Microglia and macrophages in brain injury and repair after subarachnoid hemorrhage

David C. Lauzier*, Harry V. Vinters, Shino D. Magaki

Subarachnoid hemorrhage (SAH) is a devastating condition that affects a total of 8 million people worldwide each year (Lauzier and Athiraman, 2024). Etiologies of SAH can be traumatic or non-traumatic, with the majority of non-traumatic SAH occurring due to intracranial aneurysm rupture (Rutledge et al., 2014). Patients with poor outcomes from SAH often survive the acute phase and go on to deteriorate in a delayed fashion from secondary brain injury induced by cascades of injury initiated by aneurysm rupture. Practice-level improvements such as dedicated neurocritical care units, rapid treatment protocols harnessing infrastructure from ischemic stroke care, and standardization of nimodipine therapy have resulted in improved outcomes for patients with SAH. Despite these advances, the morbidity of SAH remains unacceptably high, and novel therapeutic targets must be identified.

Modulating immune responses has provided favorable outcomes for patients suffering from numerous types of disease (Cisbani and Rivest, 2021). In the specific context of SAH, no immune targeting is utilized in clinical practice, but preclinical data has generated momentum for future studies to leverage immune targeting in SAH. Further, the recent characterization of novel classes of macrophages in the central nervous system (CNS) has highlighted additional targets for the future (Rustenhoven and Kipnis, 2022).

In this work, we turn our attention to the two most critical phagocytes in the CNS – microglia and macrophages – characterizing their ontogeny and roles in both neuroinflammation after SAH and in restorative processes. We highlight lines of evidence with the highest potential for future clinical use.

Microglia and macrophage populations of the CNS: Microglia serve as multifaceted sentinel cells of the CNS, with diverse roles at baseline and in states of pathology (Lannes et al., 2017). Indeed, the quiescent roles of microglia include regulating neural networks, controlling CNS cell populations, and clearing metabolic waste products, while their roles in disease are pathogen elimination, blood product scavenging, and recruiting inflammatory mediators (Cunningham et al., 2013). Though there is a reasonable understanding of their biological role currently, this was not always the case. Microglia were first viewed as part of the "third element" of the CNS, members of an undefined family of adendritic cells. A series of elegant experiments would go on to identify microglia as intrinsic mononuclear phagocytes that descend from early yolk sac precursors and arrive in the forming brain during the early stages of hematopoiesis (Ginhoux et al., 2010). In humans, this occurs between 4.5 and 5.5 weeks of gestation (Andjelkovic et al., 1998). Following the developmental period, mature microglia live approximately five years, though their lifespan can sometimes be up to two decades. The longevity of microglia is an important characteristic of this cell line when evaluating post-hemorrhage inflammation. While most leukocytes have life spans of days to weeks, microglia that respond to hemorrhagic cerebrovascular insults may only be a handful of divisions downstream from the earliest microglia.

Under specific pathological conditions, peripheral monocytes can traverse the blood–brain barrier and enter the CNS, adopting a microglia-like niche despite not sharing the ontogeny outlined above. Though this process is not a major source of microglia in the mature brain, it is an important consideration for patients suffering from SAH given the phenotype of blood–brain barrier hyperpermeability in this condition, which may portend a higher rate of peripheral cells contributing to disease (Huang et al., 2020). Otherwise, peripheral monocytes can undergo a classic activation process to become macrophages when they arrive at sites of SAH.

Additional cell populations to consider in the context of SAH are three major subpopulations of macrophages exclusively found in the CNS – meningeal macrophages, choroid plexus macrophages, and perivascular macrophages (Li and Barres, 2018). Meningeal macrophages and perivascular macrophages are yolk sac–derived populations, similar to microglia, whereas choroid plexus macrophages have dual ontogeny from hematopoietic and yolk sac sources. Heterogeneous transcriptomic profiles within these subpopulations have revealed regional specificity for their functions, which influences their response to injuries. This also allows for various complex interactions between the CNS and periphery since CNS macrophages can freely pass through specialized fenestrations to enter meningeal lymphatic pathways (Rustenhoven and Kipnis, 2022).

To summarize, the populations serving as key drivers of neuroinflammation in secondary brain injury are (1) microglia, (2) intrinsic CNS macrophages, and (3) recruited peripheral blood macrophages. Investigations into these cell populations will allow for a better understanding of secondary brain injury after SAH.

