Northwestern Medicine
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Microglia- Dependent Sex- Effect on Acute TBI Outcomes

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BACKGROUND

In the United States there are 638 individuals are hospitalized every day secondary to traumatic brain injury (TBI)-related injuries resulting in 176 daily deaths. Men are disproportionately represented in most studies as trauma is male predominate. This has resulted in a critical unmet research need to determine whether there is a different pathophysiology of injury between men and women. In fact, the CDC has reported that 40% of TBI is sustained by women. Women TBI patients differ in severity of injury and mortality. Indeed, women are 1.5-2 time more likely to have long-term neuropsychiatric sequelae such as anxiety and PTSD than are men. Sex hormones such as estrogen and progesterone play a critical role in protecting glial cells and neurons which subsequently protects the brain from edema, necrosis, apoptosis and inflammation. Sex differences have been largely unexplored in both preclinical and clinical TBI studies remains understudied.

RESEARCH OBJECTIVES

Recent work in our laboratory has shown that female TBI mice are disinhibited as compared to male mice suggesting greater anxiety-like deficits after TBI as compared male mice. These data suggest a different pathophysiology of injury in female subjects as compared to male subjects after TBI. Microglia, the resident innate immune cell of the brain, are complicit in this process. **To this end we hypothesized that microglia would adopt divergent, sex-dependent TBI-associated transcriptional profiles following acute brain injury.**

METHODS

Figure 1. Vaginal Smear for Estrous Cycle Staging Identification

Fig 1 **Right** showing the schematic for vaginal smear and murine estrous assessment. **Far Right** (A) showing metestrus featured by high number of leukocytes and (B) proestrus featured by the presence of large nucleated cells (indicated by a red arrow). Magnification: 20X.

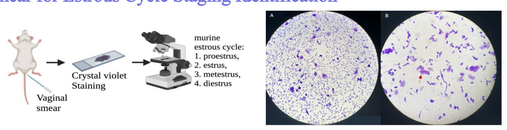


Figure 2. SSC Analysis

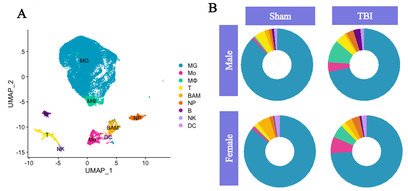


Fig 2 (A) UMAP showing population of immune cells in all samples. (B) **Top** Male Groups donut plot indicating the distribution of sequenced immune cells types **Bottom** Female Groups donut plot indicating the distribution of sequenced immune cells types

Figure 3. Dim Plot

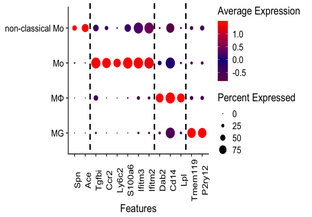


Fig 3. Dim plot showing curated markers identifying each cell type in the samples

8- HOUR MALE VS. FEMALE ANNOTATION

Figure 4. 8 hr male vs female annotation myeloid cells clustering

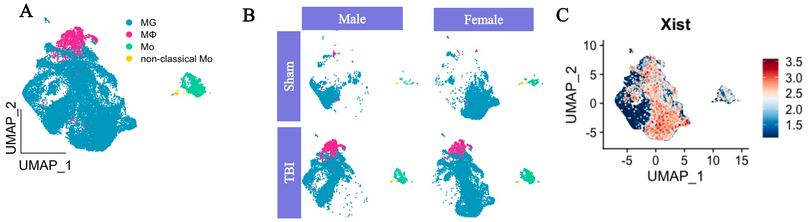


Fig 4 (A) UMAP of myeloid cell clustering of all samples ; (B). myeloid cell clustering of each group; (C) Disparate signature in microglia as female cells exclusively expresses *Xist*

MICROGLIA SCORING

Figure 5. 8 Hr Scoring Based On Function And Sex- Specific Markers

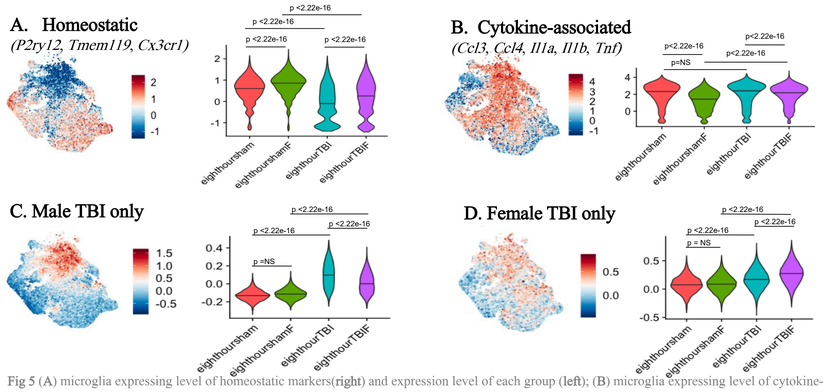
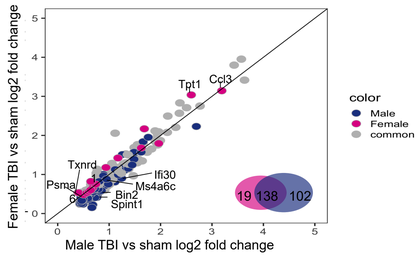


Fig 5 (A) microglia expressing level of homeostatic markers(**right**) and expression level of each group (**left**); (B) microglia expressing level of cytokine-associated markers(**right**) and expression level of each group (**left**); (C) group microglia expressing male TBI specific markers(**right**) and expression level of each group; (D) group microglia expressing female TBI specific markers(**right**) and expression level of each group.

