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# IL-1R1 Within the Meningeal Lymphatic System

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# IL-1R1 Within the Meningeal Lymphatic System

by

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**Abstract**
The meninges are made up of three membranes; the pia mater, arachnoid mater, and dura mater, that surround the brain and spinal cord. These specialized layers work in concert with cerebrospinal fluid (CSF), to protect the central nervous system by adding a layer of cushion and removing waste products from the CNS. Additionally, the meninges act as a physical barrier between the central nervous system and the periphery. The meningeal lymphatic system is a specialized group of vessels that lie within the meninges that assist in the flow of fluid and waste products from the brain. If the meningeal lymphatic system malfunctions, there is a decreased ability to remove toxins from the brain, for example amyloid beta. Interleukin-1 (IL1) is a cytokine that initiates a cascade of inflammatory pathways through the interleukin-1 receptor (IL-1R1). If IL-1R1 is localized within the meninges, it may play a role in secondary injury cascades of hemorrhages, as well as in the signaling of myeloid cells from the dura mater to the brain.

The goal of this study is to establish a meningeal extraction protocol and to establish whether and where IL-1R1 resides in the meninges. To do so, the skullcaps were extracted from the mice following transcardial perfusion. The mice used for this study were IL-1R1 reporter mice that have been genetically altered to express red fluorescent protein (RFP) in any cell that also expresses IL-1R1. This allows us to visualize the expression pattern in the brain. The leptomeninges were pulled from the skull cap and stained using immunohistochemistry for astrocytes using glial fibrillary acidic protein (GFAP) and lymphatic vessel endothelial receptor - 1 (LYVE-1) to label vasculature throughout the meninges.

The GFAP staining did reveal astrocytes within the confluence of sinuses. While it is currently unclear why astrocytes localized to the confluence of sinuses, further work will investigate these cells in the meninges. Staining for RFP revealed that there is IL-1R1 expression within the meninges. LYVE-1 staining outlined the localization of the vasculature within the meninges.

Future directions will work to determine what cell type within the meninges are expressing IL-1R1. To do so, the meninges tissue will be serial stained with GFAP, RFP, and LYVE-1.

## Introduction

1. Traumatic Brain Injury

Traumatic brain injury is defined as an external force that causes an alteration in brain function or pathology. (Manley & Maas, 2013) In adults, these injuries are commonly attributed to falls, motor vehicle accidents, contact sports, military service, and domestic violence. Intimate partner violence is an issue that has only been brought to attention recently. Previously, most TBI research was done on men who were involved in combat or contact sports. With unpublished dissertations appearing in the late 1990's, the overall attention to the topic has increased, allowing for major research studying underrepresented populations such as those with a history of intimate-partner violence (Casper & O'donnell, 2019). While much progress has been made towards treating TBI in recent years, effective treatments for primary and secondary injuries, as well as proper classification of the injury is still a large obstacle due to the heterogeneous nature of TBI.

TBI is a leading cause of injuries in the US, leading to abundant spending for treatment of TBI every year. From 2002-2006, it is estimated that 579 of every 100,000 people had an incident of traumatic brain injury per year (Faul & Coronado, 2015).According to a study done assessing 1,317 patients with mild TBI, 56% of the participants reported not seeing a medical practitioner by 3 months after their injury. Patient income and insurance status were found to not be associated with status of follow up care (Seabury et al., 2018). In 2000, cost of TBI treatment was approximately $9.22 billion. Furthermore, when estimating the overall loss of income due to the inability to work after injury, the annual cost of TBI was $51.12 billion in the United States (Stevens et al., 2006). Over the period of 2005-2014, the reports of TBI rose 40.5% per capita, with 487 index TBI visits per 100,000 people in California in 2014 (Hsia et al., 2018). According to longitudinal data produced in 2005, an estimated 3.17 million people in the US live with a TBI-related disability (Zaloshnja et al., 2005). Furthermore, 90% of admitted patients were considered mildly injured. The CDC's TBI surveillance summary reports that while TBI-related deaths are decreasing, fall-related TBI incidence is increasing among the elderly (Hsia et al., 2018). This can partly be attributed to the increasingly mobile elderly population in the United States that has been enabled by achievements in modern medicine.

