Inflammasome product IL-1β increases oligodendroglial lineage cell number and promotes their differentiation

QUEEN'S UNIVERSITY BELFAST

Ger Mullan, Marie Dittmer, Daniel Crooks, Denise Fitzgerald, Yvonne Dombrowksi

Introduction

The inflammasome is a multimeric protein complex that initiates immune responses to danger signals by releasing the cytokines interleukin-1β (IL-1β) and IL-18. Inflammasomes are thought to be involved in multiple sclerosis, an immune-mediated, demyelinating disorder that can cause serious neurological disability, including spasticity, optic neuritis and paralysis. Some studies have shown that inflammasome activity can promote CNS repair. We hypothesised that inflammasomes can be stimulated in CNS cells, and that inflammasome activity promotes oligodendrocyte progenitor cell (OPC) proliferation and/or differentiation.

Figure 1: Demyelination. Myelin facilitates neuronal conduction and provides metabolic support. The destruction of the myelin sheath via demyelination slows conduction and leads to axonal degradation.

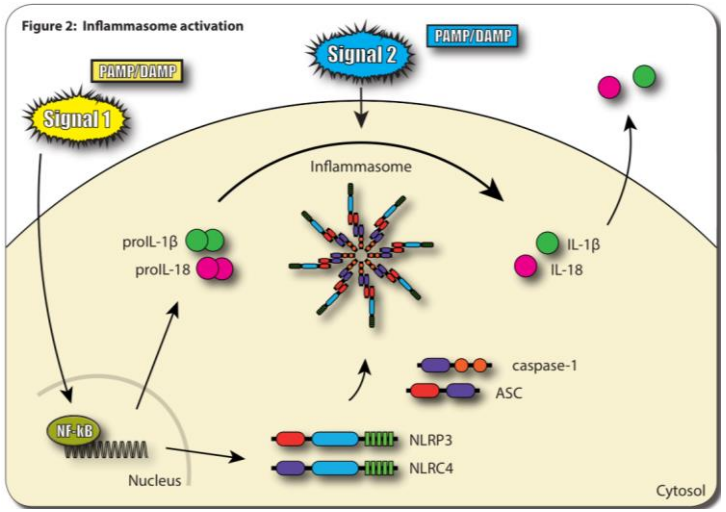
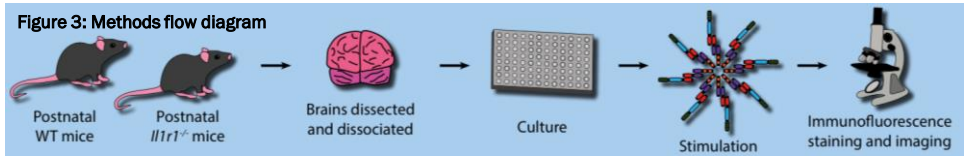


Figure 2: Inflammasome activation. Inflammasomes consist of a danger sensor, e.g. NLRP3, the adaptor protein ASC and procaspase 1. The complex cleaves the immature pro-inflammatory cytokines pro-interleukin-1β (proIL-1β) and interleukin-18 (proIL-18) to their mature forms, IL-1β and IL-18.

Methods



Inflammasome activity

Inflammasome activation was simulated in mixed glial cultures by addition of IL-1β over 5 days. Cell death, percentage of oligodendrocyte lineage cells (OLC) and total myelin area were then analysed. We found significantly increased oligodendrocyte lineage percentage and total myelin area with minimal cell death after addition of IL-1β.

