Role of resident microglia in lymphoid neogenesis after rodent cerebral ischemia

BACHELOR’S THESIS

Degree in Biotechnology

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Tarragona, 2019
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Chronic inflammation

Sustained inflammatory processes as a cause of chronification of diseases

Study of chronification mechanisms
Ectopic lymphoid-like structures (ELS)

**Structure:**
B-cell core
surrounded by T cells

**Functions:**
Local production of auto-antibodies
Maintenance of inflammatory response

**Lymphoid neogenesis:** Process of formation of ELS
Ischemic stroke

- Activation of resident microglia
- Recruitment of peripheral immune cells
- ELS
- 14 days post-stroke
- Cognitive decline
Previous experiments

Overexpression of cytokines in WT mice (d14 after stroke)
Ischemic stroke

Activation of resident microglia

Recruitment of peripheral immune cells

14 days post-stroke

ELS

Cognitive decline
Cx3cr1\textsuperscript{Cre/ERT2}-FiDTR mice

Cx3cr1-ERT2 vector: Expresses Cre-ERT2 in the presence of Tamoxifen

R21-iDTR vector: Expresses diptheria toxin receptor (DTR) once Cre eliminates the STOP codon
Cx3cr1\textsuperscript{Cre\textsubscript{ERT2}}-FiDTR mice

Expression analyses
- Pax5, Cd3e, Lta, Ltb, Cxcl13, Cxcr5, Cxcl12, and Ccl19 genes
- Ccr2, Ly6c, and Iba-1 genes

Control
- 5 WT mice
- 2 control Cx3cr1.FiDTR mice
- 2 control Cx3cr1.FiDTR mice

Test sample
- 4 Microglia-depleted Cx3cr1.FiDTR mice
- 4 Microglia-depleted Cx3cr1.FiDTR mice

Histopathology
- Control
  - 5 WT mice
- Test sample
  - 3 Microglia-depleted Cx3cr1.FiDTR mice
**Expression analyses**

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**Histopathology**

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Microglia depletion inhibits lymphocytic infiltration

- WT (day 14)
- Microglia-depleted (day 14)

Infarct volume: 18%  
Infarct volume: 25%

B220 (B-lymphocytes)  Iba-1 (microglia and macrophages)  DAPI
Microglia depletion inhibits lymphocyte infiltration

WT (day 14)  Microglia-depleted (day 14)

Infarct volume: 18%  Infarct volume: 25%

B220 (B-lymphocytes)  Iba-1 (microglia and macrophages)  DAPI
Effects on molecular pathways

Lymphotoxin

- LTa
- LTb

Lymphoid chemokines and receptors

- CXCL13
- CXCR5
- CXCL12
- CCL19

Myeloid markers

- Ly6C
- CCR2
- Iba-1
Effects on molecular pathways

- Not a clear overexpression of \( Lta \) after stroke
- \( Ltb \) shows a tendency towards a decreased expression

\( LTb \) might be the limiting component of the \( LT\alpha_1\beta_2 \) complex
Effects on molecular pathways

↓ Lymphoid chemokines → Lymphocyte infiltration → ELS
Effects on molecular pathways

Microglia depletion does not affect to other myeloid populations (infiltrating monocytes and macrophages)
Evaluation of Cx3cr1.FiDTR mouse model

- No differences in contralateral hemispheres (no stroke)
- No differences between WT and control in ipsilateral
Evaluation of Cx3cr1.FiDTR mouse model

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Evaluation of Cx3cr1.FiDTR mouse model

- No differences in contralateral hemispheres (no stroke)
- No differences between WT and control in ipsilateral
- Iba-1+ cells → Mostly infiltrating macrophages
Conclusion

- Cx3cr1.FiDTR mice is a solid model to study microglia functions after stroke
- Microglia depletion inhibits lymphocytic infiltration and ELS formation
- Microglia is involved on Cxcl13, Cxcl12, and Ccl19 overexpression after stroke in mice

Resident microglia has a crucial role in lymphoid neogenesis after rodent cerebral ischemia
Thank you for your attention