Determining the role of monocytes in Parkinson's disease-related neuroinflammation

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ABSTRACT

Parkinson's disease (PD) is a highly prevalent neurodegenerative disorder affecting 1 million people in the U.S. PD is a complex and typically idiopathic disorder characterized by motor and non-motor symptoms and loss of dopaminergic neurons in the substantia nigra. Previous studies show that systematic environmental exposure to manmade pesticides is associated with increased risk of the disorder, especially for agricultural workers. The pesticide rotenone induces cell stress by inhibiting mitochondrial complex I and the resultant cellular oxidative damage can activate the innate immune system. The NLRP3 inflammasome is a proinflammatory innate immune sensor that responds to cellular damage and stress. Past work from our lab identified a role for the NLRP3 inflammasome as a mediator of the response to rotenone in a mouse model of PD. PD has numerous systemic manifestations, but we do not know how the peripheral and central innate immune cells interact during the progression of neurodegeneration. Our laboratory uses rotenone to model systemic exposure and compares mice in which *Nlrp3* is restricted to either peripheral or central nervous system (CNS) immune cells. This project sought to characterize *Nlrp3* expression in peripheral innate immune cell populations, specifically monocytes. Wild type (WT) and *Nlrp3*$^{-/-}$ mice were exposed to systemic rotenone and their peripheral blood leukocytes (PBLs) were analyzed. Flow cytometry was used to characterize the peripheral immune response of monocytes. Rotenone treatment in WT mice led to an increase in classical monocytes (Ly6C^{hi} Ly6G^{int}), while *Nlrp3*$^{-/-}$ mice showed an upregulation of classical monocytes regardless of treatment, possibly indicating compensatory mechanisms in the absence of the NLRP3 inflammasome. Additionally, loss of *Nlrp3*$^{-/-}$ was associated with less monocytes with an antigen-presenting cell (APC) phenotype (CD11b^{+} Ly6G^{-} CD16^{int} MHCII^{hi}) relative to the WT control. *Nlrp3*$^{-/-}$ mice exhibited upregulated levels of monocytes without the APC phenotype (CD11b^{+} Ly6G^{-} CD16^{int} MHCII^{low}) compared to the WT control, suggesting that the NLRP3 inflammasome plays a role in monocyte activation.

BACKGROUND

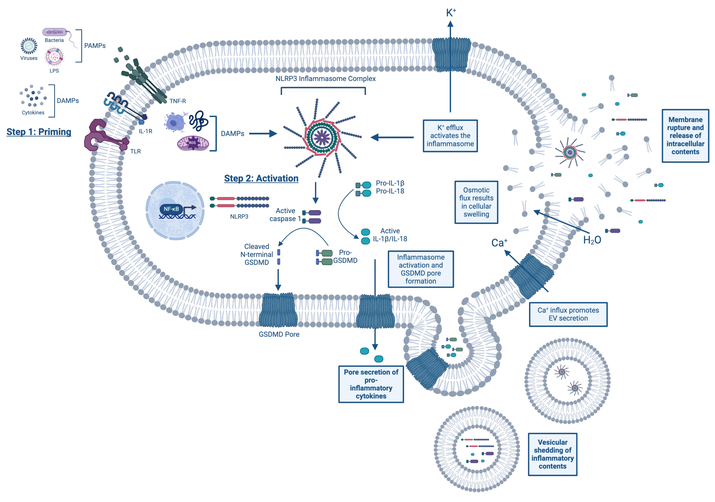

Figure 1: Overview of the activation of the NLRP3 inflammasome.
Adapted from: Faith L. Anderson, Karl E. Biggs, Brynn E. Rankin, Matthew C. Havrda, *Translational Research*, 2022.

OVERARCHING GOAL

Understand the role of the peripheral innate immune system during the progression of Parkinson's disease (PD).

METHODS

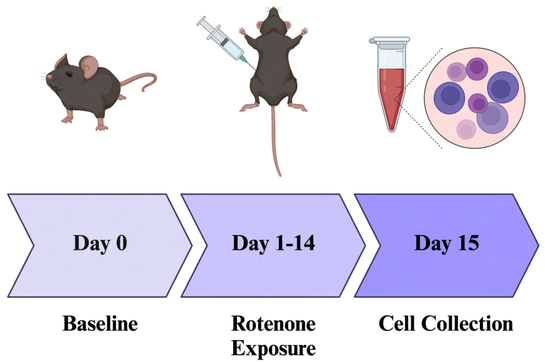

Figure 2: Timeline of rotenone exposure and sample collection. Mice were exposed to rotenone or vehicle for 14 consecutive days. Exposure was continued until blood collection, which started on Day 15.

RESULTS

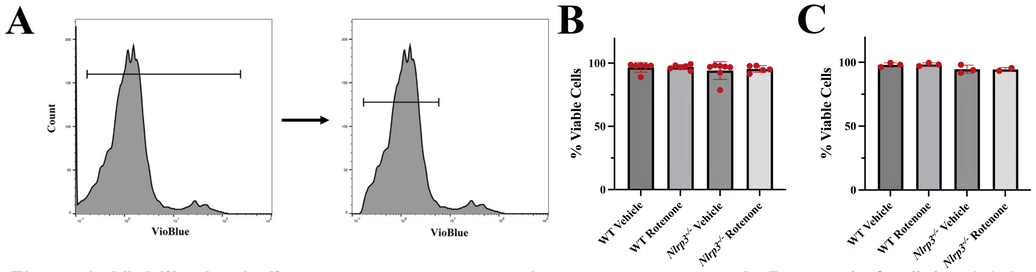

Figure 3: Viability is similar across treatment and genotype groups. A. Removal of cellular debris from the raw data to differentiate living cells from dead cells. The dead cell population is associated with a higher VioBlue staining. **B-C.** Peripheral blood leukocyte (PBL) viability across all mouse groups in panels 1 and 2. Panel 1 was repeated across 2 cohorts.

