Based on this Drp1-lipid droplet positive correlation, we hypothesized that a partial Drp1 knockout/inhibition may reduce lipid droplet accumulation. Using primary microglia from wild-type (*Dnm1l*$^{+/+}$) and Drp1 knockout (*Dnm1l*$^{+/-}$) transgenic mice, we observed that Drp1 inhibition using the small molecule peptide P110 significantly reduced LPS-Induced accumulation of lipid droplets ($p<0.05$, n=3). Similarly, primary microglia from *Dnm1l*$^{+/-}$ had a reduced amount of lipid droplets compared to *Dnm1l*$^{+/+}$ in the presence of LPS. We validated these findings in vivo where *Dnm1l*$^{+/-}$ reduced LPS-induced lipid droplet accumulation in microglia($p<0.05$, n=3). Lipid droplet accumulation in microglia is not unique to LPS because paraquat-induced-lipid droplet accumulation in microglia and *Dnm1l*$^{+/-}$ attenuated such lipid droplet accumulation. Because lipid droplets accumulating microglia have been reported to be inflammatory, we further determined whether the Drp1 knockout/inhibition-mediated reduction in lipid droplet accumulation is accompanied by decreased inflammation. Drp1 inhibition with p110 reduced LPS-induced *Tnfa*, *Il-6*, and *Il-1b* mRNA transcripts in *Dnm1l*$^{+/+}$ primary microglia. Similar results were obtained in *Dnm1l*$^{+/-}$ primary microglia compared with microglia from *Dnm1l*$^{+/+}$. **Conclusions:** Taken together, these results indicate that Drp1 plays a crucial role in microglia metabolic reprogramming and lipid droplet accumulation in different stress environments. Furthermore, Drp1 inhibition normalized toxin-induced lipid droplet accumulation and associated inflammation.

4469 Targeted Delivery of TGF-β mRNA to the Lung in Novel Lipid Nanoparticles Reduces Inflammation and Tissue Damage in a Model of Acute Lung Injury

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Background and Purpose: Acute lung injury (ALI) is characterized by an overexuberant inflammatory response that exacerbates alveolar epithelial damage. Current approaches for treatment of ALI focus on inhalation delivery, which, effective for upper-airway disease, is inadequate for ALI since therapeutics do not readily reach the lower respiratory tract. Moreover, inhalation delivery of therapeutics for ALI to the upper airways have resulted in impaired inflammation resolution and fibrosis. To bridge this clinical gap, we have developed a novel lipid nanoparticle delivery system, with specificity to the lower airways after intravenous (IV) administration. Herein we have used the novel lipid nanoparticle for targeted mRNA delivery of a pro-resolution cytokine to the lung in a model of ALI induced by intratracheal bleomycin administration (ITB). **Methods:** One-component Ionizable Amphiphilic Janus Dendrimers (IAJDs) were co-assembled with transforming growth factor beta (TGF-β) mRNA for these studies. To assess delivery and toxicity, IAJDs/TGF-β mRNA (10, 20, 30 μg) were administered to control BALB/c mice via retro-orbital injection. Lung, liver, spleen, and kidney were analyzed 24 hr later. IAJDs/TGF-β mRNA (10 μg) were next administered IV to mice treated IT with PBS control or bleomycin (3 units/kg) to assess their ability to blunt ALI. Three days later, lung tissue and bronchoalveolar lavage fluid (BAL) were collected; hematoxylin and eosin-stained sections were examined for histopathology. Cells recovered from BAL and lung digests were analyzed by flow cytometry for changes in alveolar and interstitial macrophages. **Results:** We found that 10 μg/mouse of mRNA TGF-β delivered in IAJDs selectively upregulated TGF-β protein expression in the lung parenchyma within 24 hr of administration with no evidence of toxicity. TGF-β was not detected in the liver, spleen or kidney and there was no nonspecific organ toxicity. Treatment of mice with ITB mice resulted in increased alveolar wall thickness; this was associated with an accumulation of inflammatory macrophages (CD11c-CD11b+) in the alveolar space and a reduction in resident alveolar macrophages. Administration of IAJDs/TGF-β mRNA (10 μg) to mice mitigated histopathologic changes in the lung induced by ITB; a significant reduction in inflammatory macrophages was also observed with no effect on resident alveolar macrophages. **Conclusions:** These studies demonstrate that we can successfully deliver the pro-resolution cytokine, TGF-β, to the lung parenchyma using mRNA packaged in a novel lower lung specific lipid nanoparticle delivery system. This resulted in reduced inflammatory macrophage activation and consequent ALI. These findings demonstrate that mRNA can be selectively directed to the lower lung resulting in target gene translation and protein expression. The fact that IV administered IAJDs/TGF-β mRNA reduced inflammation and ALI suggest a highly novel approach to mitigating lung injury induced by pulmonary toxicants. Supported by NIH Grants ES007148, ES005022, ES004738, ES033698, National Key Research and Development Program of China [Grant 2022YFC2403203], National Natural Science Foundation of China [Grant 22305081], and Shanghai Sailing Program [Grant 23YF1408600].

