Aido Lopez, Laboratory of Dr. Eyo Ukpong

Specific Aims: Glial cells are support cells of the brain and establish intimate relationships with the neurons, the primary functional cellular unit of the brain. These relationships give rise to the complex architecture and functionality of the brain. Specifically, astrocytes and microglia, two types of glia, engage in tightly regulated interactions, and abnormalities in their crosstalk result in both cells acquiring reactive states that arise during CNS infection, trauma, and in disease[1-7]. There is also growing suggestion that the therapeutic targeting of microglia-astrocyte interactions could be a promising avenue of research in various brain diseases[7,8]. Particularly, the microglial P2RY12 receptor has been recognized as a marker of homeostatic microglia and its downregulation has been found in neurodegenerative contexts[9,10], where microglia acquire distinctive disease-associated expression profiles[11]. While P2RY12's role in microglial identity has been initially explored, its effects on microglia heterogeneity remain unknown. Furthermore, our knowledge on how P2RY12 affects microglia-astrocyte interactions and influences astrocytic sub-states and functions remains unexplored.

Our lab recently described P2RY12KO microglia displaying fewer cell body interactions with capillaries accompanied by vascular abnormalities suggesting that microglial P2RY12 could be involved in regulating vascular features[12]. This novel microglia-vasculature interaction led me to hypothesize that microglial P2RY12 may regulate neurovascular unit cells, such as astrocytes, which are critical for homeostatic regulation of the vasculature[13,14]. Astrocytes extend large, flattened processes, called endfeet that together with pericytes cover up to ~99% of the cerebrovascular surface[15,16]. Furthermore, astrocytes support blood-brain barrier (BBB) integrity[17-20], which is essential for CNS health and its breakdown greatly contributes to neurodegenerative disorders[21]. Although there is evidence of P2RY12-positive microglial processes establishing direct contact with astrocytes[22], the role of microglial P2RY12 on astrocytic identity and function is currently unknown. To begin to address this, I performed transcriptomic analysis by bulk RNA sequencing of wildtype and P2RY12KO microglia as well as immunohistochemistry (IHC) using classical astrocytic markers in brain sections from wildtype and P2RY12KO mice. My preliminary data show that P2RY12KO microglia shift towards a pro-inflammatory profile and impact astrocytic density, reactive state, and vascular interactions. This suggests a role for the microglial P2RY12 in astrocytic identity and function which will be further explored in this proposal. Therefore, I propose to determine if the **microglial P2RY12 regulates microglial and astrocytic sub-states in the adult brain.** This hypothesis will be tested along the following aim:

- **Determine P2RY12 regulation of microglial and astrocyte sub-states using single nuclei RNA sequencing and deep learning models for transcriptomic analysis**. I hypothesize that the loss of P2RY12 skews microglia to a more pro-inflammatory sub-state and since inflammatory microglia influence astrocyte reactivity[23], I will characterize both microglial and astrocytic sub-states using single nuclei transcriptomic approaches on cells from wildtype and P2RY12KO mice. Additionally, the large-scale single nuclei data will be analyzed by implementing deep-learning models allowing a more integrative approach for robust interpretations of the underlying biological mechanisms and cellular subpopulations resulting from P2RY12 deficiency.