Globally, TBI is generally more fatal and is more common among younger people. When monitoring TBI patients in Hamedan, Iran between 2013 and 2016, it was found that 73% of patients were under the age of 40, with men having twice the risk of TBI compared to women (Saatian et al., 2018). Citizens of lower- and middle-income countries are worse off when it comes to receiving proper care after the injury. While 80% of the world's population living with disabilities live in lower- and middle-income countries, approximately 2% of those people have access to rehabilitation services (Hyder et al., 2007). Poverty and availability of transportation to a hospital are external factors that limit access to proper healthcare and can contribute to increased severity and mortality in developing countries (Hyder et al., 2007).

Considering the heterogeneity of the injuries that may cause TBI, effectively classifying the type of TBI and treating patients after TBI is challenging. The Glasgow Coma Scale (GCS) is the current clinical scale, made to assess the patient's level of consciousness after TBI (Saatman et al., 2008). While the Glasgow Outcome Scale Extended (GOSE) is a scale that reflects disability and handicap post-TBI, rather than impairment (Ann Liebert et al., 1998). According to the GOSE, "lower good recovery" is considered a score of 7 out of a possible 10. While the terminology implies that a mild TBI patient will fully recover, this is not always the case. In a study assessing the recovery of mild TBI patients, 53% of patients reported functional limitations one year after injury (Nelson et al., 2019). Furthermore, patients with mild TBI that displayed evidence of the injury on a CT scan had even higher prevalence of persistent symptoms (Nelson et al., 2019).

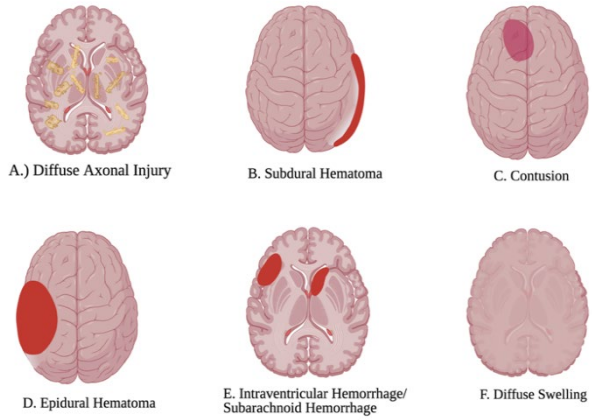


*Figure 1:* Illustration of TBI heterogeneity including diffuse axonal injury, subdural hematoma, contusion, epidural hematoma, subarachnoid hemorrhage, and diffuse swelling.

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The GCS and GOSE do not present information about the pathophysiologic mechanisms that cause neurological defects, rendering them useless for prescribing the proper therapy for different types of TBI. As seen in figure 1, each of the brains represent an injured brain with a score of 8 or more on the GCS, however they all appear to have different pathology. The heterogeneity of these pathologies serves as an example of why it is necessary to treat TBI comprehensively.

To comprehensively treat TBI, other methods of severity classification include pathoanatomic classification, the pathophysiology method and classification by physical mechanism. The heterogeneity of TBI requires multiple methods of classification and treatment (Saatman et al., 2008). When classifying TBI pathoanatomically, evidence of diffuse axonal injury (DAI) throughout the corpus callosum fibers can often be used as an indicator of TBI (Bramlett & Dietrich, 2015). While DAI was initially thought to be associated with moderate to severe injuries, advances in MRI techniques and technology has shown that DAI can be seen in patients who have experienced mild TBI (Saatman et al., 2008). Other sequelae following TBI include hematoma, contusions as well as subarachnoid and intraventricular hemorrhaging as seen in figure 1. Symptoms of moderate and severe TBI may include loss of consciousness, seizures, coma and/ or death (Faul & Coronado, 2015).

Symptoms indicative of a mild TBI may include, transient confusion, disorientation, impaired consciousness, amnesia around the time of injury, as well as neurophysiological dysfunction such as seizures, lethargy, or vomiting (Faul & Coronado, 2015). Life-long disabilities can be caused by TBI, in which case cognitive deficits lead to the inability to work or perform daily tasks without assistance. About 3.17 million US citizens have been estimated to be living with long term TBI-related injury (Zaloshnja et al., 2008). Furthermore, as a result of systemic inflammation caused by TBI, behavioral effects such as anorexia, immobility, social withdrawal, disturbed sleep and depressed mood may take place (Poon et al., 2015). Similar to those with depression, elevated levels in pro-inflammatory cytokines can be seen in the blood of mild TBI patients for months-to-years after their injury (Bodnar et al., 2018). In those with non-sports related mild TBI, psychological problems, substance abuse, or pre-existing behavioral issues could lead to an even higher risk of developing depression post-TBI (Blennow et al., 2016).

