Microglia rapidly reconstruct the damaged blood brain barrier after cerebrovascular injury

Panagiotis Mastorakos, Nicole Mihelson, Marie Luby, Scott R. Burks, Kory Johnson, Joseph A. Frank, Lawrence Latour, Dorian B. McGavern

Viral Immunology & Intravital Imaging Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland

Department of Surgical Neurology, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland
No disclosures
Introduction: Immune response to cerebrovascular injury at the capillary level

The role of the immune system in the setting of hemorrhagic stroke has long been debated. Further understanding of the role of microglia is critical for the development of immunomodulatory treatments in cerebrovascular diseases. We developed a model of cerebrovascular injury to evaluate the acute response of microglia following isolated damage to cerebral capillaries.
Methods

- We studied the dynamics of injury response using two-photon laser scanning microscopy and immunohistochemistry in microglia reporter mice.
- We used transcranial administration of small molecule inhibitors in order to identify the mechanism of microglia response.
- We evaluated blood brain barrier leakage using Evans Blue extravasation in microglia depleted mice as well as mice treated with inhibitors.
- We used a transgenic model of connexin-43 conditional knock out in astrocytes (GFAPCreER-Cx43\(^{f/f}\)) and evaluated microglia dynamics, degree of injury and survival.
Using intravital microscopy in Cx3cr1<sup>+/p/wt</sup> mice we noticed that within 10 min after injury microglia project their processes creating tube formations around damaged vessels (rosettes). Confocal microscopy confirmed microglia projections formed a barrier surrounding injured and clotted vessels.
Results 2

Microglia depletion (using CSF1R inhibitor) increased EB extravasation fibrin clot burden, endothelial cell activation and myelomonocytic cell invasion.
Transcranial inhibition of the P2RY12 receptors on microglia decreased rosette formation while inhibition of Cx43 hemichannels primarily found on astrocytes completely stopped rosette formation. Inhibition of rosette formation doubled the severity of BBB breakdown.
Knock out of Cx43 in astrocytes stopped rosette formation and increased BBB breakdown. This resulted in fatal edema within 24 hours while all naïve mice survived.
Discussion/ Summary

• We describe a new mechanism by which microglia create tube like structures encasing damaged and clotted vessels to buttress the damaged blood brain barrier after cerebrovascular injury. This mechanism is mediated by ATP secretion from astrocytes through Cx43 hemichannels.

• Rapid BBB reconstitution by microglia is necessary to prevent persistent devastating cerebral edema. Use of P2RY12 inhibitors such as clopidogrel may hamper microglia rosetting. As such use of P2RY12 inhibitors in the acute setting following stroke may need to be re-evaluated.