Overall aim:
To study the role of microglia in acute ischemic stroke.

Specific aim:

The immune receptor Mincle is a key initiator of tissue damage in ischemic stroke

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Mincle (Clec4e) is a pattern recognition receptor present on the surface of immune cells

- Macrophage Inducible C-type Lectin

- Mincle binds to pathogen-associated molecular patterns (PAMPs). In an infection, Mincle signalling results in the onset of inflammation leading to an efficient immune response

- Being a strong promoter of inflammation, the role of Mincle in neuroinflammation after stroke may be detrimental

Brown, Nat Immunology 2008
Methods used by our team

Stroke models:

- **In vivo**: Middle Cerebral Artery Occlusion

  Followed by:
  - Infarct size and neurological damage assessment.
  - Isolation of immune cells using Percoll gradient followed by flow cytometry for:
    * quantification of infiltrating immune cell types
    * assessment of cytokine production in each cell type

- **In vitro**: Oxygen and Glucose Deprivation of cultured cells of a single type (neurons, microglia, astrocytes)
Key observations over last five years

1. The Mincle KO mutant Clec4e−/− is protected from ischemic stroke

2. Mincle affects infiltration and activation of myeloid cells after stroke
Key observations over last five years

3. Expression of Mincle in the brain is the key regulator of poor outcome in ischemic stroke
Key observations over last five years

3. Mincle is expressed in primary microglia and induced by oxygen and glucose deprivation.

![Clec4e mRNA in microglia]

4. But... the response of primary Clec4e-/- microglia to OGD is no different from that of the wild-type.

![MIP2a and MMP-9 expression]

No differences in: G-CSF, CXCL2, CXCL12, CX3CR1, CD200R, TNF, IL-1b, TNF, VEGF, MMP9
Conclusion: OGD is upregulating Mincle in pure microglial cultures (Mincle is inducible by inflammatory stimuli) but it is not activating Mincle (hence the absence of KO-specific responses), possibly because Mincle's ligand comes from another cell type.

What we need now

A good model that resembles the native tissue to assess Mincle activation. Options:

- **In vivo models:**
  * MCAO: currently assessing the transcriptome of WT and KO microglia 24 hours after reperfusion.
  * Intra-hippocampal endothelin-1 injection as a more convenient model of stroke.

- **In vitro models** allow the possibility to construct in vitro chimeras:
  * Co-culture of primary neurons and microglia in vitro.
  * Tissue slices – acute (microglia activation).
  * Tissue slices – organotypic. More complex to set up, but easier to manipulate.
  * Myelinating cultures.
My most unusual collaboration is with Christine.

She and her team of bioinformaticians have the ability to help me compare my microglia transcriptome results to those of other authors, to assess the role of microglia:

- In different disease models
- Through age
- In different mutation models
- In different parts of the CNS

This ability to compare results cross-platform / cross-study is very promising. This will give us clues as to how microglia behave in different conditions to design treatments appropriate to each.
Collaborators

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I'm here till Friday!