Literature Cited

1. 12 Hasel, P., Aisenberg, W. H., Bennett, F. C. & Liddelow, S. A. Molecular and metabolic heterogeneity of astrocytes and microglia. *Cell Metab* **35**, 555-570 (2023). https://doi.org:10.1016/j.cmet.2023.03.006
2. 13 Guttenplan, K. A. *et al.* Neurotoxic Reactive Astrocytes Drive Neuronal Death after Retinal Injury. *Cell reports* **31**, 107776 (2020). https://doi.org:10.1016/j.celrep.2020.107776
3. 14 Guttenplan, K. A. *et al.* Neurotoxic reactive astrocytes induce cell death via saturated lipids. *Nature* **599**, 102-107 (2021). https://doi.org:10.1038/s41586-021-03960-y
4. 15 Guttenplan, K. A. *et al.* Knockout of reactive astrocyte activating factors slows disease progression in an ALS mouse model. *Nat Commun* **11**, 3753 (2020). https://doi.org:10.1038/s41467-020-17514-9
5. 16 Joshi, A. U. *et al.* Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration. *Nat Neurosci* **22**, 1635-1648 (2019). https://doi.org:10.1038/s41593-019-0486-0
6. 17 Hartmann, K. *et al.* Complement 3(+)-astrocytes are highly abundant in prion diseases, but their abolishment led to an accelerated disease course and early dysregulation of microglia. *Acta Neuropathol Commun* **7**, 83 (2019). https://doi.org:10.1186/s40478-019-0735-1
7. 18 Sterling, J. K. *et al.* GLP-1 Receptor Agonist NLY01 Reduces Retinal Inflammation and Neuron Death Secondary to Ocular Hypertension. *Cell reports* **33**, 108271 (2020). https://doi.org:10.1016/j.celrep.2020.108271
8. 19 Yun, S. P. *et al.* Block of A1 astrocyte conversion by microglia is neuroprotective in models of Parkinson's disease. *Nat Med* **24**, 931-938 (2018). https://doi.org:10.1038/s41591-018-0051-5
9. 20 Mildner, A., Huang, H., Radke, J., Stenzel, W. & Priller, J. P2Y(12) receptor is expressed on human microglia under physiological conditions throughout development and is sensitive to neuroinflammatory diseases. *Glia* **65**, 375-387 (2017). https://doi.org:10.1002/glia.23097
10. 21 Zrzavy, T. *et al.* Loss of 'homeostatic' microglia and patterns of their activation in active multiple sclerosis. *Brain* **140**, 1900-1913 (2017). https://doi.org:10.1093/brain/awx113
11. 22 Deczkowska, A. *et al.* Disease-Associated Microglia: A Universal Immune Sensor of Neurodegeneration. *Cell* **173**, 1073-1081 (2018). https://doi.org:10.1016/j.cell.2018.05.003
12. Bisht, K. *et al.* Capillary-associated microglia regulate vascular structure and function through PANX1-P2RY12 coupling in mice. *Nat Commun* **12**, 5289 (2021). https://doi.org:10.1038/s41467-021-25590-8
13. 7 Sofroniew, M. V. & Vinters, H. V. Astrocytes: biology and pathology. *Acta Neuropathol* **119**, 7-35 (2010). https://doi.org:10.1007/s00401-009-0619-8
14. 8 Marina, N. *et al.* Astrocytes monitor cerebral perfusion and control systemic circulation to maintain brain blood flow. *Nat Commun* **11**, 131 (2020). https://doi.org:10.1038/s41467-019-13956-y

15. Mathiisen, T. M. *et al.* The perivascular astroglial sheath provides a complete covering of the brain microvessels: an electron microscopic 3D reconstruction. *Glia* **58**, 1094-1103 (2010). https://doi.org/10.1002/glia.20990
16. Watanabe, K. *et al.* Three-dimensional organization of the perivascular glial limiting membrane and its relationship with the vasculature: a scanning electron microscope study. *Okajimas Folia Anat Jpn* **87**, 109-121 (2010). https://doi.org/10.2535/ofaj.87.109
17. Winkler, E. A. *et al.* Central nervous system pericytes in health and disease. *Nat Neurosci* **14**, 1398-1405 (2011). https:// doi:10.1038/nn.2946
18. Abbott, N. J. *et al.* Structure and function of the blood–brain barrier. *Neurobiol Dis* **37**, 13-25. (2010). https://doi.org/10.1016/j.nbd.2009.07.030
19. Abbott, N. J. *et al.* Astrocyte–endothelial interactions at the blood–brain barrier. *Nat Rev Neurosci* **7**, 41-53 (2006). https://doi:10.1038/nrn1824
20. Wolburg, H. *et al.* Brain endothelial cells and the glio-vascular complex. *Cell Tissue Res.*, **335**, 75-96 (2009). https://doi.org/10.1007/s00441-008-0658-9
21. Sweeney, M. D. *et. al.* Blood–brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. *Nat Rev Neurol* **14**, 133-150 (2018). https://doi:10.1038/nrneurol.2017.188
22. Csaszar, E. *et al.* Microglia modulate blood flow, neurovascular coupling, and hypoperfusion via purinergic actions. *J Exp Med* **219** (2022). https://doi.org:10.1084/jem.20211071
23. Liddelow, S. A. *et al.* Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* **541**, 481-487 (2017). https://doi.org:10.1038/nature21029