Vaccination against stroke

The role of microglia immune tolerance against recurrent intracerebral hemorrhage

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Step2: Acute inflammation (4-24 hours)

- The predominant type of microglia are the classically activated M1, which produces proinflammatory mediators (IL-1β, iNOS, TNFα).
 M1-like microglia can be identified by
- surface markers such as CD86,
- CD16/32.
 During this stage, microglia play a deleterious role by exacerbating inflammation and worsening damage.

Step 3: Resolving (3-7 days)

- Microglia gradually polarize towards the M2 phenotype, which produces anti-inflammatory mediators (IL-10, $TGF\beta$) and initiates phagocytosis of debris.
- M2-like microglia can be identified by surface markers CD206, CD68 and Arginase-1. This is the main period where clearance
- and maintenance occurs, where microglia action ameliorates damage

Step1: Recruitment (0 hours)

- Spontaneous rupture of intracerebral blood vessel releases
- RBCs into the brain parenchyma.
 Localized activation of microglia induced by red cells, heme and plasma proteins.

IMMUNE RESPONSE FOLLOWING INTRACEREBRAL HEMORRHAGE

A simplified step-wise diagram representing the timeline of microglia polarization after intracerebral bleed

Step 4: Long term (>14 days)

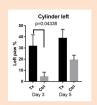
- Pro-inflammatory cytokines return to a base-line level.
- Microglia return to a resting state

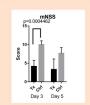
- · To observe the immune response following a moderate-sized hemorrhagic
- stroke in murine model.
 To analyze involvement of M1 and M2 polarization of microglia following hemorrhagic stroke.
- To determine whether a previous stroke may influence immune response
- To compare hematoma clearance rate, behavioral test scores, and microglia polarization within mice with a previous hemorrhagic stroke and mice with no previous exposure.

Methods:

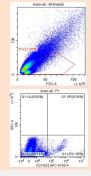
- Modified Neurological Scoring System (mNSS) comprises of four categories that assesses both motor and sensory deficits
- Rotarod tests for general motor coordination. Results are marked as duration before fall.
- Cylinder test measures spontaneous left paw and right paw usage. A higher left paw usage percentage indicates worsened outcome.
- Flow cytometry: CD45Int /CD11bHigh are identified as brain-derived microglia (to differentiate from blood-derived macrophages: CD45High/CD11bHigh).
- Histology samples: Brains are sectioned at 2mm thickness and hematoma size is measured by ImageJ threshold quantification.

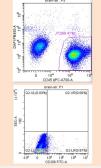
Previous injury (Tx) ameliorates damage of intracerebral hemorrhage and improves behavioral test outcomes compared to control group (Ctrl).





Flow cytometry reveals an M1 to M2 shift at 24 hour time point after intracerebral hemorrhage in mice previously exposed to similar stimuli.







Beam-walking test: left limb falls off beam (arrow), indicating left side hemiplegia caused by induction of intracerebral hemorrhage in the right basal ganglia





Original brain

Threshold gating by

Discussion and Conclusions:

- Microglia, as brain-resident derivatives of macrophages, exerts inflammatory and anti-inflammatory effects depending on
- polarization pathways.

 The hematoma clearing, alternative activation pathway (M2) is upregulated and occurs earlier in mice with previous intracerebral bleeds. This attenuates damage caused by inflammation and alters the immune cascade that follows an acute intracerebral hemorrhage, as supported by behavioral testing and hematoma size at both 24 hours and 7 days following injury.