Notice of the Final Oral Examination for the Degree of Doctor of Philosophy of

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“Investigating the Deleterious Effects of Type 1 Diabetes Mellitus on Microvascular Repair in the Mouse Cortex”

Division of Medical Sciences

Thursday, May 6, 2021
9:00 A.M.
Conducted Virtually

Supervisory Committee:
Dr. Craig Brown, Division of Medical Sciences, University of Victoria (Supervisor)
Dr. Leigh Anne Swayne, Division of Medical Sciences, UVic (Member)
Dr. Robert Chow, Department of Biology, UVic (Outside Member)

External Examiner:
Dr. Tiina Kauppinen, Department of Pharmacology and Therapeutics, University of Manitoba

Chair of Oral Examination:
Dr. Adam Con, School of Music, UVic

Dr. Stephen Evans, Acting Dean, Faculty of Graduate Studies
Abstract

Microglia and brain-resident macrophages are the sentinel immune cells of the central nervous system (CNS), and are ideally situated to respond to any damage to the brain parenchyma or vasculature. Circulating leukocytes are generally excluded from the CNS environment under homeostatic conditions but can gain access to this region in diseases that disrupt immune system function and blood-brain barrier integrity. Although these diverse immune cells exhibit properties that may engender them to be well-suited to resolve microcirculatory insults, their relative contributions to the recanalization of capillary rupture in the cortex, known as cerebral microbleeds (CMBs), has yet to be described. CMBs are particularly concerning in conditions, such as diabetes mellitus (DM), in which these insults occur more frequently and potentially underlie the onset and progression of cognitive decline.

Using in vivo 2-photon microscopy and confocal imaging, here I highlight the compromised repair of CMBs in a mouse model of type 1 DM and characterize the robust, heterogeneous macrophage response to these insults. Specifically, 20% of damaged capillaries were eliminated from the circulation in the diabetic cortex and chronic insulin treatment failed to prevent this microvascular loss. Administration of interferon-α or interferon-γ blocking antibodies to dampen inflammatory signalling, or dexamethasone to reduce global inflammation, also failed to improve repair rates of damaged microvessels in diabetic mice. In contrast, CMBs in nondiabetic mice repaired without exception. Interestingly, depletion of microglia using the colony stimulating factor-1 receptor antagonist PLX5622 resulted in microvascular elimination in nondiabetic mice. Given the robust depletion of microglial populations with this treatment, at first these data suggested that microglia were necessary for microvascular repair since their elimination produced vessel loss. However, by parsing the data I identified that microvessels repaired in all cases where macrophages were not identified at the CMB; when CX3CR1+ aggregate was localized to the injury, ~20% of microvessels were eliminated. These findings show that microglia are not necessary for microvascular repair following CMB.

Immunofluorescent co-labelling of various microglial and macrophage markers within the diabetic CMB milieu revealed a novel population of Mac2+/TMEM119- cells, distinct from homeostatic TMEM119+ microglia. These cells reliably localized to CMBs that failed to repair and rarely associated with vessels that recanalized; Mac2+/TMEM119- cells were not found
within nondiabetic CMBs. Treatment of diabetic mice with clodronate liposomes (CLR) to deplete circulating phagocytic leukocytes prevented aggregation of Mac2+/TMEM119- cells to CMBs and improved capillary repair rates. The efficacy of CLR in excluding these cells from the CMB aggregate, coincident with eradication of monocytes from circulation, indicated that these cells likely arose from the periphery. *In vivo* 2-photon imaging revealed significant increases in lipofuscin at the site of diabetic CMBs relative to the nondiabetic context; other phagocytic markers including CD68 and TREM2 were also upregulated. Mac2+/TMEM119-cells showed elevated lipofuscin content relative to homeostatic microglia; their association with CMBs may thus signal an increase in phagocytosis that contributes to capillary pruning.

Taken together, these data identify a novel Mac2+/TMEM119- macrophage associated with pathological microvascular elimination following CMB in the diabetic neocortex. These findings highlight the diversity of immune cell responses to CNS injury and provide insights into the cellular mechanisms of capillary pruning. Furthermore, these advances in our understanding of the regulation of microvascular elimination in the diabetic brain may have clinical implications for patients with DM as they provide evidence for putative adjuvant anti-inflammatory treatments, such as CLR, in mitigating cerebrovascular pathology.