How old is old? An assessment of age in traumatic brain injury

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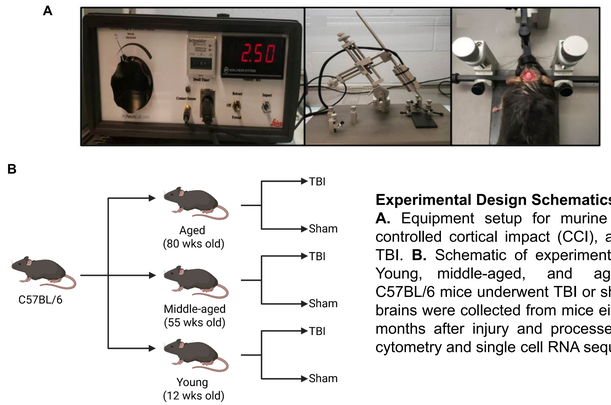
INTRODUCTION

Traumatic brain injury (TBI) affects over 3 million Americans every year leading to subsequent long-term neurocognitive morbidity. Patients over 65 years of age experience increased mortality and greater long-term neurocognitive morbidity compared to young adults after TBI. Our previously published data shows an age-specific influx of CD8+ effector T-cells into the aged brain after TBI[1,2]. These infiltrating CD8+ T-cells have been associated with worse neurocognitive outcomes, morbidity, and mortality in aged mice post-TBI compared to sham injury. However, when the transition from a "young" outcome to an "aged" outcome after TBI occurs remains unknown. Understanding the transition from a young to aged pathophysiology of injury is integral to assessing mechanisms leading to T-cell infiltration and identifying therapeutic targets aimed at reducing neurocognitive deficits and mortality in aged TBI subjects.

We hypothesized that "middle-aged" mice would have increased infiltration of effector T-cells into the brain after TBI compared to young adult mice.

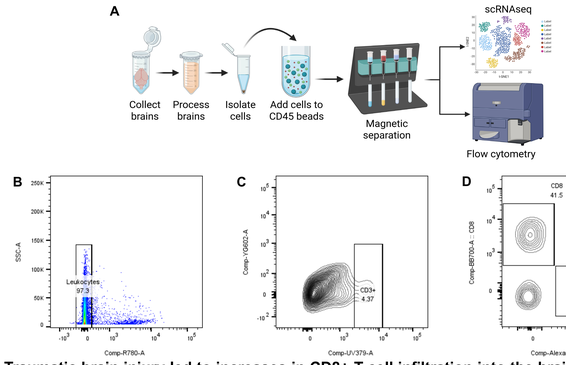
METHODS

Controlled Cortical Impact and Experimental Groups



Experimental Design Schematics
A. Equipment setup for murine model of controlled cortical impact (CCI), a model of TBI. **B.** Schematic of experimental groups. Young, middle-aged, and aged male C57BL/6 mice underwent TBI or sham injury, brains were collected from mice either 1 or 2 months after injury and processed for flow cytometry and single cell RNA sequencing

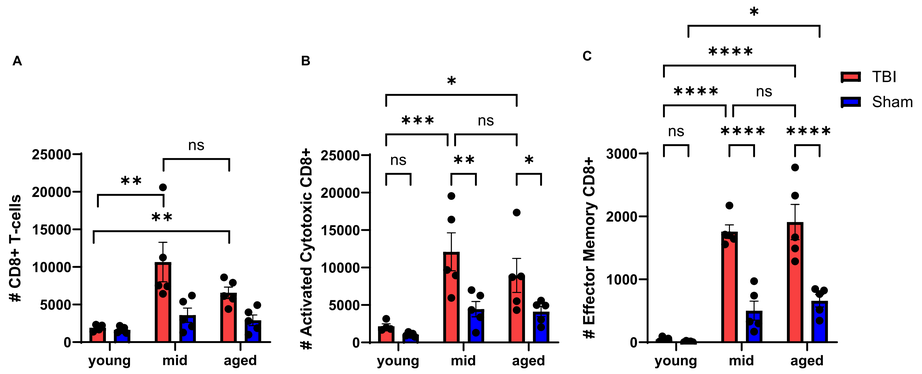
Gating Strategy for murine brain flow cytometry



