CCR2+ monocytes and pro-angiogenic microglia orchestrate repair in a rodent model of hemorrhagic stroke

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Introduction

- Stroke remains a major cause of disability worldwide. Currently, research is focused on the development of therapeutics to promote repair following injury.

- In peripheral organs, myelomonocytic cells are shown to orchestrate repair and impaired recruitment of macrophages may result in defective tissue regeneration and fibrosis.

- We evaluated the role of the immune system in the repair process following hemorrhagic stroke.

Methods

• We created a model of cerebrovascular injury using a combination of ultrasound and intravenously injected microbubbles. We allowed the repair process to occur for 10-20 days. Mice were treated with aCSF1R, aGR1 or aLFA1/VLA4 antibodies to deplete microglia, circulating myeloid cells or prevent leucocyte transmigration, respectively.

• We used intravital microscopy and immunohistochemistry to evaluate angiogenesis and neurogenesis. We also used multiparameter flowcytometry we evaluated the immune landscape in hemorrhagic stroke.

• The upregulated pathways of repair were analyzed using qPCR and bulk RNA sequencing.

• We used a Y-maze to evaluate cognitive-motor function recovery after injury.
On day 10 after injury we observed development of neo-vessels with irregular distribution and increased density.
Results 2

Preventing myelomonocytic cell diapedesis with anti-LFA1/VLA4 administration prevented angiogenesis, increased collagen deposition and reduced the neuronal population at the area of injury.

Depletion of circulating myelomonocytic cells, using anti-GR1 antibody, and targeted depletion of pro-inflammatory monocytes, using CCR2 ko mice, also prevented repair. While depletion of neutrophils, using anti-Ly6G antibodies, did not affect repair.

Real-time PCR demonstrated upregulation of genes involved in angiogenesis 6 days following injury in naïve mice, but not in mice lacking monocytes.
Results 3

Microglia depletion also prevented development of neo-vessels and decreased upregulation of angiogenesis genes.

Using immunohistochemistry and multiparameter flowcytometry we identified a new population of VEGF expressing microglia after injury.
Bulk RNA sequencing shows enrichment of the fibrosis pathway in aLFA1/VLA4 treated mice and this correlated with lack of recovery of cognitive-motor function.
Discussion/Summary

- Angiogenesis is critical for maintenance and repair of injured brain tissue.
- We show that CCR2+ monocytes and repair associated VEGF+ microglia orchestrate repair.
- Inhibition of resident or peripheral innate immunity prevents angiogenesis, leads to fibrotic healing and hampers recovery.
- These results demonstrate how aspects of the immune response are critical for recovery following cerebrovascular injury and suggest that the innate immune system should be considered as a target in the design of repair modulating therapies.