Hippocampal Microglial Senescence and its Contribution to Sexually Divergent Neurodegeneration

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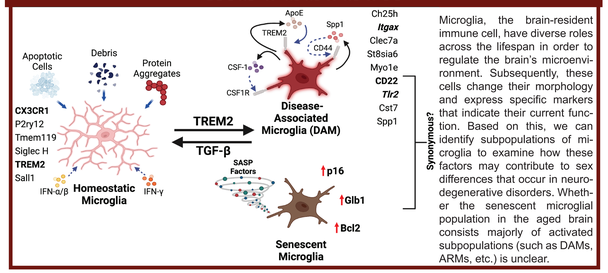
Abstract

Alzheimer's Disease (AD) is one of the most common neurodegenerative disorders and the primary risk factors for its development are sex and age. Females display greater prevalence and neuropathology of AD, while males exhibit a quicker progression to death. Sex divergence in immune-related responses observed in the mouse hippocampus with brain aging support the predicate that females have stronger immune reactions and consequently a greater occurrence of autoimmunity and "inflammaging." **Here, we test the hypothesis that female-specific neuroinflammation with brain aging is driven primarily by microglia.** To this end, we isolated hippocampal microglial transcripts using two methods: CD11b magnetic-activated cell sorting (MACS) and Cx3cr1 translating ribosome affinity purification (TRAP) from young and old mice of both sexes. We then performed RNA-Seq and downstream transcriptomic analyses. We identified an amplified induction of antigen presentation pathways and a significant increase in AD risk genes (TREM2, APOE, and PTK2B) specifically in females. Our results demonstrate that microglial reactivity is increased in females, and suggest female microglia develop an earlier senescent phenotype that contributes to the sexually divergent pathology of AD. Future directions should include assessments of the functional differences in microglia between the sexes as well as single-cell transcriptomic analyses to determine the specific microglial phenotypes that differ between sexes. This information can lead to enhanced and informed treatment options for those affected by aggressive neuropathology.

Introduction



Microglia, the brain-resident immune cell, have diverse roles across the lifespan in order to regulate the brain's microenvironment. Subsequently, these cells change their morphology and express specific markers that indicate their current function. Based on this, we can identify subpopulations of microglia to examine how these factors may contribute to sex differences that occur in neurodegenerative disorders. Whether the senescent microglial population in the aged brain consists majorly of activated subpopulations (such as DAMs, ARMs, etc.) is unclear.

Experimental Design

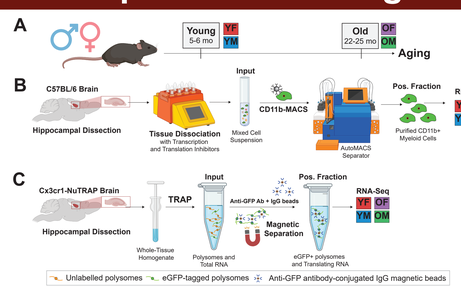


Figure 1. Isolation of hippocampal microglial transcripts by CD11b-MACS & Cx3cr1-TRAP **A)** Schematic of experimental grouping. Male and female C57BL/6N or Cx3cr1-NuTRAP mice were collected at young (5-6 months) and old (22-25 months) timepoints to form four groups: 1) young female (YF), 2) young male (YM), 3) old female (OF), and 4) old male (OM). **B)** Isolation of hippocampal microglia by CD11b-MACS. **C)** Isolation of hippocampal microglial translatome by Cx3cr1-TRAP.

Results

Sex Effects in Microglia Transcriptomics & Translatomics

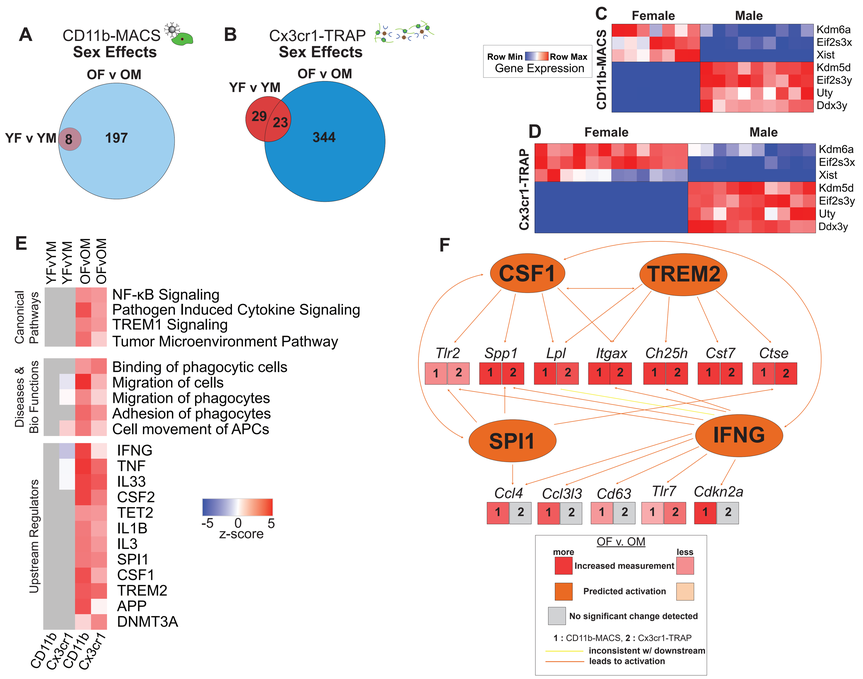


Fig 2. Analysis of microglial transcriptomic (Cd11b-MACS) & translatomic (Cx3cr1-TRAP) sex effects from young and old mouse hippocampus **A-B)** Sets of differentially expressed genes (DEGs) (GLM QLF-test, FDR<0.1) showing sex effects in young (YF v YM) or old (OF v OM) within the CD11b-MACS and Cx3cr1-TRAP dataset were compared by Venn diagram. **C-D)** Heatmap of the gene expression of seven DEGs by sex that were identified in young and old mice with both methodologies. **E)** Heatmap of z-scores for IPA canonical pathways, diseases & bio functions, and upstream regulators analyses. **F)** IPA upstream regulator comparison to assess sex effects in old hippocampal microglia.

