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T-cell Infiltrates and Microglia Adopt Long-term Gene Signature Changes Leading to Age-specific Responses to Traumatic Brain Injury in Mice

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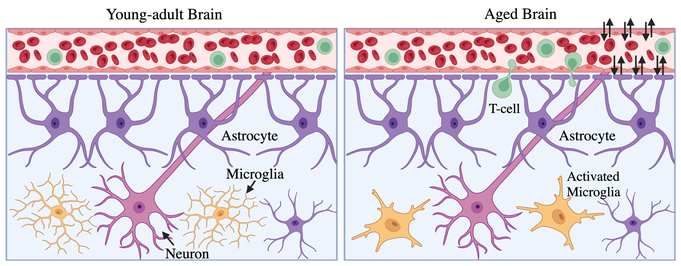
INTRODUCTION

Traumatic brain injury (TBI) has long been called the “silent epidemic”. Elders(≥ 75 years) had highest mortality rates and worst long-term neurocognitive and neuropsychiatric morbidity among all age groups. Moreover, guideline for both acute and chronic care of TBI in the geriatric population are lacking. Almost all preclinical studies of TBI exclude aged subjects. Taken together, uncovering age-linked pathophysiology after TBI is key.

Microglia, the resident macrophages of the brain, are complicit in abovementioned morbidities. Microglia can respond to injuries both acutely and chronically. During certain injuries and disease processes, microglia fail to return to homeostasis and lose normal functions, resulting in chronic, low-level neuroinflammation. Additionally, age introduces an uninvited guest in the brains - the T cells, whose functions remain understudied. Infiltrated T-cells can interact with microglia, the main antigen-presenting cell in the brain, in aging and age-associated diseases.

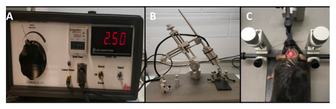


Schematic representation of the normal organization of the parenchyma and blood-brain barrier (BBB) of the young adult (left) and aged (right) brain.

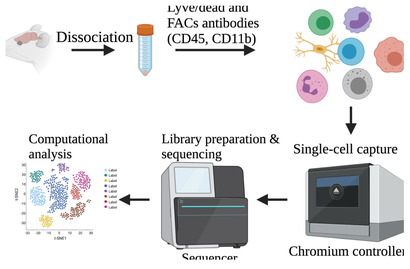
Hypothesis:
We hypothesized that aged microglia would fail to return to a homeostatic state after TBI and adopt a long-term, injury-associated state within the brain of aged mice as compared to young-adult mice after TBI.

METHODS

1. Mice of 80-wk-old (≈ 60 years of age in human) and 14-wk-old (≈ 20 years of age in human) were randomly assigned to either CCI or to sham injury (all males).



Equipment setup for the murine model of controlled cortical impact (CCI).



Schematic of 10X Genomic Single-Cell RNA-seq and CCI TBI model.

2. Brains were harvested from aged and young-adult mice 1-month post TBI or sham surgery for histology and flow cytometric analysis (n=3-5) and 4-month for single-cell RNA-Sequencing (n=2).
3. We FACSorted microglia and peripheral immune cells with CD45.
4. 10x Genomics' scRNA-seq used to obtain the transcriptome. Raw data were processed using the Cell Ranger pipeline mapped to the mm10 mouse genome. R package Seurat was used for clustering and differential expression (DE) analysis following the standard workflow from the Satija Laboratory.
5. Cell type annotation was performed using R package SingleR and canonical markers from literature.
6. Pathway analysis was performed using the Gorilla and MG-T interaction was explored using R package NicheNet. R packges ggplot2 and Seurat were used for plotting.

RESULTS

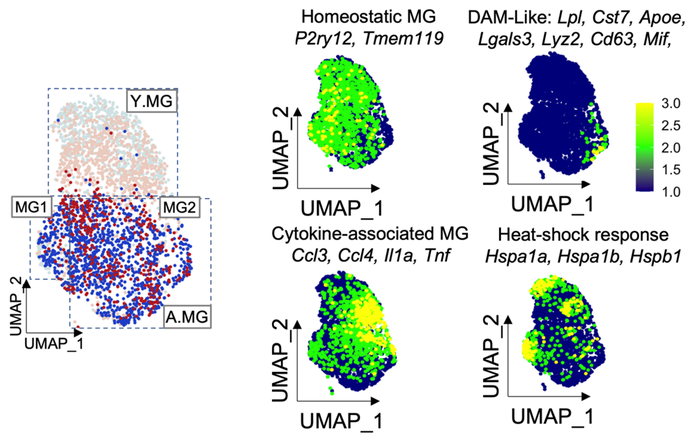


Fig. 1 Left UMAP plot demonstrating clustering obtained for microglia population, annotated by their sample origins. Microglia cluster annotations: Y.MG young mice-enriched microglial cluster, A.MG aged mice-enriched microglial cluster 1, MG1 and MG2 microglial clusters with no dominant origins, DAM disease associated microglia. **Right** microglia scoring based on functions.