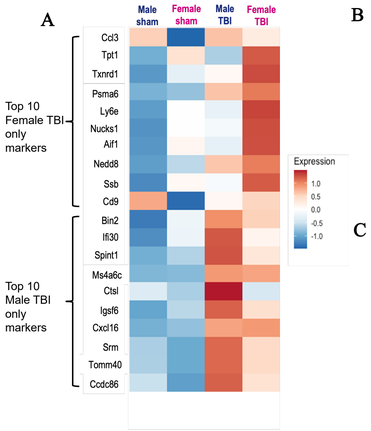
Figure 6. Differential Expressed Genes In Microglia Between Male TBI vs Female TBI

Fig 6. Scatter plot depicting the DGE. Both groups expressed 138 of the same genes. However, Male TBI expressed 102 other genes. Where as Female TBI expressed only 19 genes



TOP 10 DIFFERENTIAL EXPRESS GENES

Figure 7. 8 hr male vs female annotation myeloid cells clustering



B

Table of top 10 significant p-values and q-values for GO Biological Process 2021

	term	p-value	q-value	overlap_genes
	glial cell migration (GO:0008347)	0.000018	0.009131	[CCL3, CD9]
	cellular response to chemical stress (GO:0062197)	0.000114	0.019600	[TXNRD1, PARK7, AIF1]
	ruffle organization (GO:0031529)	0.000115	0.019600	[PLEK, AIF1]
	cellular response to oxidative stress (GO:0034599)	0.000215	0.027070	[TXNRD1, PARK7, AIF1]
	positive regulation of response to external stimulus (GO:0032103)	0.000293	0.027070	[LY86, CCL3, AIF1]
	regulation of calcium-mediated signaling (GO:0050848)	0.000318	0.027070	[PLEK, CCL3]
	modulation by host of symbiont process (GO:0051851)	0.000417	0.030377	[CCL3, NUCKS1]
	regulation of inflammatory response (GO:0050727)	0.000924	0.057222	[PSMA6, CCL3, PARK7]
	negative regulation of intrinsic apoptotic signaling pathway (GO:2001243)	0.001664	0.057222	[PARK7, TPT1]
	proteolysis involved in cellular protein catabolic process (GO:0051603)	0.002217	0.057222	[PSMA6, NEDD8]

C

Table of top 10 significant p-values and q-values for GO Biological Process 2021

	term	p-value	q-value	overlap_genes
	cytokine-mediated signaling pathway (GO:0019221)	3.078456e-10	3.598034e-07	[CD86, HSPA9, SP1, CEBPD, IFI30, SOD2, PSMA5, PSMC5, PSMA3, PSMC4, PSMD3, TCP1, BOLA2, PHF1, PSMD1, PSME2, HMOX1, BRI3, PTPN2]
	regulation of transcription from RNA polymerase II promoter in response to stress (GO:0043618)	6.011753e-10	3.598034e-07	[PSMA5, PSMA3, PSMC5, PSMC4, PSMD3, PSMD1, PSME2, HMOX1, ELOB]
	cellular response to hypoxia (GO:0071456)	1.313159e-09	5.239503e-07	[PSMA5, PLK3, PSMC5, PSMA3, PSMC4, PSMD3, PSMD1, PSME2, HMOX1, ELOB]
	regulation of mRNA stability (GO:0043488)	3.783249e-09	8.044368e-07	[PSMA5, PSMC5, PSMA3, PSMC4, SLC11A1, MAPKAPK2, PSMD3, HNRNPU, PSMD1, PSME2]
	regulation of hematopoietic progenitor cell differentiation (GO:1901532)	4.407054e-09	8.044368e-07	[PSMA5, PSMA3, PSMC5, SP1, PSMC4, PSMD3, PSMD1, PSME2]
	regulation of transcription from RNA polymerase II promoter in response to hypoxia (GO:0061418)	4.407054e-09	8.044368e-07	[PSMA5, PSMA3, PSMC5, PSMC4, PSMD3, PSMD1, PSME2, ELOB]
	Fc-epsilon receptor signaling pathway (GO:0038095)	5.355341e-09	8.044368e-07	[LYN, PSMA5, PSMC5, PSMA3, PSMC4, PSMD3, PSMD1, PSME2, CALM1]
	Fc receptor signaling pathway (GO:0038093)	5.798907e-09	8.044368e-07	[LYN, PSMA5, PSMC5, PSMA3, PSMC4, PSMD3, PSMD1, PSME2, CALM1]
	antigen processing and presentation of exogenous peptide antigen via MHC class I (GO:0042590)	6.048397e-09	8.044368e-07	[PSMA5, PSMA3, PSMC5, PSMC4, PSMD3, PSMD1, PSME2, IFI30]
	regulation of cellular amine metabolic process (GO:0033238)	7.015148e-09	8.397132e-07	[PSMA5, PSMA3, PSMC5, PSMC4, PSMD3, PSMD1, PSME2]

Fig 7 (A) Heat map of the 10 ten DGE of Male TBI and Female TBI; (B) Table of top 10 significant p-values and q-values for GO Biological Processes of Female TBI; (C) Table of top 10 significant p-values and q-values for GO Biological Processes of Male TBI

CONCLUSION

We observed a difference in transcriptional signatures within the microglia of young-adult male and female mice at baseline, which led to a divergent transcriptional response to TBI :

- The top 10 significant p-values in female TBI included glial cell migration (p>0.00002), cellular response to chemical stress (p>0.0002), and ruffle organization (p>0.0002).
- Male TBI included cytokine-mediated pathways (p=3.08 x10^{-10}), transcription regulation (p=6.01 x10^{-10}), and cellular response to hypoxia (p=1.3 x10^{-9})
- This supports the need for sex to be evaluated as an independent variable in future clinical trial design.

ACKNOWLEDGMENTS

This research was funded by NIH Grant 1R01GM130662