2. Meningeal Anatomy

The meninges are comprised of 3 layers. The dura mater is the outer most layer, and hardest layer which attaches brain to the skull, as well as supports the large venous channels in which blood can leave the brain and move back to the heart. The arachnoid mater constitutes most of the meninges' barrier function as an extension of the blood-CSF barrier. The many tight

junctions within the arachnoid barrier cellular layer help divide the CSF- filled subarachnoid space from the fenestrated capillaries within the dura. Finally, the innermost layer of the meninges is the pia mater, which is tightly adhered to the brains surface, contouring the gyri and sulci with the arachnoid mater forms the leptomeninges. The meningeal lymphatic system is a specialized group of vessels that lie within the meninges that assist in the flow of fluid and waste products from the brain. When toxins like amyloid beta accumulate in the brain, the meningeal lymphatic system is responsible for their removal (Ghannam & Kharazi, 2022).

3. Interleukin-1 Receptor 1 (IL-1R1)

Interleukin-1 is a cytokine that initiates a cascade of inflammatory pathways. The two most common forms of IL-1 are IL-1a and IL-1b (Dinarello, 2018). If IL-1R1 is localized within the meninges, it may play a role in secondary TBI cascades of hemorrhages, as well as in the signaling of myeloid cells from the dura mater to the brain. *Figure 2* represents the possible role of IL-1R1 within the meningeal lymphatic system.

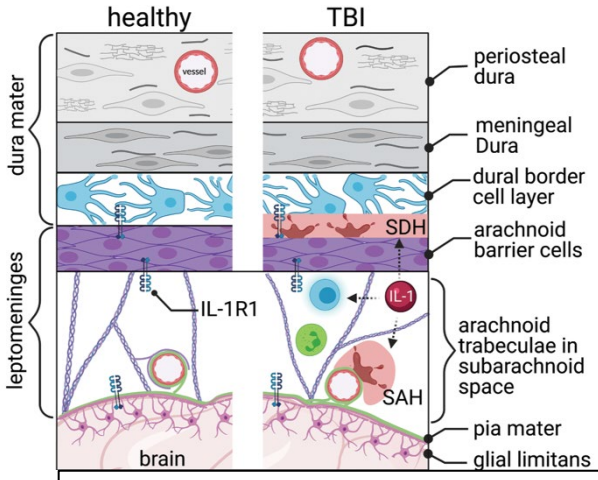


*Figure 2: Illustration of the proposed impact that IL-1R1 within the leptomeninges would have post-TBI.*

*Bodnar et. Al.*

**Objectives**

The goal of the experiments is to identify IL-1R1 within the meningeal lymphatic system and gain insight into the possible role of IL-1R1 in traumatic brain injury response.

**Methods**

To establish the presence of IL-1R1 within the meninges, mice were perfused, then the skull cap was extracted with the meninges attached to the skull cap using three cuts. The first was above the mandibles, the next cut would be across the olfactory bulb, and the last is in the rear of the skull. The skull cap is fixed in a .4% paraformaldehyde solution (PFA) for 6-8 hours, after which it is transferred to a PBS The meninges are then carefully extracted from the skull cap (Nilsson, 2022). Hematoxylin is then added onto the tissue to add color for easier mounting. The tissue is added to the slide with 0.3 ml water to allow the tissue to spread out on the slide. After being mounted, the tissue is left to dry for 3 days at room temperature. The mounted meningeal tissue then underwent immunohistochemical (IHC) and immunofluorescent (IF)

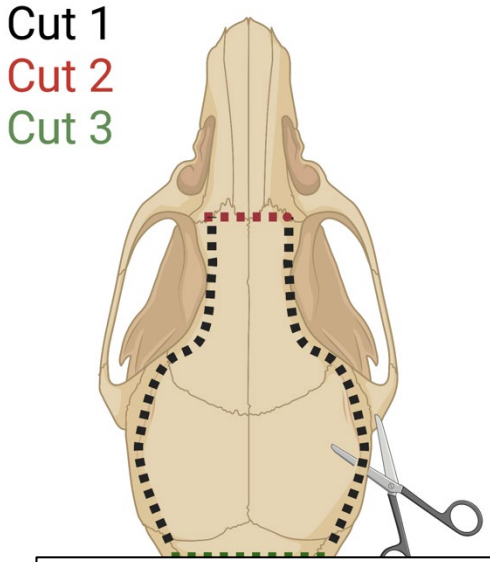


*Figure 3: Method used to dissect the skullcap of mice. The skullcap was cut following the pattern shown to ensure that the meninges would remain intact.*

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staining for astrocytes (GFAP), IL-1R1 (RFP), and vasculature (LYVE-1).