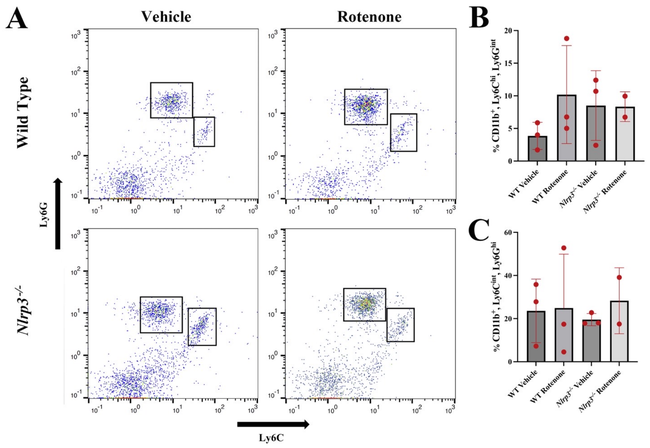

Figure 4: Rotenone increases the number of classical monocytes (Ly6C^{hi}) in mice PBLs. A. Pseudocolor plots exhibiting the Ly6C^{hi} Ly6G^{int} and Ly6C^{int} Ly6G^{hi} cell clusters in each mouse group. **B.** Percentage of Ly6C^{hi} Ly6G^{int} monocytes within CD11b^{+} myeloid cells. Rotenone appears to increase the number of classical monocytes within myeloid cells in the peripheral immune system. **C.** Percentage of Ly6C^{int} Ly6G^{hi} neutrophils within CD11b^{+} myeloid cells.

RESULTS

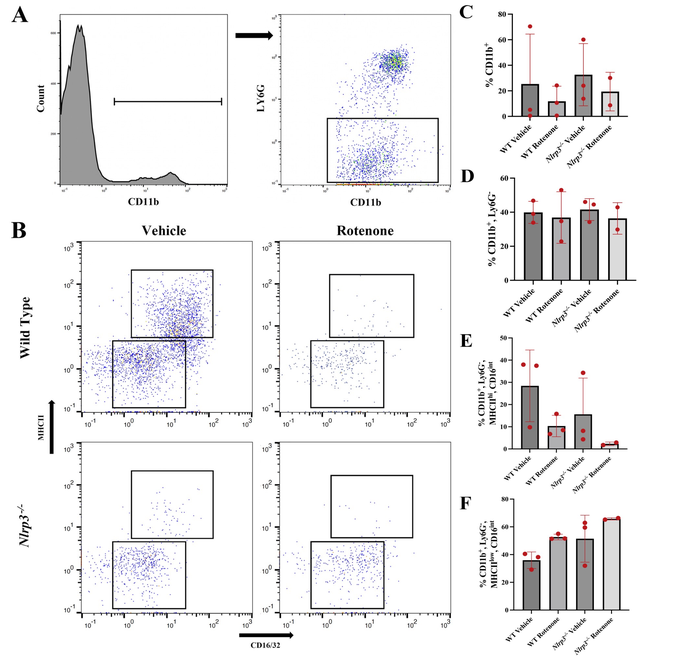

Figure 5: Rotenone and loss of *Nlrp3* reduces the number of monocytes with an antigen-presenting cell (APC) phenotype in mouse PBLs. A. Separation of the CD11b^{+} and Ly6G^{-} cells from the raw data set. **B.** Pseudocolor plots exhibiting the CD11b^{+} Ly6G^{-} CD16^{int} MHCII^{hi} (APC monocyte) and CD11b^{+} Ly6G^{-} CD16^{int} MHCII^{low} (non–APC monocyte) cell clusters in each mouse group. **C.** Percentage of CD11b^{+} cells, **D.** CD11b^{+} Ly6G^{-} cells, **E.** APC monocytes, and **F.** non-APC monocytes in mouse PBLs.

FUTURE DIRECTIONS

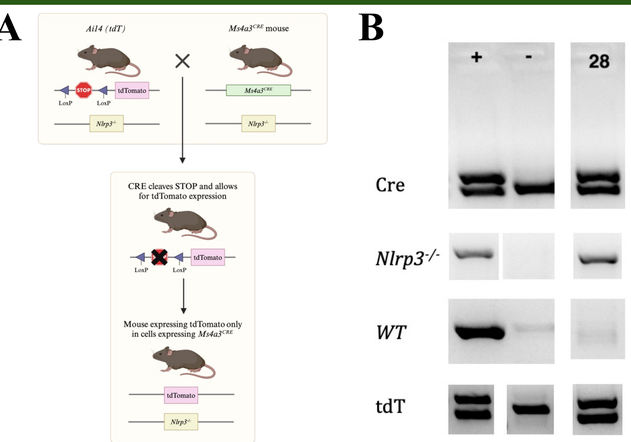

Figure 6: Development of a fluorescently labeled-granulocyte monocyte progenitor (GMP) mouse model. A. Breeding of the Ai14 reporter mice to the *Ms4a3*^{CRE} mice results in cleavage of the loxP-bound STOP codon via Cre-recombinase activity, promoting tdTomato (tdT) expression only in cells expressing *Ms4a3* (GMPs). **B.** PCR genotyping shows that Mouse 28 expresses a *Ms4a3*$^{CRE+}$ *Nlrp3*$^{-/-}$ tdT^{+} genotype.

CONCLUSIONS

In a mouse model of Parkinson's disease:

- Rotenone **increases** the proportion of classical monocytes within PBLs
- Rotenone and loss of NLRP3 **decreases** the number of monocytes with an APC phenotype

ACKNOWLEDGEMENTS

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