Female-Biased Induction of Senescence Markers

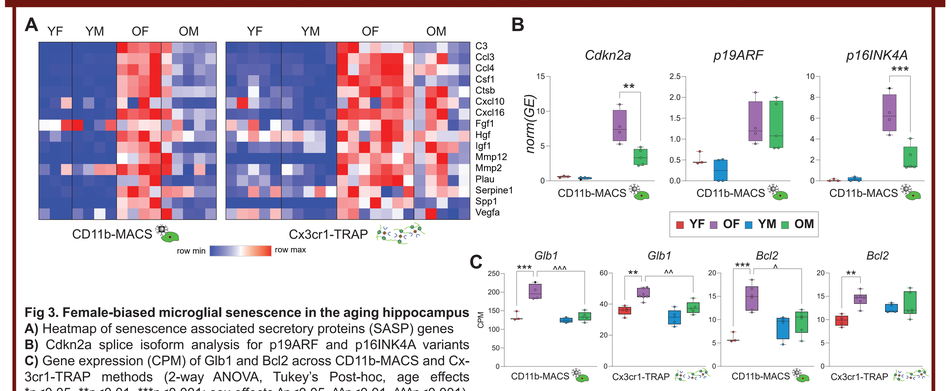


Fig 3. Female-biased microglial senescence in the aging hippocampus **A)** Heatmap of senescence associated secretory proteins (SASP) genes **B)** Cdkn2a splice isoform analysis for p19ARF and p16INK4A variants **C)** Gene expression (CPM) of Glb1 and Bcl2 across CD11b-MACS and Cx3cr1-TRAP methods (2-way ANOVA, Tukey's Post-hoc, age effects *p<0.05, **p<0.01, ***p<0.001; sex effects ^p<0.05, ^^p<0.01, ^^^p<0.001).

Female-Biased Induction of DAMs

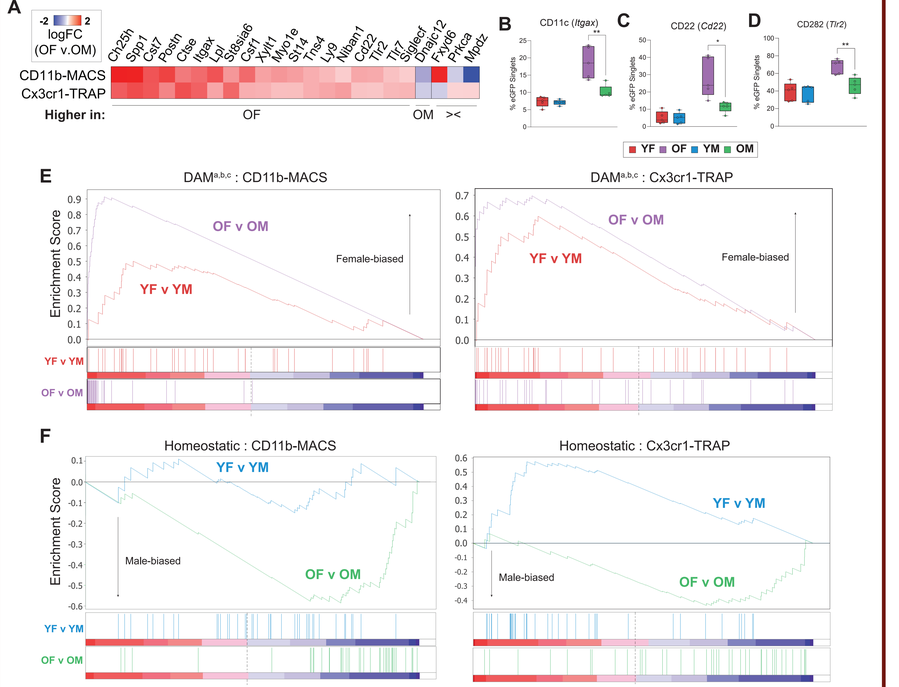


Fig 3. Sexually divergent microglial gene expression in transcriptomic (CD11b-MACS) & translatomic (Cx3cr1-TRAP) analyses **A)** Heatmap of the logFC(OF/OM) from the 23 sex DEGs identified in the old age groups across both the transcriptome (CD11b-MACS) and translatome (Cx3cr1-TRAP). **B-D)** Flow cytometry confirmations of the percentage of **B)** CD11c (Itgax), **C)** CD22 (Cd22), and **D)** CD282 (Tlr2) positive cells among the eGFP+ cells from Cx3cr1-NuTRAP hippocampus. **G-H)** GSEA enrichment plots for **G)** DAM and **H)** homeostatic marker genes.

Conclusions & Future Directions

- **Mouse hippocampal transcriptomic and translatomic analyses indicate sex-specific development of inflammatory and senescent phenotypes with aging.**
- **Females display greater induction of disease-associated microglial transcripts (DAMs) and senescence characteristics.**
- **These divergent neuroimmune responses may explain the sex differences observed in susceptibility and disease expression in neurodegenerative diseases such as Alzheimer's Disease.**
- **Future studies will aim to reveal the interactive effects of sex chromosomal and sex hormonal mechanisms in mediating the sex divergences seen in hippocampal microglial reactivity with aging.**
- **Conclusions from these studies will provide the insight necessary to identify novel sex-informed, disease-modifying therapies.**

Full Article:

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