**Results/Creative Output**

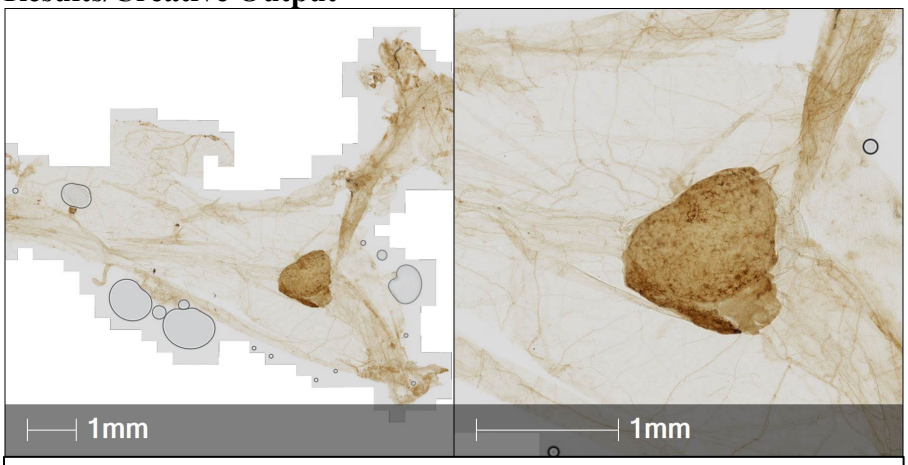


*Figure 4:* Meningeal *GFAP stain that reveals the location of astrocytes.*

The GFAP staining revealed astrocytes withing the confluence of sinus. While it is currently unclear why astrocytes localized to the confluence of sinuses, further work will be done to investigate these cells in the meninges.

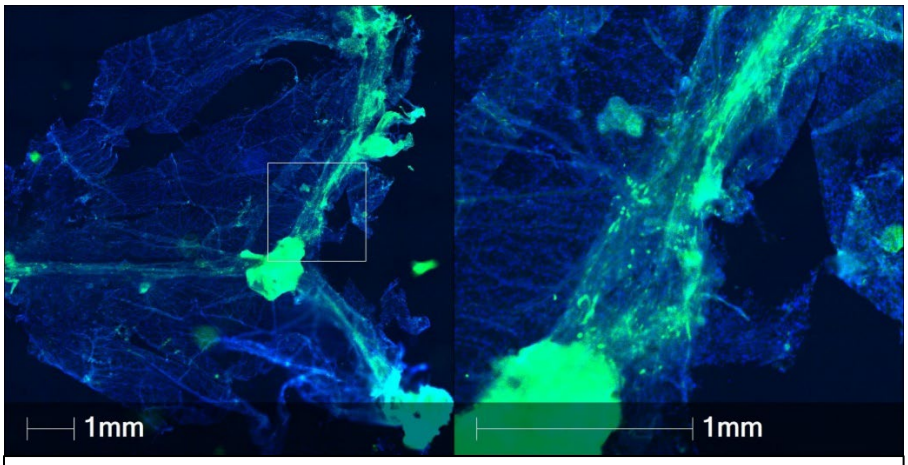


*Figure 5: Meningeal RFP stain that reveals the location of IL-1R1.*

The green highlighted regions represent areas in which IL-1R1 is being expressed. Most of the expression takes place within the transverse sinus as well as the confluence of sinus, however it is unclear what pattern that the IL-1R1 is being expressed throughout the rest of the leptomeninges.