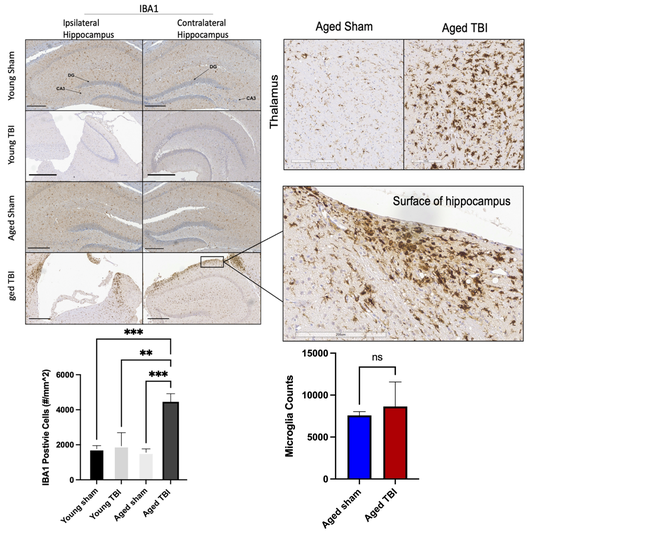
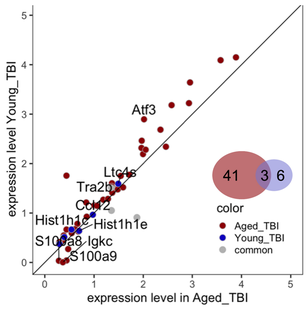


Fig. 2 CCI results in greater ionized calcium-binding adaptor molecule 1 (IBA1) staining in aged mouse brains as compared to young mouse brains after TBI.

In the scRNA-seq analysis, four microglia cluster were identified: young mice-enriched Y.MG, aged mice-enriched A.MG, microglia with no dominant origins MG1 and MG2. DE analysis demonstrated that a subcluster in A.MG adopts gene signature of DAM, a microglial type associated with neurodegenerative diseases. With additional upregulation of *Lgals3*, *Lyz2*, *Cd63*, *Mif*, *Cd74*, this DAM-like subcluster in A.MG is likely associated with immune-inflammatory response in aged brains (**Fig 1**). Furthermore, IBA1 is a pan-microglia marker staining both resting and activated microglia. Since the number of microglia remains unchanged in aged sham vs TBI brains, we reasoned that the substantial increase in IBA1 coverage in aged mice post TBI was attributable to increased microglial activation (**Fig 2**). DE and pathway analysis of genes upregulated in the microglia from aged TBI brain indicate enriched pathways involving in leukocyte recruitment (**Fig 3**).



Aged_TBI only

Pathways (FDR < 0.05)	Genes
Leukocyte (e.g., T, Mo, NP) migration and chemotaxis	*S100a9, S100a8, Ccl2, Ccl3, Pdgfb*
Phagocytosis	*Igkc, Ighm, Abca1*
Cellular response to TNF	*Ccl2, Ccl3, Cebpa*
Regulation of ERK1/2 cascade	*Ccl2, Ccl3, Cd74, Atf3, Klf4, Pdgfb, Dusp1*
Positive regulation of cell death	*Atf3, Ddx3x, Fox, Cdkn1a, Ccl3, Egr1, S100a8, S100a9, Dusp1*
Positive regulation of macromolecular metabolic process	*Ccl2, Ccl3, Klf4, Hspa5, Calr, Fox, Foxb, Cebpa, Zfp36, Pdgfb, Cebpa, Zfp36, Pdgfb, Klf6, S100a8, S100a9, Cd74, Atf3, Ddx3x Tra2b, Kdm6b, Ighm, Cdkn1a, Ier2, Egr1*

Young_TBI only

Pathways (FDR < 0.05)	Genes
Histone H3-K27 trimethylation	*Hist1h1e, Hist1h1c*
Histone H3-K4 trimethylation	*Hist1h1e, Hist1h1c*

Fig. 3 Left Scatter plot depicting expression levels of differentially upregulated genes in Aged TBI (greater than Aged sham) and Young TBI (greater than Young sham). **Right** Pathways analysis of 41 upregulated genes expressed by microglia isolated from aged TBI mice only and 6 upregulated genes expressed by microglia isolated from young TBI mice only with top enriched pathways shown.