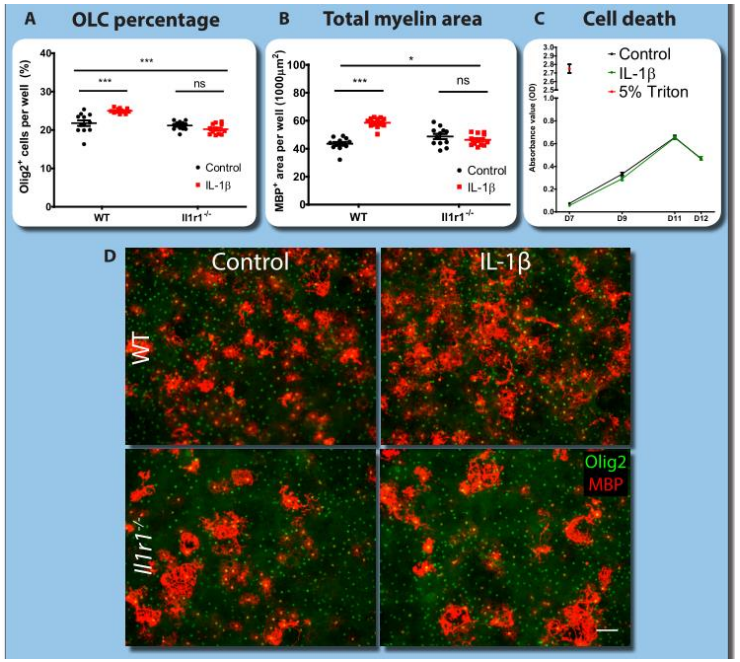


Figure 4: Inflammasome product IL-1β increases OLC number and promotes their differentiation, mediated via IL-1R1. WT and Il1r1-/- glial cells were cultured over 7div and subsequently treated with or without 100ng/mL IL-1β. Cultures were immunofluorescently stained for Olig2 (green) and MBP (red), imaged and analysed using a CellInsight CX5 microscope. **A)** Percentage of mature oligodendrocytes Olig2+MBP+/Olig2+ count), **B)** total MBP+ area, **C)** LDH absorbance values, 5% triton death control. **D)** Representative images: WT n=3 experiments; Il1r1-/- n=1. n = 12 wells (25 FOV/well), mean ± SEM, two-way ANOVA with Bonferroni post-test, * $p < 0.05$, *** $p < 0.001$. Scale bar = 100μm.

Inflammasome activation

Mixed glial cultures were stimulated with inflammasome triggers over 6 hours to test endogenous inflammasome responses in glial cells. Levels of inflammasome activation and cell death were then analysed. We found high levels of inflammasome activation in dual-stimulation conditions.

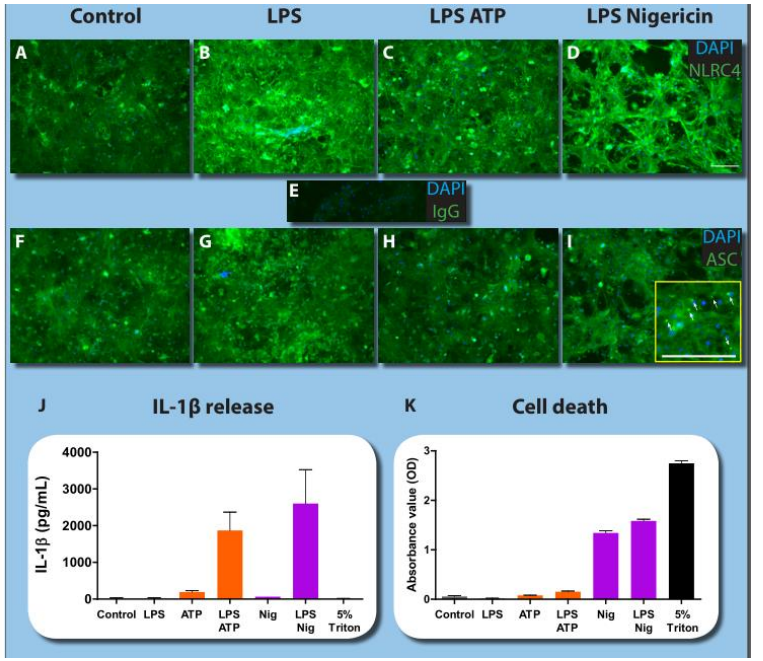


Figure 5: Inflammasome triggers stimulate inflammasome activation in glial cells in vitro. Mixed glial cultures at 7div were primed with LPS for 4 hours (100ng/mL) and subsequently stimulated with ATP (5mM) and/or nigericin (20μM) for 2 hours. NLRC4 immunofluorescence staining in **A)** untreated control, **B)** LPS, **C)** LPS + ATP, **D)** LPS + nigericin. **E)** rabbit IgG. ASC immunofluorescence staining in **F)** untreated control, **G)** LPS, **H)** LPS + ATP, **I)** LPS + nigericin, putative ASC specks (white arrows). Supernatant was analysed by **J)** IL-1β ELISA and **K)** LDH absorbance values, 5% triton death control. Representative images of 3 independent experiments. Scale bar = 100μm.

Conclusions and future work

Our results suggest a role for inflammasomes in glial cells. Figure 4 demonstrates that IL-1β can increase OLC number and promote differentiation to oligodendrocytes. Figure 5 shows endogenous inflammasome activation in glial cells via danger signalling. Future work will investigate the impact of endogenous inflammasome activation on oligodendrocytes.

Acknowledgements

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