Increased Response of Primary Adult Microglia from 5xFAD Mice to Pro-Inflammatory Stimuli

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BACKGROUND

The importance of microglia in neurodegenerative diseases is well-known and these cells are therefore frequently used as a target for new pharmacological interventions.
To study this cell type, isolation of early postnatal microglia from mice is a great tool but does not properly reflect conditions in aged or diseased individuals. Isolation of viable microglia from adult mouse brains of specific disease models via Magnetic Cell Sorting (MACS) opens new possibilities to assess the efficacy of microglia targeting treatments *in vitro*.
Here we investigated the *in vitro* response of isolated adult microglia from 5xFAD mice to various stimuli in comparison to age-matched non-transgenic (nTg) microglia.

MATERIAL & METHODS

Microglia were isolated from 9-months old 5xFAD mice and nTg littermates. Purity of isolated microglia was assessed by qRT-PCR. Cultivated cells were stimulated with 50 ng/mL LPS or 10 µM Aβ1-42 for 24 h in presence or absence of the anti-inflammatory agent dexamethasone (Dexa) and cytokine release (TNF-α, IL-6 and IL-1β) into the supernatant was measured. Additionally, cells were treated with 10 µM Aβ1-42, followed by determination of phagocytosis via pHrodo™ Red staining.

RESULTS

While a strong enrichment of Itgam- (CD11b) positive cells in the microglial fraction compared to the flow through was detected, GFAP expression was extremely low or absent.

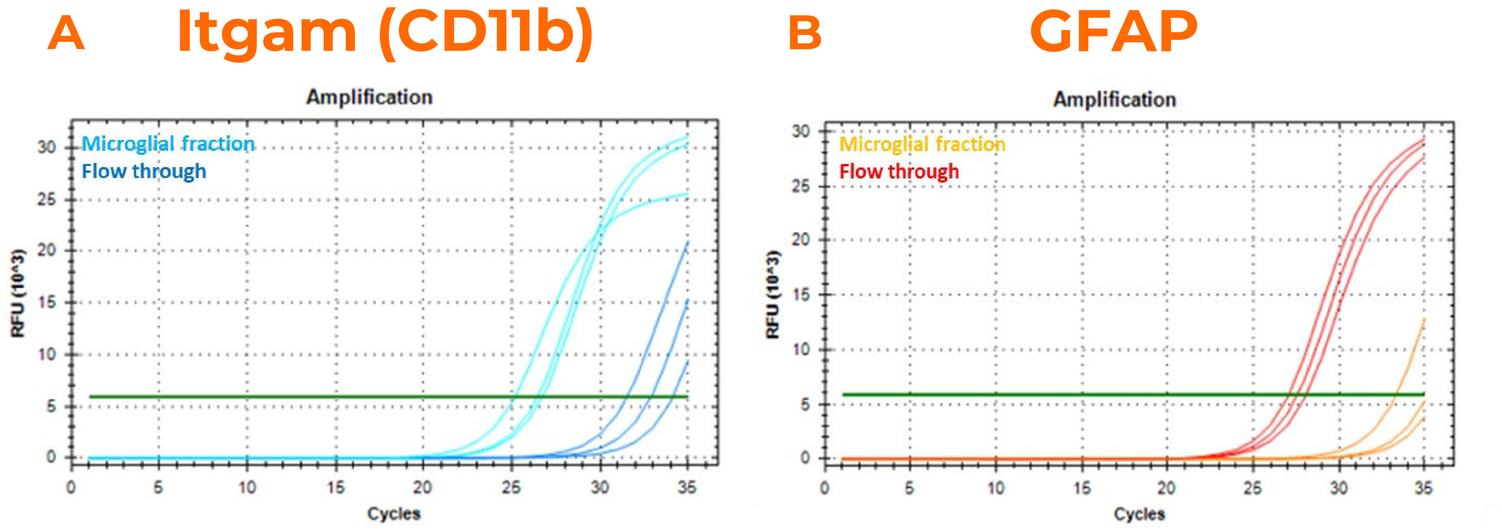

Figure 1: Purity of isolated microglia. RT-PCR amplification graphs of Itgam (A) and GFAP (B) SYBR green signal in microglia and flow through fractions prepared from 3 separate 9.5 months old mouse brains. The graphs show relative fluorescence units (RFU) of the SYBR green signal per cycle.

The response to pro-inflammatory stimuli was tremendously stronger in 5xFAD microglia revealing 10-fold higher levels of secreted cytokines compared to nTg microglia stimulated with the same stressor. Increased phagocytosis accompanied with reduced Aβ1-42 levels in the supernatant was observed in 5xFAD microglia compared to nTg microglia.