Microglia and macrophages in secondary brain injury: ***secondary brain injury:*** SAH induces secondary brain injury across previously described timeframes. These are the acute injury period, the early brain injury period, the delayed cerebral ischemia period, and the chronic injury period (Lauzier and Athiraman, 2024). The actions of microglia, intrinsic CNS macrophages, and peripheral blood macrophages are present in all stages of secondary brain injury (Lauzier et al., 2023). Intrinsic CNS macrophages have roles that are only beginning to be elucidated and their role in secondary brain injury must be further characterized (Rustenhoven and Kipnis, 2022). However, our understanding of the role of microglia and macrophages in these periods of injury has become increasingly refined in recent years.

Activation or recruitment to the CNS: Microglia are present in the CNS at the time of rupture and may detect the earliest alterations to cerebral homeostasis such as intracranial pressure elevations and distress signals released by injured glial cells and neurons, whereas macrophages localize to the CNS following SAH to mediate the scavenging of toxic blood products in a CD163-mediated process (activation of microglia after SAH shown in **Figure 1**; Lauzier et al., 2023). The localization of peripheral macrophages to the CNS is a necessity, as the volume of blood extravasation in the setting of intracranial hemorrhages rapidly saturates the heme-scavenging capacity of microglia and also promotes phenotypic transformation of phagocytosing cells (Lauzier and Athiraman, 2024).

In the early stages of SAH, the homeostatic functions of meningeal lymphatic drainage also become disrupted, which provides an opportunity for intrinsic CNS macrophages to partake in the pathological cascades following acute rupture (Lauzier and Athiraman, 2024). Indeed, these lymphatic pathways can become completely congested in high-grade SAH, prolonging the exposure of microglia, macrophages, and the neuroaxis to toxic blood products that can drive secondary brain injury (Lauzier et al., 2023). Further validation of the role of these meningeal lymphatic drainage pathways in SAH is necessary.

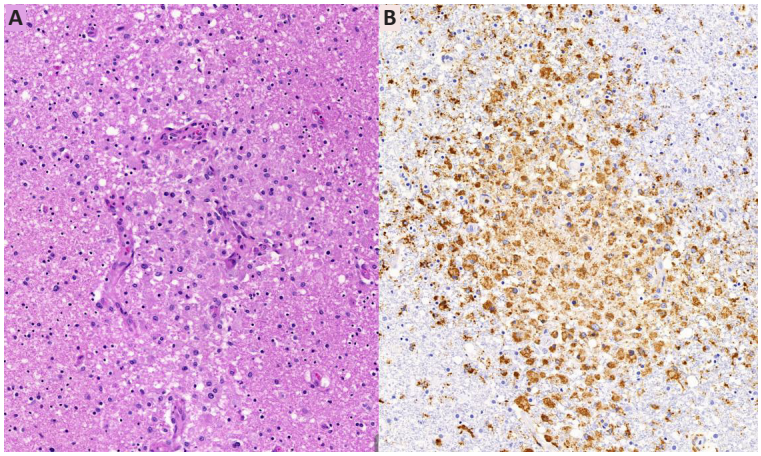

Figure 1 | **Results from hematoxylin and eosin staining and immunohistochemistry.**
Hematoxylin and eosin (A) and CD68 immunohistochemistry (B) sections of the brain were obtained from an unfortunate male patient in his 40s who developed a left frontoparietal subarachnoid hemorrhage and expired 7 days after his hemorrhage. CD68 staining identifies microglial activation. Unpublished data.

Functional evolution across brain injury periods: Microglia and peripheral macrophages develop similar roles as secondary brain injury progresses after hemorrhage, with microglia functioning within the parenchyma, and macrophages more active in the subarachnoid space. Indeed, both demonstrate polarization in SAH, that is, the ability to adopt pro-inflammatory and/or anti-inflammatory phenotypes based on local signaling. Traditionally, pro-inflammatory macrophages and microglia were classified as "M1-polarized" and anti-inflammatory macrophages and microglia as "M2-polarized", though modern definitions have added greater nuance to this classification schema. The reason for the change in classification is pertinent to SAH, as there is a spectrum of phenotypes that can be adopted by microglia and macrophages ranging from pro-inflammatory to anti-inflammatory. Microglia and macrophages are not confined to a single "polarization" state and adopt varying expression profiles over time. This is essential to the overall progression of brain injury from the acute phases to the chronic phases of SAH. Both groups of cells are overall pro-inflammatory at the initial phases of SAH, and progress to anti-inflammatory phenotypes as brain injury progresses.

