The complement system: a target for therapy of spinal cord injury

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Mechanism of Injury

Primary Injury:
- Mechanical Injury at impact site
- Necrotic cell death
- Can’t treat initial trauma

Secondary Injury:
- Inflammation
- Cell death
- Further impairs recovery
- A target for therapy

The complement system is involved in propagating secondary injury

Outline of complement cascade

Classical

Lectin

Alternative

Opsonins

Cytolytic complex

Peptides

C3a

C5a

C3b

iC3b

C3dg

C3d

Cell

amplification
Complement inhibition

• **Limited success in clinical trials**
  • Systemic approach
  • High levels of target proteins (eg. C3)
  • Poor bioavailability
  • Protective roles of complement in health and disease

**Targeted complement inhibition**

• Targeting complement inhibition to sites of disease
  • Improve efficacy
  • Improve safety
Targeted complement inhibitor fusion proteins

Classical
Lectin
Alternative
Opsonins

C3

Crry
fH

CR2
CR2

C3b → iC3b → C3dg → C3d

Cell
Targeted complement inhibition improves locomotor recovery and reduces tissue injury after SCI

CR2-Crry or CR2-fH injected 30 min after traumatic injury

Similar data for CR2-fH treatment
Targeting neoepitopes expressed on ischemic/injured tissue
Model for complement activation following ischemia and reperfusion (intestine, heart, liver)

IgM mAbs that bind ischemic neoepitopes

C2 mAb - Subset of phospholipids
B4 mAb – Annexin IV
Does IgM play a role in SCI?

SCI

Rag1 -/- (Ab deficient)

B4 or control IgM tail vein inj.

30 min

72 hr

Histology IgM and complement staining

Locomotor score for 21 days
Rag1-/- mice have improved recovery after SCI and injury is restored with B4 IgM mAb

\[ p<0.0001 \]
\[ n=6 \]
Immuno-staining of spinal cord sections 72 hours after SCI

Wild type mice

Rag1-/- mice + B4 mAb

Rag1-/- mice + control mAb
Targeting a complement inhibitor using a B4 single chain antibody construct

B4 IgM mAb

Single chain Ab (ScFv)

Complement inhibitor

fH

Crry
Injury → Annexin IV neoepitope

C1q → C3 → C3 convertase → C5 → MAC

C3a → C5a

Targeting via B4 single chain
Significant improvement in locomotor recovery observed with B4-Crry treatment

![Graph showing BMS score over days after injury for WT and B4-Crry groups with p<0.0001 significance level.]
B4-Crry reduces injury and hemorrhage at impact site (72 hours after SCI)

Wild type + PBS

C3-/- mice

Wild type + B4-Crry (inj. 30 min after SCI)
Summary/conclusions

• CR2- and IgM scFv-targeted inhibition effective at reducing SCI
• scFv-targeted inhibitor has dual function (block self reactive-Ab and C)
• Short circulatory half life, long tissue half life
• Human CR2 inhibitors in clinical development
• For IgM scFv inhibitors, similar recognition system/specificities in humans
South Carolina Spinal Cord Injury Research Fund

Data from studies supported by the fund contributed directly to:

• **Startup company based on CR2-targeted inhibitors**
  – Now in clinical development by Alexion Pharmaceuticals

• **IgM scFv targeted inhibitors**
  – Provisional patent issued (based in part on SCI work)
  – Commercialization effort underway
  – R21/33 ($750,000) NIH NINDS application for preclinical development of B4-Crry/fH SCI/stroke therapeutic (will be submitted June 2015).
What is the treatment window for CR2-Crry/fH?

CR2-fH

Label with fluorescent probe

Inject 30 min after SCI

Inject 3 hour after SCI

Sacrifice and remove spinal cords at different time points post SCI

ex vivo imaging of spinal cord
CR2-fH is targeted to the site of injury at similar levels whether injected 30 min or 3 hours after injury, and retained for up to 7 days.

**30 min post-SCI injection of fluorescent CR2-fH**

- SCI  SCI  Sham
  - 24 h
- SCI  SCI  Sham
  - 72 h
- SCI  SCI  Sham
  - 7 d

**3 hour post-SCI injection of fluorescent CR2-fH**

- SCI  SCI  Sham
  - 24 h
- SCI  SCI  Sham
  - 72 h
- SCI  SCI  Sham
  - 7 d
Collaborators

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