Traumatic brain injury led to increases in CD8+ T-cell infiltration into the brain of middle aged and aged mice only.
A. Schematic displaying methods of cell isolation protocol. Representation of flow cytometry gating strategy, isolating **B.** leukocyte, **C.** CD3+ and **D.** CD4+ and CD8+ T-cell brain infiltration 1-month post-TBI. N=5

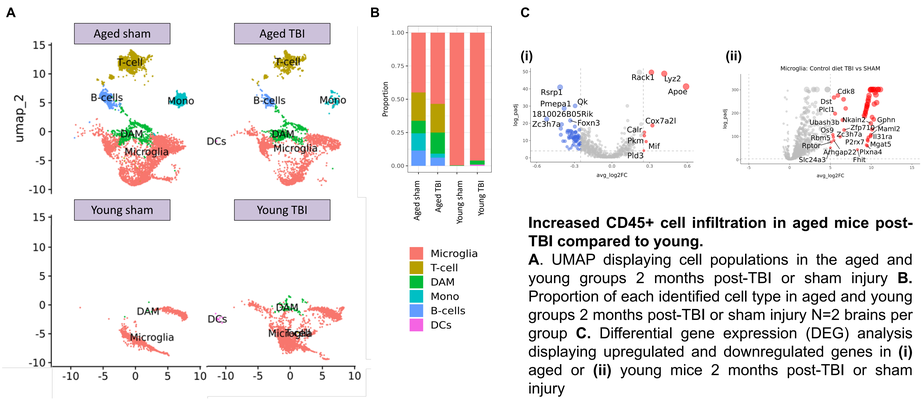
RESULTS

TBI increases CD8+ T-cell infiltration into the brain in middle-aged and aged mice



Several CD8+ T-cell subsets are significantly increased in middle aged and aged mice 1-month post-TBI.
Flow cytometry results displaying the infiltration of **A.** CD8+ **B.** Activated cytotoxic, **C.** effector memory T-cells into the aged, middle-aged and young brain 1-month post-TBI. N=5

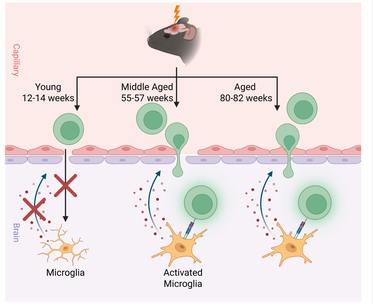
scRNAseq displays increased CD45+ cells in aged mice compared to young mice



Increased CD45+ cell infiltration in aged mice post-TBI compared to young.
A. UMAP displaying cell populations in the aged and young groups 2 months post-TBI or sham injury **B.** Proportion of each identified cell type in aged and young groups 2 months post-TBI or sham injury N=2 brains per group **C.** Differential gene expression (DEG) analysis displaying upregulated and downregulated genes in **(i)** aged or **(ii)** young mice 2 months post-TBI or sham injury

CONCLUSIONS

- We hypothesized that middle-aged mice would have increased CD8+ T-cell infiltration compared to young adult mice due to inflammaging.
- Middle-aged mice had increased CD8+ T-cell infiltration compared to young
- Immune infiltration associated with inflammaging begins before 55 weeks of age in mice, earlier than previously reported[1-3].



- Our previous data suggests worse neurocognitive outcomes and mortality related to age-specific CD8+ T-cell infiltration[4] and that the age-dependent response to TBI is mediated by aged microglia sending recruitment signals to T-cells[2].
- Ongoing studies are focused on therapeutic blockade of microglia-T-cell signaling as a novel treatment paradigm in aged TBI.

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