Role for Microglial NLRP3 Inflammasome and Intestinal Hyperpermeability in Parkinson's Disease

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Introduction

Research Objective:
To establish the intestinal microbiota as a regulator of neuroinflammation in Parkinson's Disease.

- Parkinson's Disease (PD) is the second most common neurodegenerative disease and is characterized by neuroinflammation, nigrostriatal dopaminergic (DA) cell loss, an accumulation of alpha synuclein (α-syn) containing Lewy bodies, and motor impairments.
- Though the most notable symptoms are neurological, there are gastrointestinal (GI) dysfunctions as well. The pathophysiological significance of these GI problems has not been explored thoroughly for its diagnostic/therapeutic potential.
- As of now, it is unclear if the release of peripheral toxins and endotoxins, such as lipopolysaccharide (LPS) contributes to neuroinflammation in PD.
- The effects of LPS are mediated by the toll-like receptor 4 (TLR4) which is found on microglia, and the TLR4 receptor is significantly increased in the substantia nigra (SN) of PD patients.
- Recent studies in PD patients also support a critical role for the microglial inflammasome protein complex: NOD-, LRR-, and pyrin domain-containing 3 (NLRP3). The NLRP3 inflammasome can be activated by LPS-TLR4 signaling, resulting in caspase-1 activation and increased activation of IL-1β, a key cytokine of inflammation. In recent post mortem PD samples, SN microglia exhibit significantly increased NLRP3 staining [1].
- Our group, and others, have shown that newly diagnosed PD patients exhibit intestinal α-syn staining, inflammation, hyperpermeability (leaky gut), endotoxemia (systemic LPS), as well as gut microbiota dysbiosis with an increase in LPS-producing bacteria [2,3].
- Here, we will test the mechanistic hypothesis that gut-derived LPS exacerbates PD pathology via activation of SN microglia through a LPS-TLR4-Notch1-NLRP3-dependent mechanism.
- In addition, we investigate how leaky gut-derived microbial products, especially LPS, promote PD-like pathology and behavior in an α-syn overexpressing (ASO) PD mouse model.

Central Hypothesis

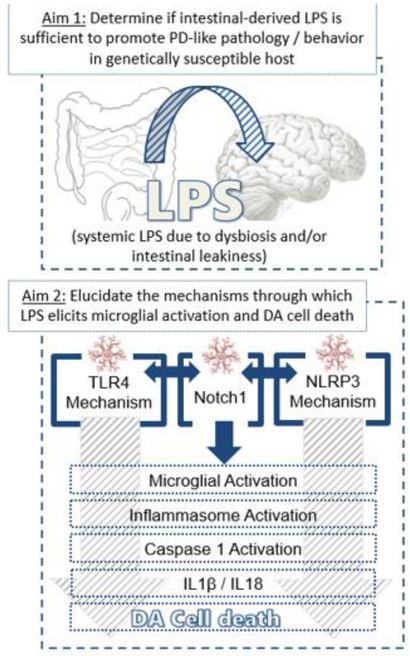

Figure 1: Hypothesis

Experimental Design

Experiment 1: Dextran sodium sulfate-induced intestinal permeability (*in vivo*)

Working hypothesis: Intestinal hyperpermeability promotes PD-like pathology and behavior.

- Dextran sodium sulfate (DSS) will be administered in drinking water to ASO murine PD model for three cycles. These tests will be analyzed through longitudinal tracking of intestinal permeability and motor function and a post-mortem analysis of microglial inflammation markers.

Experiment 2: Microglial response to microbial products (*in vitro*)

Working Hypothesis: The Notch1 receptor (expressed by microglia) regulates activation of the NLRP3 inflammasome and IL-1β/IL-18 production by microglia in response to microbial (LPS, Lipoteichoic acid (LTA)).

- SIM-A9 microglial cell line and use primary microglia from ASO and control mice to elucidate how microbial products activate microglia and determine whether Notch1 regulation of NLRP3/IL-1β activation by TLR2/4 stimulation is the molecular mechanism involved and to investigate whether microglial from the ASO genetically susceptible host is primed to microbial product-induced activation.

Results

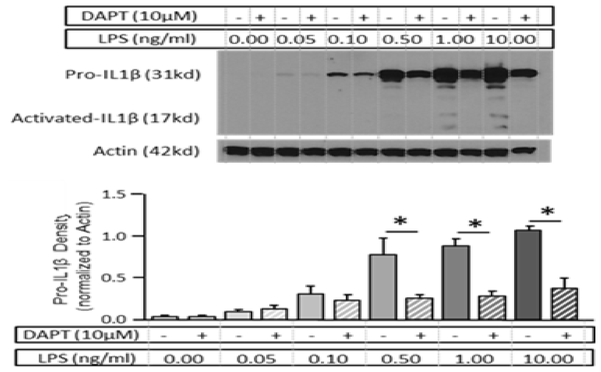

Figure 2: LPS-induced increase in pro-IL-1β activation by activated Nlrp3 is inhibited by treatment with Notch1 inhibitor, DAPT.

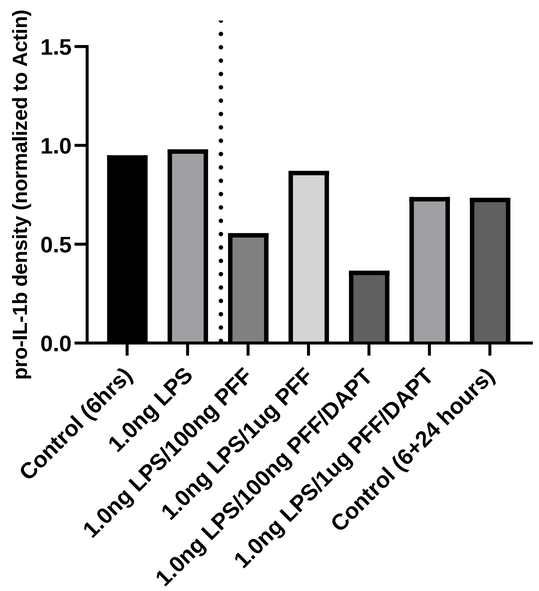

Figure 3: Higher dose of PFF with LPS pretreatment induced an increase in pro-IL-1β and is inhibited by treatment with Notch1 inhibitor, DAPT.

Summary and Conclusions

- Data show that LPS induces a dose-dependent increase in pro-IL-1β.
- The LPS-induced increase in pro-IL-1β was decreased by treatment of microglia with DAPT, Notch1 inhibitor.
- This decrease in pro-IL-1β when Notch1 is inhibited tells us that Notch1 has a positive role in promoting IL-1β expression during the first signal of NLRP3 inflammasome activation.
- The lack of active IL-1β expression when Notch1 is inhibited supports that Notch1 activation is also essential for the second signal required for in NLRP3 inflammasome assembly and caspase activation of IL-1β.
- However, PFF stimulated increases in pro-IL-1b that was not blocked by DAPT.

Future Directions

Short-Term

- Analyze the effect microglia have on neurons within these models.

Long-Term

- Mechanistic studies to evaluate potential preventative and therapeutic targets of neuroinflammation-related diseases.

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