Microglia orchestrate several inflammatory responses when they shift towards the "M1-polarized" phenotype. It is believed that mast cells located in the meninges are the earliest recognizers of blood-derived toxins, and then induce degranulation and tryptase secretion, which binds to the PAR-2 receptor on microglia and activates these cells (Gris et al., 2019). This signaling induces a pro-inflammatory environment via the secretion of tumor necrosis factor-α, interleukin-1β, and interleukin-6 (Gris et al., 2019). Microglia first recruit neutrophils, which arrive within 10 minutes of experimental SAH (Zeyu et al., 2021). Neutrophils serve many functions in this early time frame such as the release of neutrophil extracellular traps, which further stimulate microglial inflammation and promote the pro-inflammatory phenotype in microglia (Zeyu et al., 2021). This leads to the recruitment of numerous peripheral macrophages via CCL2-CCR2 signaling, and other adjunct pathways (Gris et al., 2019). During this period, M1-polarized microglia express surface markers including CD14, CD16, CD32, CD 86, and major histocompatibility complex-II while M1-polarized macrophages express CD80, CD86, and major histocompatibility complex-II.

As blood products are digested by macrophages and the irritative effects of blood products begin to wane, patients with SAH transition to the delayed cerebral ischemia period, which is characterized by an overall attenuation of inflammation and a transition towards M2 polarization by microglia and macrophages. These cells begin to partake in restorative processes that include cellular repair, regeneration, angiogenesis, and enhanced debris clearance. Still, these phagocytes promote deleterious processes including vasospasm, which is associated with poor outcomes in the delayed cerebral ischemia period (Lauzier and Athiraman, 2024). This is speculated to be related to endothelial reactions induced by sustained pro-inflammatory cytokine signaling (Romoli et al., 2023). As more restorative phenotypes are adopted, both microglia and macrophages express surface markers including CD163 and CD206.

Patients who recover from the acute and subacute phases of SAH, unfortunately, go on to display impaired cognitive function in areas of language, memory, and executive function due to brain injury from sustained inflammation as a consequence of their brain injury (Lauzier and Athiraman, 2024). At this point, the functions of peripheral macrophages have concluded, and blood products have been appropriately sequestered. Dysfunction is now confined to the parenchymal regions. In this chronic injury period, one of the major irritants is cortical iron deposits, which can stimulate local immune responses in resident microglia. Synaptic dysfunction is also a likely underlying mechanism for cognitive impairments, which can be favorably remodeled by microglia that have adopted restorative, anti-inflammatory states. Therefore, in chronic phases of brain injury and recovery, the heterogeneity of microglial responses based on external stimuli is demonstrated.

Clinical applications and concluding remarks: Secondary brain injury after SAH has gained increasing attention as an area to target for improving patient outcomes. This has occurred in light of clinical trials that showed no clinical benefit to attenuating what was believed to be the initial culprit for poor outcomes after SAH – large vessel vasospasm. Across several periods of brain injury, inflammation is a constant feature that likely unites deleterious pathways that range from intracranial pressure elevations to oxidative cascades. In the context of inflammation, phagocytes play both pro- and anti-inflammatory roles, making them promising therapeutic targets for future investigations. Given the duality of microglia and macrophages as actors that promote and attenuate secondary brain injury depending on timing after stroke, targeting these cell populations is challenging. Targeting microglial activation during early aneurysm rupture is not possible given the clinical presentation time course for patients. Targeting pro-inflammatory signaling axes including nuclear factor-κB, MyD88, TIR-domain-containing adapter-inducing interferon-β, and Toll-like receptors has the potential to attenuate both microglial activation and peripheral macrophage recruitment in aneurysm rupture. However, the restorative role of these phagocytes at later phases of secondary brain injury may be hindered by targeting these cell populations early in secondary brain injury. Therefore, some have considered targeting pro-inflammatory microglia and macrophages to promote a transition to the M2 state and create a more restorative environment. No clinical trials have been conducted to date on microglia/macrophage-specific targeting in SAH, and future studies would do well to pilot such therapeutics in animals.

