The Implications of TNF-α and Microglia Activation on the Progression of Multiple Sclerosis

Kate Barrett, Blake Dilda, Mansi Gupta, Ishmeet Maharaj, Dr. Shveta Parekh, PhD
Department of Psychology and Neuroscience, University of North Carolina at Chapel Hill

Introduction

- Multiple sclerosis (MS) is a disease localized to the brain and spinal cord where the immune system mistakenly attacks the myelin sheath on neurons.
- Women with MS tend to outnumber men with the condition by nearly 4 to 1.[1]
- TNF-α is a cytokine known to modulate the progression neurodegenerative disorders such as MS.
- In the short-term, TNF-α negatively affects the myelin sheath; however, in the long run, TNF-α plays a beneficial role in stimulating remyelination.[2]
- In microglia, an immune defense cell, TNF-α has been shown to exhibit a positive feedback loop, causing sustained pro-inflammatory activation.[3]
- Thalamic atrophy caused by demyelination of axons is one of the most important indicators of cognitive impairment associated with MS.[4]
- The thalamus is essential for relaying sensory information to the brain, impacting perception and consciousness.

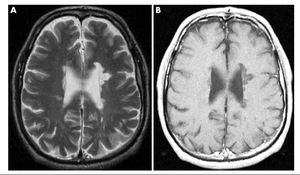

Figure 1. MRI Scans of Brain for MS. Left MRI scan shows brain with MS. The decrease in white gradient in the image depicts the degradation of myelin sheath. The right image depicts a normal brain.

Aims

Aim: Investigate the expression of TNF-α in microglia in the thalamus of female rats after inducing an inflammatory response with lipopolysaccharide (LPS), a component of gram negative bacteria that triggers the release of inflammatory cytokines, as it may offer insight into acute demyelination and increased prevalence of MS.

Hypothesis: There will be an increase in TNF-α expression in rat microglia within the thalamus after an LPS induced inflammatory response.

Methods

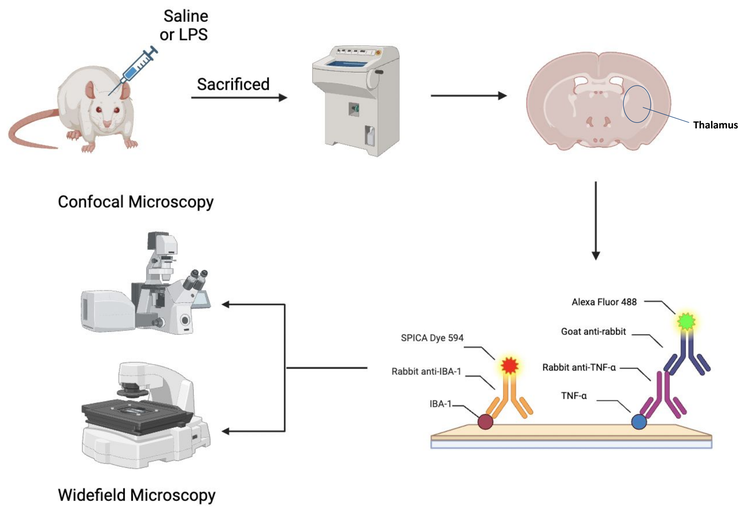

Figure 1. General Schematic for Experimental Design. 16 female rats were injected with either LPS or saline and further cryosectioned. Rat brain slices were stained for Iba-1 and TNF-α using immunohistochemistry. Rat brain slices were then imaged using confocal microscopy (microglial soma area, microglia processes) and widefield microscopy (percent area, colocalization, and cell counts).

Results

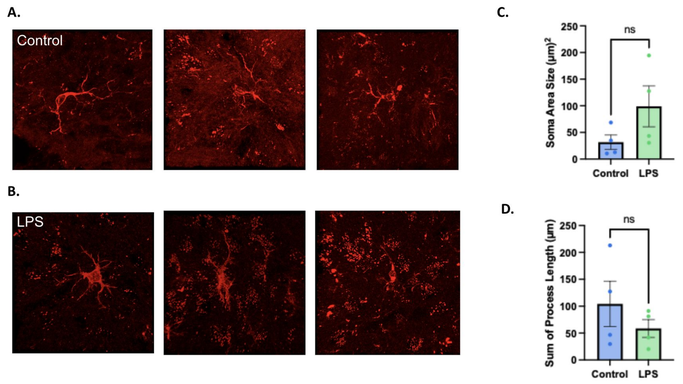

Figure 2. Microglia Soma Area Size (μm2) and Sum of Processes Length (μm) in LPS vs Saline Rat Thalamus. Panel A depicts images of microglia of the rat brain tissue at 63x that received a saline injection. Panel B shows the images of microglia of rats at 63x that received the LPS injection. Panel C shows the difference in soma area in control (saline) versus LPS-subjected rats. Panel D shows the difference in the sum of processes length for the control (saline) versus LPS-subjected rats. There was no significant difference in either soma area or sum of processes length according to the unpaired t-test. Error bars signify the standard error of mean.