LPS-Induced Inflammation

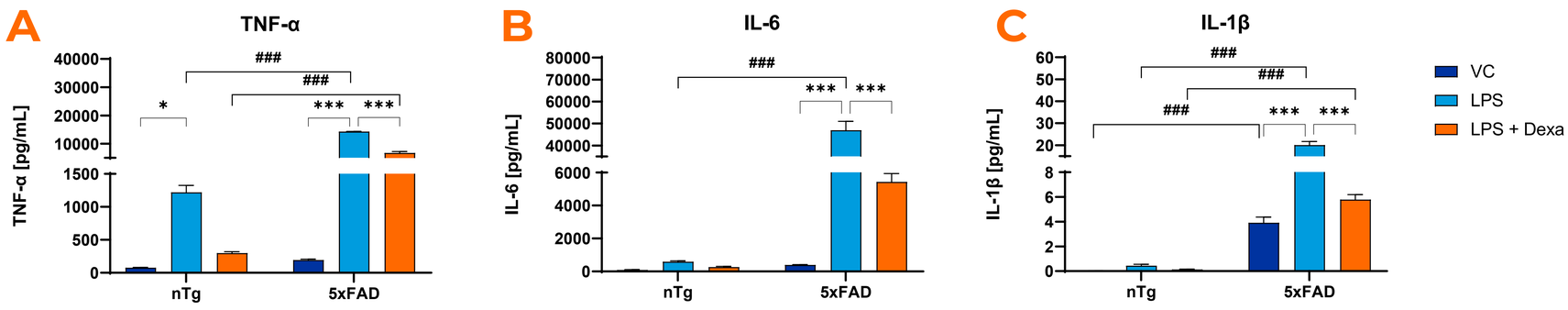

Figure 2 : Quantification of TNF-α, IL-6 and IL-1β levels in the supernatant of LPS-stimulated microglia. Isolated microglia of 5xFAD mice and nTg littermates were treated with Vehicle (VC), 50 ng/mL LPS or LPS plus dexamethasone (Dexa) and TNF-α (A), IL-6 (B) and IL-1ß (C) secretion was measured. Data are presented as bar graphs with mean + SEM (n=4-6 per group). Two-Way ANOVA followed by Bonferroni's Multiple Comparison *post hoc* test compared to LPS control; *p<0.01; ***p<0.001 or nTg versus 5xFAD ###p<0.001.

Aβ1-42-Induced Inflammation and Phagocytosis

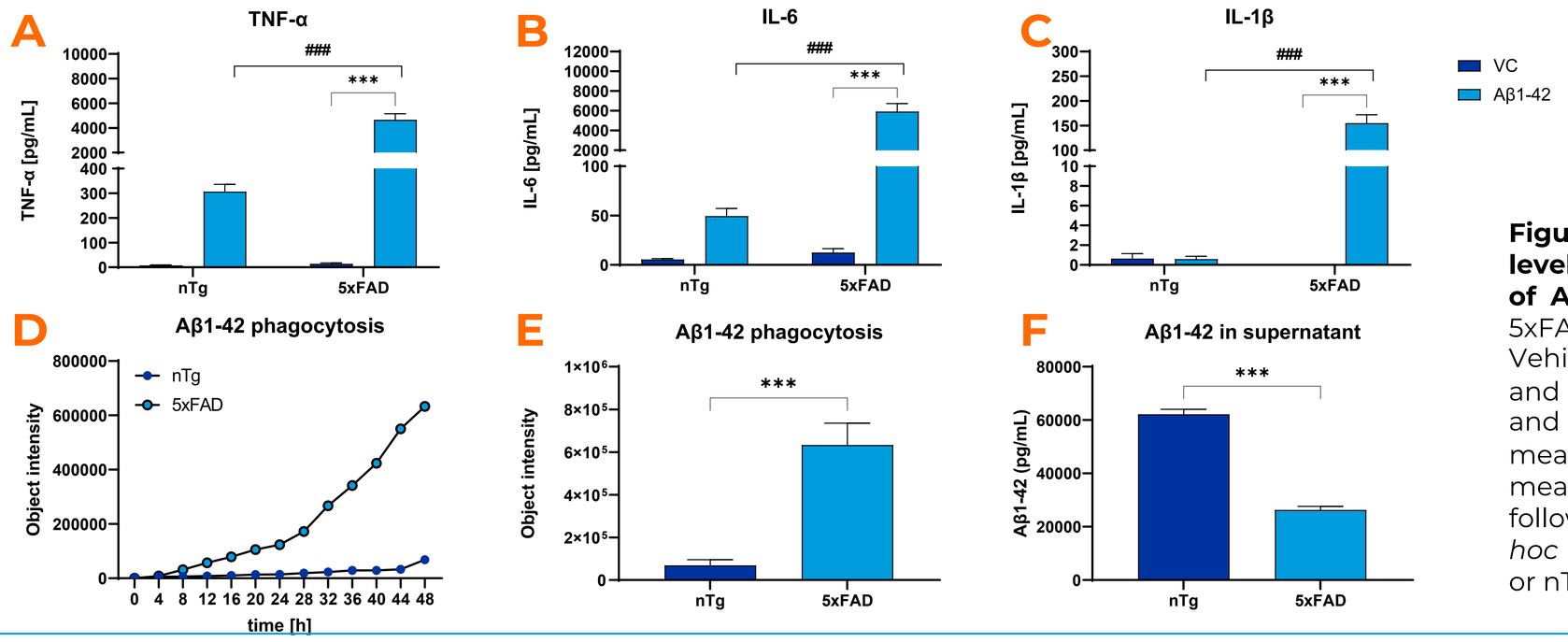

Figure 3 : Quantification of TNF-α, IL-6 and IL-1β levels in the supernatant as well as phagocytosis of Aβ1-42-treated microglia. Isolated microglia of 5xFAD mice and nTg littermates were treated with Vehicle (VC) or 10 µM Aβ1-42 and TNF-α (A), IL-6 (B) and IL-1ß (C) secretion as well as phagocytosis (D,E) and Aβ1-42 levels in the supernatant (F) were measured. Data are presented as bar graphs with mean + SEM (n=5 per group). (A-C) Two-Way ANOVA followed by Bonferroni's Multiple Comparison *post hoc* test; (E-F) Two-tailed unpaired *t-test;* ***p<0.001 or nTg versus 5xFAD ###p<0.001.

SUMMARY and CONCLUSION

Generation of a pure microglial fraction from adult brains of transgenic mice opens a variety of new possibilities to assess the efficacy of treatments in diseased microglia.

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