antiCD49d Ab treatment ameliorates age-associated inflammatory response and mitigates CD8+ T-cell cytotoxicity after traumatic brain injury

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Introduction

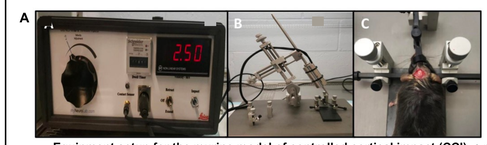
Traumatic brain injury (TBI) afflicts approximately sixty-nine million people worldwide yearly [1]. While TBI affects individuals of all ages, the elderly (aged 65 years and older) experience higher mortality and more severe consequences than younger individuals with similar injury severity. Recently, studies have found that age introduces an uninvited guest in the brains – the T cells likely due to the structural and functional alterations of the blood brain barrier (BBB), which leads to the pass of T cells. Infiltrated T cells can interact with microglia, the gatekeepers in the central nervous system and the main antigen-presenting cell in the brain, in age-associated neurodegenerative diseases [2,3]. Previously, we have observed that aged mouse brains showed significant T cells two months post-TBI. These T cells were largely $CD8^+$ T effector memory (EM) cells. They were more activated and inflammatory. Microglia from these aged, injured mouse brains were also activated and upregulated genes involved in leukocyte chemotaxis, partially explaining the significant presence of T cells in the brains.

Previously Published Findings and Hypothesis

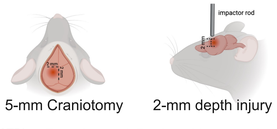
Previously, we utilized anti-CD49d antibody (aCD49d Ab), an FDA approved drug for treating multiple sclerosis and Crohn disease (also known as Natalizumab), to reduce the invasion of circulating lymphocytes. We found that **aCD49d Ab treatment 1). significantly improved survival, neurocognition and motor function in aged but not young TBI mice, and 2). specifically reduced cytotoxic CD8+ T cells within the brains of aged mice [4].** To study the underlying mechanism, we hypothesized that *aCD49d Ab treatment would mitigate age-associated T cell functional decline after TBI in aged mice*.

Methods

Use of aCD49d Ab to reduce the invasion of circulating lymphocytes to the injured brains



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



Young: 3-4 months
vs
Aged: 18-20 months

Day 0
Injury

aCD49d Ab/isotype (*IP*) given 2 hours post-injury; repeated biweekly

Behavioral testing — Behavioral testing

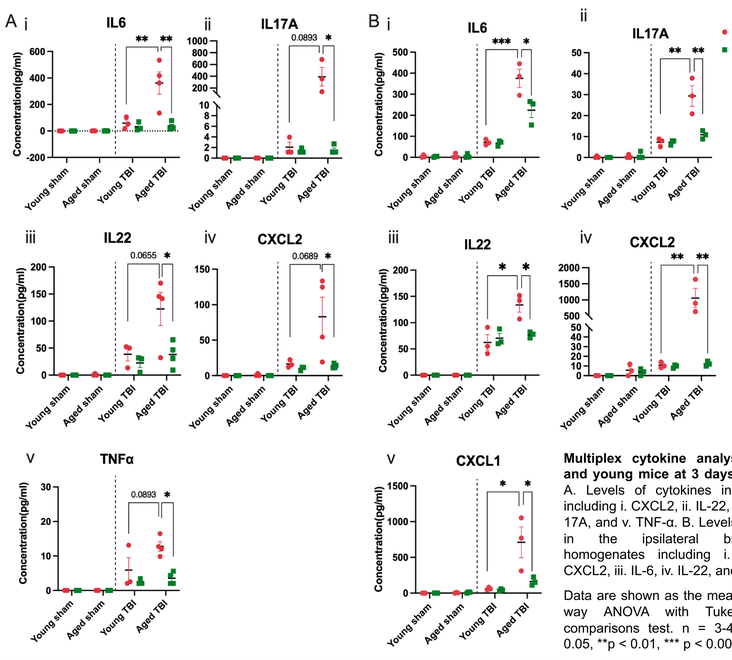
3D post-injury
- Cytokine assessment of brain and plasma samples
- EdU proliferation assay
- CD45 Ab labeling peripheral immune cells

2M post-injury
- Cytokine assessment of brain and plasma samples
- EdU proliferation assay
- CD45 Ab labeling peripheral immune cells
- scRNAseq combined with TCRseq

Experimental Design. Young and aged male C57BL/6 mice were subjected to CCI vs sham injury. 300 ug of aCD49d Ab or isotype control were administered 2 hours post-injury and repeated every 2 weeks. *In vivo* cerebral cell proliferation: 50 mg/kg body weight of 5-ethynyl-2´-deoxyuridine (EdU) via intraperitoneal injection for 3 consecutive days. *In vivo* labeling infiltrating immune cells: 3ug BUV661-CD45 via intravenous injection 2 hours before euthanasia.

