ABSTRACT NUMBER: 5065 **Poster Board Number:** P166
TITLE: Trem2 Regulates Ozone-Induced Immune Cell Trafficking in the Lung-Brain Axis

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KEYWORDS: Neurotoxicology

ABSTRACT: Alzheimer's disease (AD) is the leading cause of dementia, and reports implicate a potential role for disrupted immune cell trafficking in the disease. However, the role of the peripheral immune system in the pathology of AD is poorly understood. Evidence supports that air pollutants play a role in AD etiology, and epidemiology reports have linked ozone (O_3) exposure to increased AD incidence. How O_3 affects the brain or interacts with AD risk factors is not well understood. O_3 is a reactive oxidant that is confined to the respiratory tract after inhalation and is unable to translocate to the bloodstream or the brain, highlighting a potential role for the pulmonary immune response in the central nervous system (CNS) effects (The Lung-Brain Axis). Recently, genome-wide association studies have identified several loss of function *Trem2* (Triggering Receptor Expressed on Myeloid Cells 2) mutations, indicating myeloid cell-specific mutations and loss of function can convey AD risk. We have previously shown O_3 disrupts the chemotactic microglial response to amyloid plaques, elevates plaque load, and disturbs the disease-associated microglia phenotype in 5xFAD mice. These are processes regulated by TREM2, supporting a role for TREM2 in the CNS effects of O_3. However, the role of TREM2 in the lung-brain axis has yet to be directly tested, and whether TREM2 regulates the O_3-induced peripheral immune mechanisms that could impact the brain is unknown. To begin to address this, male *Trem2*$^{-/-}$ mice and *Trem2*$^{+/+}$ control mice were exposed to either filtered air or 1 ppm O_3 for 4 hours, and bronchoalveolar lavage fluid (BALF), plasma, cervical lymph nodes (deep and lateral, CLNs), and brains were collected 24 hours later. While data revealed O_3 exposure caused an increase in neutrophils in the BALF in *Trem2*$^{+/+}$ mice, there were no genotype differences. While *Trem2*$^{+/+}$ mice showed no change in BALF eosinophils in response to O_3 exposure, *Trem2*$^{-/-}$ mice exhibited a significant reduction in BALF eosinophils with O_3 exposure when compared to controls, supporting a potential role for TREM2 in eosinophil responses. CLNs collect immune cells that are present in the cerebral spinal fluid and meninges. These cells are stationed outside the brain parenchyma and are hypothesized to regulate parenchymal neuroimmune homeostasis. As such, CLN transcriptional changes were analyzed with the NanoString immunology panel and revealed that O_3 exposure changed gene expression patterns indicative of modified immune cell trafficking, which was dependent upon *Trem2* genotype. For example, *Trem2*$^{-/-}$ mice exposed to O_3 showed changes in gene expression patterns indicative of an increase in T-cells, which did not occur in *Trem2*$^{+/+}$ mice. *Trem2*$^{-/-}$ mice also showed CLN transcriptional changes indicative of modified chemokine or cytokine secretion, such as impaired *Cxcr3, Cxcl10, Cxcl12, Ccr2* and *Xcl1.* Collectively, these findings indicate TREM2 regulates O_3-induced immune cell trafficking in the lung-brain axis at two levels: the lung and CLNs, illustrating TREM2's impact in the periphery may affect the CNS neuroimmune milieu and the glymphatic system to regulate CNS health and disease.