David C. Lauzier*, Harry V. Vinters, Shino D. Magaki

Division of Neuropathology, Department of Pathology, University of California Los Angeles, Los Angeles, CA, USA (Lauzier DC, Vinters HV, Magaki SD)

Department of Neurology, University of California Los Angeles, Los Angeles, CA, USA (Vinters HV)

Brain Research Institute, University of California Los Angeles, Los Angeles, CA, USA (Vinters HV)

***Correspondence to:** David C. Lauzier, MD, davidlauzier1@gmail.com.

https://orcid.org/0000-0003-2825-3360 (David C. Lauzier)

Date of submission: September 4, 2024

Date of decision: October 12, 2024

Date of acceptance: November 1, 2024

Date of web publication: December 7, 2024

https://doi.org/10.4103/NRR.NRR-D-24-01037

How to cite this article: *Lauzier DC, Vinters HV, Magaki SD (2026) Microglia and macrophages in brain injury and repair after subarachnoid hemorrhage. Neural Regen Res 21(1):308-309.*

Open peer reviewer: *Peeyush Kumar Thankamani Pandit, The University of Texas Health Science Center at Houston, USA.*

Additional file: *Open peer review report 1.*

References

Andjelkovic AV, Nikolic B, Pachter JS, Zecevic N (1998) Macrophages/microglial cells in human central nervous system during development: an immunohistochemical study. Brain Res 814:13-25.

Cisbani G, Rivest S (2021) Targeting innate immunity to protect and cure Alzheimer's disease: opportunities and pitfalls. Mol Psychiatry 26:5504-5515.

Cunningham CL, Martinez-Cerdeno V, Noctor SC (2013) Microglia regulate the number of neural precursor cells in the developing cerebral cortex. J Neurosci 33:4216-4233.

Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330:841-845.

Gris T, Laplante P, Thebault P, Cayrol R, Najjar A, Joannette-Pilon B, Brillant-Marquis F, Magro E, English SW, Lapointe R, Bojanowski M, Francoeur CL, Cailhier JF, Canadian Critical Care Translational Biology G (2019) Innate immunity activation in the early brain injury period following subarachnoid hemorrhage. J Neuroinflammation 16:253.

Huang Y, Chen S, Luo Y, Han Z (2020) Crosstalk between inflammation and the BBB in stroke. Curr Neuropharmacol 18:1227-1236.

Lannes N, Eppler E, Etemad S, Yotovski P, Filgueira L (2017) Microglia at center stage: a comprehensive review about the versatile and unique residential macrophages of the central nervous system. Oncotarget 8:114393-114413.

Lauzier DC, Jayaraman K, Yuan JY, Diwan D, Vellimana AK, Osbun JW, Chatterjee AR, Athiraman U, Dhar R, Zipfel GJ (2023) Early brain injury after subarachnoid hemorrhage: incidence and mechanisms. Stroke 54:1426-1440.

Lauzier DC, Athiraman U (2024) Role of microglia after subarachnoid hemorrhage. J Cereb Blood Flow Metab 44:841-856.

Li Q, Barres BA (2018) Microglia and macrophages in brain homeostasis and disease. Nat Rev Immunol 18:225-242.

Romoli M, Giammello F, Mosconi MG, De Mase A, De Marco G, Digiovanni A, Ciacciarelli A, Ornello R, Storti B, Iay (2023) Immunological profile of vasospasm after subarachnoid hemorrhage. Int J Mol Sci 24:8856.

Rustenhoven J, Kipnis J (2022) Brain borders at the central stage of neuroimmunology. Nature 612:417-429.

Rutledge WC, Ko NU, Lawton MT, Kim H (2014) Hemorrhage rates and risk factors in the natural history course of brain arteriovenous malformations. Transl Stroke Res 5:538-542.

Zeyu Z, Yuanjian F, Cameron L, Sheng C (2021) The role of immune inflammation in aneurysmal subarachnoid hemorrhage. Exp Neurol 336:113535.

P-Reviewer: Thankamani Pandit PK; C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y