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Co-targeting of Pathogenic Postischemic Self-Recognition by Natural Antibodies and Complement Activation Limits Chronic Neuroinflammation and Improves Outcomes After Experimental Stroke

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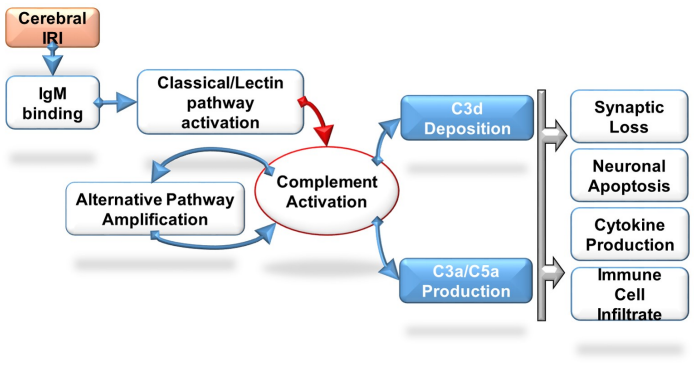
Introduction

After stroke, penumbral cells express neo-epitopes that are recognized by natural IgM antibodies (NIgM) leading to complement activation and propagation of inflammation and injury. We previously identified NIgMs, namely B4IgM, that only recognizes ischemic cells, and developed a site-targeted complement inhibitor by fusing a single chain antibody derived from B4mAb to the complement inhibitor Crry. We hypothesize that the fusion construct B4Crry inhibits neurodegenerative effects of IgM and complement and improves outcome after stroke.

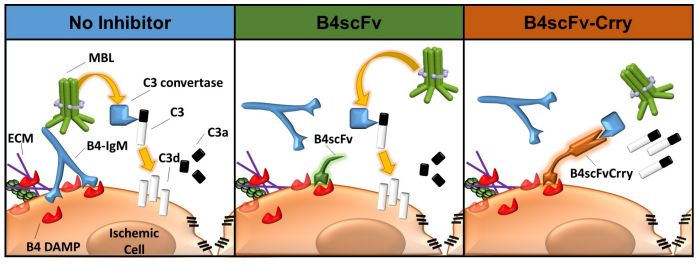
Complement Activation after Stroke



Methods

Stroke was induced in adult and aged mice by 60 min right MCA occlusion. B4Crry was administered IV as a single dose at 2-24 hours after stroke. Animals were tested for infarct volume and motor and cognitive recovery. Brains were used for histological analyses.

Targeted approach to inhibit IgM & complement



Results

A single dose of B4Crry 6-24 hours post-ischemia specifically targeted to the ischemic hemisphere and inhibited complement and IgM deposition in the penumbra, yielding significant reduction in infarct volumes, neuronal loss and neurological deficits ($p<0.01$) at 24hrs in adult males, females and aged mice. B4Crry-mediated neuroprotection persisted throughout 15 days of recovery yielding reduced forelimb laterality ($p<0.01$), improved spatial learning and memory (Barnes maze, $p<0.05$), and improved skilled handling (Pasta task, $p<0.01$) compared to vehicle. Vehicle treated animals showed a sustained inflammatory response with continuous IgM and complement deposition and robust proinflammatory microglial activation detected 15 days post-stroke. Acute B4Crry therapy interrupted the chronic neuroinflammatory cycle by significantly inhibiting complement and IgM deposition, and shifted microglial polarity to anti-inflammatory phenotypes resulting in preserved penumbral neuronal density. Simultaneously, B4Crry resulted in pronounced increase in neurogenesis and neuronal migration. Finally, we show that B4Crry bound specifically to the ischemic penumbra of postmortem brain samples obtained from acute stroke patients, but not to normal brain tissue from the same patient.

Conclusions

B4Crry is a novel translational therapeutic agent that interrupts neurodegenerative inflammatory cascades and improves outcomes after stroke.

Learning Objectives

By the conclusion of this session, participants should be able to:

- Identify the role of natural IgM antibodies and

Figure 1

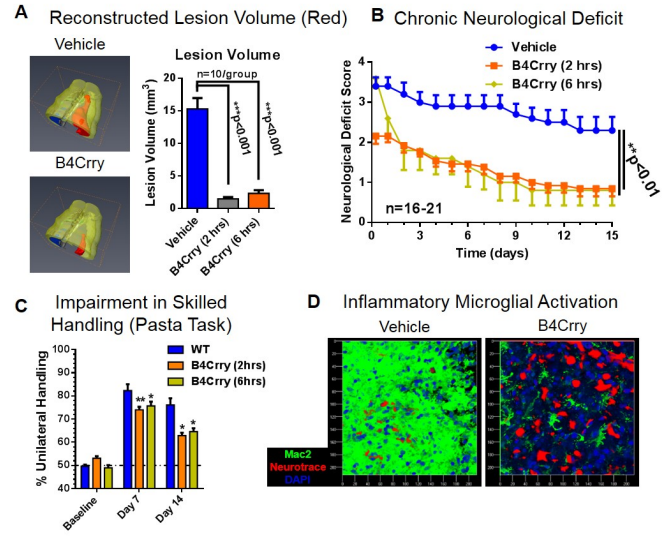


Figure 1. (A) B4Crry significantly reduces lesion volume (rendered in red on 3D brain reconstructions) compared to vehicle at 15 days after murine MCAO occlusion. ANOVA. n=10/group. ***p<0.001. (B) B4Crry significantly reduces neurological deficit scores in animals throughout 15 days after MCA occlusion compared to vehicle. Two-way ANOVA with Bonferroni. n=16-21/group. **p<0.01. (C) B4Crry significantly improves skilled forearm performance as assessed by reduction in unilateral handling on pasta task after MCA occlusion. Two-way ANOVA, Bonferroni. n=8/group. *p<0.05.**p<0.01. (D) Reduction in penumbral inflammatory microglial activation by B4Crry assessed by super-resolution immunofluorescence microscopy 15 days after MCA occlusion. Red: neurons. Green: inflammatory microglia (Mac2+). Blue: DAPI.

Acknowledgments

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References

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Alawieh, A, and S Tomlinson. Immunological Rev (2016).