4470 Nitroalkenes regulate myeloid cell effector function to promote inflammation resolution

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Background and Purpose: Myeloid cell effector function has emerged as a target of therapeutic intervention in inflammatory disease. Nitro-oleic acid (NO_2-OA) is a reversible, electrophilic fatty acid derivative that initiates diverse, anti-inflammatory signaling. Myeloid-derived cell populations, notably macrophages and eosinophils, are poised as initiators and regulators in many pulmonary pathologies. As such, understanding the metabolic changes mediated by NO_2-OA has broad implications for its therapeutic potential. This work aims to investigate NO_2-OA-mediated shifts in metabolism and the myeloid-specific proteome both *in vitro* and *in vivo*. **Methods:** RAW 264.7 macrophages were stimulated with lipopolysaccharide (LPS, 10 ng/mL) and treated with oleic acid (OA, 5 μM) and NO2-OA (5 μM) in the presence of universally labeled ^{13}C glucose or glutamine. Quantification of intracellular metabolites was performed via liquid chromatography-high resolution mass spectrometry (LC-HRMS). To translate *in vitro* discoveries to a more complex, myeloid cell-driven model of disease, a murine model of allergic airway disease (AAD) was utilized by exposing 15 week-old male C57BL6/J mice to the environmental allergen house dust mite (HDM, 2 μg) with cholera toxin (CT, 0.05 μg) adjuvant. During allergen challenge (d14-18), mice were treated via oral gavage with vehicle (glycerol trioleate, 100 μL) or NO2-OA (25 mg/kg, 100 μL). Analysis of lung function, inflammation, and assessment of myeloid (CD45+) cell dynamics in AAD via metabolomics and proteomics was performed using LC-HRMS. **Results:** NO_2-OA significantly (*$p<0.05$ by one-way ANOVA) diminished LPS-induced increases in metabolites such as succinate (Analyte/Standard: $5.3x10^{-2}±7x10^{-4}$*v $3.2x10^{-2}±1x10^{-3}$) and itaconate (Analyte/Standard: $3.1x10^{-1}$* ±$4x10^{-3}$ v $2.1x10^{-1}±6x10^{-3}$) that are associated with pro-inflammatory polarization. ^{13}C-glutamine tracing experiments demonstrated that NO_2-OA redirects glutamine utilization as indicated by a decrease in the fractional synthesis of succinate from ^{13}C glutamine with NO_2-OA compared to LPS alone (%Analyte/Standard compared to vehicle-treated: 176.8±0.4%* v 160.7±3%). It was determined that glutamine is redirected towards glutathione (GSH) biosynthesis, as GSH was increased with NO_2-OA treatment after stimulation compared to LPS alone (Analyte/Standard: $3.5x10^{-1}±6x10^{-2}$v $4.3x10^{-1}±2x10^{-2}$). *In vivo*, NO_2-OA treatment alleviated AAD-induced airway hyperresponsiveness (R_N: 1.2±0.2* cmH_2O-sec/mL v 1.8±0.3 cmH_2O-sec/mL), respiratory resistance (R_{rs}: 2.7±0.3* cmH_2O-sec/mL v 4.9±1.0 cmH_2O-sec/mL) and elastance (E_{rs}: 59±5.2* cmH_2O-sec/mL v 86.6±19.8 cmH_2O-sec/mL) when mice were challenged with 50 mg/mL of methacholine. Furthermore, myeloid cell dynamics were assessed via proteomics analysis on pulmonary CD45+ cells, where AAD mice had a significant upregulation (MSstats R package; log fold-change > 2, p value <0.05) of proteins involved in redox signaling. **Conclusions:** NO_2-OA is a pleotropic therapeutic that alters energy substrate utilization in myeloid cells and improves lung function in AAD *in vivo*. This use of advanced analytical techniques to interrogate both metabolite levels and protein dynamics helps to illuminate cell-specific changes contributing to disease and can assist in therapeutic target identification. This on-going work assists in defining the connections between the bioenergetic demands of inflammatory cells and their effector function in the context of disease.

4471 Rapid Activation of Prostaglandin Synthesis Pathways in Mouse Lungs Exposed to Multi-Walled Carbon Nanotubes

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Background and Purpose: Pulmonary exposure to respirable particles elicits acute inflammation with concurring tissue damage that may progress to chronic outcomes. The initiation and resolution of inflammation are critically regulated by potent proinflammatory and pro-resolving mediators in a time-dependent manner. Here we examined the synthesis of lipid mediators (LMs) in acute inflammation in mouse lungs exposed to fibrogenic multi-walled carbon nanotubes (MWCNTs). **Methods:** Adult C57BL/6J mice were exposed to MWCNTs (Mitsui-7) at 1860.4 μg/kg body weight by oropharyngeal aspiration. Lung tissue was collected at 24 hours post-exposure. Lipidomic profiling of LMs was performed to identify and quantitate major LMs by liquid chromatography-tandem mass spectrometry using Waters ACQUITY Xevo TQ-XS. Quantification of LMs was performed using both external and deuterium-labeled internal standards. **Results:** Exposure to MWCNTs induced acute inflammation and elevated phospholipase A2, cyclooxygenase 2, and prostaglandin (PG) E synthase at the protein and enzyme activity levels, indicating rapid activation of PG synthesis in mouse lungs. MWCNTs significantly elevated the levels of PGs including PGE2, PGD2, PGF2a, and thromboxane B2, as well as PG intermediate metabolites 5-, 12-, and 15-hydroxyeicosatetraenoic acids. Additionally, MWCNTs increased levels of protectin DX and 14(S)- and 17-hydroxydocosahexaenoic acids from the docosahexaenoic acid pathway, and 12-, 15-, and 18-hydroxyeicosapentaenoic acids from the eicosapentaenoic acid pathway. **Conclusions:** The results revealed rapid activation of synthesis pathways of LMs, particularly, PGs, in mouse lungs by nanoparticles. The study demonstrates the utility of lipidomics of LMs in the study of nanoparticle-induced sterile inflammation and pulmonary toxicity.

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