RESULTS

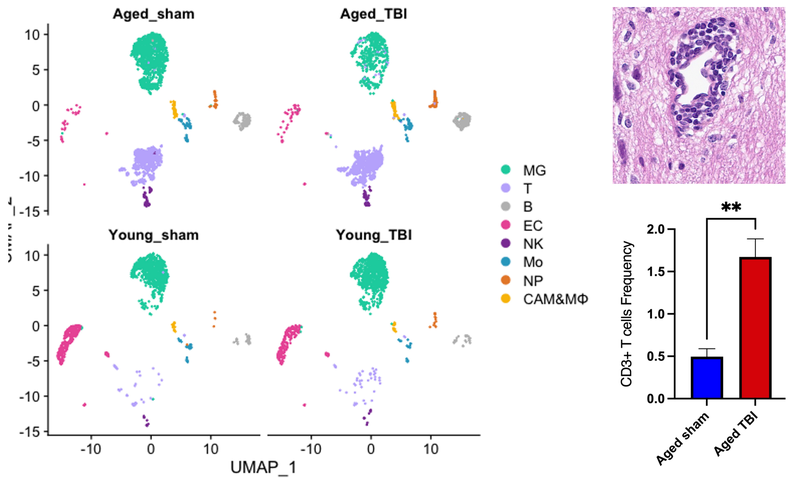


Fig. 4 UMAP plot demonstrating clustering obtained for each group. Cluster annotations: MG microglia, EC epithelial cells, NK natural killer cells, Mo monocytes, NP neutrophils, CAM CNS border-associated macrophages, MΦ monocytes/monocytes-derived macrophages. scRNA-seq, histological, and flow cytometric analyses show that aged brains have substantial T-cell infiltrates.

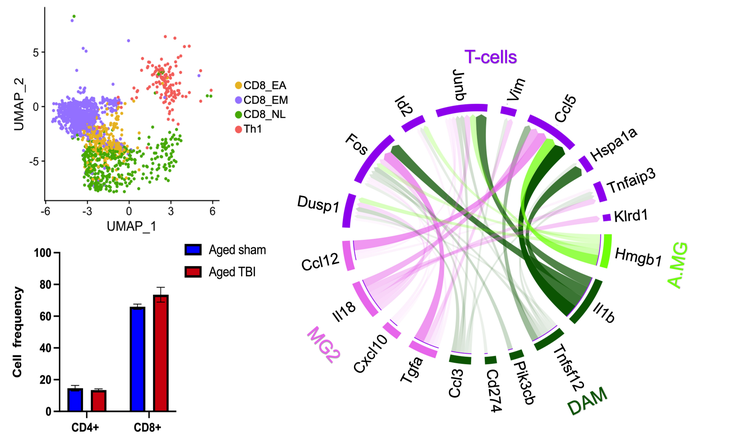


Fig. 5 Left T-cell infiltration is predominated by CD8⁺ T and CD4⁺ cells. UMAP plot demonstrating clustering obtained for T subtypes. Annotations: CD8⁺ EM CD8⁺ effector memory T cells, CD8⁺ EA CD8⁺ early active T cells, CD8⁺ NL CD8⁺ naive-like (century memory and naive) T cells, Th1 T helper type 1 cells. **Right** Circus plot viasualizing predictions of MG-T cells interaction in aged TBI brain.

Concurrently, we did notice amplified lymphocytic infiltrates in aged brain post TBI, which primarily comprised CD8⁺ and CD4⁺ T cells (**Fig 4**). Of note, CD8⁺ effector memory, early active, naive-like T cells and T helper type 1 cells were identified. These T-cell infiltrates might interact with age-associated microglia such as DAM via predicted mediators such as IL1B and IL18 (**Fig 5**).

CONCLUSIONS

1. Young and aged brains of mice have distinct molecular mechanisms of injury in that aged mice have enriched inflammatory, which corresponded to DAM and immune response, which corresponded to the leukocyte infiltration predominated by CD8⁺ T and Th1 cells. Whereas, young-adult microglia returned to homeostasis.
2. Age should be an a priori consideration in future trial design.

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