An In Vitro Model To Explore the Effects of an Inflammatory Environment on Mouse Astrocytes

Ryan Morris, Lisa Conti, PhD, Kelly Crusio, PhD

Introduction

Inflammation has been implicated in worsening brain damage in many central nervous system diseases such as Alzheimer's, which as of 2020, affected as many as 5.8 million Americans[1] or stroke, which affects over 795,000 people yearly.[2]

Increases in systemic inflammation has been correlated with increases in chronic psychosocial stress.[3] The effects that inflammation and chronic stress can contribute to central nervous system (CNS) diseases is currently not included in treatment options and remains an important area to research. For example, by 2060, the number of Alzheimer's disease cases is expected to rise to around 14 million people, with minority populations being the most affected.[1] The interplay between how inflammation and chronic stress interact in situations such as these needs to be studied.

One of the potential contributors to such inflammation and responders to stress in the brain are glial cells. Specifically, astrocytes can be tissue-protective or can contribute to damage of the central nervous system, playing a vitally important role. A receptor that responds to stress is corticotropin releasing factor 1 (CRF1), a major modulator of the body's response to stress. It is located throughout the body, including astrocytes.

The main purpose of this experiment was to examine the effects of an inflammatory environment on the release of cytokines/chemokines from mouse astrocytes. CRF mRNA levels were also assessed.

Methods

Ċultured astrocytes from mouse cortex were treated with S100A9, a calcium binding protein actively involved in modulating the inflammatory response, and IL-1B, a cytokine that is also an important mediator of the inflammatory response were added to the media of the experimental plates.

There were three collection timepoints of 6, 12, and 24 hours.

This experiment was repeated a total of three times.

A cytokine array was used to measure the levels of cytokines and chemokines expressed from the supernatant collected.

ImageJ was used to analyze the data from these arrays and to determine the relative light units (RLU). RLU was used to reflect the level of expression of each cytokine/chemokine.

Real-time PCR was used to determine the level of expression of corticotropin releasing factor receptors by the astrocytes.

Results

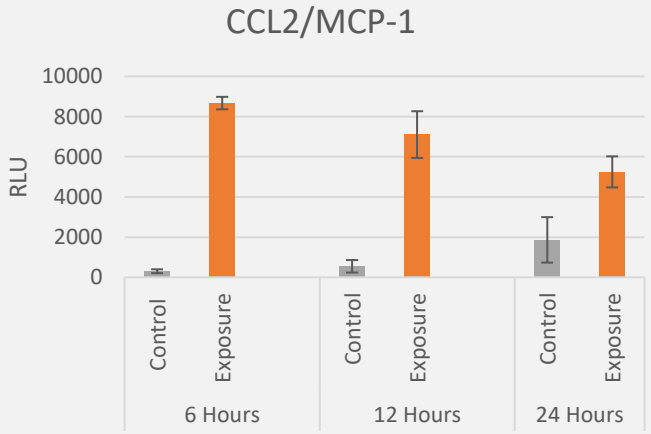


Figure 1: An example of the differences in expression seen between the cytokines/chemokines in the exposure group versus the control group from all three experiments.

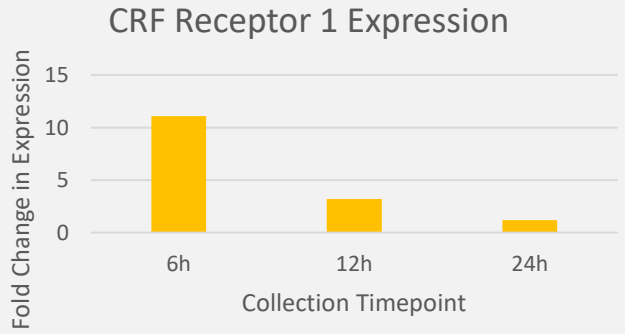
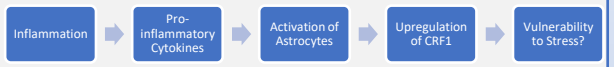


Figure 2: Fold change in CRF expression from control group compared to exposure group. Realtime RT-PCR was used (delta/delta Ct method).The mean of all three experiments was taken.

Results/Discussion

Eight cytokines showed a significant difference in expression between the experimental and control groups

1. CXCL10/IP-10/CRG-2
2. CCL2/MCP-1
3. CCL5/RANTES
4. CxCL1
5. CCL3/MIP1alpha
6. CCL4/Mip1beta
7. CXC12/SDF1
8. TNF-alpha



Summary and Conclusion

There were eight cytokines/chemokines that showed a significant increase in expression at all three timepoints. The CRF1 receptor was increased within the first 6 hours following exposure to an inflammatory environment.

There appears to be a significant relationship between chronic stress, inflammation, and the progression of common CNS diseases, such as Alzheimer's. A few studies done on patients with Alzheimer's disease have shown that treatment with SSRIs have delayed decline in cognitive function.[4] The cause of this delay is likely multifactorial, but this experiment shows that there may be a significant interplay between inflammation and the natural stress response, possibly worsening these diseases. Due to the increase in CRF1 at the first timepoint, this experiment shows that inflammation may leave people more vulnerable to subsequent stress. The impact of this vulnerability and the likely increase in stress needs to be studied.

Further research will be done to determine the impacts of these cytokines/chemokines on the brain. Future research is needed to investigate primary care interventions that can prevent or delay the progression of inflammation mediated CNS disease.

References

1.Matthews, K. A., Xu, W., Gaglioti, A. H., Holt, J. B., Croft, J. B., Mack, D., & McGuire, L. C. (2018). Racial and ethnic estimates of Alzheimer's disease and related dementias in the United States (2015–2060) in adults aged≥ 65 years. *Alzheimer's & Dementia*. https://doi.org/10.1016/j.jalz.2018.06.3063
2.Tsao CW, Aday AW, Almarzooq ZI, Beaton AZ, Bittencourt MS, Boehme AK, et al. Heart Disease and Stroke Statistics—2023 Update: A Report From the American Heart Association. *Circulation*.2023;147:e93–e621
3.Liu YZ, Wang YX, Jiang CL. Inflammation: The Common Pathway of Stress-Related Diseases. Front Hum Neurosci. 2017 Jun 20;11:316. doi: 10.3389/fnhum.2017.00316. PMID: 28676747; PMCID: PMC5476783.
4. Dolotov OV, Inozemtseva LS, Myasoedov NF, Grivennikov IA. Stress-Induced Depression and Alzheimer's Disease: Focus on Astrocytes. *International Journal of Molecular Sciences*. 2022; 23(9):4999. https://doi.org/10.3390/ijms23094999