Results

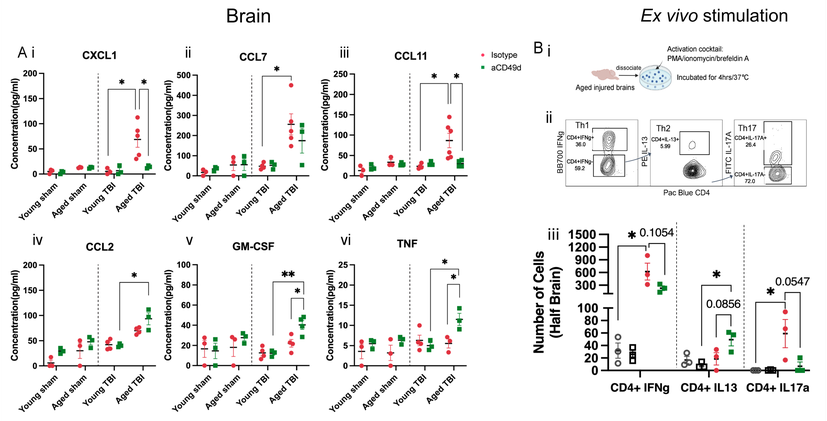
aCD49d Ab treatment functions through suppression of age-associated activated cytokine/chemokine response during acute TBI



Multiplex cytokine analysis in aged and young mice at 3 days post injury. A. Levels of cytokines in the plasma including i. CXCL2, ii. IL-22, iii. IL-6, iv. IL-17A, and v. TNF-α. B. Levels of cytokines in the ipsilateral brain tissue homogenates including i. CXCL1, ii. CXCL2, iii. IL-6, iv. IL-22, and v. IL-17A.

Data are shown as the mean ± SEM, 2-way ANOVA with Tukey's multiple comparisons test. n = 3-4/group, *p < 0.05, **p < 0.01, *** p < 0.001.

aCD49d Ab Treatment Suppressed Immune Cell Chemotaxis but Induced GM-CSF in Aged Brains with Chronic TBI



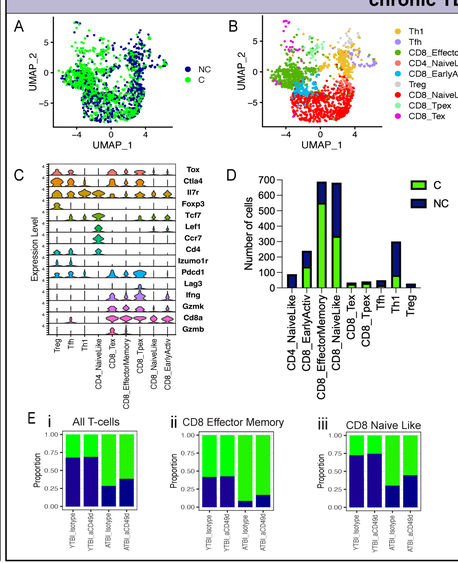
Multiplex cytokine analysis in aged and young mice at 2 months post injury.

A. Levels of cytokines in the ipsilateral brain tissue homogenates including i. CXCL1, ii. CCL7, iii. CCL11, iv. CCL2, v. GM-CSF, and vi. TNF-α.

B. Cytokine response in brain-derived T cells isolated from aged TBI mice i. *ex vivo* stimulation. ii. Gating and iii. number of IFNg, IL13, and IL17a expressing CD4+ T cells indicative of Th1, Th2, and Th17 response.

2-way ANOVA with Tukey's multiple comparisons test was used for A and the Student's t test for B. n =3-8/group, *p < 0.05, **p < 0.01.

aCD49d Ab treatment leads to fewer clonally expanded CD8+ T cells in aged brains with chronic TBI



Clonally expanded CD8+ T cells patrolled aged mouse brains post-TBI and aCD49d Ab treatment induced transcriptional changes largely in CD8+ T Cells.

A. single-cell TCR analysis overlaid on UMAP projections showing distribution of T cell clonality. C: clonally expanded. NC: nonclonally expanded.

