDE* thus contributing to the upregulation of *hsa-miR-381-3p*-mediated regulations in MØs.

4144 Prenatal phthalate exposure and DNA methylation trajectories in childhood and adolescence in a Mexican birth cohort

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Background and Purpose: Phthalates are a class of human-made chemical compounds used to make plastics flexible and to enhance fragrances. Phthalates are endocrine disrupting chemicals, and gestational exposures to these chemicals are associated with reproductive health, hormone homeostasis, and metabolic dysregulation. Less is known about how exposure to phthalates during the gestational period impacts health trajectories throughout childhood. Gestational phthalate exposure is associated with epigenetic changes including to DNA methylation in the offspring, which may be a mechanistic link between exposure and later-in-life outcomes. DNA methylation also changes over time at some, but not all, genes. There is evidence in animal models that early life exposures can alter the trajectory of age-related epigenetic changes, a process called environmental deflection. We investigated the relationship between gestational phthalate exposure and DNA methylation at three time points: birth, childhood, and adolescence. The purpose of this study was to first examine the change in DNA methylation across the epigenome from birth to adolescence, and then identify CpG sites with evidence for environmental deflection by phthalates. We hypothesize that gestational phthalate exposure significantly alters the DNA methylation trajectory during this time period. **Methods:** This study included participants in the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) study, a multi-generational prospective cohort study which began in 1994 in Mexico City. Eighty-nine participants had DNA methylation measured from cord blood and venous blood from at least 1 of the following age periods: childhood and adolescence; 70 of these participants also had gestational phthalate exposure data. Methylation was measured via the EPIC array. After quality control, approximately 780,000 CpG sites remained for analysis. Beta values, which represent the percent methylation of each CpG site, were used as the outcome of interest in this study. Phthalate (9 metabolites) concentrations were measured via isotope-dilution liquid chromatography-tandem mass spectrometry in urine samples collected during each trimester, and the geometric mean across all available samples was used for each participant. Participants' exposure to di(2-ethylhexyl) phthalate (DEHP), a high-molecular weight phthalate, was approximated by summing 4 known metabolites of DEHP: mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono(2-ethyl-5-oxohexyl) phthalate (MEOHP). Linear mixed effects models were used to first identify CpG sites that significantly changed across up to 3 age periods, adjusting for cell type composition and batch and with a random intercept for each individual. We then include the summed DEHP metabolite concentration and an interaction term for this concentration and participant age in the linear mixed effects models to assess environmental deflection. **Results:** The median log transformed summed DEHP urinary metabolite concentration was 15.15 µg/L. DNA methylation changed across time at over 290,000 CpG sites (q-value <0.05). In the interaction model to assess environmental deflection, there was evidence for interaction between gestational DEHP exposure and age on levels of DNA methylation at 21 CpG sites (p-value < 0.00001), six of which were significant at q<0.05. These CpG sites mapped to the genes SLC6A9, BAT3, and HLA-DPA1. We are investigating this interaction relationship with 5 additional phthalate metabolites. **Conclusions:** In this epigenome-wide association study, we demonstrate that DNA methylation changes over time from birth to adolescence. Phthalate (DEHP) exposure during the gestational period significantly interacts with age to influence repeat measures of DNA methylation at multiple loci. This is one of the first examples of environmental deflection in a human study. We are continuing to investigate whether additional phthalates modify the relationship of age with DNA methylation, and continued research should examine how this influences child health.

SOT 64TH ANNUAL MEETING & ToxExpo • March 16–20, 2025 ORLANDO, FLORIDA

The Toxicologist

Supplement to *Toxicological Sciences*

SOT | Society of Toxicology

OXFORD UNIVERSITY PRESS

Toxicological Sciences

The Official Journal of the Society of Toxicology

ISSN 1096-6080 Volume 204, Issue S1 (March 2025)
www.academic.oup.com/toxsci

Publication Date: March 5, 2025