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Fecal Microbiota Transplant (FMT) with Youthful Gut Microbiota Increases Cellular Metabolism in Microglia from Aged Mice Post Traumatic Brain Injury (TBI)

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Background

- Traumatic brain injury (TBI) affects nearly 3 million people annually in the United States each year.
- is well-recognized to have long-term TBI neurocognitive consequences in patients aged 65+.
- The brain-gut-microbiota axis is a complex, bidirectional communication network, and disruptions to this system can disturb its finely tuned balance

Prior data

Fecal microbiota transplant (FMT) post-TBI in adult mice (12-15 weeks old):

- restores injury-induced dysbiosis (Fig 1a)
- downregulates inflammatory gene expression in microglia (Fig 1b)

Aged mice (78 – 80 weeks old) display:

- aging-induced dysbiosis
- TBI exacerbates existing dysbiosis
- constitutively active microglia³





Figure 1. (a) Boxplots summarizing the restoration of gut microbiota with FMT following TBI. Fecal microbiome biodiversity was measured by species richness and evenness from operational taxonomic units (OTU) generated from 16s rRNA-sequencing¹. Each box representing groups with N=5. ($P \leq 0.05(*)$, 0.001(**), and 0.0001(***) (b) Heatmap demonstrating a reduction in inflammatory gene expression in microglia 60 days post-TBI after FMT. The number of microglia within each group: FMT = 2,046, FMT_TBI = 3,435, sham = 2,941, and TBI = $3,093^2$.

Hypothesis

Restoration a youthful gut microbial community structure in aged mice after TBI will attenuate activation of microglia.





Figure 3. (a) Scheme of the experimental workflow. Aged C57BL/6 mice (84 weeks old) underwent severe TBI or sham injury via open-head controlled cortical impact. The mice received oral gavage of either vehicle (PBS), stool from uninjured young donors (10-14 weeks old) or aged donors weekly. Brains were harvested 60 DPI and processed using CD45 microbeads to deplete neurons. CD45+ cells then were prepared for single cell RNA-seq using the 10x Genomics Chromium Single Cell 3' v3.1 Reagent Kit. (b) UMAP plot of CD45⁺ cells from each group. Number of cells following QC from each group from two biological replicates: sham-PBS = 5,276, sham-agFMT = 4,363, sham-ygFMT = 7,926, TBI-PBS = 18,769, TBI-agFMT = 6,156, TBI-ygFMT = 11,342.



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Figure 6. Cell-cell communication analysis of TGFβ signaling pathway using CellChat. (a) Heatmap identifying key signaling roles across cell types from each condition. Importance, or centrality, is a quantification of the interconnectedness within the pathway. (b) Representative chord diagram across groups visualizing a ligand-receptor pair: Tgfb1 (ligand) with its receptor complex, *Tgfbr1* and *Tgfbr2*.

Conclusion

- Our hypothesis was not supported suggesting age-dependent differences in pathophysiology
- Interestingly, FMT from young donors increased mitochondrial metabolism in microglia, potentially reflecting a shift from glycolytic metabolism—characteristic of DAM—to oxidative phosphorylation.
- FMT from young donors attenuated residual, injury-induced, TGFβ pathway suggesting potential mechanism as to how youthful gut microbiota may affect microglia from aged mice.

References

¹ Davis, B. T. et al. Fecal Microbiota Transfer Attenuates Gut Dysbiosis and Functional Deficits After Traumatic Brain Injury. Shock (2022). ² Davis, B. T. et al. Postinjury Fecal Microbiome Transplant Decreases Lesion Size and Neuroinflammation in Traumatic Brain Injury. Shock (2022). ³ Chen, Z. et al. Microglia and Infiltrating T-cells Adopt Long-Term, Age-Specific, Transcriptional Changes After Traumatic Brain Injury in Mice. Shock (2023)



Table 2. Common gene ontology (GO) and pathway terms up-regulated in ygFMT mice compared to agFMT with both sham- & TBI-injured **microglia.** Combined score computed using the log of the p-value from the Fisher exact test and multiplying that by the z-score of the deviation from the expected rank. Adjusted p-value using Benjamini-Hochberg method for multiple hypothesis testing. Terms were curated using *EnrichR* from differentially up-regulated genes: GO (*biological processes, cellular* component, and molecular function) and pathways (Reactome 2024 Pathway, WikiPathway 2024 Mouse, and MSigDB Hallmark).