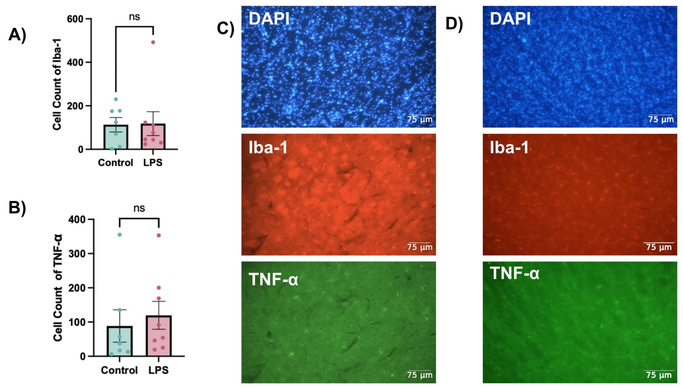

Figure 3. Cell counts of Iba-1 and TNF-α within the thalamus. Panel A shows the cell counts of Iba-1 within the thalamus for the control rats vs. the LPS injected rats. Panel B shows the cell counts of TNF-α within the thalamus for the control rats vs. the LPS injected rats. There was no significant difference in the cell counts for either Iba-1 and TNF-α according to the unpaired t-test. Panel C shows widefield microscopy images taken at 20x of the thalamus within a saline injected rat (rat 19) and (D) a LPS injected rat (rat 5). Error bars signify the standard error of mean.

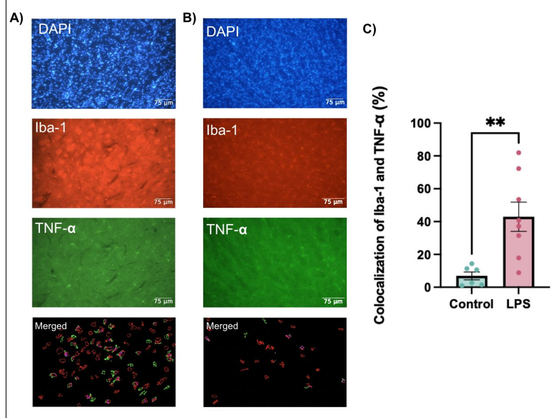

Figure 4. Colocalization of Iba-1 and TNF-α within the thalamus. Panel A shows sample images demonstrating the overlap of Iba-1 and TNF-α within a saline injected rat (rat 19) taken at 20x with widefield microscopy. Panel B shows sample images demonstrating the overlap of Iba-1 and TNF-α within an LPS injected rat (rat 5) taken at 20x with widefield microscopy. Panel C demonstrates the percent of microglia (Iba-1) that colocalize with TNF-α for both the saline injected and LPS injected rats. There was a significant difference (represented by **) in the percent colocalization according to the unpaired t-test. Error bars show the standard error of mean.

Discussion

- Previous research says the thalamus is a common region of interested related to MS progression.[2]
- LPS rats showed increased thalamic soma area and decreased process length, which is in agreeance with known literature.
- The lack of an increase in TNF-α for the LPS-induced rats does not support our hypothesis as we predicted there would be more TNF-α released by the microglia when subjected to an inflammatory response.
- Significant differences were found regarding colocalization of TNF-α and microglia in LPS induced mice.
- Our data suggests that microglia produced the TNF-α that has a role with response to neuroinflammation.
- According to previous research, the presence of TNF-α in the thalamus is thought to be closely associated with the primary symptom of MS, neurodegeneration. [5]
- Limitations such as sample size, sex bias, limited trials, and absence of MS-affected rats decreased the strength of our results.

Conclusion

- No significant differences were found for microglia soma area size and sum of processes length between the LPS and saline group.
- Trends were seen with a larger soma area and shorter processes in the LPS, an amoeboid shape typically seen with microglial activation.
- There was no significant difference in cell counts for Iba-1 and TNF-a between the LPS and saline group.
- There was a significant difference in the co-localization of TNF-a and Iba-1 for the LPS and saline group.
- The data acquired represents the earlier stages of MS development, as the high levels of TNF-α and activated microglia morphology suggest that a similar pro-inflammatory response associated with an acute MS response was induced.

Future Studies

- Long-term effects of LPS on microglia activation and TNF-α expression in order to better understand the acute vs. long-term role of microglial TNF-α in MS.
- Sex-difference studies for microglia, TNF-α, and MS using both male and female rats.
- Role of estrogen on microglia, TNF-α, and MS. Specifically, looking into possible reasonings behind sex-differences in MS.
- Use MS-induced rats in order to collect direct data on the role of TNF-α in MS (compared to making generalizations).

References and Acknowledgements

1. Harbo, H. F., Gold, R., & Tintoré, M. (2013). Sex and gender issues in multiple sclerosis. Therapeutic advances in neurological disorders, 6(4), 237–248. https://doi.org/10.1177/1756285613488434
2. Arnet, H. A., Mason, J., Marino, M., Suzuki, K., Matsushima, G. K., & Ting, J. P. Y. (2001). TNF-α promotes proliferation of oligodendrocyte progenitors and remyelination. Nature Neuroscience, 4, 1116-1122. https://doi.org/10.1038/nn738
3. Rawji, K. S., & Yong, V. W. (2013). The Benefits and Detriments of Macrophages/Microglia in Models of Multiple Sclerosis. Journal of Immunology Research, Article 948976. https://doi.org/10.1155/2013/948976
4. Amin, M., & Ontaneda, D. (2021). Thalamic injury and cognition in multiple sclerosis. Frontiers in Neurology, 11. https://doi.org/10.3389/fneur.2020.623914
5. Popic, J., Pesic, V., Milanovic. D., Loncarevic-Vasiljkovic, N., Smiljanic, K., Kanazir, S., Ruzdijic, S. (2015). Induction of TNF-α signaling cascade in neonatal rat brain during propofol anesthesia. International Journal of Developmental Neuroscience, 44. https://doi.org/10.1016/j.ijdevneu.2015.05.003

Thank you to Dr. Parekh and the Neuroscience department for allowing us to conduct our research.