ABSTRACT NUMBER: 5066 **Poster Board Number:** P167
TITLE: Sex Differences in the Th2 Lung-Brain Axis Response to *Aspergillus versicolor* Inhalation in C57BL/6J Mice

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KEYWORDS: Exposure, Environmental; Respiratory Toxicology; Neurotoxicology; Alzheimer's disease

ABSTRACT: Alzheimer's disease (AD) is a devastating form of dementia that includes a dysregulated microglial phenotype and maladaptive immune cell trafficking. Yet the identity of environmental exposures that are culpable in this disruption, and in peripheral immune responses regulating the brain remain poorly understood. *Aspergillus versicolor is* a common filamentous fungi found in damp indoor environments, where human inhalation exposure has been linked to asthma, a respiratory disease. Asthma is also associated with an increased AD risk. Previously, we have demonstrated that *A. versicolor* exposure results in transcriptional neuroimmune and neurochemical changes in the brain. According to the lung-brain-axis hypothesis, the pulmonary response to environmental exposures dysregulates microglia through changes in peripheral immune cells and circulating factors that regulate CNS health and disease. However, the role of sex differences in this process is largely unknown. Consistent with reports from female B6C3F1/N mice, male 5xFAD mice exposed to nose-only filtered air or *A. versicolor* (3×10^5 spores) two times a week for 13 weeks, resulted in *A. versicolor*-induced humoral changes indicative of a Th2 pulmonary response. Unfortunately, the estrogen response element in the promotor of the 5xFAD mouse confounds and precludes any analysis of females in this strain. As such, the C57BL/6J control strain was used to explore sex differences in the humoral and microglial responses to *A. versicolor*. Here we found sex differences in the changes in cortical microglial morphology, where male mice showed a significant increase in microglia volume after 13 weeks of *A. versicolor* exposure when compared to filtered air; but female mice failed to show a significant response. Furthermore, data revealed a significant increase in circulating factors IL-5, IP-10, IL-17, and TNFα in male mice exposed to *A. versicolor*, whereas these effects were absent in the female mice. Interestingly, during reproductive years, females often have a more robust immune response than males. However, when reproductive females do experience adverse reactions, the severity is often greater than affected males. Taken together, these findings support that the humoral and microglial response to *A. versicolor* is dysregulated in female C57BL/6J, highlighting the presence of sex differences in the Th2 Lung-Brain axis. Given the increased female risk in AD, further studies are needed to explore how these differences translate to amyloid and tau pathology.

ABSTRACT NUMBER: 5067 **Poster Board Number:** P168

TITLE: Low Concentrations of Manganese Potentiate Rotenone-Induced Production of Reactive Oxygen Species in Primary Astrocytes by Increasing Mitochondrial Calcium Levels

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KEYWORDS: Glia; Neurotoxicology; Neurotoxicity; Metals

ABSTRACT: Rotenone is a broad-spectrum insecticide, pisicide, and pesticide that continues to be used worldwide. This toxin is known for its capacity to uncouple mitochondrial complex I, as well as induce regionally distinct a-synuclein aggregation in C57Bl/6 mouse models causing symptoms that mirror those found in Parkinson's disease (PD). Manganese (Mn) is an essential trace mineral needed for cellular enzymatic function, however, excess accumulation in the basal ganglia results in Manganism, a disease characterized by Parkinsonian-like symptoms. Genetic mutations or environmental perturbations leading to mitochondrial dysfunction remain a driving factor in numerous neurodegenerative diseases, including PD and Manganism. Recent research conducted by our lab and others, have implicated glial cell activation and inflammatory involvement in the progression of disease, yet exact mitochondrial mechanisms leading to inflammation in glia remains unknown. To address this question, primary murine glial cultures were exposed to subacute concentrations of Mn (10μM) and rotenone (100nM) and respiratory capacity and extra-mitochondrial hydrogen peroxide generation were investigated using by polarography and fluorometry, respectively. In addition, the determination of Mn priming exacerbating the inflammatory response caused by secondary rotenone exposure was determined by isolation of primary C57Bl/6 glial cells from neonatal (P0-P1) mouse pups and grown to confluency in thin-walled glass chamber slides. Subacute concentrations of Mn (10uM) were used to prime cells for 24 hours prior to additions of 100nM rotenone in respective comparative experimental groupings (Control, 10μM Mn, 100nM rotenone, 10μM Mn+

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Publication Date: March 15, 2023