B. UMAP showing distribution of T cell subtypes based on T cell atlases.

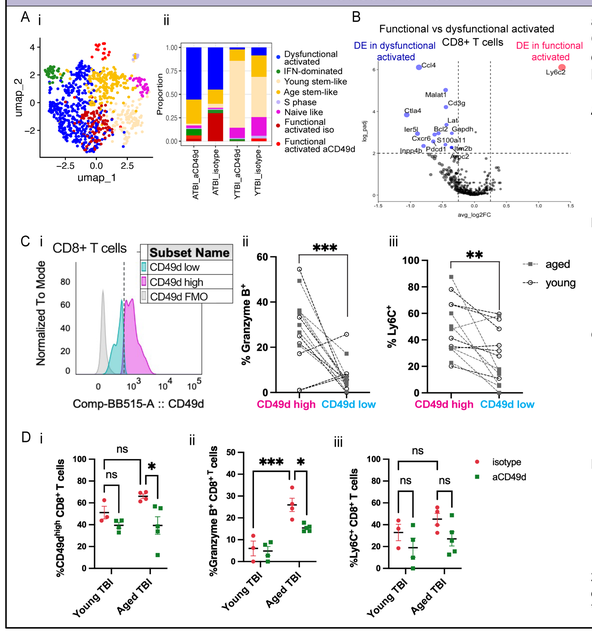
C. Dot plot showing the markers to identify different T cell subtypes.

D. Bar graph demonstrating number of clonality across T cell subtypes.

E. Bar graph demonstrating proportion of i. all T-cell, ii. CD8 effector memory T-cell, and iii. CD8 naïve like T-cell clonality across samples.

F. The proportion of clones belong to each clone size category. ATBI: aged TBI mouse brains. YTBI: young TBI mouse brains.

aCD49d Ab Treatment Reduces CD8+ T cells Exhibiting Higher Effector Status in Aged Mouse Brains with TBI



aCD49d Ab treatment reduces CD8+ T cells that exhibit higher effector status in aged mouse brains after TBI.

A. i. UMAP showing distribution of CD8+ T cell subtypes based on various T cell differentiation states. ii. Bar graph demonstrating proportion of different T cell differentiation states across samples.

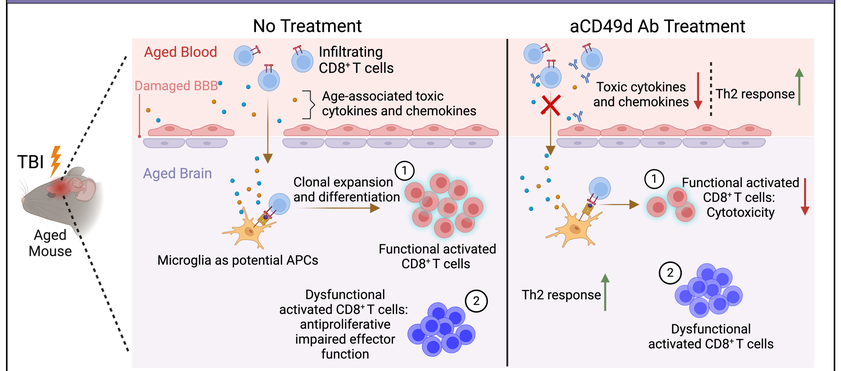
B. Volcano plot depicting DEGs in functional vs dysfunctional activated CD8+ T cells within the brains of aged, injured mice two months post TBI. ATBI: aged TBI mouse brains. YTBI: young TBI mouse brains.

C. i. Histogram showing cutoff for CD49d high vs CD49d low in CD8+ T cells. ii. Comparison of % granzyme B high in CD49d high vs CD49d low CD8+ T cells. iii. Comparison of % Ly6C high in CD49d high vs CD49d low CD8+ T cells.

D. i. % CD49d high in CD8+T cells across TBI samples. ii. % granzyme B high in CD8+ T cells across TBI samples. iii. % Ly6C high in CD8+ T cells across TBI samples.

2-way ANOVA with Tukey's multiple comparisons test for D, n=3-5/group, *p < 0.05, **p < 0.01, ***p < 0.001.

Conclusions



References

[1]Maiden et al., *Am J Respir Crit Care Med.* 2020 [2]Zhang et al, *Immun Ageing.* 2022 [3]Schetters et al., *Front. Immunol.* 2018 [4]Chen et al., *Shock* 2024

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