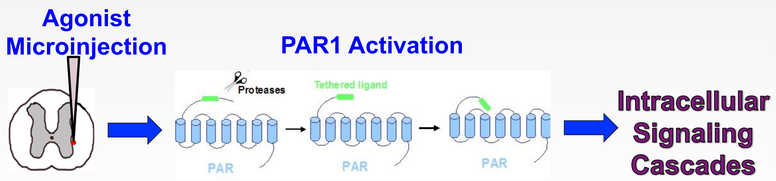
The Role of Indirect PAR1 Activation in Tissue Repair after SCI

Lindsay M. Milich, F. Rezan Sahinkaya, Dana M. McTigue
Department of Neuroscience, Ohio State University, Columbus, OH 43210

Introduction

- **Protease Activated Receptor 1 (PAR1)** is a G-protein coupled receptor present on many central nervous system cell types.
- When cleaved by serine proteases, PAR1 can initiate many different signaling cascades based on the activator.

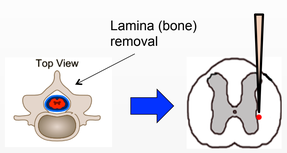


- The activators of PAR1 are found in blood, and get into the spinal cord after injury.
- Microinjection of a synthetic PAR1 agonist into normal rat spinal cord stimulated marked **oligodendrocyte progenitor cell (OPC)** proliferation, while the endogenous activator thrombin did not.
- OPCs are important for repair after spinal cord injury.
- Our goal is to determine the mechanism of PAR1-mediated OPC proliferation: Is it direct or indirect?
  - If it is indirect, which cell/cells are responsible for secreting factors that stimulate OPC proliferation?

Methods

Microinjection of PAR1 Agonist

T8 Laminectomy to expose spinal cord → Microinjection of factor into lateral white mater of cord → Tissue Perfusion and spinal cord harvest → Stain tissue for CD11b expression

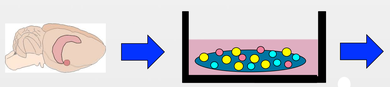


- Tissue perfused with 4% Paraformaldehyde
- Tissue is processed for histology

Antibody	Cell Type
OX42 (CD11b marker)	Activated Microglia

Co-culture OPC's with Astrocytes and Microglia

Isolation and Culture of Mixed Glial Cells from neonatal rat cortex → Re-plate OPC's, microglia, and astrocytes → Treat Cells → 16h → Apply BrdU to media → 24h → Fix Cells and stain for proliferating OPC's

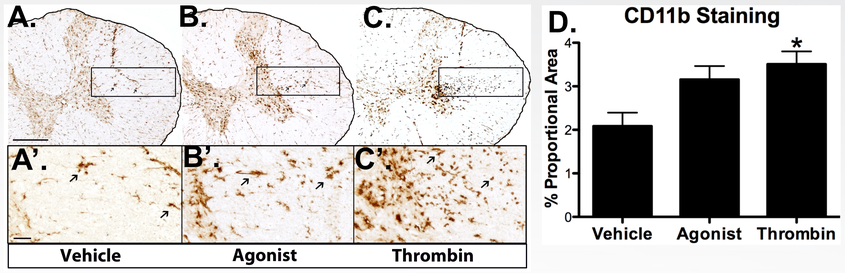


Treatments
Vehicle (PBS)
PAR1 Agonist (NH_2-TFLLR-$CONH_2$)
Recombinant Thrombin (0.01U/mL)

Antibody	Cell Type
A2B5	Glial progenitors (OPC's)
BrdU	Proliferating cells in S-phase of Mitosis

Results

Fig.1: Different Modes of PAR1 Activation Affect Microglia Morphology and Accumulation



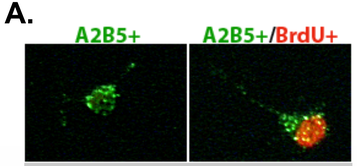
A-C) PAR1 Agonist and thrombin stimulated different patterns of microglia activation within the microinjection area (boxes) centered at the base of the needle track.

A'-C') Arrows identify examples of CD11b+ cells (a marker for microglia). Microglia with activated morphology (rounder, less processes) can be seen in PAR1 Agonist and Thrombin.

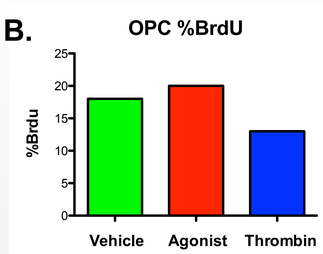
D.) CD11b area was quantified in microinjection areas (boxes in A-C). Thrombin stimulated increased accumulation of microglia.

Fig.2: Direct Activation of PAR1 in Cultured Oligodendrocyte Progenitor Cells Does not Affect Their Proliferation

A.



B.

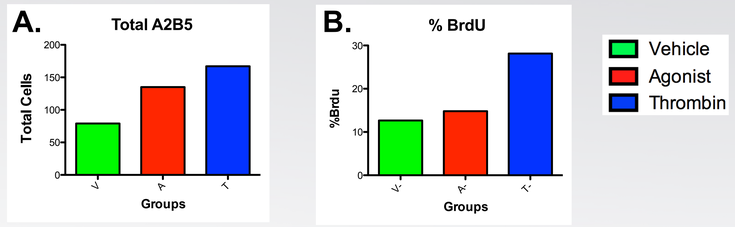


- **Vehicle:** PBS (phosphate buffered saline)
- **PAR1 Agonist (TFLLR):** Synthesized peptide that selectively activates protease activated receptor 1
- **Thrombin**: Serine protease, found in blood. Endogenous activator of PAR1, but can activate the other PARs as well. 1000X more potent than TFLLR

A.) Immunoflourescence was used to identify proliferating OPCs. Example of a non-proliferating OPC vs. a proliferating OPC. Proliferating OPCs were identified by bright red BrdU+ nuclei surrounded by green speckled A2B5 staining.

B.) Activation of PAR1 directly on OPCs does not increase proliferation.

Fig.3: PAR1 Activated Microglia Promote Survival and Proliferation of OPC Cultures

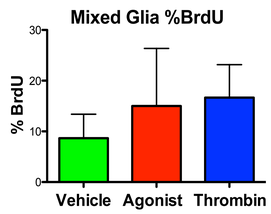


"Non-contact" co-cultures grown in transwell inserts, allowing for microglia secretions to indirectly affect OPCs.

A.) PAR1 activated microglia in transwells increased OPC survival. Indicating a possible protective role for PAR1 activated microglia.

B.) Thrombin activated microglia increased OPC proliferation.

Fig.4: PAR1 Activation in Mixed Cultures Does not Increase OPC Proliferation



PAR1 activation in mixed cultures (OPCs, microglia, and astrocytes all together) did not show a robust effect in OPC proliferation.

Summary & Future Directions

- Different types of PAR1 stimulation promotes differences in microglia activation.
- Direct activation of PAR1 on oligodendrocyte progenitor cell cultures does not affect proliferation.
- Activating PAR1 on all glial cells at the same time in mixed glial cultures also does not change OPC proliferation.
- **Factors from PAR1 activated microglia that are not in contact with OPCs increase OPC proliferation, and survival.**
- **PAR1 may be playing a role in promoting OPC proliferation through activated microglia.**
- **This makes PAR1 a potential target for the development of treatments for tissue repair after spinal cord injury.**
- ***Follow up study***: Analyze the phenotypes of PAR1 agonist vs. thrombin activated microglia in culture and *in vivo*.