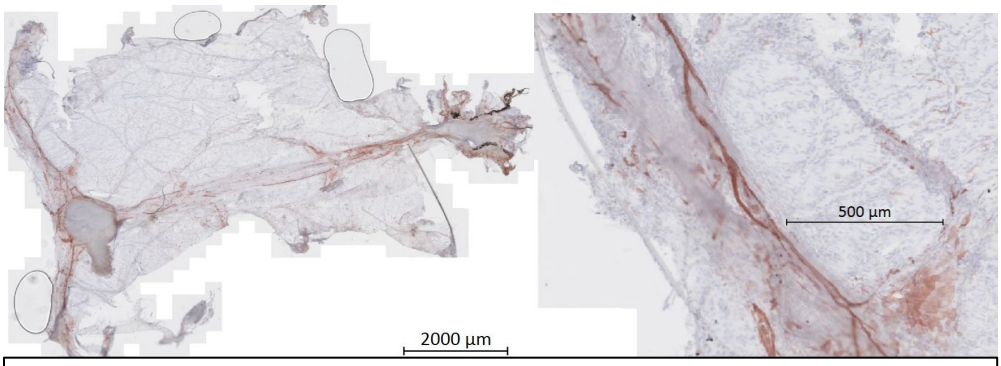


*Figure 6: Meningeal LYVE-1 stain that reveals the location of vasculature.*

The red regions depict areas in which vascular runs throughout the meninges. Conversely to GFAP and IL-1R1, there is little signal within the confluence of sinus.

**Discussion**

The staining in *figure 3* was performed to establish a protocol for the experimental design. While the location of the astrocytes is not significant to our data, their presence represents successful staining which enabled our experiment to move forward.

Evidence from *figure 4* confirms that there is IL-1R1 receptors in the meninges of our mice models. Furthermore, the location of the IL-1R1 is mostly in the sinus' which motivated our study to investigate the location of the vasculature.

Evidence from *figure 5* outlines the location of the vasculature within the meninges. The outline of the vasculature in *figure 5* and the location IL-1R1 in *figure 4* are assessed in two different animals however they are both located within the sinus.

**Conclusions and Recommendations**

In conclusion, our data supports the idea that the meningeal lymphatic system may be impacted by traumatic injury. Future experiments will continue assessing the lymphatic system using a serial staining method in which multiple stains are used then washed from the tissue. After each staining, the tissue will be scanned, and the images will be superimposed to assess the location of each IL-1R1 and vasculature. This would allow for the direct comparison of the location of the IL-1R1 and the vasculature on the same tissue. Furthermore, to assess the impact that TBI will have on the meninges, closed head injuries (CHI) could also be performed on the mice. Analyzing the possible changes, the structure of the tissue and the location of IL-1R1 following the TBI may allow for a higher understanding of the meningeal role in TBI cascades,

When extracting meningeal tissue from the skullcap, care must be taken to avoid tearing the tissue. To remove the tissue most efficiently, start by gently tearing the meningeal tissue from each corner of the skullcap. Gradually peel off the tissue, being cautious not to tear it. Continue to peel until the only remaining attached areas are those that are connected to the sinuses.

Use a drop of hematoxylin on the tissue before mounting because it makes the folds of the tissue more defined. This allows for more accurate mounting with less folds. Also, while mounting the

tissue, add 0.5 ml of water to hydrate the tissue. If the tissue is dry, it is likely tear on the slide during mounting.

Several drying periods were tested, including 2 hours, 12 hours, 1 day, 2 days, 3 days at room temperature, 2 hours, and 6 hours on the slide warmer. The results revealed that the tissues that dried for 2 days at room temperature consistently demonstrated superior staining quality as compared to the other drying methods tested. These findings suggest that 2 days at room temperature could be the optimal drying period for improving tissue staining quality. It is important to note that the drying period may vary depending on staining protocol, and further experimentation may be warranted to determine the most ideal drying period for specific staining techniques.

Antigen retrieval was performed on the tissue at the beginning of each staining protocol to restore immunoreactivity to the meningeal tissue after the fixation process. If the tissue was not properly dried on the slide, the tissue would shrink and become very damaged. While the antigen retrieval was generally successful the first time, if tissue were to undergo antigen retrieval for a second time they were destroyed every time. This caused an abundance of issues during the staining process and prevented successful serial staining.

The principle aim of the experiment was to determine the role that the meningeal lymphatic system plays in interleukin signaling post TBI. While IL1-R1 was successfully located within the leptomeninges, the next step in the experiment would be to analyze the changes in IL1-R1 in the leptomeninges post-injury.

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